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Review

Enhancing efficiency and precision in CRISPR genome editing for plants using computational tools

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ABSTRACT

Integrating computational tools into CRISPR-Cas9 genome editing has significantly enhanced the precision and efficiency of plant genetic modifications. This review explores bioinformatics resources' development, functionality, and application in designing, optimizing, and analyzing CRISPR-based experiments. From initial discoveries of CRISPR arrays to their evolution as powerful gene-editing technologies, computational advancements have played a pivotal role in predicting guide RNA (gRNA) efficiency, minimizing off-target effects, and streamlining editing processes. This article highlights key web-based platforms, such as CHOPCHOP, CRISPOR, CRISPR-P, Benchling, and Deskgen, comparing their features for gRNA design and off-target prediction. Tools like TIDE and TIDER for downstream analysis for evaluating editing outcomes are also discussed. By leveraging bioinformatics, researchers can overcome the complexities of plant genomes, enhance experimental accuracy, and accelerate crop improvement initiatives. This review underscores the transformative impact of computational tools in improving the efficiency of CRISPR-Cas mediated genome editing technologies for sustainable agriculture.

Keywords: Computational platforms, Efficiency, Features, Limitations, Off-target effects Precision, sgRNA designing

The global population is projected to reach 10 billion by 2050 (FAO, 2017), presenting a significant challenge in sustainably feeding this burgeoning world population. Thanks to the Green Revolution and advances in plant breeding tools, the current crop yields are sufficient for much of the population. However, agricultural productivity is plateauing or even declining due to climate change and the shrinking availability of arable land. To meet future demand, a 60% increase in crop yields will be necessary (Springmann *et al.,* 2018). Enhancing agricultural productivity and sustainability is crucial, requiring urgent scientific and technological innovations to secure the global food supply for the future.

Genetic variation is fundamental to agricultural advancements, serving as the foundation of plant breeding efforts. The primary goal of plant breeding is to generate and utilize these variations to develop improved crop varieties. Over the extensive history of plant breeding, four key breeding techniques have been employed: conventional cross-breeding, mutation breeding, transgenic breeding and the most recent method, genome editing (Chen *et al.,* 2019) (Fig. 1).

Traditional cross-breeding, which entails the selective and deliberate crossing of plants to combine favourable traits through sexual recombination, and then selecting the plants with a desirable combination of traits (with or without the help of molecular markers) has historically been instrumental in enhancing agricultural productivity. The best shown example is the Green Revolution of the late 1950s, during which mutations in "dwarfing" genes were introduced into staple crops like wheat (*Triticum aestivum*) and rice (*Oryza sativa*), leading to high-yielding varieties (Khush, 2001). However, this method is limited by the genetic diversity present in the plants used as parents, restricting its potential when working with elite germplasms comprising low variability (Gao, 2021).

Mutation breeding, on the other hand, expands genetic variation by inducing random mutations using chemicals or radiation. While this approach broadens the genetic pool, the changes are not directed and identifying the rare mutant plants with desirable traits from a vast population of mutagenized individuals is both labor-intensive and time-consuming (Holme *et al*., 2019).

A major advancement in plant breeding came with the development of transgenic breeding, in which genes from other organisms are introduced into crops to enhance traits such as yield, pest and disease resistance, and nutritional quality. However, the widespread adoption of genetically modified crops has been limited due to regulatory constraints and public concerns regarding their safety. Additionally, the random integration of foreign DNA into plant genomes presents challenges in precision breeding (Gao, 2021).

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More recently, precise modification of genomic regions (commonly referred to as genome editing) through techniques such as meganucleases, zinc finger nucleases (ZFNs), and TALENs (Transcription Activator-Like Effector Nucleases)-which are all based on the use of site-directed nucleases (SDNs)-has addressed many of the limitations in inducing desirable changes in elite plant breeding lines. These modifications enable improvements in key agronomic traits such as yield, stress tolerance, and disease resistance. Notable examples include the reduction of phytate content in corn using ZFNs (Shukla *et al.,* 2009) and the creation of fragrant rice by disrupting the OsBADH2 gene using TALENs, which resulted in the synthesis of 2-acetyl-1-pyrroline (2AP), a key fragrance compound (Shan *et al.,* 2015) (Fig.1).

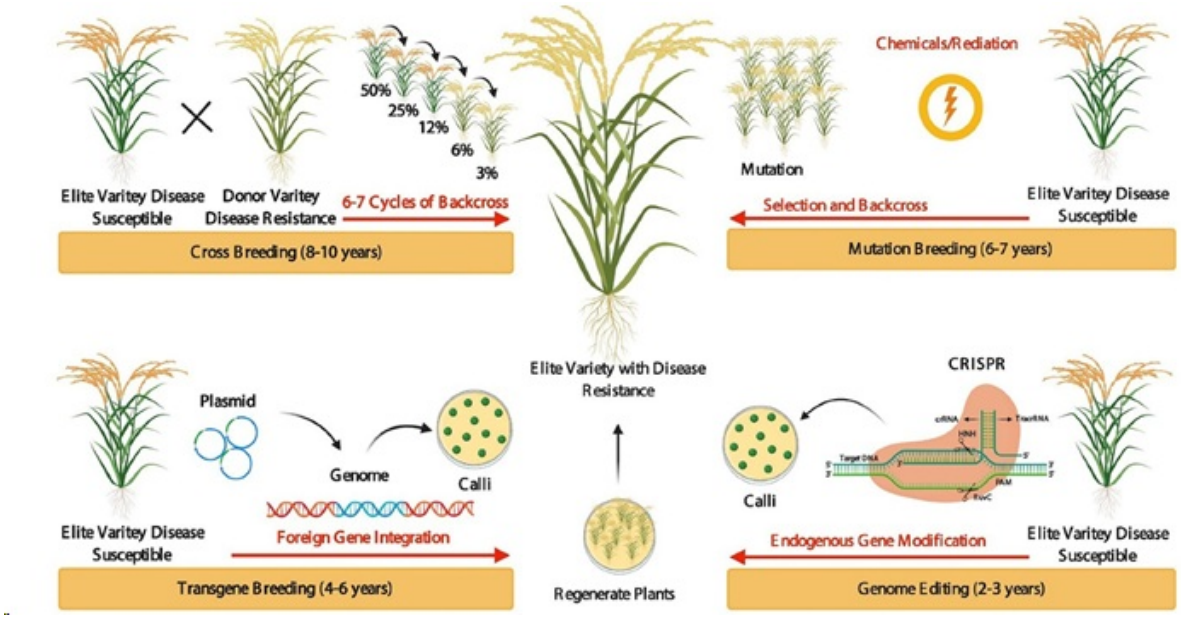

Fig. 1. Plant breeding approaches to improve desirable characters/traits (e.g. disease resistance) in an elite variety

In cross breeding the time required could be 8-10 years and still the linkage drag cannot be completely avoided. In mutation breeding, traits are imparted through mutations, either using the chemical of physical mutagen, and the process might take 6-7 years if the desired mutation for the target trait happens in the random mutagenesis process. Transgenic breeding is easy and well-known, improving crop traits within (4-6 years) by the exogenous transformation of genes into economically important elite varieties, but has to cleared through rigorous biosafety assessment process. In genome editing the target trait could be realized within 2-3 years and will be free of outside genetic material (adopted from the review article Wang *et al*., 2022 which is under Creative Commons Attribution license).

Genome editing technologies have transformed plant breeding by enabling precise and targeted genetic modifications and thus engineering the crop genomes with unprecedented accuracy to improve the desirable traits. Among these techniques, the emergence of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) mediated genome editing has revolutionized the fields of molecular biology, genetics, and biotechnology. Originally discovered as a component of the adaptive immune system in bacteria and archaea, CRISPR-associated systems have been repurposed for targeted genetic modifications across various organisms. Due to its precision, efficiency, and versatility, CRISPR has become an essential tool in scientific research and agricultural innovation.

Due to its simplicity, precision, and versatility, the CRISPR-Cas9 system has emerged as the most widely adopted tool among CRISPR-based genome editing technologies. It enables targeted modifications in a cost-effective and time-efficient manner, making it highly suitable for crop improvement programs. CRISPR-Cas9 has been successfully employed across several economically important oilseed and pulse crops to modify traits such as oil composition, disease resistance, flowering time, plant architecture, and herbicide tolerance. These advancements

not only contribute to yield enhancement and stress adaptation but also support the development of climate-resilient and nutritionally superior varieties. An indicative list of its applications in some oilseeds and pulses is presented in Table 1.

Advancements in CRISPR-based tools, including base editing and prime editing, have further enhanced the precision of genome modifications by allowing for specific nucleotide substitutions and controlled DNA insertions or deletions. The integration of CRISPR-Cas technologies with modern breeding strategies is set to play a crucial role in the next generation of crop improvement programs, facilitating the development of resilient, high-yielding, and nutritionally enhanced crops to meet global food security demands (Chen *et al*., 2023).

Table 1 Some examples of CRISPR-Cas9 applications in the genetic improvement of oilseed and pulse crops

Crop	Gene(s) Targeted	Trait Modified	Source
Cover Cress (*Thlaspi* spp.) [Pennycress]	Genes for erucic acid synthesis & seed-shattering	Reduced erucic acid to <2% and decreased seed shattering by ~90%	Innovative Genomics, 2024 (Innovative Genomics Institute)
Camelina (*Camelina sativa*)	TT8 (three homoeologs)	Created yellow-seed coat; boosted total fatty acids from ~32% to ~38% of seed weight and +21% oil yield	Cai *et al*., 2024
Soybean (*Glycine max*)	FAD2-2	Increased monounsaturated fatty acids in seed oil	Amin *et al*., 2021
Canola (*Brassica napus*)	BnWRKY70	Improved resistance to *Sclerotinia* stem rot	Sun *et al*., 2018
Peanut (*Arachis hypogaea*)	AhFAD2A, AhFAD2B	Elevated oleic acid content	Yuan *et al*., 2019
Camelina (*Camelina sativa*)	FAD2	Reduced PUFA; increased oleic acid	Morineau *et al*., 2017
Soybean (*Glycine max*)	SPL9	Optimized plant architecture	Bao *et al*., 2019
Oil Palm (*Elaeis guineensis*)	IFR, MT	Enhanced fungal disease resistance	Budiani *et al*., 2018
Flax (*Linum usitatissimum*)	EPSPS (T178I/P182A)	Conferred glyphosate resistance	Sauer *et al*., 2016

Originally discovered as a component of the adaptive immune system in bacteria and archaea, CRISPR-associated systems have been repurposed for targeted genetic modifications across various organisms. These modifications enable improvements in key agronomic traits such as yield, stress tolerance, and disease resistance as mentioned in Table 1. However, the success of application of CRISPR in plants requires addressing several biological, technical, and environmental challenges (Fig.2).

The figure illustrates the natural function of the CRISPR-Cas9 system as an adaptive immune mechanism in bacteria against viral infections. The process involves key stages; a) infection; b) spacer recognition; c) acquisition of the spacer sequence into CRISPR array d) reinfection; e) DNA hybridisation and cleavage (Figure drawn using Microsoft PowerPoint).

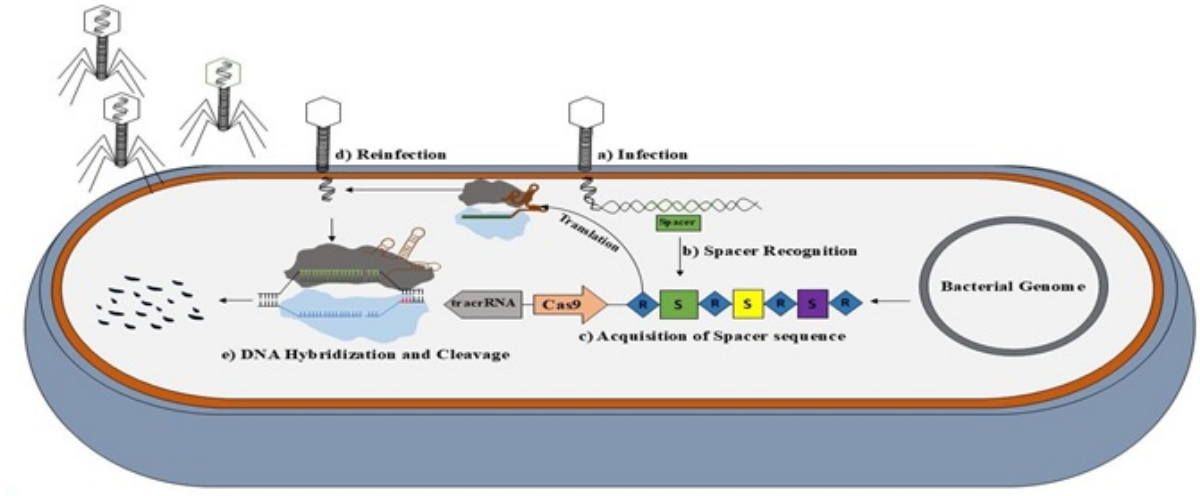

Fig. 2. Mechanism of CRISPR-Cas9 adaptive immunity in bacteria

In its natural context, the CRISPR-Cas9 system functions as an adaptive immune mechanism in bacteria and archaea, providing defence against mobile genetic elements such as viruses. This system records past infections within the prokaryotic genome in the form of CRISPR arrays, which consist of viral DNA fragments (spacers) separated by palindromic repeat sequences. During an immune response, these arrays undergo transcription and processing to generate CRISPR-associated RNA (crRNA), also called guide RNA (gRNA). The spacer region within the crRNA is complementary to the corresponding viral genomic sequence, known as the protospacer (Wiedenheft *et al.*, 2012; Bhaya *et al.,* 2011; Terns *et al.,* 2011) (Fig. 2). The crRNA binds to a trans-activating crRNA (tracrRNA), and forms a ribonucleoprotein (RNP) complex upon binding with the Cas9 nuclease for the functional activation of Cas9. The resultant complex is directed to the complementary sequence of crRNA, located adjacent to a trinucleotide called protospacer-adjacent motif (PAM) within the invading viral genome. The PAM sequence varies for different Cas9 systems eg., NGG in the case of Streptococcus pyogenes Cas9 (SpCas9), (Wiedenheft *et al.,* 2012; Bhaya *et al.,* 2011; Terns *et al.,* 2011). The PAM sequence, located immediately downstream of the protospacer, plays a critical role in distinguishing self from non-self DNA, thereby preventing autoimmune reactions within the host. Once the target sequence is recognized, the Cas9 nuclease induces a double-strand break (DSB) three base pairs upstream of the PAM, leading to the inactivation of the invading genetic element. Cas9 basically has two lobes - the NUC (nuclease) lobe and REC (recognition) lobe. NUC lobe has three domains - HNH domain that cleaves the target DNA strand, the strand that is complementary to the guide RNA, RuvC domain that cleaves the non-target DNA strand, and PAM interacting domain that facilitates binding of the complex the PAM on the target strand. The REC lobe contains multiple alpha helical domains that facilitate binding of Cas9 to RNA and DNA. The cartoon representation of the different domains is provided in Fig. 3.

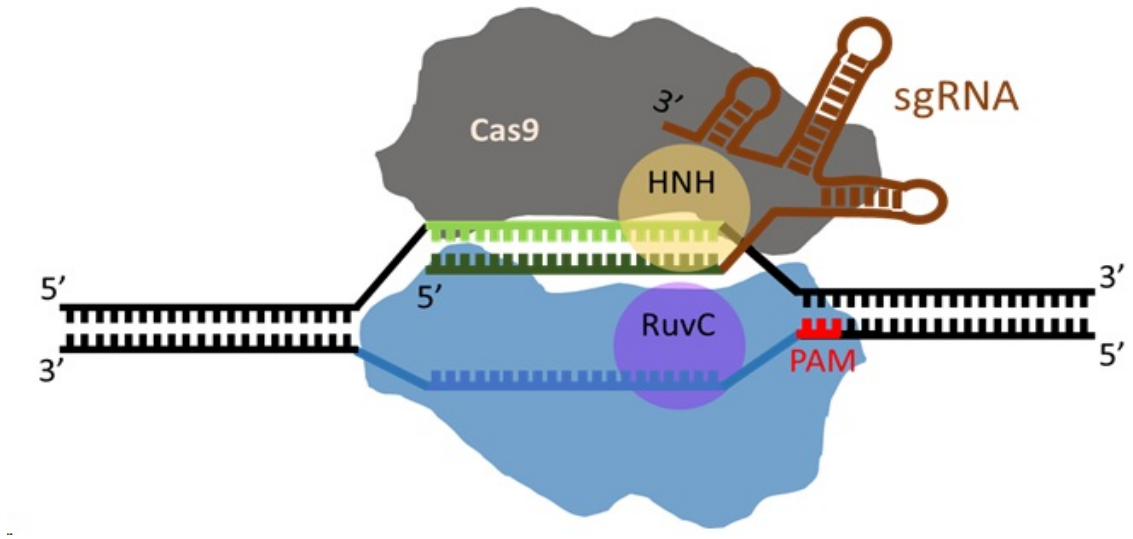

Fig. 3. CRISPR-Cas9 mechanism of DNA targeting and cleavage

The figure illustrates the CRISPR-Cas9 system, a gene-editing tool used for precise DNA modification. The Cas9 protein (black) is guided by a single-guide RNA (sgRNA, brown) to a complementary DNA target. The DNA sequence (green and blue) is recognized based on base-pair complementarity, with the protospacer adjacent motif (PAM, red) serving as a crucial recognition site. The two nuclease domains within Cas9, HNH (yellow) and RuvC (purple), induce site-specific double-strand breaks in the DNA, facilitating genome editing (Figure drawn using Microsoft PowerPoint).

After understanding the basic mechanism of DSB under natural conditions in the bacterial system, experiments were conducted to explore whether this process could be adopted under laboratory conditions. These experiments indicated that the CRISPR-Cas9 system can be streamlined by utilizing a chimeric RNA molecule known as single guide RNA (sgRNA), which combines the functions of crRNA and tracrRNA. This simplification allows efficient genome editing since both the sgRNA and Cas9 nuclease can be co-expressed from a single plasmid in the target cells. The system requires only these two essential components to

function (Jinek *et al.,* 2012; Cong *et al.,* 2013; Mali *et al.,* 2013).

By modifying the nucleotide sequence of the guide RNA (gRNA), researchers can direct Cas9 to virtually any genomic location, where it induces a site-specific double-strand break (DSB). The cell then repairs the break through one of two major pathways: nonhomologous end joining (NHEJ) or homology-directed repair (HDR) (Gan and Ling, 2022). NHEJ is an error-prone repair mechanism that often results in small insertions or deletions (indels) at the break site. While these mutations were initially considered random, recent studies suggest a degree of predictability. This approach is frequently used to introduce frameshift mutations, effectively disrupting gene function and leading to gene knockout (KO). Conversely, HDR enables precise genomic modifications by incorporating a homologous DNA template during repair. This pathway facilitates the introduction of point mutations or the insertion of specific gene fragments. The repair template can be supplied exogenously, such as through plasmids or single-stranded oligodeoxynucleotides (ssODNs), or it can be derived from endogenous sources like a sister chromatid (Mali *et al*., 2013).

The specificity of the CRISPR-Cas9 system is primarily determined by the complementarity between the guide RNA (gRNA) and the target DNA sequence. However, a major challenge in genome editing is off-target activity, where Cas9 induces unintended mutations at sites with partial sequence complementarity to the gRNA. Research has shown that certain genomic loci can tolerate multiple mismatches, increasing the risk of off-target effects (Hsu *et al*., 2013; Fu *et al.,* 2013; Pattanayak *et al.,* 2013). Moreover, even at fully complementary target sequences, cleavage efficiency may vary significantly due to sequence context and chromatin structure (Doench *et al.,* 2016; Wu *et al.,* 2014).

Another complication arises from the recognition of non-canonical protospacer-adjacent motifs (PAMs) by Streptococcus pyogenes Cas9 (SpCas9), such as NAG and NGA, which can contribute to off-target effects. To address these challenges, various modifications, including inducible expression systems, paired nickases (Cas9n), alternative Cas9 orthologs, rationally engineered Cas9 variants with enhanced specificity, and different delivery strategies, have been developed. However, despite these advancements, completely eliminating off-target activity remains an unsolved issue. As a result, one of the key challenges for researchers using CRISPR-Cas systems is designing experiments that achieve a favorable balance between on-target and off-target editing.

Computational tools play a crucial role in optimizing CRISPR experiments by aiding in gRNA design, assessing editing efficiency, and minimizing unintended (off-target) modifications. These tools can be broadly divided into three categories. The first category of computational tools focuses on predicting gRNA activity by evaluating on-target efficiency and potential off-target sites. These tools have evolved from basic sequence alignment methods to sophisticated scoring systems that consider several parameters such as mismatch tolerance, sequence features, chromatin accessibility, and genetic variation. The second category of tools utilizes observed biases in DNA repair mechanisms to predict the most probable outcomes of CRISPR-induced edits. This approach reflects the rapid progress in genome engineering and the expanding landscape of bioinformatics tools that support experimental design and data interpretation. The third class of computational tools is designed to analyze the sequencing data-both Sanger sequencing and next-generation sequencing (NGS)-to assess the accuracy and outcomes of genome editing experiments.

In plant genome editing, computational tools are vital for identifying optimal target sites, predicting off-target activity, and refining editing strategies. Advances in bioinformatics and machine learning have significantly enhanced sgRNA design, improved editing precision, and minimized unintended genetic modifications. Furthermore, these tools enable the analysis of complex plant genomes, including polyploid species, where genome editing presents additional challenges (Scheben *et al.,* 2017).

This section explores the role of computational tools in advancing CRISPR-based plant genome editing. By integrating these tools with experimental workflows, researchers can overcome key technical barriers, enhance editing efficiency, and accelerate the application of CRISPR technology in plant science and agricultural improvement.

From CRISPR's Origins to Modern Innovations: **The Pivotal Role of Bioinformatics and computational tools**

Bioinformatics has been instrumental in both the discovery and advancement of CRISPR-Cas as a gene-editing technology. The journey began in 1987 when Yoshizumi Ishino first identified unusual DNA repeats interspersed with spacer sequences in bacterial genomes. However, due to the lack of advanced bioinformatics tools at the time, he was unable to determine their function or identify conserved domains across different genomes (Ishino *et al.,* 1987).

The development of genome sequencing databases and bioinformatics tools, such as BLAST, revolutionized the field. In 2005, Francis Mojica utilized these resources to reveal that the mysterious spacer sequences within CRISPR arrays were not of bacterial origin but belonged to foreign

genetic elements, such as viruses (Mojica *et al.,* 2000). Subsequent bioinformatics analyses confirmed that these spacers were derived from bacteriophages, leading to the realization that the CRISPR-Cas system might be functioning as an adaptive immune defense in bacteria, allowing them to recognize and combat viral infections (Mojica *et al.,* 2005).

Building on this foundation, Jennifer Doudna and Emmanuelle Charpentier, who were awarded the 2020 Nobel Prize in Chemistry, refined the CRISPR-Cas system by engineering CRISPR-derived RNA (gRNA) to direct Cas nucleases to specific genomic sites. Their ground-breaking work established CRISPR-Cas9 as a precise RNA-guided gene-editing tool applicable to prokaryotic and eukaryotic cells alike.

Bioinformatics has continued to play a crucial role in expanding the CRISPR toolbox. Computational analyses have enabled the identification of CRISPR spacer sequences across diverse genomes and facilitated the discovery of various Cas nucleases, such as Cas12, Cas12a, and Cas10, each with unique targeting capabilities for DNA, RNA, and even proteins. These advancements have significantly enhanced the efficiency and versatility of CRISPR-based genome editing (Alkhnbashi *et al.,* 2020).

Thus, bioinformatics has been fundamental in the discovery, characterization, and evolution of CRISPR-Cas based technology, which has driven the transformation of the technology into one of the most powerful tools in modern genetic research and biotechnology.

Classification of CRISPR-Cas systems

Over the last twenty years, bioinformatics tools have been developed along two major directions: one focused on analyzing protein-coding genes and the other on predicting nucleic acid interactions at the genomic level. With the breakthrough discovery and implementation of the CRISPR/Cas9 system in both mammalian and crop genomes, computational methods initially designed for coding gene analysis have been repurposed to classify various Cas protein variants. This classification, achieved through heuristic optimization techniques and genomic annotations, has led to the identification of distinct CRISPR-Cas system types.

CRISPR-Cas systems are categorized into three major types: Type I, Type II, and Type III, each distinguished by a signature Cas protein. Cas proteins exhibit diverse domain architectures that define their functional mechanisms. These were further subdivided into multiple subtypes based on differences in gene composition and functional architecture. Later, additional CRISPR-Cas types, Type IV, V, and VI, were identified, further enriching the classification (Makarova *et al.,* 2020). The most widespread CRISPR-Cas system found in bacteria and archaea is the Type I CRISPR-Cas Systems (Cas3 as a Signature Protein). This system uses a Cascade (CRISPR-associated complex for antiviral defense) complex for recognizing and binding target DNA. The Cas3 protein, which contains a helicase and DNase domain, is responsible for degrading the target DNA after interference. Type I is further divided into seven subtypes (I-A to I-G), with variations in CRISPR array architecture and effector complex composition (Shmakov *et al.,* 2015; Zetsche *et al.,* 2015; Koonin *et al.,* 2017).

The Type II CRISPR-Cas Systems (Cas9 as a Signature Protein) is the simplest and most well-known system, exemplified by Streptococcus pyogenes Cas9, widely used in genome editing. This makes use of a single Cas9 protein that contains RuvC and HNH nuclease domains, both essential for precise DNA cleavage. Additionally this requires a tracrRNA for processing pre-crRNA into mature crRNA. The system is further divided into three subtypes (II-A, II-B, II-C) based on differences in tracrRNA processing and associated accessory proteins (Abudayyeh *et al.,* 2016; Koonin and Makarova, 2019).

The Type III CRISPR-Cas Systems (Cas10 as a Signature Protein) is capable of both DNA and RNA cleavage, allowing more flexible antiviral defense. The signature protein contains a Palm domain (for cyclic oligoadenylate production) and two DNA/RNA-targeting nuclease domains. It is associated with Csm (III-A) or Cmr (III-B) complexes, which cleave RNA rather than DNA. The system is unique in activating non-specific immune responses, such as cyclic oligoadenylate signaling to degrade viral RNA.

A relatively rare and less characterized system is the Type IV CRISPR-Cas Systems (Cas5 as a Signature Protein). The system lacks adaptation modules, suggesting dependence on other CRISPR types for spacer acquisition. It uses Cas5 instead of Cas3 or Cas9 for target cleavage (Fig. 4).

Type V CRISPR-Cas Systems (Cas12 as a Signature Protein) is a single-effector CRISPR system with diverse genome-editing applications. Cas12 proteins (formerly known as Cpf1, C2c1, C2c3) contain a single RuvC-like nuclease domain, capable of both double-stranded and single-stranded DNA cleavage. The system exhibits collateral activity, degrading nearby nucleic acids after target recognition. It includes subtypes V-A, V-B, and V-C, each showing variations in processing and targeting mechanisms.

Type VI CRISPR-Cas Systems (Cas13 as a Signature Protein) is specialized in RNA targeting rather than DNA cleavage. The Cas13 protein contains two HEPN (Higher

Eukaryotes and Prokaryotes Nucleotide-binding) domains, responsible for RNA degradation. It is known for its collateral RNase activity, making it useful for RNA-targeted applications like viral diagnostics. Further, it includes subtypes VI-A, VI-B, and VI-C, differing in accessory proteins and target specificity (Makarova *et al.,* 2002; Abudayyeh *et al.,* 2016; Koonin and Makarova, 2019).

Advancements in comparative genomics and metagenomic analysis of archaeal, bacterial, and viral genomes have expanded the number of known Cas protein families to 27, with ongoing discoveries continuously enriching this classification. As new variants emerge, bioinformatics remains essential for understanding Cas protein diversity, functional mechanisms, and potential applications in genome editing and therapeutic interventions.

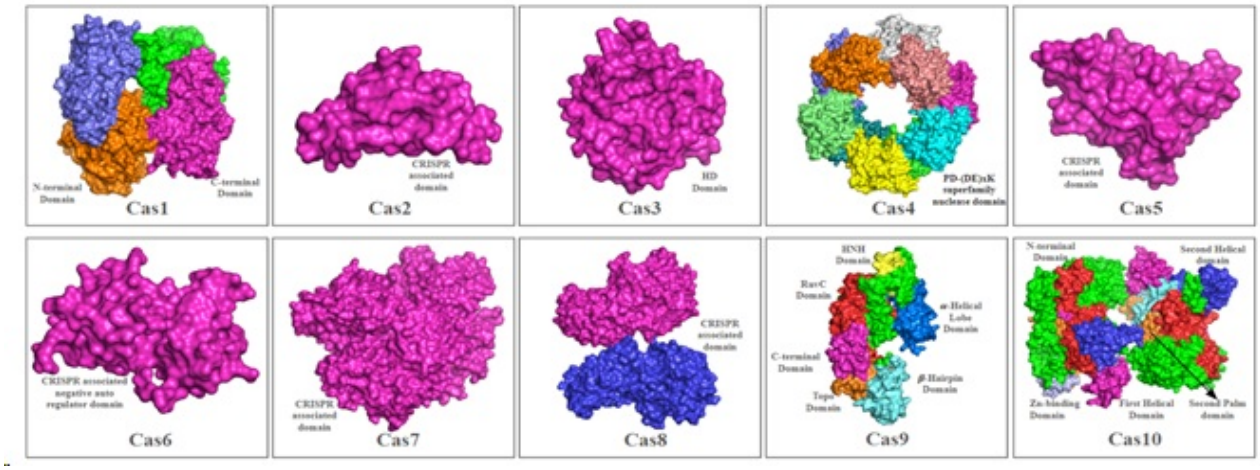

Fig. 4. Various CAS nucleases and their respective domains

Tools harnessed to streamline CRISPR/Cas system prediction

Several bioinformatics tools have been developed for the identification and characterization of CRISPR arrays. These tools primarily rely on recognizing repeat-spacer structures, leveraging sequence similarity, and refining predictions through filtering algorithms. The section below is a comparison of four widely used tools: CRISPRFinder/CRISPRCasFinder, PILER-CR, CRISPR Recognition Tool (CRT), and CRISPRDetect.

1. CRISPRFinder & CRISPRCasFinder

Functionally, they are the most widely used tools for identifying CRISPR arrays based on direct repeat (DR) sequence similarity. They make use of an enhanced suffix array via the Vmatch tool to efficiently detect putative repeats in large genomic datasets. They filter candidate repeats based on length (23-55 nt), repeat similarity (>80%), and relative offset (0.6-2.5 times repeat size). Further, incorporates MUSCLE, multiple sequence alignment, for refining predicted spacer sequences. The two major advantages of the system being: 1. High sensitivity in detecting CRISPR arrays due to advanced filtering and 2. Incorporates truncated repeat search to enhance prediction accuracy. However, these systems may produce false positives in repetitive genomic regions. They also do not directly predict associated Cas proteins; require additional tools for that.

2. PILER-CR

This tool identifies CRISPR repeats using local self-alignment searches within a genome. It introduces the concept of a pile (a set of bases covered by local alignments) to detect repetitive elements. Constructs a connectivity graph, where piles are nodes and alignments are edges, to refine candidate CRISPR arrays. Further, filters partial matches and merges arrays with highly similar (>95%) repeat sequences. This tool is effective for detecting short, imperfect CRISPR repeats, and it provides a graphical representation of repeat connectivity. However, the tool is sensitive to sequence conservation levels-if repeat conservation is below 90%, data may be discarded. Also, merging arrays with highly similar repeats might cause misidentifications.

3. CRISPR Recognition Tool (CRT)

Designed for low-memory usage, unlike suffix array-based methods, this tool uses a sliding window approach to scan DNA sequences for repetitive elements. It identifies exact matches within user-specified repeat and spacer length ranges. Extends repeat sequences by allowing a user-defined fraction of mismatches. Advantages of the tool include, Platform-independent Java implementation, available as both CLI and GUI; Customizable parameters for repeat length, spacer size, and mismatch tolerance, and More memory-efficient than tools like CRISPRFinder. The tool is less sensitive than suffix array-based methods and lacks advanced sequence alignment-based refinement, and therefore leads to lower specificity.

4. CRISPRDetect

This tool works on a five-step detection process including regular expression searches and repeat refinement. It uses pattern-based identification (?3 repetitions of an 11 bp sequence) and filters out tandem repeats by aligning candidate spacers and checking for excessive similarity. Further, extends repeats based on 75% identity and searches for missing repeats in flanking regions. It is available as both a Perl command-line tool and a web server, and it is a more refined filtering approach compared to CRT, reducing false positives. The requirement of a Linux environment for command-line use limits it. And also, relies on a fixed threshold for repeat extension, which may not generalize across diverse genomes.

Each tool has strengths and weaknesses, making them suitable for different cases and contexts. The choice of tool depends on the specific genomic dataset and computational resources available. Integrating multiple tools often improves the accuracy of CRISPR-Cas system annotation.

Key Steps in Designing a CRISPR-Cas Experiment

The design and execution of a CRISPR-Cas experiment require meticulous planning to ensure high precision, minimal off-target effects, and reproducibility. This process follows a systematic workflow that includes selecting an appropriate target, designing guide RNAs (gRNAs), choosing a suitable CRISPR-Cas system and delivery method, validating editing efficiency, and conducting downstream analyses.

1. Defining the Experimental Objective

The first and most critical step in designing a CRISPR-Cas experiment is establishing the specific objective. CRISPR-Cas technology can be utilized for various genetic modifications, including Gene knockout (KO) by introducing insertions or deletions (indels) to create loss-of-function mutations. Gene knock-in where precisely sequences are inserted into the genome via homology-directed repair (HDR). Base editing approach uses the modified Cas enzymes (e.g., Cas9-nickase fused to a deaminase) to introduce targeted nucleotide changes without creating double-strand breaks (DSBs). Prime editing utilizes a Cas9-nickase fused to a reverse transcriptase for precise sequence modification. Epigenetic modifications and transcriptional regulation where catalytically inactive dCas9 (dead Cas9) is fused to transcriptional activators (e.g., VP64) or repressors (e.g., KRAB) are employed to modulate gene expression. Clearly defining the experiment's goal ensures the appropriate selection of components, such as the CRISPR-Cas variant and editing strategy.

2. Target Gene Selection and Sequence Analysis

Once the objective is established, the gene of interest (GOI) is selected. A high-confidence reference sequence is crucial, obtained from well-annotated genomic databases such as: NCBI GenBank, Ensembl or UCSC Genome Browser. The selection process involves identifying functional regions such as exons, conserved domains, or regulatory sequences, ensuring that the intended modification produces the desired biological outcome while avoiding unintended transcript variations.

3. Guide RNA (gRNA) Design and Optimization

The efficiency and specificity of CRISPR-mediated genome editing largely depend on the guide RNA (gRNA) sequence. Key factors to consider when designing gRNAs include their proximity to a Protospacer Adjacent Motif (PAM), on-target cleavage efficiency, minimization of off-target effects, and strategic selection of the target region. For gene knockout (KO) experiments, it is advisable to target functionally relevant exonic sequences while avoiding repetitive elements and known single-nucleotide polymorphisms (SNPs), which may compromise targeting accuracy or efficacy (Doench *et al.,* 2016; Tsai and Joung, 2016).

To identify highly efficient sgRNAs, both *in vitro* and in-cell validation methods are employed. *In vitro* methods, such as *in vitro* cleavage assays and electrophoretic mobility shift assays (EMSAs), help assess the interaction between Cas9-sgRNA complexes and DNA substrates. Meanwhile, in-cell assays like the T7 Endonuclease I assay, Surveyor assay, Sanger sequencing followed by TIDE/ICE

analysis, and next-generation sequencing (NGS) are widely used to evaluate editing efficiency, mutation types, and specificity within the cellular context (Brinkman *et al.,* 2014; Tsai and Joung, 2016).

4. Selection of CRISPR-Cas System

The choice of Cas protein depends on the experimental application: The most commonly used Cas variant, SpCas9, is suitable for general genome editing, while other variants such as Cas12a (Cpf1) offer distinct advantages such as a T-rich PAM sequence and staggered cuts. Cas13 is employed for RNA editing, while catalytically inactive dCas9 is fused to transcriptional regulators for gene activation or repression. Additionally, base editors and prime editors provide precise nucleotide modifications without generating double-strand breaks, making them ideal for applications requiring single-nucleotide changes.

5. Construct Assembly and Vector Selection

After selecting the appropriate Cas system, the next step involves constructing the CRISPR-Cas delivery system. This can involve cloning the gRNA sequence into vectors containing the Cas protein and selectable markers or preparing synthetic gRNAs and ribonucleoprotein (RNP) complexes for direct delivery. Vector selection depends on experimental requirements, with plasmid-based expression suitable for stable cell lines, virus (such as TSV, TRV, PEBV, BSMV etc) based vectors used for efficient gene delivery in non-dividing cells, and RNP-based delivery preferred for transient genome editing with minimal cellular toxicity.

6. Delivery Method Selection

CRISPR-Cas constructs must be effectively delivered into the target cells or organism, with the method dependent on cell type. Common delivery approaches include electroporation for mammalian cells, microinjection for embryos, Agrobacterium-mediated transformation or particle bombardment for plant systems.

7. Validation of Genome Editing Efficiency

Once CRISPR-Cas constructs are introduced into cells, assessing genome editing efficiency is essential. Various assays are employed to validate editing outcomes, including T7 endonuclease I (T7E1) and Surveyor assays for detecting indels, as well as deep sequencing techniques such as Sanger sequencing or Next-Generation Sequencing (NGS) for high-resolution verification of modifications. In cases of gene knock-in, additional validation techniques such as junction PCR and Southern blotting may be necessary to confirm precise insertions.

8. Functional Analysis of Edited Cells or Organisms

To validate the biological impact of genome editing, researchers commonly perform gene expression analysis using qPCR (Livak and Schmittgen, 2001), assess protein changes via techniques like Western blot (Mahmood and Yang, 2012), and evaluate phenotypic traits depending on the intended outcome (Manghwar *et al.,* 2019).

9. Optimization and Reproducibility

After initial validation, optimization of experimental conditions ensures reproducibility. Hence, the experiment is optimized by testing multiple gRNAs, refining off-target predictions, and repeating the experiment with independent clones or biological replicates. Computational tools are used to refine off-target predictions and improve overall editing efficiency.

10. Data Analysis and Documentation

The final step involves comprehensive data analysis and proper documentation of all experimental procedures, including gRNA sequences, transformation efficiency, sequencing results, and phenotypic observations. Statistical analysis is performed to ensure the reliability of the results, and sequencing data may be deposited in public repositories such as NCBI, ENA, or GEO for transparency and future reference.

A well-structured CRISPR-Cas experiment involves precise target selection, rational guide RNA design, appropriate delivery strategy, and rigorous validation. The integration of bioinformatics tools, experimental optimization, and robust data analysis ensures reliable and reproducible genome editing outcomes, enabling advancements in genome editing. In the next section, we will discuss the tools that help in each of these steps.

How to choose which gene to edit?

Computational tools play a crucial role in narrowing down target genes for editing within large gene families, such as the MLO (Mildew Locus O) genes responsible for powdery mildew resistance. These tools facilitate the identification and characterization of gene family members, enabling researchers to pinpoint the most relevant candidates for editing. This is illustrated better by considering the MLO as a target gene for genome editing

in sunflower. Genome annotation software helps catalogue all the HaMLO homologs in the sunflower genome, while transcriptome analysis using RNA-seq data or qPCR analysis of the expression of MLO genes, at selected stages of powdery mildew development, highlights the homologs that are highly expressed in tissues susceptible to infection. Functional predictions derived from domain analysis and phylogenetic relationships with the homologs of MLO genes that are already implicated in powdery mildew susceptibility in other crops, help identify critical HaMLO genes that might contribute to mildew susceptibility in sunflower. Furthermore, computational platforms for network analysis provide insights into gene co-expression and protein-protein interactions, revealing the broader functional context of each homolog (Wang *et al.,* 2023). Tools designed for CRISPR/Cas9 editing further refine the selection by predicting off-target effects, ensuring precision in gene-editing experiments. Additionally, in silico simulations, such as mutagenesis and protein modelling, predict the functional consequences of specific edits, while the crop modelling tools (APSIM, DSSAT etc) estimate the impact of gene modifications on resistance and yield. By integrating these computational approaches, researchers can efficiently select specific MLO genes for editing, accelerating the development of crops with enhanced resistance to powdery mildew.

Designing Guide RNA (gRNA) for CRISPR-Cas Genome Editing

The efficiency and specificity of guide RNA (gRNA) are critical parameters that determine the success of CRISPR-Cas-mediated genome editing. Efficiency refers to the ability of a single-guide RNA (sgRNA) to accurately target and direct the Cas enzyme to the intended genomic locus, typically quantified as the percentage of cells successfully edited. Specificity, on the other hand, ensures that the genome editing events are precise and do not induce unintended modifications at off-target sites.

The CRISPR-Cas9 system is capable of cleaving any target site that precedes a specific Protospacer Adjacent Motif (PAM) sequence. However, the distribution and variability of PAM sites across the genome pose challenges for achieving precise editing. In some instances, the presence of non-canonical PAM sequences can contribute to off-target effects, particularly when sequence similarity exists between the intended target site and other loci in the genome. Such off-target modifications are primarily attributed to the tolerance of nucleotide mismatches between the crRNA component of sgRNA and the genomic target. Based on specificity and efficiency, sgRNA molecules can be broadly classified into two categories: efficient sgRNAs, which exhibit high target specificity with minimal off-target effects, and non-efficient sgRNAs, which have lower specificity and a higher probability of off-target interactions. The ability to design highly efficient sgRNAs is therefore a crucial step in ensuring the accuracy of genome editing.

The efficiency of an sgRNA is largely influenced by both its unique nucleotide composition and its secondary structural conformation. While nucleotide sequence preferences for sgRNAs have been well-documented in animal systems, similar trends have not been extensively observed in plant species (Zhang *et al.,* 2019). One key determinant of sgRNA efficiency is its GC content, which has been shown to play a pivotal role in target recognition and cleavage efficiency. A study by Liang *et al.* (2016) analyzed a large set of sgRNAs and found that approximately 97% of functionally validated sgRNAs contained a GC content within the range of 30-80%, suggesting that this range is optimal for efficient target recognition.

Beyond nucleotide composition, the secondary structure of sgRNA is another critical determinant of its functionality. Functional sgRNAs typically exhibit three conserved stem-loop structures, particularly the intact Repeat and Anti-Repeat (RAR) regions along with stem loops 2 and 3. These structural elements are essential for proper sgRNA processing, as the RAR region facilitates crRNA maturation via RNase III, enabling the Cas9 protein to initiate DNA cleavage at the target site. The secondary structure of sgRNA is also influenced by internal base pairing (IBP) within the guide sequence, which can interfere with target recognition. Empirical analyses suggest that functional sgRNAs tend to maintain a total base pairing (TBP) score of no more than 12 base pairs, with a conservative base pairing (CBP) threshold of seven and an IBP limit of six base pairs (Xie and Yang, 2013).

In conclusion, the design of efficient sgRNAs for precise genome editing requires careful consideration of multiple parameters, including GC content (optimal range: 30-80%), secondary structural integrity (RAR and stem loops 2 and 3), and base pairing constraints (TBP $\leq$12, CBP $\leq$7, IBP $\leq$6). Understanding these factors enables researchers to optimize gRNA selection, thereby improving editing efficiency while minimizing off-target effects. As CRISPR-Cas technology continues to evolve, the integration of computational tools for sgRNA design will further enhance the precision and applicability of genome editing across diverse biological systems.

To facilitate designing of efficient sgRNAs, there are different established algorithms. The development of computational algorithms for the design of single-guide RNAs (sgRNAs) has significantly improved the specificity

and efficiency of CRISPR-Cas9 genome editing. One of the earliest studies in this area was conducted by Hsu *et al.* (2013), who characterized the targeting specificity of Streptococcus pyogenes Cas9 (SpCas9) in human cells. They evaluated over 700 sgRNA variants, assessing insertion-deletion (indel) mutation rates at both target sites and predicted off-target loci. Their findings demonstrated that Cas9 tolerance for mismatches between the sgRNA and the target sequence varied depending on mismatch distribution, position, and frequency. Furthermore, they observed that modulating the concentrations of Cas9 and sgRNA could minimize off-target effects. Based on these insights, Hsu *et al.* (2013) also proposed a set of web-based tools to assist in target sequence selection, validation of sgRNA specificity, and off-target prediction.

To enhance the specificity of Cas9-mediated editing, four key constraints were proposed for identifying off-target genomic sequences. First, off-target sites should not be followed by a PAM sequence (5' NGG or 5' NAG), as these motifs are crucial for Cas9 recognition. Second, sequence similarity between the target and potential off-targets should be minimized, with a preference for sgRNAs that exhibit at least three mismatches from unintended genomic loci. Third, off-target sequences should contain more than two mismatches in the PAM-proximal region, as this region is critical for Cas9 binding. Finally, when mismatches occur, they should be either consecutive or positioned less than four bases apart. Using these criteria, a frame-scoring algorithm was developed to compute potential off-target sites and assess sgRNA validity, which was later implemented as a computational tool known as the CRISPR Design Tool (MIT CRISPR tool), available at www.genome-engineering.org. This tool generates specificity scores ranging from 0 (highly non-specific) to 100 (highly specific), with a score above 50 indicating a well-designed sgRNA with minimal off-target binding.

In 2014, Doench *et al.* (2014) further refined sgRNA design methodologies by selecting endogenous human and mouse genes to generate a comprehensive set of sgRNAs targeting coding sequences (CDS). They assessed the ability of these sgRNAs to produce null alleles, allowing for functional gene knockout. Their study identified several sequence-dependent features that influenced SpCas9 activity, leading to the development of a predictive model for sgRNA activity. Analysis of 1,841 sgRNAs revealed significant nucleotide preferences at key positions: guanine was strongly favored at position 20 (adjacent to the PAM), cytosine was disfavored at position 3 but preferred at position 16, and adenine was preferred at the central region of the sgRNA. Additional sequence determinants included nucleotide composition at the PAM-proximal variable site. These sequence-based parameters were integrated into a predictive model using a logistic regression classifier, which ranked sgRNAs based on their predicted activity. The ranking system assigned a "percentage rank" score, where the least efficient sgRNAs were assigned a value of 0 and the most efficient ones approached a value of 1. The resulting guidelines for sgRNA selection were collectively termed "Rule Set 1" and have since been widely adopted for CRISPR-based genome editing (Yang *et al.,* 2014).

Building on these findings, Doench *et al.* (2016) introduced further refinements to improve sgRNA design, optimizing both on-target activity and off-target avoidance. Their revised approach, termed "Rule Set 2," incorporated additional parameters such as sgRNA target location within the gene, position-independent nucleotide counts, and localized thermodynamic properties. Unlike "Rule Set 1," which focused primarily on sequence composition, "Rule Set 2" integrated machine learning models trained on large CRISPR datasets. These advancements enabled the development of highly optimized sgRNA libraries for human and mouse genomes, named Brunello and Brie, respectively. The introduction of "Rule Set 2" also extended predictive capabilities to CRISPR activation (CRISPRa) and CRISPR interference (CRISPRi) applications. Experimental validation of these libraries was conducted using well-established screening systems, ranking sgRNAs based on log2 fold changes in their abundance within plasmid DNA pools.

Despite these improvements, early predictive models for sgRNA design were limited by their reliance on a small number of genomic loci and a single Cas9 variant. To overcome these constraints, an in vivo library-on-library methodology was developed, allowing for simultaneous assessment of sgRNA activity across 1,400 genomic loci. This approach facilitated the development of more sophisticated predictive models, such as an elastic net regression algorithm, which integrated oligonucleotide design principles and nucleosome occupancy data to enhance sgRNA efficiency predictions. The model also incorporated position-dependent dinucleotide features, highlighting the importance of T and TT dinucleotide frequencies in determining sgRNA activity.

Beyond these advancements, additional computational tools have been developed to refine sgRNA selection. One notable example is CCTop (CRISPR-Cas9 Target Online Predictor), introduced in 2015, which prioritizes sgRNAs based on their off-target quality and predicted efficiency. By integrating multiple scoring criteria, CCTop has become a valuable resource for researchers seeking to balance specificity and efficacy in their CRISPR experiments.

Collectively, these studies and computational advancements have significantly enhanced the precision of sgRNA design, allowing for more accurate genome editing

with minimized off-target effects. As CRISPR technology continues to evolve, future improvements will likely incorporate deeper machine learning approaches and expanded datasets to further refine the sgRNA prediction models across diverse genetic backgrounds and Cas enzyme variants. It has been suggested that as there are several algorithms available for sgRNA designing, a researcher has to use multiple tools and then decide the efficient one by choosing those which are commonly listed under each of the tools (references)

Online available sgRNA-designing web tools

A wide range of computational tools is currently available to design precise sgRNA sequences for efficient genome editing. These tools play a pivotal role in selecting the most effective sgRNAs by predicting both on-target efficiency and off-target activity-the two critical parameters that influence the accuracy and success of CRISPR-based gene editing. The curation of highly efficient sgRNAs primarily depends on algorithms that evaluate the target site based on sequence features, thermodynamic properties, chromatin accessibility, and the potential off-target binding sites across the genome. Several web-based platforms have been developed to automate sgRNA design by integrating these prediction models. Here, we will discuss some widely used web tools, especially those used in plants that are still functional and actively maintained, offering comprehensive features for the design and evaluation of sgRNAs for various genome editing applications. Table 2 lists the comparative characteristic features of these tools.

Table 2. Characteristic features of the widely used web tools for designing sgRNA

Tool	Key Features	Off-target Analysis	Species Support	Advanced Editing Support	Limitations	Website/ Reference
CRISPR RGEN	Cas-Designer, Cas-Analyzer, Cas-Offinder; supports Cas9, Cas12a; cleavage site prediction; base/prime editing support	Cas-Offinder	Multiple model and non-model organisms	Base editing, Prime editing	Limited visualization of off-targets	http://www.rgenome.net
CHOPCHOP	Beginner & Advanced modes; primer design; visual guide layout; supports Cas9, Cas12a	Bowtie algorithm	Broad (many model & non-model species)	No	No secondary structure, Tm, or dimerization analysis	https://chopchop.rc.fas.harvard.edu
CRISPR-P	Plant-specific; secondary structure output; restriction sites display	Batmis algorithm	~20 plant genomes	No	No primer design or self-complementarity check; limited genome support	http://cbi.hzau.edu.cn/crispr
CRISPOR	Efficiency scoring (Doench 2016, Moreno-Mateos 2015); UCSC browser integration; primer design	Bowtie-based, ranked off-targets	>150 genomes	No	No secondary structure, Tm, or dimerization analysis	http://crispor.org
Benchling	Collaborative workspace; BLAST & Primer3 integration; dual sgRNA design	Alignment-based scoring	Numerous genomes (focus on model organisms)	Base editing, CRISPRi, CRISPRa	No structure, Tm, or dimerization info; basic off-target model	https://www.benchling.com/crispr
CRISPRdirect	Jellyfish k-mer algorithm; evaluates 20mer, 12mer, and 8mer; TTTT motif filter	k-mer match scanning	Multiple (human, rat, yeast, fly, rice, etc.)	No	No secondary structure, dimerization, or editing-type awareness	http://crispr.dbcls.jp
E-CRISP	SAE scoring (Specificity, Annotation, Efficiency); CpG island avoidance; Multi-CRISP for gene families	Bowtie2	Human, mouse, fly, worm, Arabidopsis, Leishmania, etc.	Multi-targeting	No Tm, GC%, or secondary structure; no primer design	http://www.e-crisp.org/E-CRISP/
CRISPR MultiTargeter	Finds common/unique sgRNAs; multiple sequence alignment	Uses Cas-Offinder (external)	Limited (human, fly, rat, Arabidopsis, worm)	No	No structure, primer design, or full off-target integration	http://multicrispr.net
CRISPick	CRISPRko, CRISPRa, CRISPRi; gnomAD SNP filtering; high-throughput design	Alignment-based + variant filtering	Human, mouse, rat	CRISPRko, CRISPRa, CRISPRi	No structure, Tm, or GC%; limited to 3 organisms	https://portals.broadinstitute.org/gpp x/crispick/public
Fly CRISPR	Designed for Drosophila; HDR-focused; large DNA insertions	Not detailed	Drosophila, some insects	Homology-directed repair	Limited to fly; no general applicability	http://flycrispr.molbio.wisc.edu
CASPER	On/off-target scoring independent of experimental conditions; multi-target and population analysis	Internal scoring algorithm	Not specified	Yes	No structure, Tm, or primer design	https://github.com/TrinhLab/CASPER

CRISPR RGEN

CRISPR RGEN is a comprehensive web server offering a suite of tools for designing, validating, and analyzing sgRNAs, including off-target prediction. Among its diverse functionalities, Cas-Designer is the core tool for sgRNA design, allowing users to input target sequences and select from a wide range of Cas protein options (e.g., Cas9, Cas12a, etc). This flexibility enables researchers to tailor their experiments to specific Cas preferences. The server boasts extensive genome data for numerous species, making it a valuable resource for both model and non-model organism studies. Beyond basic sgRNA design, Cas-Designer provides detailed information such as mismatch locations, sgRNA positioning, and importantly, the predicted cleavage site within the target sequence. For in-depth sgRNA analysis, CRISPR RGEN offers Cas-Analyzer, which evaluates various sgRNA properties. Furthermore, the platform extends its utility to advanced genome editing techniques by incorporating tools for designing and analyzing sgRNAs for base editing and prime editing. Off-target analysis is facilitated by Cas-Offinder, a robust tool that efficiently identifies potential off-target sites based on user-defined parameters. CRISPR RGEN distinguishes itself by its integration of diverse functionalities, from basic sgRNA design to complex editing analysis, all within a single, user-friendly web interface. While the tool offers a wide range of features, it may benefit from enhanced visualization of off-target sites and more detailed guidance for advanced editing techniques. Overall, CRISPR RGEN is a powerful and versatile platform that streamlines the CRISPR workflow, providing researchers with the necessary tools for accurate and efficient genome editing.

CHOPCHOP

CHOPCHOP (https://chopchop.rc.fas. harvard.edu/) is a versatile web-based tool designed for designing guide RNAs for both CRISPR and TALEN genome editing systems (Montague *et al.,* 2014). It accepts a wide range of input formats, including genomic regions, DNA sequences, gene names, and chromosomal coordinates, making it highly flexible for different types of experiments. The tool offers two distinct user modes: Optimized Mode for beginners and Advanced Mode for experienced users. The advanced mode allows users to target specific sub-regions of the gene, such as the 5' UTR, 3' UTR, and splice sites, which makes it particularly useful for fine-tuned gene regulation studies.

CHOPCHOP uses the Bowtie algorithm to search for off-target sites in the genome. Bowtie performs short-read alignments using the Burrows-Wheeler Transform (BWT), which enables fast and memory-efficient sequence searches. The identified target sites are scored based on their predicted efficiency, with higher scores indicating more efficient sgRNAs. The tool visually highlights the target sites according to their scores, making it easier for users to select the best candidate guides.

A significant advantage of CHOPCHOP is its additional functionalities, including primer design tools for amplifying the targeted region and downstream validation. It provides comprehensive information about sgRNA properties, such as GC content, self-complementarity scores, and efficiency scores based on the number of off-target sites and mismatches. Users can customize several parameters, including the size of primers, amplicons, sgRNA length, type of PAM sequence, primer melting temperature (Tm), and Cas protein type (Cas9 or Cas12a). Furthermore, the tool supports a wide range of species, making it a valuable resource for researchers working on both model and non-model organisms.

CHOPCHOP also provides an interactive graphical visualization showing the location of sgRNAs, designed primers, genomic regions (exons, introns), and restriction sites present in the input gene sequence. This feature makes it easier to interpret the distribution of guides along the target gene.

CHOPCHOP remains one of the most comprehensive sgRNA design tools available, offering multiple customization options and detailed visual outputs. Integrating secondary structure prediction and custom genome uploads would further enhance its usability and accuracy.

CRISPR-P

CRISPR-P is the first web-based sgRNA design tool specifically developed for plant species (Yang *et al.,* 2014) (http://cbi.hzau. edu.cn/crispr). It facilitates the identification of highly specific target sites for CRISPR-based genome editing across various plant genomes. The tool supports more than 20 plant species, including *Arabidopsis thaliana, Brassica napus, Oryza sativa, Chlamydomonas reinhardtii,* and Sorghum bicolor, making it a highly valuable resource for plant genome editing research.

CRISPR-P allows users to search for Cas9 target sites in both genome-wide and user-defined DNA sequences. The tool performs off-target site prediction by integrating "Off-target rules" and the Batmis algorithm (Basic Alignment Tool for Mismatches), a C/C++-based alignment program that efficiently identifies mismatches between sgRNAs and genomic sequences. The off-target sites are

visually displayed along with detailed mismatch information, making it easier to select highly specific sgRNAs. Each designed sgRNA is assigned a guide score based on its on-target efficiency and off-target specificity, enabling users to prioritize the best candidate guides.

One of the unique features of CRISPR-P is its ability to display restriction enzyme cutting sites for each designed sgRNA, which facilitates the experimental validation of genome editing events. Additionally, the tool provides the secondary structure of sgRNAs, which helps users assess the stability and folding properties of the guides-an important factor often overlooked by other sgRNA design tools.

CRISPR-P also lets users download the predicted sgRNA sequences along with their off-target information and restriction enzyme sites in tabular format. The tool's intuitive graphical output makes it easier to interpret the distribution of sgRNA target sites along the input sequence. CRISPR-P remains one of the most widely used sgRNA design tools for plants, especially due to its comprehensive off-target prediction, secondary structure analysis, and user-friendly interface. Further improvements, such as adding self-complementarity analysis, melting temperature calculations, and support for more plant genomes, would enhance its utility in plant genome editing research.

CRISPOR

The CRISPOR is a widely used web-based sgRNA designing tool for CRISPR/Cas9 genome editing (Concordet and Haeussler, 2018) (http://crispor.org/). It is a comprehensive platform that assists researchers in selecting highly efficient and specific sgRNA target sites by integrating both on-target and off-target activity scores. CRISPOR is compatible with more than 150 genomes, including widely studied model organisms such as *Homo sapiens, Mus musculus, Danio rerio, Drosophila melanogaster, Arabidopsis thaliana,* and many others, making it one of the most versatile CRISPR guide RNA design tools.

It predicts off-target effects by aligning each sgRNA against the entire genome and scoring mismatches using the Bowtie alignment algorithm. The off-target sites are ranked and highlighted based on their degrees of similarity to the target sequence, enabling researchers to select the most specific sgRNAs. CRISPOR also integrates various efficiency scoring algorithms, such as Doench 2016 and Moreno-Mateos 2015, to estimate the likelihood of successful genome editing.

One of the notable features of CRISPOR is its primer design functionality, which helps users generate primers for validating the editing outcomes using PCR-based methods. It also provides options for identifying restriction enzyme cutting sites, which can be used for genotyping the edited sequences. Additionally, the tool allows direct export of target sites to the UCSC Genome Browser, enabling users to visualize the genomic context of their selected sgRNAs. CRISPOR provides output data in tabular format with various parameters, including: Target sequence, PAM sequence, Off-target mismatches, On-target and off-target activity scores, Efficiency prediction scores and Primer pairs for validation.

CRISPOR remains one of the most widely used CRISPR sgRNA design tools due to its large genome database, comprehensive off-target prediction, and integration with external genome browsers. Adding features such as self-complementarity analysis, secondary structure prediction, and sgRNA-specific primer design would significantly enhance its functionality and improve its applicability for genome editing experiments.

Benchling

Benchling is an advanced web-based platform that facilitates the design, management, and sharing of sgRNAs for CRISPR genome editing experiments (Pellegrini, 2016) (https://www.benchling.com/crispr). It is widely used by researchers in pharma, biotech industries, and academic labs due to its collaborative features that allow users to share experimental designs and results with team members. Benchling offers CRISPR sgRNA design for single and paired guide RNAs, supporting various genome editing applications such as gene knockout, base editing, and CRISPR interference (CRISPRi).

It designs sgRNAs based on several critical parameters, including target location, specificity, and on-target efficiency. The scoring system evaluates sgRNAs within seconds, displaying both on-target and off-target effects side by side to help users select the most efficient guides. This feature significantly reduces the time required for manual cross-referencing of candidate sgRNAs.

A unique feature of Benchling is its organization system, which allows users to tag, store, and organize potential sgRNAs in folders. These sgRNA sequences can be easily exported along with their scores and off-target information for further analysis in spreadsheet applications. The platform also provides built-in bioinformatics tools like Primer3 for designing primers and NCBI BLAST for aligning sgRNAs to reference genomes, making it a one-stop solution for CRISPR experiment planning.

Additionally, Benchling supports paired sgRNA design, enabling users to plan dual sgRNA experiments for creating larger deletions or performing targeted gene excisions. The platform also allows users to design sgRNAs for base

editing applications, making it highly versatile for different CRISPR-based modifications.

Benchling is a popular choice due to its user-friendly interface, collaborative workspace, and integration of various molecular biology tools. Further improvements, such as including secondary structure prediction and supporting more plant genomes, would enhance its utility in plant genome editing research.

CRISPR direct

This web tool is developed to facilitate the design of guide RNA (gRNA) sequences with reduced off-target effects, a crucial factor in CRISPR-based genome editing (Naito *et al.,* 2014) (http://crispr.dbcls.jp/). The tool aims to identify highly specific sgRNA target sites within genomic sequences of various organisms by minimizing the probability of unintended off-target cleavage. It employs the Jellyfish algorithm, a k-mer counting evaluation method that rapidly scans large genomic datasets to identify perfect matches between guide RNA sequences and target sites. The Jellyfish algorithm functions as a command-line program compatible with 64-bit Linux systems and processes DNA sequences provided in FASTA file format. The tool analyzes all potential sgRNA sequences from the input genomic data and provides detailed outputs in three primary columns - 20mer+PAM, 12mer+PAM, and 8mer+PAM. The 20mer+PAM column represents perfect matches between the entire 20-nucleotide gRNA sequence and the target DNA site, offering the highest specificity. The 12mer+PAM and 8mer+PAM columns display perfect matches limited to the seed regions (12 or 8 nucleotides adjacent to the PAM sequence), which are critical for CRISPR specificity. These outputs allow users to evaluate the likelihood of off-target binding, as shorter seed region matches are more prone to off-target effects.

Additionally, the tool provides important parameters for each sgRNA, including GC content, melting temperature (Tm), and the presence of TTTT motifs, which are undesirable as they may cause premature transcription termination. Sequences containing TTTT motifs are highlighted in gray and recommended to be avoided.

Despite its utility, the tool has several limitations that affect its comprehensive functionality. It does not assess self-complementarity of gRNAs, which is essential for predicting potential secondary structures that could hinder Cas9 binding or activity. Furthermore, the absence of secondary structure visualization prevents users from evaluating whether the designed gRNAs may fold into hairpin loops or other inhibitory conformations. The tool also does not consider the type of genome editing experiment (knockout, knock-in, or base editing) when designing guides, as it focuses solely on target region selection without accounting for repair outcomes. Moreover, primer design for sgRNA cloning or validation experiments is not provided, which could streamline downstream experimental workflows. The visualization of guide RNA positions across target sequences is also less intuitive compared to other advanced tools, making it difficult for users to interpret the spatial distribution of sgRNAs within genomic regions.

The tool remains a valuable resource for preliminary sgRNA design, particularly for organisms with available genomic datasets such as Homo sapiens, Rattus, Saccharomyces, Gallus, Rana, Drosophila, Bombyx, Helianthus annuus, and Oryza sativa.

E-CRISP

E-CRISP is a web-based tool developed to design sgRNA sequences for CRISPR/Cas-based genome editing (Heigwer *et al.,* 2015) (http://www.e-crisp.org/E-CRISP/). The tool identifies complementary target sequences ending with the 3' NG PAM motif, which is essential for Cas9 recognition. E-CRISP utilizes the widely used alignment tool Bowtie2 to search for potential off-target sites across the genome and provides a comprehensive list of sgRNA target sites along with detailed information regarding their genomic context. This tool not only assists in the initial design of sgRNAs but also enables the re-evaluation of CRISPR constructs to identify possible off-targets.

One of the unique features of E-CRISP is its ability to assess the genomic environment surrounding the target site, including the presence of exons, introns, and CpG islands. This feature helps in designing sgRNAs that are more likely to target functionally relevant regions of the genome while avoiding highly methylated regions, which could hinder CRISPR efficiency.

The designed sgRNAs are ranked based on the SAE scoring system, which integrates three key parameters: Specificity Score (S) - Measures the likelihood of off-target binding, Annotation Score (A) - Prioritizes target sites located within exonic regions and avoids repetitive elements or CpG islands, and Efficiency Score (E) - Predicts the cleavage efficiency of the sgRNA.

This ranking system improves the selection of highly specific and efficient sgRNAs while minimizing off-target effects. Additionally, E-CRISP offers a Multi CRISP feature that allows the design of sgRNAs capable of targeting multiple gene copies or paralogous genes within the genome. This makes the tool particularly useful for targeting gene families or regions with high sequence redundancy.

Despite its advantages, E-CRISP has several limitations. Similar to CRISPRdirect, the tool does not perform self-complementarity checks or secondary structure prediction, both of which are critical for predicting sgRNA folding patterns that may affect Cas9 binding. It also lacks primer design options and does not provide information about the melting temperature, GC content, or other physicochemical properties of the designed sgRNAs.

E-CRISP supports a wide range of model organisms, including *Homo sapiens, Mus musculus, Macaca mulatta, Arabidopsis thaliana, Brachypodium distachyon, Drosophila melanogaster, C. elegans, Leishmania, Chlamydomonas reinhardtii,* and *Neurospora crassa.* Despite its limitations, E-CRISP remains a valuable tool for designing sgRNAs for both single and multi-gene targeting, particularly in organisms with complex or repetitive genomes. However, integrating additional features like self-complementarity analysis, secondary structure visualization, and primer design modules would further enhance its functionality and usability.

CRISPR MultiTargeter

The CRISPR MultiTargeter is generally used for finding the common (highly similar) and unique sgRNAs in the input sequences corresponding to queries (Prykhozhij *et al.,* 2015) (http://multicrispr.net/). The multiple sequence alignment is used for common target search while the string comparison algorithm is used for identification of unique sequence(s) among all input sequences. The key point of this alignment approach is that it searches for the sequence that does not show pairing with other sequences. This tool also provides information about melting temperature (Tm) for DNA: RNA hybrid.

However, this tool does not predict off-targets and it has an additional tool named as Cas-OFFinder for this purpose. It lacks self-complementarity checks, secondary structure prediction, and primer design options. The tool supports only a limited range of species, including *H. sapiens, Drosophila, Arabidopsis, Rattus,* and *C. elegans.*

Despite these drawbacks, CRISPR MultiTargeter remains valuable for designing sgRNAs for paralogous genes and isoform-specific editing, but integrating off-target prediction and structure analysis would enhance its functionality.

CRISPick

CRISPick is a web-based tool developed by the Broad Institute for designing sgRNA sequences for various CRISPR-based genome editing applications, including CRISPR knockout (CRISPRko), CRISPR activation (CRISPRa), and CRISPR interference (CRISPRi) (https://portals.broadinstitute.org/gppx/crispick/public). It is an upgraded version of the earlier GPP sgRNA Design tool, offering a streamlined interface with improved sgRNA selection algorithms. The tool identifies target sites based on the 3' NGG PAM sequence and ranks the designed sgRNAs based on predicted on-target efficiency and potential off-target activity.

CRISPick integrates comprehensive genomic data to minimize off-target effects by using alignment algorithms. For human (GRCh38) genome designs, it incorporates gnomAD variation filtering, which deprioritizes sgRNAs that overlap known genetic variations such as SNPs and indels. This feature helps improve the reliability of sgRNA designs by reducing the risk of non-specific binding in polymorphic regions of the genome.

The tool supports sgRNA design for the CRISPR applications: **CRISPRko (Knockout): Induces double-stranded breaks for gene inactivation, CRISPRa (Activation):** Upregulates gene expression by recruiting transcriptional activators to the target site, and CRISPRi (Interference): Represses gene expression by blocking transcriptional machinery.

CRISPick provides ranked lists of sgRNAs based on their predicted specificity and efficiency scores. It also allows users to select multiple target genes at once, making it particularly useful for high-throughput CRISPR screening experiments.

However, despite its robust performance, CRISPick has several limitations. The tool only supports reference genomes for Homo sapiens, Mus musculus, and Rattus norvegicus, limiting its utility for researchers working on non-model organisms. Additionally, like many other web-based sgRNA design tools, CRISPick does not offer features such as secondary structure prediction, self-complementarity analysis, or primer design for sgRNAs. It also does not provide critical sequence properties such as GC content, melting temperature (Tm), or warnings about repetitive motifs like TTTT sequences, which could impair sgRNA functionality.

Despite these limitations, CRISPick remains a widely used tool due to its integration of comprehensive genomic datasets and its versatility across multiple CRISPR applications. However, expanding its support to more organisms and incorporating additional features like structure prediction and primer design would significantly improve its utility for genome editing research.

Fly CRISPR

Fly CRISPR is an exclusively available web tool for utilization of the CRISPR-Cas9 system in Drosophila and

some other insects based upon homology-directed repair approach. It facilitates the incorporation of large DNA sequences with the aim of genome engineering (Bassett and Liu, 2014) (http://fycrispr.molbi o.wisc.edu/).

CASPER

CRISPR-associated software for pathway engineering and research (CASPER) implements an algorithm for prediction of both on- and off-target activities irrespective of experimental conditions of sgRNA. For on-target activities, the formulated scores are given by dividing CRISPR scan score with penalty score (Mendoza and Trinh, 2018) (https://github.com/TrinhLab/CASPER). This scoring method depends only upon target sequence and hence does not rely on experimental conditions. This tool has expanded functions for multi-targeting analysis and multipopulational analysis.

Post-Editing Analysis Tools for CRISPR Genome Editing

After CRISPR-based genome editing, verifying and analyzing genetic modifications is crucial to confirm successful edits, evaluate efficiency, and detect unintended mutations (off-target effects or indels). Several computational and web-based tools facilitate sequence analysis, mutation detection, editing efficiency assessment, and off-target validation. Here a brief account of these tools including their key features and the limitations, is given to provide a comprehensive work flow for the genome editing exercise (Table 3).

Table 3 ……

Tool	Key features	Limitations
1. TIDE (Tracking of Indels by DEcomposition) https://tide.nki.nl/ TIDE is a widely used web-based tool for quantifying CRISPR-induced insertions and deletions (indels) from Sanger sequencing data (Brinkman et al., 2014). Different versions of this work-flow have been made available to analyse the edited sites resulting from NHEJ and HDE.	Compares edited vs. unedited DNA sequences to detect indel frequency and size. Provides editing efficiency estimation without requiring a clonal population. Uses sequence decomposition algorithms to predict editing patterns. Works with Sanger sequencing reads, making it cost-effective.	Cannot detect large deletions or complex genomic rearrangements. Limited to single-nucleotide resolution. Less accurate when high sequence variability is present.
2.ICE (Inference of CRISPR Edits) https://www.synthego.com/guide/how-to-use-crispr/ice-analysis-guide Developed by Synthego, ICE is an alternative to TIDE that provides higher accuracy in analyzing CRISPR-induced mutations using Sanger sequencing data	Uses Bayesian inference models for precise indel analysis. Supports multiplex CRISPR editing detection. Provides percentage-based efficiency estimation for different editing events. Generates automated visual reports.	Cannot detect single-nucleotide substitutions. Requires high-quality sequencing data for accurate predictions.
3. CRISPResso2 https://github.com/pinellolab/CRISPResso2 CRISPResso2 is a powerful next-generation sequencing (NGS)-based tool for deep analysis of CRISPR edits (Clement et al., 2019)	\Analyzes indels, substitutions, and large deletions from NGS data. Provides quantitative assessment of HDR (homology-directed repair) vs. NHEJ (non-homologous end joining) outcomes. Detects allelic modifications and off-target events. Includes visualization tools such as sequence alignment plots and mutation frequency histograms	Requires bioinformatics expertise for proper usage. Computationally demanding for large datasets.
4. CRISP-ID https://gbiomed.kuleuven.be/english/research/50488876/51819059/crisp-id CRISP-ID is an NGS-based tool for detecting and quantifying CRISPR edits, specifically designed to handle mixed editing events (Dehairs et al., 2016)	Identifies single-nucleotide polymorphisms (SNPs), insertions, and deletions in pooled or clonal samples. Works efficiently with multiplex editing scenarios. Provides graphical representations of editing outcomes.	Less frequently updated than CRISPResso2. Requires NGS data preprocessing before providing the input.
5. CRISPR-Analyzer https://www.crispr-analyzer.org/ CRISPR-Analyzer is an interactive online platform for evaluating CRISPR mutations in amplicon sequencing data	Identifies mutation types and frequencies. Provides sequence alignment and variant analysis. Works with Illumina sequencing data.	Requires data upload, which might not be suitable for sensitive genetic data. Not optimized for long-read sequencing technologies.
6. EditR https://moriaritylab.shinyapps.io/editr_v10/ EditR is a Sanger sequencing-based tool that specifically analyzes base-editing outcomes (Kluesner et al., 2018)	Quantifies A-to-G and C-to-T base conversions in single-nucleotide editing experiments. Does not require specialized NGS data. Provides mutation frequency estimates.	Cannot analyze indels or large deletions. Does not assess multiplex editing events
7. BE-Analyzer http://www.rgenome.net/be-analyzer/# BE-Analyzer is a base-editing analysis tool designed for Adenine and Cytosine base editors (ABE & CBE)	Identifies precise base substitutions from NGS data. Quantifies editing efficiency and purity of base changes. Supports multiplex base-editing detection.	Cannot analyze indel formation or large deletions. Requires sequencing data preprocessing before analysis.
8. AmpliconSeq https://github.com/ctb/ampliconseq AmpliconSeq is a high-throughput NGS analysis pipeline for genome editing assessment	Processes amplicon sequencing data for CRISPR-edited samples. Provides mutation frequency analysis across multiple samples. Supports custom genome input.	Requires command-line expertise. Not optimized for Sanger sequencing data.

Post-editing analysis tools play a critical role in validating CRISPR experiments, helping researchers to confirm the results of their targeted experiments. They basically help in:

- Confirm successful edits (TIDE, ICE, CRISPResso2).
- Assess editing efficiency and detect off-target mutations (CRISP-ID, CRISPR-Analyzer).
- Evaluate base-editing accuracy (EditR, BE-Analyzer).

For small-scale CRISPR validation, TIDE and ICE are excellent choices. For deep sequencing-based analysis, CRISPResso2 and CRISP-ID provide comprehensive mutation profiling. Base-editing researchers should consider EditR and BE-Analyzer for precise mutation frequency analysis.

Gene-edited plant mutant database

The Plant Genome Editing Database (PGED) (http://plantcrispr.org/), developed by the Boyce Thompson Institute (BTI), is currently the only publicly available repository dedicated to cataloging plants modified using CRISPR-Cas technology for the investigation of economically important traits [Zhang *et al.,* 2021]. This database provides comprehensive information on various aspects of genome editing experiments, including the targeted trait, the transformation method employed, plant variety, DNA constructs, guide RNA sequences, and the primers used to induce specific genetic modifications.

In addition to detailing the experimental components, PGED also documents the characteristics of the resulting mutant lines, such as the nature of the gene alteration, zygosity (heterozygous or homozygous), and associated phenotypic changes. Researchers can contribute their own CRISPR-edited plant data to the database, promoting community-driven data sharing. The platform supports multiple search functionalities, allowing users to query by plant species, gene features (using gene IDs or descriptions), batch gene ID lists, or specific guide RNA sequences (Saha *et al.,* 2024).

Conclusions and perspectives

CRISPR-Cas-based genome editing has emerged as a transformative tool in plant biology, offering unprecedented precision for functional gene studies and rapid crop improvement. It has progressively replaced conventional transgenic approaches, enabling targeted modifications in diverse plant species. The widespread adoption of CRISPR in both model and non-model crops underscores its versatility and efficiency.

Currently, successful genome editing in plants relies on key factors such as the availability of high-quality reference genomes, the selection of an appropriate CRISPR-Cas system, efficient delivery methods (e.g., plasmid, viral vectors, or RNP complexes), and robust tissue culture protocols for regeneration. In parallel, a significant leap has been made through computational tools that support gRNA design, off-target prediction, and mutation analysis. Platforms such as CRISPR RGEN, CRISPOR, and CRISPR Direct are now widely used, hosting numerous plant genomes and facilitating rapid in silico screening of target sites. Integration with high-throughput sequencing allows precise post-editing analysis, while standalone versions of these tools support customization for species with unannotated genomes.

Despite these advancements, several improvements can further enhance the utility and reach of genome editing tools. First, expanding the database coverage to include more underutilized and orphan crops is essential to ensure broader application in global agriculture. Second, the development of tools that allow gene copy-specific sgRNA design within multigene families would address current challenges in achieving selective editing. Additionally, incorporating transcriptomic and functional gene annotation data (e.g., from RNAi, mutant, and transgenic studies) into sgRNA design platforms would enable users to prioritize biologically relevant targets. The application of machine learning and artificial intelligence holds great promise in improving sgRNA prediction accuracy, minimizing off-target risks, and optimizing editing outcomes across varied genomic contexts.

With these enhancements, CRISPR-bioinformatics platforms will become more comprehensive, user-friendly, and impactful. They will not only facilitate efficient genome editing but also empower researchers-especially those working in resource-limited settings-to design better experiments, interpret results more confidently, and accelerate the development of improved crop varieties tailored for future agricultural needs."

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Review

Ricin detoxification in castor (*Ricinus communis* L.) seeds: Current advances, integrated strategies, and future directions

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ABSTRACT

Castor bean (*Ricinus communis* L.) is a key industrial crop valued for its non-edible oil rich in ricinoleic acid. However, the presence of ricin - a type II ribosome-inactivating protein (RIP)-in the seed endosperm remains a major bottleneck for valorizing the protein-rich de-oiled cake (~20-25% protein) as animal feed. Ricin is highly toxic, with an LD_{50} of ~1-5 µg/kg in humans, and poses significant risks to human and animal health, thereby limiting the crop's commercial potential. Traditional detoxification methods, including physical (thermal), chemical (alkali/acid), and enzymatic treatments offer limited efficacy, often compromising protein quality or leaving toxic residues. Recent advances in plant genomics and molecular biology have facilitated the identification and characterization of ricin and RCA gene families, enabling targeted gene silencing and editing approaches. RNA interference (RNAi), antisense suppression, and CRISPR/Cas9-mediated knockouts have demonstrated promising results in reducing ricin expression while maintaining normal seed development and oil composition. Novel strategies such as promoter engineering, tissue-specific expression control, and base editing are emerging as powerful tools for generating transgene-free, low-ricin castor lines. Additionally, microbial degradation using enzymes and biotransformation pathways offers eco-friendly and scalable alternatives. This review discusses the current knowledge on the structure and biosynthesis of ricin, its mechanisms of toxicity, and highlights the latest strategies and technological interventions aimed at detoxifying castor bean seeds. We also evaluate the advantages, limitations, and regulatory challenges of these approaches and propose future research directions for sustainable, safe, and economically viable detoxification of castor products.

Keywords: Castor bean, Cas9, Detoxification, Genome editing, RCA. Ricin, RNAi

The castor bean plant (Ricinus communis L.) is an important non-edible oilseed crop known for its unique fatty acid composition, particularly ricinoleic acid, which makes castor oil indispensable in a wide range of industrial sectors including pharmaceuticals, lubricants, biofuels, polymers, cosmetics, and surface coatings (Anjani, 2014; Ogunniyi and David, 2006). India, Brazil, and China are the primary producers of castor, with India accounting for over 80% of global exports (FAOSTAT, 2023). Despite its economic potential, the utilization of the residual seed cake (de-oiled meal) post oil extraction is severely limited due to the presence of ricin-a highly potent ribosome-inactivating protein (RIP) (Sousa *et al.,* 2017). Ricin, a type II RIP, irreversibly inhibits eukaryotic protein synthesis by depurinating a specific adenine residue in the 28S rRNA of the 60S ribosomal subunit. It is lethal at low doses, with an estimated human LD_{50} of ~1-10 µg/kg body weight when injected or inhaled, and ~1 mg/kg when ingested. This extreme toxicity renders castor meal unsuitable for use in livestock feed or food applications, despite its high protein content (>20%) and favourable amino acid profile (Vila *et al.,* 2010). Moreover, ricin is considered a Category B bioterrorism agent by the Centre for Disease Control and Prevention (CDC), making its elimination from castor seed derivatives a pressing safety concern.

Conventional detoxification techniques, such as heat treatment, chemical hydrolysis (alkali, acids), organic solvent extraction, and enzymatic degradation, have been employed with variable success (Singh *et al.,* 2003; Soares *et al.,* 2015). These methods often compromise nutritional quality, exhibit incomplete inactivation, or are economically unfeasible at industrial scale. Consequently, there is growing interest in advanced, targeted, and sustainable detoxification strategies. Recent breakthroughs in genomics, transcriptomics, and proteomics have enhanced understanding of ricin biosynthesis and gene regulation, facilitating targeted gene silencing or editing approaches (Chan *et al.,* 2010; Brown *et al.,* 2012). Genetic engineering strategies-including RNA interference (RNAi), antisense RNA, and more recently CRISPR/Cas9 genome editing-have shown promise in downregulating or knocking out ricin and RCA genes (Wang *et al.,* 2012; Rivarola *et al.,* 2011). CRISPR-based approaches allow precise and heritable mutations, offering a stable route to develop

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ricin-free or ricin-null castor lines without altering oil or yield traits (Zhang *et al.,* 2019). However, the challenges in implementing these biotechnological solutions remain considerable. These include the genetic recalcitrance of castor to transformation, regulatory hurdles, biosafety concerns, intellectual property issues, and the lack of publicly available ricin-null cultivars. Concurrently, microbial detoxification using protease-secreting bacteria and fungi capable of degrading ricin and RCA is gaining attention for its eco-friendly, scalable potential (Fernandes *et al.,* 2012; Baldini *et al.,* 2020). Additionally, breeding efforts for low-ricin genotypes have been explored, although these are limited by the lack of natural variation and challenges in castor's breeding

In this review, we provide a comprehensive synthesis of the current status of ricin detoxification in castor, exploring both traditional and emerging strategies, their scientific basis, efficacy, and limitations. We particularly emphasize genome editing technologies, microbial biocatalysis, and integrated molecular breeding approaches that could enable the safe, sustainable utilization of castor bean meal. Finally, we outline future directions and research priorities for developing ricin-free castor varieties that balance economic potential with biosafety imperatives.

Anti-Nutritional and Toxic Factors in Castor

Despite its high protein content, castor meal contains several potent anti-nutritional and toxic compounds that significantly impair its safe use in animal feed or human nutrition.

Ricin

Ricin is a highly potent type II ribosome-inactivating protein (RIP), composed of an enzymatic A-chain (RTA) and a lectin B-chain (RTB) (Tumer *et al*., 2019). RTA irreversibly depurinates a critical adenine residue in the 28S rRNA of the 60S ribosomal subunit, halting protein synthesis at extremely low concentrations-even one molecule can be lethal per cell. RTB facilitates cell entry by binding to galactose residues on the cell surface. Ricin's median lethal dose (LD_{50}) is 5-10 µg/kg for injection or inhalation (Tumer *et al.,* 2019), and its high toxicity has been the main barrier to utilizing castor cake safely.

Ricinus Communis Agglutinin (RCA)

The hemagglutinin RCA (also known as RCA120) is a tetrameric glycoprotein closely related to ricin, sharing ~90% sequence identity in the A-chain and 84% in the B-chain (Brandon *et al.,* 2016). Although RCA is less potent than ricin, its agglutinating activity toward mammalian erythrocytes contributes to its toxicity. Being heat-labile, RCA can be partially neutralized by sufficient thermal treatment (Brandon *et al.,* 2016).

Allergenic 2S Albumins

Castor meal contains 2S albumin proteins such as Ric C1 and Ric C3, which are resilient to heat and enzymatic degradation. These proteins can elicit IgE-mediated hypersensitivity responses, posing allergenic risks (Salim *et al*., 2023).

Ricinine

Ricinine, a pyridone alkaloid unique to castor, exerts various toxic effects: nausea, vomiting, gastrointestinal hemorrhage, hepatic and renal damage, seizures, hypotension, respiratory depression, and death at high doses (Zhu *et al.,* 2018). Though less toxic than ricin, its cumulative presence in meal contributes significantly to overall toxicity.

Other Anti-Nutritional Compounds

Phytic acid chelates essential minerals such as calcium, iron, and zinc, decreasing their bioavailability and potentially inducing mineral deficiencies (Salim *et al.,* 2023). Tannins form indigestible complexes with proteins, reducing digestibility and palatability, thereby further diminishing feed value (Salim *et al.,* 2023).

Ribosome-Inactivating Proteins (Rips): Structure, Mechanism, and Biological Roles

Ribosome-inactivating proteins (RIPs) are a diverse group of enzymatic proteins predominantly found in plants, although members have also been isolated from fungi and bacteria. These proteins possess the remarkable ability to inhibit protein synthesis by depurinating a specific adenine residue from ribosomal RNA (rRNA), thereby arresting translation. RIPs are broadly categorized into two major classes based on structural organization: Type I RIPs consist of a single polypeptide chain with RNA N-glycosidase activity, while Type II RIPs are composed of an enzymatically active A-chain and a lectin-like B-chain connected via a disulfide bond (Iglesias *et al.,* 2022). The Type II RIPs, such as ricin and abrin, are significantly more cytotoxic due to their B-chain, which facilitates cell surface binding and internalization via endocytosis. In contrast, Type I RIPs, including saporin and trichosanthin, lack the B-chain and are less efficient at cell entry, although they

remain potent once inside the cytoplasm (Stirpe and Battelli, 2006). A less common category, Type III RIPs, consists of an enzymatically inactive precursor requiring proteolytic activation, as seen in maize RIPs (Bass *et al.,* 2004).

Mechanism of Action

The central biochemical function of RIPs is the irreversible inactivation of eukaryotic ribosomes through the depurination of a specific adenine residue (A4324 in rat 28S rRNA) located in the universally conserved α-sarcin/ricin loop (SRL) of the large ribosomal subunit. This reaction is catalyzed by RIPs' RNA N-glycosidase activity, which cleaves the N-glycosidic bond of adenine, thereby inhibiting the binding of elongation factor 2 (EF-2) and halting polypeptide elongation (Endo and Tsurugi, 1988). (Iglesias *et al.,* 2022). Crucial active site residues such as Tyr80, Glu177, Arg180, and Trp211 in the ricin A-chain (RTA) have been implicated in catalysis. Among them, Tyr129 plays a pivotal role, as its mutation results in a ~7-fold decrease in enzymatic activity, highlighting the critical nature of this residue in rRNA interaction (Ready *et al.,* 1991). Structural studies have shown that the 43-amino acid motif encompassing these residues forms a unique cleft that specifically.

Biological Functions and Applications

RIPs are hypothesized to serve as part of the innate defense arsenal of plants, acting against a spectrum of biotic threats including insects, fungi, bacteria, and viruses. They are often induced by abiotic stressors and pathogen attack, suggesting their role in systemic acquired resistance (SAR) and stress signalling pathways (Chen *et al.,* 2022; Hartley and Lord, 2004). For instance, the barley RIP JIP60 is induced by methyl jasmonate and UV-B radiation, implicating its role in abiotic stress tolerance (Chaudhry *et al.,* 1994). Many RIPs exhibit antiviral properties by directly depurinating viral RNA genomes or inactivating host ribosomes to arrest virus replication. Trichosanthin, a Type I RIP from *Trichosanthes kirilowii,* has been shown to inhibit HIV-1 replication in vitro (Lee-Huang *et al.,* 1991). Similarly, PAP (pokeweed antiviral protein) from *Phytolacca americana* exhibits broad-spectrum antiviral activity and has been used experimentally to engineer virus-resistant plants (Taylor *et al.,* 1994). The antifungal and insecticidal activities of RIPs have opened avenues for their use in crop protection. For example, transgenic tobacco plants expressing the barley RIP have demonstrated resistance to fungal pathogens like *Fusarium oxysporum,* and *Rhizoctonia solani,* thus highlighting their biotechnological utility in integrated pest management (Liu *et al.,* 2002). The unique mode of action and high specificity of RIPs have attracted interest in pharmaceutical and biotechnological applications. Immunotoxins constructed by fusing the A-chain of RIPs with monoclonal antibodies targeting cancer-specific antigens are being explored for cancer therapy. Ricin A-chain and saporin-based immunoconjugates have shown cytotoxic effects on tumor cells *in vitro* and in animal models (Frankel and Roberts, 2003). However, systemic toxicity and immunogenicity remain significant hurdles in clinical translation. Extended research into engineering hypo toxic variants through site-directed mutagenesis (e.g., at Tyr129) is ongoing, offering paths toward safer therapeutic uses. However, while strategically valuable, the inherent toxicity of RIPs-particularly type 2 variants-raises biosecurity concerns, such as ricin's classification as a biothreat agent given its lethal activity at doses as low as 1-10 µg/kg in humans. As a result, rigorous safety protocols and regulatory oversight are imperative during research and application development.

Detoxification of Castor Seed Meal: Strategies and Efficacy

Efficient detoxification of castor meal is critical for enabling its safe use as animal feed or fertilizer. Current methods can be categorized into physical, chemical, biological (microbial/enzymatic), and biotechnological approaches. Biotechnological approaches are discussed in length.

Physical Methods: Heat-based treatments such as autoclaving or moist cooking are the simplest detoxification routes: Autoclaving castor meal at 15 psi for 60 minutes has been demonstrated to abolish detectable ricin activity in seed cake (Anandan *et al.,* 2005). Moist-heating of flaked meal, sometimes with alkali addition, denatures ricin, reducing its toxicity (Gardner *et al.,* 1960). Despite their effectiveness, thermal methods may incompletely eliminate other toxins (e.g., RCA, ricinine) and can degrade essential amino acids, reducing nutrient quality. Additionally, these approaches demand high energy input, which may limit scalability (Fernandes *et al.,* 2012).

Chemical Methods: Chemical treatments using calcium-based compounds have shown encouraging results. 8% CaO or $Ca(OH)_2$ treatment of castor meal rendered it non-toxic to Vero cells. This treatment also mitigates allergenic responses, possibly via modification of IgE-binding epitopes (Fernandes *et al.,* 2012). However, chemical detoxification must be finely optimized to avoid

residual chemicals and ensure minimal impact on protein digestibility and nutritive value. Moreover, effluent treatment and environmental safety are major considerations.

Biological Methods

A. Microbial Detoxification (Solid-State Fermentation): Solid-state fermentation (SSF) using fungi, especially *Aspergillus niger,* can remove nearly all ricin activity (~98% reduction) within 24 hours (Fernandes *et al.,* 2012). Such methods utilize fungal enzymatic systems to degrade anti-nutritional proteins, offering cost-effective and scalable detoxification. Research into microbial consortia and engineered bacteria has also begun, indicating potential enhancements in biodegradation efficiency for complex toxin mixtures (De Oliveira *et al.,* 2010).

B. Enzymatic Detoxification: Enzymatic processing with proteolytic enzymes (e.g., amylases, glucoamylases, selected proteases) can specifically hydrolyze ricin chains, decreasing toxicity without excessive heat and preserving nutritional integrity (Melo *et al.,* 2008). Enzyme-based detoxification can be further enhanced by combination treatments, layering enzymatic hydrolysis with chemical treatments (e.g., calcium salts) to target both protein toxins and allergenic glycoproteins (Fernandes *et al.,* 2012). Challenges of biological methods include the need for stringent process control, longer detoxification times, and ensuring complete toxin removal in mixed compound matrices. A comparison of physical, chemical and biological methods of detoxification of ricin and RCA is presented in Table 1.

Table 1 Comparison of Detoxification Methods

Method	Ricin/RCA elimination	Nutritional retention	Scalability	Limitations
Physical (heat)	High	Moderate loss	Moderate-High	Nutrient loss; energy use
Chemical (Ca-based)	High	Preserved	Moderate	Residuals; effluent treatment
SSF fermentation	Very high	Good	High	Biocontrol needed; process time
Enzymatic	High	Excellent	Moderate	Cost; enzyme specificity

Integrated Detoxification: Strategy Emerging evidence favours integrated methods, combining physical, chemical, and biological stages for robust, scalable detoxification: Pre-treatment (heat or mild alkali) to reduce ricin, Enzymatic digestion to break protein toxins, Ca-based treatment to neutralize residual proteins/allergens, Final SSF polishing with fungi or bacterial cultures to minify residual enzymes and anti-nutrients.

This sequential approach has the potential to ensure safe, high-nutrient castor meal suitable for global feed markets. By integrating physicochemical and biological detoxification under controlled conditions, castor meal can be transformed into a viable and valuable protein source, supporting both food security and economic sustainability.

Biotechnological Detoxification Of Ricin In Castor Bean: Advanced Strategies and Future Directions

RNA Interference (RNAi): Silencing Ricin at the Transcript Level: RNA interference (RNAi) has emerged as a highly promising strategy for detoxifying ricin in castor bean (*Ricinus communis* L.), offering high specificity, stability, and the potential for durable gene silencing confined to seed tissues. RNAi exploits a conserved post-transcriptional gene silencing mechanism wherein double-stranded RNA (dsRNA) is processed into small interfering RNAs (siRNAs), which are incorporated into the RNA-induced silencing complex (RISC) to cleave complementary target mRNAs, thereby preventing translation. By designing constructs to target conserved regions of ricin and RCA (*Ricinus communis agglutinin*) gene transcripts, researchers have successfully developed transgenic plants with significantly reduced or undetectable levels of these toxic proteins (Ashfaq *et al.,* 2018; Kumaraswamy *et al.,* 2020; 2022). At ICAR-IIOR, extensive efforts have focused on achieving endosperm-specific silencing of ricin and RCA genes using native promoters (Ashfaq *et al.,* 2019, 2010). Multiple silencing approaches-including intron-hairpin RNA (ihpRNAi), transitive RNAi, and artificial microRNA strategies-have been deployed, with constructs validated initially in model systems such as tobacco (Sai-Kumar *et al.,* 2009; Soma-Sekhar *et al.,* 2009; 2010).

Transgenic castor lines expressing these RNAi constructs exhibited complete absence of hemagglutination activity and ricin toxicity in seeds. Sousa *et al.* (2017) demonstrated the absence of ricin and RCA120 in engineered castor seeds, which were proven safe in both in vitro (rat intestinal epithelial cells) and *in vivo* (Swiss Webster mice) toxicity assays. The same group later

reported that the ricin-silenced phenotype was stably inherited across three generations in the transgenic line TB14S-5D (Sousa *et al.,* 2022). Notably, this line also incorporated the ahas gene conferring tolerance to the herbicide imazapyr, thereby offering dual benefits of detoxification and weed management in castor cultivation. Although the RNAi strategy has shown molecular efficacy, the application has been constrained by the difficulty of stable transformation in castor due to its recalcitrance to in vitro regeneration. Recent advances at IIOR have addressed this challenge by developing a robust regeneration protocol using hypocotyl explants derived from mature embryo seedlings, which now supports ongoing optimization of Agrobacterium-mediated transformation (Ushakiran *et al.,* 2020; IIOR Annual Reports, 2021; 2022). Complementary efforts with in planta seed inoculation have also yielded promising, albeit limited, transformation success (Lakshmidevi *et al.,* 2023). Importantly, RNAi-mediated detoxification has been demonstrated to preserve vital agronomic traits such as oil content and seed yield (Ashfaq *et al.,* 2018; Sousa *et al.,* 2022). This biotechnological breakthrough unlocks significant economic potential, as detoxified castor meal-previously discarded as hazardous waste-can now be repurposed as a high-protein livestock feed, organic fertilizer, or bioenergy resource, adding substantial post-oil-extraction value.

Challenges and Future Perspectives for RNAi Mediated Silencing

Despite its potential, the RNAi approach faces several biological and regulatory challenges:

Gene Silencing Stability: Though studies demonstrate multi-generational silencing, epigenetic modifications or environmental triggers could attenuate silencing efficacy over time, potentially reactivating toxic gene expression.

Regulatory Oversight: RNAi-based transgenics fall under GMO regulations in many countries. Compliance with biosafety protocols, environmental risk assessments, and public acceptance are non-trivial hurdles to deployment (Kumaraswamy *et al.,* 2020).

Off-Target Effects: siRNAs may exhibit partial complementarity to unintended mRNA targets, raising concerns about off-target gene silencing, which could impact seed development or stress responses (Gao *et al.,* 2015).

Field-Scale Efficacy: Most demonstrations have been in greenhouse conditions. Field performance under biotic and abiotic stressors remains underexplored and critical to commercial validation.

RNAi Resistance: Similar to pest resistance in transgenic crops, there is a theoretical risk of adaptation that could bypass RNAi-mediated silencing, necessitating combinatorial or layered silencing strategies (Bally *et al.,* 2018).

CRISPR/Cas9 Genome Editing: Permanent Detoxification via Precision Mutagenesis

The CRISPR/Cas9 system has revolutionized plant biotechnology by enabling site-specific genome modifications. In the context of castor (*Ricinus communis* L.), where ricin and RCA (Ricinus communis agglutinin) are potent toxins, CRISPR/Cas9 offers a promising route to genetically eliminate or mitigate their biosynthesis. However, the implementation of genome editing for ricin detoxification is complicated by the gene family's structure, regulatory elements, and potential physiological roles of these proteins.

Molecular and Functional Complexities of Ricin/RCA Genes

Gene Redundancy and Sequence Homology: Ricin and RCA genes share high sequence similarity, particularly in regions encoding the conserved A-chain (toxic domain) and B-chain (lectin-binding domain). This homology poses challenges for designing guide RNAs (gRNAs) that can specifically target individual paralogs without cross-targeting (Lord *et al.,* 2003). Furthermore, the presence of multiple copies organized in tandem clusters on chromosome 8 necessitates multiplexed or combinatorial gRNA strategies for efficient editing.

Regulatory Control and Tissue-Specific Expression: The expression of ricin and RCA is tightly regulated during seed development through endosperm-specific promoters. Disrupting these genes could inadvertently affect related seed developmental pathways. Therefore, it is essential to preserve seed viability and oil biosynthesis while eliminating toxicity (Kumar *et al.,* 2021).

Potential Pleiotropic Effects: Emerging evidence suggests ricin-like proteins may play roles in defense or stress signaling in castor. Functional knockouts, especially via constitutive expression of Cas9, could result in unintended agronomic consequences unless strategies are carefully optimized (Ghosh *et al.,* 2012).

CRISPR-Based Genome Editing Strategies for Ricin Detoxification

1. Targeted Gene Knockout via Non-Homologous End Joining (NHEJ)

Double-stranded breaks (DSBs) induced by Cas9 can activate the error-prone NHEJ repair pathway, leading to small insertions or deletions (indels) that disrupt gene function. This approach can be used to knock out ricin and RCA by targeting their coding exons, particularly those encoding catalytic residues in the A-chain or carbohydrate-binding residues in the B-chain.

Ricin A-chain knockout: Disruption of the A-chain catalytic domain via frame shift mutations can abolish enzymatic activity, thus detoxifying the protein. Multiplexed gRNAs targeting conserved motifs improve knockout efficiency and reduce escape mutants.

B-chain editing: Disabling the B-chain's lectin-binding domain can block cellular uptake, rendering ricin biologically inert even if enzymatic activity remains (Miller *et al.,* 2019).

Advantages:

- Rapid, efficient, and applicable in multiple genotypes.
- Enables functional gene disruption without template requirements.

Limitations:

- Potential off-targets if gRNAs are not highly specific.
- May generate mosaic mutations in early generations

2. Simultaneous Deletion of Gene Clusters

Given the tandem arrangement of ricin and RCA gene families on chromosome 8, dual-gRNA systems can be designed to induce DSBs at flanking sites. This strategy enables large-fragment deletions, effectively eliminating entire gene clusters in a single transformation event (Xie *et al*., 2011). However, this requires careful off-target analysis due to the repetitive nature of the gene loci.

Advantages:

- Removes functional redundancy among paralogs.
- Prevents compensation by nearby homologs.

Limitations:

- Risk of unintended loss of neighboring genes.
- Designing unique gRNAs in highly repetitive regions is challenging.
- Requires thorough off-target analysis due to sequence homology.

3. Base Editing for Functional Mutagenesis

Base editors, such as cytosine or adenine deaminases fused to catalytically dead Cas9 (dCas9), allow precise nucleotide substitutions without DSBs. This approach is particularly useful for:

- Altering catalytic residues in the A-chain to render ricin enzymatically inactive.
- Modifying splicing or regulatory sequences to suppress expression without affecting other seed traits (Komor *et al*., 2016).

Base editing minimizes genomic disruption and is suitable for trait refinement in elite lines.

Advantages:

- Highly specific and DSB-free.
- Minimal genomic disruption; ideal for fine-tuning detoxification.
- Compatible with elite castor genotypes.

Limitations:

- Editing window is narrow (~4-6 bp).
- Requires PAM-proximal target motifs.
- May still trigger unintended edits at bystander bases.

4. Regulatory Element Engineering

CRISPR/Cas9 can be applied to edit cis-regulatory elements such as enhancers or promoters of ricin/RCA genes, allowing transcriptional downregulation without disrupting coding regions. This method preserves gene structure and reduces the likelihood of pleiotropic effects while achieving tissue-specific suppression.

Advantages:

- Lower risk of pleiotropic effects.
- Preserves coding gene structure.
- Can allow seed-specific suppression using endogenous regulatory cues.

Limitations:

- Requires prior identification of key regulatory elements.
- Effects may be quantitative rather than absolute (i.e., reduced, not eliminated expression).

5. Homology-Directed Repair (HDR)-Mediated Replacement

HDR enables precise replacement of ricin genes with non-toxic orthologs or inactivated versions. While HDR efficiency is low in plants, recent advancements such as geminivirus-based vectors (Borphukan *et al.,* 2025) and synchronized cell-cycle editing may enhance HDR outcomes. This approach can generate detoxified castor with traceable genetic modifications suitable for regulatory approval.

Advantages:

- Allows controlled, precise sequence insertion.
- Suitable for generating elite non-toxic lines with single-copy insertions.

Limitations:

- Extremely low efficiency in plants.
- HDR is mostly active during S/G2 phase-rare in most plant tissues.
- Delivery of large donor templates remains a technical challenge.

6. Incorporation of Anti-CRISPR Proteins for Temporal Control

Anti-CRISPR (Acr) proteins, derived from bacteriophages, inhibit Cas9 by blocking its DNA-binding or cleavage domains (e.g., AcrIIA4 inhibits SpCas9). Co-expression with Cas9 allows temporal or inducible control of editing, reducing off-target risks and mosaicism. It can be combined with chemical inducers or tissue-specific promoters to fine-tune editing windows (Zhang *et al.,* 2019).

Advantages:

- Enhances biosafety by limiting Cas9 activity.
- Improves precision in multi-copy gene editing scenarios like ricin/RCA.

Limitations:

- Additional cloning and regulatory elements needed.
- May suppress on-target editing if timing is not optimized.

The successful application of CRISPR/Cas9 to detoxify ricin in castor depends on continued innovation in delivery systems, editing precision, and biosafety evaluation. Integrating seed-specific promoters with inducible editing systems could minimize developmental side effects. Furthermore, combining CRISPR with RNAi or epigenome editing may allow multi-layered gene regulation. With increasing public and regulatory acceptance of genome-edited crops, especially those without transgenes, a ricin-free castor line with retained oil quality and agronomic performance appears achievable soon. Overall comparison of the different methods of biotechnological approaches is provided in Table 3.

Table 2 Advantages of CRISPR/Cas9 for Ricin Detoxification

Advantage / Limitation/ Challenges	Description
Advantages	
High specificity	Allows targeted mutagenesis of toxic genes without altering beneficial traits
Multiplexing capability	Enables simultaneous editing of gene clusters or multiple paralogs
No foreign DNA	Edited plants may be non-transgenic, facilitating regulatory clearance in some countries
Preserves oil traits	Targeted editing minimizes impact on seed development and oil content
Faster breeding cycles	Direct gene knockouts are faster than conventional breeding or transgenics
Limitations and Challenges	
Low HDR efficiency	Precise gene replacement is still inefficient in most plant systems
Gene family redundancy	Requires editing of multiple paralogs due to functional overlap
Off-target effects	Risk of unintended mutations, though minimized with optimized gRNA design
Transformation barriers	Castor remains recalcitrant to tissue culture and transformation
Regulatory concerns	Edited plants may still face regulatory scrutiny depending on jurisdiction

Table 3 Comparative summary and integration potential of different techniques

Strategy	Key Features	Advantages	Challenges
RNAi	Post-transcriptional silencing	Proven efficacy, seed-specific control	Variable silencing, regulatory hurdles
CRISPR Knockouts	Targeted gene disruption via NHEJ	Permanent, heritable edits	Gene redundancy, off-target risks
Base Editing	Precise point mutations	No DSBs, transgene-free potential	Limited scope of base conversions
Cluster Deletion	Whole-locus excision	Eliminates all toxin genes	Requires high transformation efficiency
Promoter Editing	Regulatory silencing	Retains gene integrity	Risk of incomplete suppression

Future Directions and Applications

1. Integration of Multidisciplinary Detoxification Strategies

Future efforts should focus on synergistic combinations of genetic editing (e.g., CRISPR/Cas9), microbial fermentation, enzymatic treatments, and chemical processes for ricin removal. For instance, genome-edited lines with traits such as ricin/RCA knockout can be further processed using fungal solid-state fermentation or calcium-based chemical detoxification to minimize residual allergens and anti-nutritional factors while preserving amino acid integrity. This integrated pipeline could drastically improve detoxification efficacy, cost-efficiency, and scalability.

2. Robust Toxicological and Nutritional Validation

To ensure safety and regulatory compliance, systematic in vitro and in vivo assessments of detoxified products are needed. Standardized protocols for detecting ricin and RCA activity-such as ELISA-based assays and cell viability tests-are critical (Sousa *et al.,* 2017; Akande *et al.,* 2016). Animal feeding trials-such as those in goats, sheep, broilers, and steers-have shown that detoxified castor meal replacing up to 90% of soybean meal in diets does not negatively impact digestion or performance (Menezes *et al.,* 2016; Melgar *et al.,* 2024). However, comprehensive toxicokinetics, residue analysis in meat/milk, and allergenicity studies remain gaps.

3. Analytical Tools and Traceability

Simplified, affordable assays for quantifying ricin and RCA in feed, serum, and animal tissues are urgently needed (Akande *et al.,* 2016). Innovations such as rapid ELISA kits, field-deployable immunoassays, or portable molecular sensors would support both safety monitoring and regulatory transparency throughout the value chain.

4. Field Trials and Crop Optimization

Detoxification technologies must be integrated into elite castor cultivars via field-testing. GM ricin-free lines (e.g., TB14S 5D) and other edited genotypes must undergo agronomic evaluation under various climatic conditions to validate yield, oil content, and seed viability (Sousa *et al.,* 2022; Embrapa report). Participatory trials with farmers and industrial partners-especially in India, Brazil, and China-are essential for adoption.

5. Regulatory and Biosafety Frameworks

A coordinated framework involving scientists, regulators, and industry is vital to standardize requirements for detoxified castor products. Robust toxicological assessments of detoxified castor products are essential. Establishing standardized protocols for testing ricin levels across global regulatory frameworks (e.g., Codex Alimentarius, EFSA, FSSAI) will ensure consistent safety and quality. Policies should articulate safety thresholds for ricin/RCA, validated analytical methods, environmental biosafety of GM or edited lines, and guidelines for commercialization (Akande *et al.,* 2016). Public engagement, transparency, and labelling regulations will build trust and acceptance.

6. Industrial and Agricultural Utility

Animal Feed: Detoxified castor meal with high protein content (>30%) can substitute soy in livestock diets, improving feed economy and sustainability (Santos *et al.*, 2023; Lima *et al.*, 2020)

Renewable Energy: Detoxified residual cake can be used as high-quality biofertilizer or as biomass for biofuel production.

Pharmaceutical Use: Protein isolates free of ricin/RCA could serve as safe biochemical feedstock or for protein engineering in industrial enzymes.

Detoxification of ricin in castor beans has progressed significantly-from conventional heat/chemical methods to sophisticated biotech approaches like RNAi and CRISPR editing (Sousa *et al.,* 2017; 2022; Akande *et al.,* 2016).

Each detoxification strategy offers distinct benefits: physical and microbial methods provide practical and low-tech solutions; RNAi and CRISPR/Cas9 approaches offer targeted and heritable elimination of toxic proteins. The most promising pathway forward is an integrated, multi-platform detoxification pipeline that ensures safety, nutritional integrity, scalability, and regulatory compliance. By combining optimized transformation protocols, precise genome editing, microbial fermentation, and chemical detoxification steps, ricin free castor cultivars can become commercially viable. This innovation has transformative potential for animal nutrition, sustainable agriculture, renewable energy, and industrial biotechnology, expanding the global utility and acceptance of castor bean-derived products.

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Review

Application of "push pull strategy" in pest management: An overview

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ABSTRACT

The "push-pull" strategy is an emerging tool in sustainable and integrated pest management (IPM) that effectively reduces pest pressure in various cropping systems, including pulses and oilseeds. This method utilizes a combination of repellent (push) and attractant (pull) stimuli to divert pests away from the main crop towards trap crops or designated removal zones. The mechanism largely depends on semiochemicals-plant volatiles that modify insect behaviour. The push-pull technique minimizes pesticide usage, thereby reducing health hazards, resistance development, and environmental contamination. This review highlights the principles, practical implementation, and relevance of push-pull strategies in managing insect pests of pulses and oilseed crops.

Keywords: Behavioural manipulation, Companion cropping, Pulses, Push-pull, Oilseeds, Repellent crops, Semiochemicals, Trap crops

The push-pull strategy is an ecological pest management approach that combines behaviour-modifying stimuli to repel (push) pests away from the main crop while simultaneously attracting (pulling) them towards trap crops or attract stimuli. Initially developed for cereal stemborer control in Africa, this strategy is now gaining relevance in diverse cropping systems, including oilseeds (e.g., groundnut, mustard) and pulses (e.g., pigeonpea, chickpea) (Khan *et al.,* 2016).

This method was first conceptualized by Pyke *et al.* (1987) in Australia for Helicoverpa spp. management in cotton. Later, Miller and Cowles (1990) refined the concept in the USA while studying Delia antiqua in onions. The technique found its most successful application in Africa, particularly in cereal-based systems (Khan *et al.,* 2008), where Desmodium repelled stem borers while Napier grass acted as a trap crop. In groundnut (*Arachis hypogaea*) and mustard (*Brassica juncea*), insect pests such as *Spodoptera litura* (tobacco caterpillar) and *Aphis craccivora* (aphids) cause significant yield losses. Intercropping with repellent plants like marigold (Tagetes spp.) or coriander (*Coriandrum sativum*) has been shown to deter aphid populations (Push), while castor (*Ricinus communis*) serves as a trap crop (Pull) to attract ovipositing moths of Spodoptera (Patel *et al.,* 2019). In soybean (*Glycine max*), infestation by whiteflies (*Bemisia tabaci*) and green semilooper (*Chrysodeixis acuta*) poses significant yield threats. Intercropping soybean with repellent plants like garlic (*Allium sativum*) or ginger (*Zingiber officinale*) (Push) has been observed to reduce whitefly colonization due to the release of strong volatile compounds. At the same time, okra (*Abelmoschus esculentus*) serves as a pull crop, attracting Bemisia adults for oviposition, thus acting as a trap crop to protect the soybean (Patel *et al.,* 2021). In sunflower (*Helianthus annuus*) fields, *Helicoverpa armigera* (sunflower head borer) is a persistent pest. Intercropping sunflowers with repellent plants like basil (*Ocimum basilicum*) or coriander (*Coriandrum sativum*) (Push) reduces moth attraction to the main crop. Concurrently, castor (*Ricinus communis*) can function as a pull crop, luring Helicoverpa moths for egg laying, diverting them away from sunflower heads (Kumar *et al.,* 2018). In chickpea (*Cicer arietinum*), pod borer (*Helicoverpa armigera*) is a key pest. Linseed (*Linum usitatissimum*) has been effectively used as a push crop owing to its repellent properties, while trap crops like marigold (*Tagetes erecta*) or sunflower (*Helianthus annuus*) (Pull) attract Helicoverpa adults, thereby reducing egg-laying on chickpea plants (Singh *et al.,* 2014). In pigeonpea (*Cajanus cajan*), pod borer (*Helicoverpa armigera*) infestation is a major concern. Studies have demonstrated that intercropping pigeonpea with attractant crops like marigold or cowpea (Push) reduces pest colonization on crop. Simultaneously, sunflower (*Helianthus annuus*) can act as a pull crop, luring Helicoverpa moths away from pigeonpea (Srinivasan and Krishna Moorthy, 1992). Additionally, border rows of sorghum or pearl millet have also been used as physical barriers to reduce pest influx (Sharma *et al.,* 2017). The details of Integrated Mechanisms and Components of Push-Pull Strategy given in Table 1.

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Table 1 Integrated mechanisms and components of push-pull strategy

Component Type	Push Components (Mechanism & Examples)	Pull Components (Mechanism & Examples)	Natural Enemies / Tools	Key References
Host Plant Volatiles (Pull)	-	Bait traps or trap crops emitting host volatiles to lure pests	-	Njihia *et al*., 2014
Visual Stimuli (Pull)	-	Yellow sticky traps attract pests like whiteflies and aphids	-	Cook *et al*., 2007
Oviposition Stimulants (Pull)	-	Trap crops that stimulate oviposition away from main crops	-	Witzgall *et al.,* 2010
Non-Host Volatiles (Push)	Volatiles from non-host plants deter pests	-	-	Cook *et al.,* 2007
Herbivore-Induced Volatiles (Push)	Volatiles signaling pest presence repel pests and attract natural enemies	-	-	Pickett *et al.,* 2007
Alarm Pheromones (Push)	Aphid alarm pheromones (Eβf) repel pests and attract predators	-	-	Pickett *et al*., 2007
Antifeedants (Push)	Neem, Pongamia, Melia, Annona extracts deter pest feeding	-	Suppresses larval feeding (e.g., *Helicoverpa armigera*)	Cook *et al*., 2006
Oviposition Inhibitors (Push)	Neem-based inhibitors prevent egg laying	-	-	Miller and Cowles, 1990
Volatile Compounds	Methyl isonicotinate, Carvacrol	-	-	Fabrick *et al*., 2020; Pickett, 2019
Companion Plants	Marigold (*Tagetes* spp.), Mustard, Geranium (visual & olfactory repellents)	Okra (*Abelmoschus esculentus*), Cotton (serve as pull crops for pest diversion)	-	Silveira *et al.,* 2009; Mansour, 2015
Trap Crops	Garlic, Castor, Neem (push due to repellence)	Pigeon pea (*Cajanus cajan*), Okra (pull by attracting pests)		Karavina *et a*l., 2014; Sharma *et al.,* 1993
Sticky Traps	Yellow-coloured traps serve as visual deterrents and monitoring tools			Cook *et al*., 2007
Banker Plants	Buckwheat (conserves beneficial insects through nectar resources)		Enhances parasitoids and predators	Bruce *et al*., 2005
Natural Enemies (N.E.)	Push via habitat manipulation, visual and olfactory cues		*Cyrtopeltis tenuis, Orius laevigatus*, Ladybird beetles	Frank, 2010; Lee *et al*., 2019
Pheromones	Alarm pheromones repel pests (e.g., aphids)	Aggregation & sex pheromones used in mass trapping	Beneficial for early IPM intervention	Pickett *et al.,* 2007; Witzgall *et al.,* 2010

The push-pull strategy is an eco-friendly approach that enhances biological control, reduces pesticide residues, delays pest resistance development, and supports sustainable farming practices (Cook *et al.,* 2007). The success of push-pull in oilseed and pulse crops lies in manipulating pest behavior through semiochemicals (volatile organic compounds) released by companion and trap crops, thereby enhancing natural enemies and reducing pest populations. This strategy also fosters agro-biodiversity, leading to improved soil health, microclimate moderation, and reduced dependency on chemical pesticides (Cook *et al.,* 2007). The Figure 1 explains the push-pull model. While promising, the large-scale adoption of push-pull in oilseeds and pulses is limited due to lack of location-specific companion/trap crop combinations and farmer awareness. Research focusing on the identification of suitable volatile-emitting plants, coupled with extension efforts, can significantly enhance the implementation of this eco-friendly pest management approach in diverse agro-ecosystems.

In oilseed crops such as groundnut (*Arachis hypogaea*) and mustard (*Brassica juncea*), insect pests like *Spodoptera litura* (Tobacco caterpillar) and *Aphis craccivora* (Aphids) cause substantial yield losses. The Push-Pull Strategy has been effectively utilized to manage these pests by manipulating their host-finding behavior through intercrops.

Push Crops: Marigold (Tagetes spp.) and coriander (*Coriandrum sativum*) act as repellent crops, releasing allelochemicals that interfere with the pest's ability to locate the main crop.

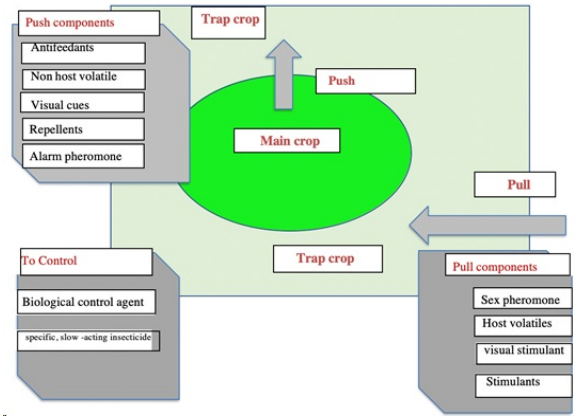

Fig. 1. Push-pull model

Pull Crops: Castor (*Ricinus communis*) serves as a trap crop, highly attractive to Spodoptera moths for oviposition. This helps divert the pest population away from groundnut and mustard.

This push-pull system, when combined with biological control agents (such as *Trichogramma* spp. and coccinellid beetles) and cultural practices like border cropping and timely sowing, enhances the effectiveness of Integrated Pest Management (IPM) in oilseed ecosystems. In oilseed and pulse pest management, several key pests are effectively managed through push-pull strategies. For the Gram Pod Borer (*Helicoverpa armigera*), push crops like marigold (*Tagetes* spp.), garlic (*Allium sativum*), and neem (*Azadirachta indica*) repel the pest, while pull crops such as pigeon pea (*Cajanus cajan*), okra (*Abelmoschus esculentus*), and castor (*Ricinus communis*) attract the pest away from the main crop (Manjunath *et al.,* 1989; Reddy, 1998; Silveira *et al.,* 2009; Patel *et al.,* 2019). For Spotted Pod Borer (*Maruca testulalis*), repellent plants like marigold, garlic, and basil are used as push components, while cowpea (*Vigna unguiculata*) and okra act as pull crops (Margam *et al*., 2011; Yadav & Patel, 2015; Atachi & Djihou, 1994). The Blue Butterfly (*Lampides boeticus*) is managed using marigold and mint (*Mentha* spp.) as repellents, while broad bean (*Vicia faba*) and garden peas (*Pisum sativum*) serve as attractants (Varshney *et al.,* 2012; Durairaj, 1999; Lohman *et al.,* 2008). For Bean Aphid (*Aphis craccivora*), push components include marigold, mustard (*Brassica juncea*), and geranium, whereas okra and cotton are used as trap crops (Singh & van Emden, 1979; Dorge *et al.,* 1966). In the case of Whitefly (*Bemisia tabaci*), repellents such as marigold, basil (*Ocimum basilicum*), and mint are effective, while pull crops like okra, cowpea, and chickpea help lure pests away (Oliveira *et al.,* 2001; De Barro *et al.,* 2011). For Leafhopper (*Empoasca kerri*), marigold and neem act as push crops, while cowpea and green gram are used as pull crops (Saxena, 1973). The mechanisms that drive these push-pull effects include host plant volatiles that lure pests (Njihia *et al.,* 2014), visual stimuli like yellow sticky traps for monitoring and controlling pests (Cook *et al.,* 2007), and oviposition stimulants enhancing pest egg-laying on trap crops (Witzgall *et al.,* 2010). Non-host plant volatiles repel pests (Cook *et al.,* 2007), and herbivore-induced volatiles signal pest presence, deterring colonization and attracting natural enemies (Pickett *et al.,* 2007). Alarm pheromones released by social pests act as repellents and attract predators (Pickett *et al.,* 2007). Additionally, plant-derived antifeedants like neem extracts deter pest feeding (Cook *et al.,* 2006), and neem-based oviposition inhibitors prevent egg-laying (Miller and Cowles, 1990). The application of push-pull strategy in oilseed and pulse has been given in Table 2 and 3.

Table 2 Application framework of push-pull strategy in oilseeds and pulses

Component	Description	Key References
Main Crops	Groundnut, Mustard, Sunflower, Soybean, Chickpea, Pigeonpea, Black gram	Patel *et al*., 2019; Reddy, 1998
Push Components	Repellent intercrops: Marigold, Garlic, Neem, Mustard, Basil; Non-host volatiles masking host cues; Antifeedants (Neem extracts); Visual repellents (reflective mulches)	Cook *et al*., 2007; Miller and Cowles, 1990; Pickett *et al*., 2007
Pull Components	Trap crops: Castor, Sunflower, Okra, Cowpea, Pigeonpea; Oviposition stimulants; Host plant volatiles in bait traps; Visual lures (yellow sticky traps)	Silveira *et al.,* 2009; Witzgall *et al.,* 2010; Njihia *et al*., 2014
Natural Enemy Conservation	Banker plants (e.g., buckwheat); Use of VOCs to attract parasitoids and predators (e.g., Trichogramma chilonis, Coccinellids, Chrysopids)	Bruce *et al*., 2005; Frank, 2010
IPM Integration	Timely sowing, crop rotation, sanitation, monitoring Economic Threshold Levels (ETL), minimal selective pesticide use	Reddy, 1998; Cook *et al*., 2007
Outcomes	Reduced pest infestation, enhanced beneficial arthropod populations, lower pesticide residues, sustainable pest management	Oliveira *et al*., 2001; Pickett *et al.,* 2007

The push-pull strategy embodies a sustainable and ecologically sound pest management paradigm that effectively manipulates pest behavior using a blend of repellents and attractants, thereby reducing dependency on chemical pesticides. Its integration into IPM frameworks not only mitigates pest-induced crop losses but also fosters biodiversity, ecological resilience, and farm sustainability (Cook *et al.*, 2007; Khan *et al.*, 2016). To realize its full potential in oilseed and pulse agroecosystems, targeted research for refining crop combinations and enhancing semiochemical-based interven t ion s, alongside comprehensive farmer outreach, remains imperative.

Table 3 Application of push-pull strategy in management of major pest oilseeds and pulses

Pest Name	Push Crops / Components	Pull Crops / Components	References
Gram pod borer (*Helicoverpa armigera*)	Marigold (*Tagetes* spp.), Garlic (*Allium sativum*), Neem (*Azadirachta indica*)	Pigeon pea (*Cajanus cajan*), Okra (*Abelmoschus esculentus*), Castor (*Ricinus communis*)	Manjunath *et al*., 1989; Reddy, 1998; Silveira *et al.*, 2009; Patel *et al*., 2019
Spotted ood borer (*Maruca testulalis*)	Marigold, Garlic, Basil	Cowpea (*Vigna unguiculata*), Okra	Margam *et al*., 2011; Yadav and Patel, 2015; Atachi and Djihou, 1994
Blue butterfly (*Lampides boeticus*)	Marigold, Mint (*Mentha* spp.)	Broad bean (*Vicia faba*), Garden peas (*Pisum sativum*)	Varshney *et al*., 2012; Durairaj, 1999; Lohman *et al*., 2008
Bean aphid (*Aphis craccivora*)	Marigold, Mustard (*Brassica juncea*), Geranium	Okra, Cotton	Singh & van Emden, 1979; Dorge *et al*., 1966
Whitefly (*Bemisia tabaci*)	Marigold, Basil (*Ocimum basilicum*), Mint	Okra, Cowpea, Chickpea	Oliveira *et al.*, 2001; De Barro *et al*., 2011
Leafhopper (*Empoasca kerri*)	Marigold, Neem	Cowpea, Green gram	Saxena, 1973

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Character association and gene action for yield and its contributing traits in Indian mustard [*Brassica juncea* (L.) Czern & Coss]

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ABSTRACT

To enhance productivity, genetic restructuring of Indian mustard germplasm is needed to develop high-yielding cultivars or hybrids. In the present investigation, 55 genotypes (10 parents, and 45 F1s) of Indian mustard were assessed to study the character association among traits and to know the gene action, which aids the breeder to evaluate the proportional contributions of various components towards genetic diversity. The field experiment was laid out in a Randomized Block Design with three replications during *rabi* 2017-18 and 2018-2019. The correlation study revealed that seed yield per plot showed a positive and significant correlation with days to 50% flowering, plant height, primary branches/plant, secondary branches per plant, secondary branches per plant, and number of siliquae per plant. The path coefficient analysis revealed that seed yield per plot, days to 50% flowering and plant height have a positive direct effect on seed yield per plant, which suggests that direct selection for these traits can be done for improvement of yield. As a consequence of path coefficient analysis, the highest direct positive effects were observed for number of siliquae per plant followed by days to maturity, main shoot length and seeds per siliquae. Both additive and non-additive gene action were important in controlling days to 50% flowering, days to maturity, plant height (cm), primary branches per plant, main shoot length, siliquae on the main shoot, siliquae length (cm), number of siliquae per plant, seeds per siliquae and oil content. Symmetrical proportion of positive and negative genes (H2/4 H1) was observed for all the characters. In the case of heritability, narrow sense showed the highest values in plant height and moderate in days to maturity and remaining characters showed low values of narrow sense heritability. The predominance of non-additive gene action for seed yield per plant and its components could be explored through heterosis breeding.

Keywords: *Brassica juncea,* Correlation, Diallel analysis, Gene action, Mustard, Path coefficient

Brassica juncea (L.) Czern and Coss often known as Indian mustard, is a species of the Cruciferae family. It is an amphidiploid (2n = 36, AABB) as reported by Vaughan (1977). Oilseeds are the major source of vegetable oil in the country. Rapeseed & mustard are important oilseed crops occupying more than five million hectares of area under cultivation, contributing 27% of total oilseed production in the country (Anonymous, 2021). It is grown throughout the country, seven states, namely Haryana, Rajasthan, Gujarat, Uttar Pradesh, Madhya Pradesh, West Bengal and Assam, account for more than 90% of its output and acreage. The seed oil content ranges from 38 to 46% and is made up of unsaturated fatty acids (Smooker *et al.,* 2011). The protein-rich oil cake is primarily used as feedstuffs for animals (Vaughan and Hemingway, 1959). Unseasonal rains during the flowering of the mustard plants followed by early heat waves have affected mustard cultivation. Farmers are complaining of wilting crops and poor quality mustard seeds, which will cause yield decline. In addition, non-availability of quality seed is also to be found one of the major causes of low crop yield. It is important to increase the yields of mustard crop by improving the available germplasm lines, for that we need to know various yield contributing characters and the relationship among them and with the seed yield. Thus, character association studies become a necessity for initiating a successful breeding programmer. Yield is a complex quantitative trait that is subject to environmental fluctuations requiring indirect selection of simply highly heritable traits for its improvement. This is possible through estimates of correlations, which help in determining the degree to which various yield contributing characters are associated (Wright, 1921). Path analysis, Dewey and Lu (1959) further reveals the associations of these characters with yield are due to direct impact on yield or may be a consequence of their indirect effects via other characters. Simple correlation and path coefficient analyses have been frequently utilized to assess trait relationships and help genotype selection for desirable economic attributes (Abraha *et al.,* 2016). The direct and indirect effects of one or more causative variables on a response variable are differentiated using path coefficient analysis. The most significant predictor variable(s) on dependent variables can be determined via path analysis (Teklu *et al.,* 2021). Path analysis studies in Indian mustard reported that 1000 seed weight had a positive direct effect on seed yield per plant indicating the importance of this trait for the selection of high seed yielding genotypes by Tiwari (2019). Diallel mating design has been extensively used in both self and cross pollinated species to understand the nature of gene action involved in

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the expression of quantitative traits. In the breeding of high yielding varieties of crop plants, the breeder is confronted with the problem of choice of parents. Elimination of poor yielding crosses on the basis of their performance in early generations had been recommended, but it was felt that knowledge of the genetic architecture of yield and its attributes would help to sort out the better crosses more efficiently. Several reports in the past have appeared that indicate that diallel analysis is the quickest method of understanding the genetic nature of quantitatively inherited traits and ascertaining the prepotency of parents. Kearsey (1965) noted that Hayman and Jinks' diallel analysis provides more information than other methods, but has more necessary assumptions. The analyses proposed by Griffing (1956) do not provide any test to detect epistasis or linkage. Hayman and Jinks' analysis does provide such test. For this, it is necessary to identify gene action involved in the expression of various yield contributing characters. The economic character of prime importance i.e. yield is an outcome of the multiplicative interaction of component characters. For breeding high yielding varieties of crop plants, breeders usually face the problem of selecting desirable parents. In general, parents are selected on the basis of their per se performance but many times high yielding genotype(s) may/may not transmit their superiority to the progeny. Hence, the critical choice of parents is of utmost importance, particularly for the improvement of complex quantitative characters such as yield. In this experiment, we have studied the correlation or mutual association among different yield contributing characters and their direct and indirect effects were also estimated through path coefficient analysis. The inter-relationship between the yield components will be helpful to a breeder for assessing the nature, extent and direction of selection pressure on characters. In the present investigation, efforts have also been made to understand the nature and estimate the genetic component of seed yield and its contribution in Indian mustard.

MATERIALS AND METHODS

The experimental material comprising the ten diverse genotypes of Indian mustard, NRCHB-101, DRMR-IJ-31, Kanti, Urvashi, Pusa mustard-25 (NPJ-112), Pusa Mustard-26 (NPJ-113), Pusa mustard-27 (EJ-17), CS 54, RH 406 and RH 749. These parents maintained as pure lines by selfing for several generations were crossed in half diallel fashion at Agriculture Research Farm R B (PG) College Agra. Fifty-five treatments consisted of 10 parents, and 45 F_1s were evaluated in a Randomized Block Design with three replications during *rabi* season of 2017-2018, and 2018-2019 at the Agriculture Research Farm of School of Agricultural Sciences and Engineering, IFTM University, Moradabad (Uttar Pradesh), India. Each parents and F_1s were grown in single row in two rows of five-meter length spaced at 45 cm × 15 cm apart. All the recommended agronomic practices were adopted for raising a good crop. Ten plants were randomly selected from parents and F_1s for recording the observations on fourteen characters. The coefficients of phenotypic and genotypic correlation among seed yield and its component traits were calculated, respectively, using the formula suggested by Johnson *et al.* (1955). Path co-efficient analysis was carried as recommended by Wright (1921) and further illustrated by Dewey and Lu (1959) for partitioning of genotypic correlations into direct and indirect effect upon seed yield per plant. The direct and indirect effects are rated as follows by Lenka and Mishra (1973). The data were subjected to genetic analysis of following Hayman (1954 a, b) and Jinks (1954).

RESULTS AND DISCUSSION

Before subjecting the data for various genetic analyses, the analysis of variance was done for all the characters for testing the significant differences among genotypes and their hybrids. Highly significant differences were observed among the treatments for all the characters.

Character association analysis gives us an estimate of degree of association among two or more variable or characters. The dependence of yield on different yield attributing characters can be known by correlation coefficients. The genotypic and phenotypic correlation coefficients were estimated between all possible pairs of characters involving 10 parents and their F_1s generation. The data presented in Table 2 indicated that the magnitude of genotypic correlation was greater than phenotypic correlation for all the traits in all the combination. Because the genotypic correlation only represents the genetic relationship between traits, but the phenotypic correlation is influenced by both genetic and environmental factors, the genotypic correlation is frequently greater than the phenotypic correlation. The positive and significant correlation was observed between seed yield per plant and days to 50% flowering, plant height, primary branches per plant, secondary branches per plant, secondary branches per plant, and number of siliquae per plant. The similar results were reported by Jat *et al.* (2019), and Pandey *et al.* (2020). Pal *et al.* (2019) and Shar *et al.* (2020) for number of siliquae per plant, Yadev and Pandey (2018), and Kumar *et al.* (2019) for number of secondary branches per plant. Some characters *viz.,* days to maturity, main shoot length (cm), siliquae on main shoot, seeds per siliquae, 1000 seed weight, oil content and fiber content found to have positive and non-significant association with seed yield per plant.

However, one of the characters *viz.,* siliquae length exhibited non-significant negative correlation with seed yield per plant. The similar results for siliquae length were reported by Pal *et al.* (2019). Path coefficient analysis was conducted to portioning the correlation coefficient of the characters studied with seed yield per plant into direct and indirect effects. Path coefficient analysis along with correlation coefficient analysis will be very efficient in the selection. The results of genotypic path coefficient were presented in Table 3. In the present study, the highest direct positive effects were observed for no. of siliquae per plant followed by days to maturity, main shoot length and seeds per siliquae. Such direct and positive effects were also observed by Rauf and Rahim (2018) and Pal *et al.* (2019) for no. of siliquae per plant. As far as, the findings revealed that remaining traits have negative direct effects on seed yield per plant. The results were similar to the findings of Devi (2018) and Pal *et al.* (2019) for plant height and Lakra *et al.* (2020) for number of primary branches per plant.

A comparative evaluation for nature and magnitude of genetic parameters has been presented in Table 4. The estimates of genetic components revealed that the highly significant additive (D) gene action was observed for all the characters except secondary branches per plant, 1000 seed weight and seed yield per plant. Dominance (H) gene action was noticeable for all the characters except fiber content and seed yield per plant. This indicated that both additive and non-additive gene action were important in controlling days to 50% flowering, days to maturity, plant height, primary branches per plant, main shoot length, siliquae on main shoot, siliquae length, number of siliquae per plant, seeds per siliquae and oil content. Both D and H component were played important role in genetic components of different traits in mustard (Avtar *et al.,* 2019). The magnitude of dominance components (H1 and H2) as compared to additive component (D) were found greater for all the characters in both the generations thus indicating predominance of non-additive type of gene action for these characters. This finding is in confirmatory with reports from Kumar *et al.* (2017) for different characters. Significant and positive values of (F) were noticeable for days to 50% flowering, days to maturity, plant height, primary branches per plant, main shoot length, siliquae on main shoot, seeds per siliquae and oil content. These findings suggested that dominant genes were more frequent than the recessive ones except secondary branches per plant, siliquae length, 1000 seed weight, fiber content and seed yield per plant in both the generations. The values of (H1/D) indicated over dominance for most of the characters. Symmetrical proportion of positive and negative genes (H2/4 H1) was observed for all the characters. The ratio [{(4 D H1)0.5+F}/ {(4 D H1) 0.5-F}] which reflected the negative values of dominant and recessive genes were more than an unity of all the characters indicating that dominant genes were more pronounced for all the characters. In case of heritability, narrow sense showed highest values in plant height and moderate in days to maturity and remaining characters were showed low values. The predominance of non-additive gene action for seed yield per plant and its components could be explored through heterosis breeding or population improvement by inter-mating the improved genotypes.

Character association study revealed that number of siliquae per plant exhibited positive direct effect on seed yield per plant with positive and significant correlations. Hence, the direct selection for this trait through simple breeding procedure will be helpful in improving the yield of the mustard genotypes. Presence of both additive and non-additive gene action for most of traits indicated the possibility of developing variety and hybrids. The predominance of non-additive gene action for seed yield per plant and its components could be explored through population improvement or heterosis breeding.

Table 1 Analysis of variance for parents and their hybrids of Indian mustard

Source of variance	D.F.	Days to 50% flowering	Days to maturity	Plant height (cm)	Primary Branches/ plant	Secondary Branches/ plant	Main shoot length (cm)	Siliquae on main shoot	No of siliquae/ plant	Siliquae length (cm)	Seeds per siliquae	1000 seed weight (g)	Oil content (%)	Fiber content (%)	Seed yield per plant (g)
Replication	2	8.49	13.69	15.94	22.34	41.46	27.25	106.00	2175.52	0.00	0.15	0.02	2.42	0.11	4.35
Parent	54	24.51**	304.01**	1924.75**	8.91**	315.63**	881.26**	559.52**	558978.88**	1.71**	14.04**	2.52**	1.52**	2.07**	420.07**
Error	108	7.19	8.40	2.46	0.64	0.58	2.31	6.79	2019.31	0.07	0.56	0.01	0.20	0.10	5.64

* Significant at 5% level, ** Significant at 1% level

Table 2 Genotypic and phenotypic correlations among traits of parents and F1 hybrids in Indian mustard

Parameters		Days to 50% flowering	Plant height (cm)	Days to maturity	Primary Branches per plant	Secondary Branchesper plant	Main shoot length (cm)	Siliquae on main shoot	No of siliquaeper plant	Siliquae length (cm)	Seeds per siliquae	1000 seed weight (g)	Oil content (%)	Fiber content (%)	Seed yield per plant (g)
Days to 50% flowering	G	1.000	0.316**	0.688**	0.026	0.216**	0.370**	0.357**	0.226**	0.094	0.320**	0.163*	0.297**	0.347**	0.224**
	P	1.000	0.213**	0.433**	0.064	0.148	0.244**	0.219**	0.140	0.070	0.177*	0.106	0.149	0.216**	0.141
Plant height (cm)	G			0.321**	0.335**	0.212**	0.519**	0.589**	0.530**	-0.130	0.028	0.149	0.124	-0.030	0.299**
	P			0.305**	0.301**	0.212**	0.518**	0.576**	0.525**	-0.120	0.025	0.149	0.104	-0.026	0.293**
Days to maturity	G				0.040	0.013	0.269**	0.286**	-0.010	0.243**	0.298**	0.270**	0.228**	-0.049	0.137
	P				0.030	0.011	0.260**	0.267**	-0.008	0.210**	0.272**	0.256**	0.173*	-0.043	0.129
Primary Branches per plant	G					0.250**	0.257**	0.220**	0.484**	-0.007	-0.051	-0.063	-0.157*	0.242**	0.226**
	P					0.236**	0.239**	0.217**	0.429**	0.027	-0.033	-0.052	-0.098	0.196*	0.206**
Secondary Branches per plant	G						0.143	0.110	0.658**	-0.153	-0.113	-0.160*	0.099	0.317**	0.392**
	P						0.144	0.113	0.652**	-0.136	-0.103	-0.159*	0.085	0.295**	0.380**
Main shoot length (cm)	G							0.899**	0.387**	-0.112	0.358**	0.185*	0.474**	0.015	0.084
	P							0.882**	0.384**	-0.103	0.337**	0.183*	0.388**	0.014	0.082
Siliquae on main shoot	G								0.456**	-0.179*	0.267**	0.141	0.489**	-0.072	0.093
	P								0.445**	-0.161*	0.269**	0.138	0.401**	-0.065	0.082
No of siliquae per plant	G									-0.124	-0.079	-0.052	0.218**	0.171*	0.649**
	P									-0.121	-0.080	-0.051	0.175*	0.162*	0.633**
Siliquae length (cm)	G										0.294**	0.115	-0.019	-0.142	-0.061
	P										0.267**	0.113	-0.023	-0.122	-0.056
Seeds per siliquae	G											0.236**	0.094	0.012	0.007
	P											0.221**	0.074	0.004	-0.009
1000 seed weight (g)	G												-0.164*	-0.100	0.006
	P												-0.139	-0.092	0.006
Oil content (%)	G													0.057	0.095
	P													0.053	0.087
Fiber content (%)	G														0.093
	P														0.089
Seed yield per plant (g)	G														1.000
	P														1.000

* Significant at 5% level, ** Significant at 1% level

Table 3 Path coefficient analysis among traits of parents and F1 hybrids in Indian mustard

Parameters	Days to 50% flowering	Plant height (cm)	Days to maturity	Primary Branches per plant	Secondary Branches per plant	Main shoot length (cm)	Siliquae on main shoot	No of siliquae per plant	Siliquae length (cm)	Seeds per siliquae	1000 seed weight (g)	Oil content (%)	Fiber content (%)	Seed yield per plant (g)
Days to 50% flowering	-0.065	-0.010	0.257	-0.004	-0.066	0.091	-0.264	0.272	-0.018	0.046	-0.005	-0.001	-0.008	0.224**
Plant height (cm)	-0.021	-0.031	0.120	-0.058	-0.065	0.128	-0.436	0.637	0.025	0.004	-0.005	0.000	0.001	0.299**
Days to maturity	-0.045	-0.010	0.374	-0.007	-0.004	0.066	-0.212	-0.013	-0.047	0.043	-0.009	-0.001	0.001	0.137
Primary Branches per plant	-0.002	-0.010	0.015	-0.174	-0.077	0.063	-0.162	0.582	0.001	-0.007	0.002	0.000	-0.006	0.226**
Secondary Branches per plant	-0.014	-0.007	0.005	-0.044	-0.306	0.035	-0.081	0.791	0.030	-0.016	0.005	0.000	-0.007	0.392**
Main shoot length (cm)	-0.024	-0.016	0.101	-0.045	-0.044	0.246	-0.665	0.465	0.022	0.052	-0.006	-0.001	0.000	0.084
Siliquae on main shoot	-0.023	-0.018	0.107	-0.038	-0.034	0.221	-0.740	0.549	0.035	0.038	-0.005	-0.001	0.002	0.093
No of siliquae/plant	-0.015	-0.016	-0.004	-0.084	-0.201	0.095	-0.338	1.202	0.024	-0.011	0.002	-0.001	-0.004	0.649**
Siliquae length (cm)	-0.006	0.004	0.091	0.001	0.047	-0.027	0.133	-0.149	-0.195	0.042	-0.004	0.000	0.003	-0.061
Seeds per siliquae	-0.021	-0.001	0.111	0.009	0.034	0.088	-0.197	-0.095	-0.057	0.144	-0.008	0.000	0.000	0.007
1000 seed weight (g)	-0.011	-0.005	0.101	0.011	0.049	0.045	-0.104	-0.062	-0.022	0.034	-0.033	0.000	0.002	0.006
Oil content (%)	-0.019	-0.004	0.085	0.027	-0.030	0.117	-0.361	0.263	0.004	0.014	0.005	-0.003	-0.001	0.095
Fiber content (%)	-0.023	0.001	-0.018	-0.042	-0.097	0.004	0.054	0.206	0.028	0.002	0.003	0.000	-0.023	0.093

Note: Residual = 0.389; Values in bold are direct effect

Table 4 Genetic components analysis of Indian mustard

Parameters	Days to 50% flowering	Days to maturity	Plant height (cm)	Primary Branches per plant	Secondary Branches per plant	Main shoot length (cm)	Siliquae on main shoot	Number of siliquae per plant	Siliquae length (cm)	Seeds per siliquae	1000 seed weight (g)	Oil content (%)	Fiber content (%)	Seed yield per plant (g)
D	9.56**	203.60**	1964.44**	2.45*	19.54	326.95**	268.66**	164067.4**	0.60**	3.19**	0.24	0.58**	0.37*	72.21
SE±	3.77	32.63	247.13	1.65	50.13	83.55	76.32	59357.7	0.11	1.57	0.30	0.07	0.19	59.30
F	17.89**	294.70**	2837.97**	5.81**	50.33	631.11**	517.27**	218827.2**	0.04	6.59**	0.28	0.82**	0.34	2.76
SE±	8.71	75.28	570.20	3.82	115.67	192.78	176.10	136956.0	0.25	3.61	0.69	0.16	0.43	136.81
H1	34.89**	433.98**	2803.75**	15.14**	450.77**	1410.62**	918.86**	827499.3**	1.22**	20.56**	3.51**	2.00**	2.64	439.04
SE±	8.03	69.45	526.03	3.52	106.71	177.85	162.46	126348.4	0.23	3.33	0.64	0.15	0.40	126.22
H2	25.76**	314.77**	1675.46**	10.93**	353.86**	1054.61**	607.36**	732613.6**	1.16**	15.66**	3.05**	1.60**	2.29**	379.93**
SE±	6.83	59.02	447.07	2.99	90.69	151.15	138.07	107382.1	0.20	2.83	0.54	0.13	0.34	107.27
h2	12.18**	257.95**	1054.53**	0.21	104.49*	1175.71**	477.31**	138245.3**	-0.01	19.76**	0.40	1.88**	0.12	8.26
SE±	4.57	39.51	299.25	2.00	60.70	101.18	92.42	71877.4	0.13	1.90	0.36	0.08	0.23	71.80
E	2.40	2.80	0.82	0.21	0.19	0.77	2.26	673.1	0.02	0.19	0.00	0.07	0.03	1.88
SE±	1.14	9.84	74.51	0.50	15.12	25.18	23.01	18897.0	0.03	0.47	0.09	0.02	0.06	17.88
(H1/D)1/2	1.91	1.46	1.19	2.48	4.80	2.08	1.85	2.25	1.42	2.54	3.82	1.85	2.67	2.47
H2/4H1	0.18	0.18	0.15	0.18	0.20	0.19	0.17	0.22	0.24	0.19	0.22	0.20	0.22	0.22
{(4 D H1)0.5+F}/{(4 D H1)0.5-F}	2.92	2.97	4.06	2.82	1.73	2.74	3.17	1.84	1.05	2.37	1.36	2.23	1.42	1.02
h^2/H2	0.47	0.82	0.63	0.02	0.30	1.11	0.79	0.19	-0.01	1.26	0.13	1.18	0.05	0.02
R value	-0.36**	-0.69**	0.02	-0.36	-0.44*	-0.77**	-0.42*	0.60**	0.50**	-0.80**	-0.05	-0.14	-0.20	0.60**
Heritability (narrow)	26.44	57.50	85.60	19.42	4.64	29.47	39.55	21.16	32.08	17.80	6.91	28.75	13.16	13.99

* Significant at 5% level, ** Significant at 1% level

D = additive genetic variance; F = relative frequency of dominant to recessive alleles; H_1 = variation due to dominant effect of genes

H_2 = variation due to dominant effect of gene correlated with gene distribution; h^2 = Over all dominant effect of heterozygous loci; $(H_1/D)^{1/2}$ = mean degree of dominance

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Effects of gamma irradiation on growth and productivity in dose-dependent response of Indian Mustard (*Brassica juncea* L.) genotypes [P13RGN-303 and RH-761]

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ABSTRACT

Gamma irradiation is a widely used mutagenic technique in plant breeding, employed to induce genetic variations for crop improvement. In this study, the effects of different doses of gamma irradiation (0, 5, 10, 15, 20 and 25 kR) on the morphological, phenological, yield, and quality traits of two Indian mustard (*Brassica juncea* L.) genotypes, P13RGN-303 and RH-761, were investigated. The study focused on key parameters such as field emergence, survival rate, plant height, days to 50% flowering, siliqua length, siliqua per plant, number of seeds per siliqua, seed yield, test weight and oil content. Significant variations were observed across the different radiation treatments, with the highest performance noted at 20 kR for both genotypes. The optimal doses of gamma irradiation (20 kR) resulted in increased field emergence and survival rates, indicating improved seed vigor. Furthermore, plant height and days to 50% flowering were favorably affected, suggesting enhanced growth and early maturity. Siliqua traits, including siliqua length and seed number, as well as seed yield (per plant, plot, and hectare), were all significantly improved, reflecting increased reproductive efficiency. The highest test weight and oil content were recorded at 20 kR, signaling enhanced seed quality. Conversely, higher doses (25 kR) led to a reduction in growth and yield, likely due to excessive radiation stress. Overall, the study demonstrates the potential of gamma irradiation at 20 kR for improving mustard traits, providing valuable insights for crop improvement initiatives.

Keywords: *Brassica juncea,* Gamma irradiation, Growth, Indian mustard, Mutagenesis, Yield

Brassica species, including *Brassica napus, B. rapa,* and *B. juncea,* are essential oilseed crops cultivated across 11 million hectares worldwide under diverse climatic conditions. Among these, *Brassica juncea* (L.) Czern. & Coss. is extensively grown in the Indian subcontinent, where it serves multiple purposes, including as a source of edible oil, a condiment, a lubricant, and a component of cattle feed and fertilizers. Given its agronomic and economic significance, *B. juncea* breeding programs focus on enhancing yield potential, disease resistance, and quality traits. Conventional breeding methods have played a crucial role in genetic improvement; however, recent advancements in nonconventional approaches such as mutation induction, tissue culture, and molecular genetics have significantly contributed to trait enhancement (Tiliouine *et al.,* 2018; Gupta, 2019; Lal *et al.,* 2020).

One of the most effective nonconventional breeding techniques is gamma radiation, a form of ionizing radiation that induces cytological, biochemical, physiological, and morphological changes in plant cells and tissues. Gamma irradiation has been widely used in plant breeding programs to generate novel genetic variations, leading to improved stress tolerance, enhanced growth, and increased yield potential (Moghaddam *et al.,* 2011; Celik and Atak, 2017). The interaction of gamma rays with plant cells triggers the production of reactive oxygen species (ROS) such as superoxide radicals, hydroxyl radicals, and hydrogen peroxide, which alter cellular structures and metabolic functions (Esfandiari *et al.,* 2007. While high doses of gamma radiation can cause severe physiological damage, including chromosomal aberrations and impaired photosynthesis, low doses have been shown to stimulate growth, enhance secondary metabolite production, and improve stress resilience by activating specific physiological pathways (Aly, 2010; Vardhan and Shukla, 2017).

In mustard (*B. juncea*), gamma radiation has been explored as a means to address key challenges such as low genetic diversity, vulnerability to abiotic stresses, and limited yield potential. Studies have demonstrated that low doses of gamma irradiation can improve photosynthetic efficiency, increase chlorophyll content, and enhance stress tolerance mechanisms (Kulandaivelu and Noorudeen, 1983; Wi *et al.,* 2005). Additionally, chlorophyll fluorescence analysis has emerged as a reliable tool for assessing plant health and photosynthetic performance under stress conditions (Sousaraei *et al.,* 2021; Esmaeili *et al.,* 2022).

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Given the high nutritional and economic value of mustard oil, which is rich in omega-3 and omega-6 fatty acids with significant health benefits, the development of improved mustard varieties through gamma irradiation is of paramount importance (Gupta *et al.,* 2014; Bhatia *et al.,* 2021).

This study aims to evaluate the mutagenic effectiveness and efficiency of different doses of gamma radiation on in vitro regeneration and physiological traits of *B. juncea.* By examining dose-dependent responses, the research seeks to determine the optimal radiation levels that induce beneficial genetic variations while minimizing deleterious effects. The findings will contribute to the development of superior mustard genotypes with enhanced agronomic performance, thereby supporting sustainable agricultural practices and global food security.

MATERIALS AND METHODS

This study aimed to evaluate the effect of gamma irradiation on growth, yield, and oil content in two mustard (Brassica juncea) genotypes, P13RGN-303 and RH-761, under field conditions. The research was conducted during the *rabi* season of 2023-24 at the Field Experimentation Centre, Department of Genetics and Plant Breeding, Naini Agricultural Institute, Sam Higginbottom University of Agriculture, Technology and Sciences (SHUATS), Prayagraj, Uttar Pradesh, India. The mustard seeds were procured from ICAR-Directorate of Rapeseed-Mustard Research (ICAR-DRMR), Bharatpur, Rajasthan, India. The seeds were irradiated with gamma rays at the National Botanical Research Institute (CSIR-NBRI), Lucknow, using a GIC-1200 model gamma irradiator equipped with a Cobalt-60 (^60Co) radioactive source. The treatments included a control (T0 - unirradiated seeds) and five gamma irradiation doses: 5 kR (T1), 10 kR (T2), 15 kR (T3), 20 kR (T4), and 25 kR (T5).

The experiment was laid out in a Randomized Block Design (RBD) with six treatments, each replicated three times. Each plot had a uniform size of 2 × 2 m² with a seed rate of 5 kg per hectare and a row-to-row spacing of 30 cm, while the plant-to-plant spacing was 10 cm to ensure optimal growth conditions. The experimental soil was sandy loam in texture, with a pH of 7.1, electrical conductivity of 0.37 dS/m, organic carbon content of 0.50%, available nitrogen of 189.6 kg/ha, available phosphorus of 12.96 kg/ha, and available potassium of 225.7 kg/ha. Standard agronomic practices, including irrigation, thinning, weeding, and pest management, were uniformly followed across all plots.

The survival rate of mustard plants under different gamma irradiation doses was assessed using the exponential survival model (FAO, 2013):

$$S(D) = S_0.e^{-kD}$$

where S(D) represents the survival rate at a given radiation dose D, S0 is the survival rate in the control treatment, and k is the survival decline constant. Oil content was estimated using the AOAC method, where oil was extracted using a Soxhlet apparatus with petroleum ether as the solvent. The oil percentage was calculated using the formula:

Oil Content (%) = (Weight of Extracted Oil / weight of seed sample) * 100

Data for survival rate, growth parameters, yield, and oil content were statistically analyzed using analysis of variance (ANOVA) for a randomized block design (RBD), with significance determined at a 5% probability level (P = 0.05) using the Least Significant Difference (LSD) test. Statistical software was used for precision in data analysis. Observations were systematically recorded at critical growth stages, including germination, seedling establishment, vegetative growth, flowering, pod formation, and physiological maturity. The recorded parameters included germination percentage, plant survival rate, seedling vigor, plant height, number of branches per plant, days to 50% flowering, pod formation percentage, seed yield per plant and per plot, and oil content percentage. The study provides insights into the impact of gamma irradiation on mustard genotypes and its potential application in crop improvement through induced mutagenesis.

RESULTS AND DISCUSSION

Gamma irradiation is widely utilized in mutation breeding to induce genetic variability, enhance stress tolerance, and improve agronomic traits in crops. Its impact on plant growth and yield is mediated through complex physiological, biochemical, and molecular changes rather than a direct effect on seed yield. The extent of these alterations depends on the radiation dose, with moderate levels often stimulating beneficial mutations and metabolic processes, while excessive doses may induce oxidative stress, DNA damage, and metabolic dysfunctions. This study evaluated the response of two mustard genotypes, P13RGN-303 and RH-761, to varying doses of gamma irradiation, with a focus on growth attributes, reproductive traits, and seed yield potential (Tables 1 to 4).

Field emergence (FE) and survival rate (SR) showed significant variation among treatments, reflecting the effect of gamma irradiation on seed viability and seedling vigor (Tables 3 and 4). In P13RGN-303, the highest FE (85.0%) and SR (82.7%) were recorded at 20 kR, whereas the lowest values (76.67% and 74.00%, respectively) occurred at 25

kR. A similar pattern was observed in RH-761, where 20 kR resulted in the highest FE (78.33%) and SR (75.67%). The reduction at 25 kR can be attributed to excessive radiation-induced oxidative stress and impaired cellular function, as described by Alikamanoglu *et al.* (2007). In contrast, moderate doses may enhance germination and seedling establishment by activating DNA repair pathways and antioxidant defense mechanisms (Hamideldin and Eliwa, 2015).

Table 1 Analysis of variance on effect of different doses of Gamma irradiation on Indian mustard (*Brassica juncea* L.) Genotype P13RGN-303

Characters	Mean sum of square		
	Treatment (d.f=5)	Replication (d.f=2)	Error (d.f=10)
Field emergence	23.3*	2.00	4.40
Survival rate	26.93*	2.16	7.50
Plant height	22.04*	34.26	4.61
Days to 50% flowering	48.35*	34.72	6.79
Siliqua length	0.27*	0.00	0.01
Siliqua/plant	363.68*	338.74	1,088.61
Number of seeds/ Siliqua	0.27*	0.00	0.01
Seed yield/Plant	363.68*	338.74	108.86
Seed yield/Plot	3484.06*	504.39	211.79
Seed yield/Hectare	3.87*	0.56	0.24
Test weight	0.07*	0.34	0.02
Days to maturity	33.55*	7.39	7.92
Oil content	8.29*	4.66	2.07

*Indicates significant at 5% level of significance

Table 2 Analysis of variance on effect of different doses of Gamma irradiation on Indian mustard (*Brassica juncea* L.) Genotype RH-761

Characters	Mean sum of square		
	Treatment (d.f=5)	Replication (d.f=2)	Error (d.f=10)
Field emergence	10.23*	0.67	2.60
Survival rate	6.22*	8.22	1.49
Plant height	22.58*	102.13	2.31
Days to 50% flowering	6.89*	9.06	1.46
Siliqua length	0.10*	0.01	0.56
Siliqua/plant	159.45*	1,474.46	47.16
No. of seeds/ Siliqua	1.58*	0.11	0.47
Seed yield/Plant	16.05*	22.07	3.46
Seed yield/Plot	34,491.97*	52.67	99.13
Seed yield/Hectare	3.88*	0.06	0.11
Test weight	0.28*	0.05	0.09
Days to maturity	13.69*	5.06	2.86
Oil content	9.83*	1.50	2.83

*Indicates significant at 5% level of significance

Plant height and days to 50% flowering were significantly affected by gamma irradiation. The tallest plants (177 cm) in P13RGN-303 were observed at 15 kR, while the shortest plants (169 cm) occurred at 25 kR. Similarly, RH-761 exhibited maximum height (158 cm) at 20 kR, with a significant reduction at 25 kR (150 cm). These findings align with earlier reports by Sharma *et al.* (2017), indicating that moderate radiation doses enhance auxin biosynthesis and promote cell elongation, whereas excessive exposure disrupts mitotic activity and reduces

plant height. Early flowering was recorded at 20 kR for both genotypes (51 days in P13RGN-303; 73 days in RH-761), while 25 kR delayed flowering. Gamma irradiation at optimal levels may modulate hormonal homeostasis, particularly gibberellins and cytokinins, accelerating floral initiation and shortening the vegetative phase (Guangyaol *et al.,* 2007).

Siliqua-related traits, including siliqua length (SL), siliqua per plant (SPP), and the number of seeds per siliqua (NSPS), were significantly influenced by irradiation. In P13RGN-303, the highest SL (5.8 cm), SPP (272.08), and NSPS (12.85) were recorded at 20 kR, while similar improvements were observed in RH-761. However, 25 kR caused a decline in these traits, likely due to radiation-induced disruptions in reproductive development, such as ovule abortion and reduced pollen viability, as reported by Garg *et al.* (2022). Moderate doses, however, may induce beneficial genetic variations that enhance seed set and assimilate partitioning.

Seed yield per plant (SYPP), seed yield per plot (SYPPl), and seed yield per hectare (SY) exhibited significant improvements at 20 kR. In P13RGN-303, the highest SYPP (24.66 g), SYPPl (614.00 kg) and SY (20.47 q/ha) were recorded at 20 kR, whereas 25 kR resulted in the lowest values. A similar pattern was observed in RH-761, with peak yield recorded at 20 kR. These findings corroborate earlier studies by Yassein and Amina, 2014, which demonstrated that moderate gamma irradiation enhances photosynthetic efficiency, nutrient uptake, and carbon assimilation, leading to improved seed productivity. However, excessive radiation exposure negatively impacts chloroplast function and disrupts source-sink relationships, ultimately reducing yield potential.

Table 3 Mean performance on effect of different doses of Gamma irradiation on morphological and yield components of mustard (*Brassica junceae*) Genotype P13RGN-303

Treatment	FE (%)	SR (%)	PH (cm)	50% DOF	SL (cm)	SPP	NSPS	SYPP (g)	SYPP (kg)	SY (ha)	TW (g)	DM	OC (%)
T0	81.67	79.33	175.04	58.67	5.45	271.28	12.17	23.74	560.00	18.67	4.13	138	38.48
T1	79.67	77.00	174.29	55.67	5.23	267.17	11.83	21.75	550.67	18.36	3.63	139	37.65
T2	80.00	80.00	174.07	60.00	5.25	262.55	12.47	24.31	566.67	18.89	3.93	141	39.32
T3	82.00	77.00	177.81	53.33	5.33	271.47	12.72	23.42	576.67	19.22	4.17	138	39.69
T4	85.00	82.67	177.51	51.33	5.80	272.08	12.85	24.66	614.00	20.47	4.33	136	41.29
T5	76.67	74.00	169.82	61.67	4.88	243.44	11.34	21.66	509.67	16.99	3.57	146	36.54
CD (5%)	5.07	5.45	5.28	7.31	0.55	20.03	1.04	2.32	62.00	2.07	0.56	6.37	3.02
CV (%)	3.45	3.83	1.66	7.08	5.66	4.16	4.68	5.49	6.05	6.05	7.76	2.51	4.28

Legends: FE-Field emergence, SR-Survival rate, PH-Plant height, DOF-Days of flowering, SL-Siliqua length, SPP-Siliqua per plant, NSPS Number of seeds per siliqua, SYPP-Seed yield per plant, SYPP-Seed yield per plot, SY-Seed yield, TW-Test weight, OC-Oil content, DM-days to maturity, T0-Control, T1-5kR, T2-10kR, T3-15kR, T4-20kR, T5-25kR treatment of Gamma rays

Table 4 Mean performance on effect of different doses of Gamma irradiation on morphological and yield components of mustard (*Brassica junceae*) Genotype RH-761

Treatment	FE (%)	SR (%)	PH (cm)	50% DOF	SL (cm)	SPP	NSPS	SYPP (g)	SYPP (kg)	SYPH	TW (g)	DM	OC (%)
T0	76.00	73.33	155.40	77.33	5.26	131.22	12.17	22.92	493.66	16.46	3.03	143	34.33
T1	74.67	72.67	154.11	76.67	5.20	135.53	12.17	24.38	475.33	15.84	3.00	143	32.33
T2	76.00	72.33	155.50	75.33	5.30	131.08	11.59	22.67	481.00	16.03	3.27	142	33.00
T3	77.00	74.00	152.00	76.00	5.32	136.78	12.50	24.20	492.33	16.41	3.17	141	35.33
T4	78.33	75.67	158.39	73.00	5.34	140.33	13.50	26.00	561.66	18.72	3.17	140	36.33
T5	73.00	70.33	150.70	76.33	5.13	119.33	11.50	19.18	467.00	15.57	2.83	146	31.67
CD (5%)	4.87	5.22	5.10	6.95	0.48	11.36	1.02	2.15	56.70	1.94	0.41	5.23	2.97
CV (%)	3.25	3.67	2.14	6.89	4.92	4.08	4.52	5.12	5.87	5.79	6.72	2.27	4.11

Legends: FE-Field emergence, SR-Survival rate, PH-Plant height, DOF-Days of flowering, SL-Siliqua length, SPP-Siliqua per plant, NSPS-Number of seeds per siliqua, SYPP-Seed yield per plant, SYPP-Seed yield per plot, SY-Seed yield, TW-Test weight, OC-Oil content, DM-days to maturity, T0-Control, T1-5kR, T2-10kR, T3-15kR, T4-20kR, T5-25kR treatment of Gamma rays

Test weight (TW), oil content (OC), and days to maturity (DM) were also significantly affected by irradiation. The highest TW (4.33 g) and OC (41.29%) in P13RGN-303 were observed at 20 kR, while the lowest values (3.57 g and 36.54%, respectively) were recorded at 25 kR. Similarly, RH-761 exhibited the highest TW (3.17 g) and OC (36.33%) at 20 kR, with a significant decline at 25 kR. The improvement in oil content at moderate doses suggests a positive influence on lipid biosynthesis, possibly through the up-regulation of genes involved in fatty acid metabolism (Mohurle *et al.,* 2017). In contrast, excessive irradiation may impair metabolic pathways, leading to reduced oil accumulation and seed deterioration.

Days to maturity were slightly reduced at 20 kR for both genotypes, indicating an accelerated reproductive phase. This effect may be attributed to radiation-induced modulation of flowering genes and hormonal signals, promoting early transition to reproductive growth. These findings highlight the potential of moderate gamma irradiation as a strategic tool for improving mustard yield and seed quality while emphasizing the need to avoid excessive doses that may induce deleterious effects.

The results of this study highlight the potential of gamma irradiation as an effective tool for improving the morphological and yield characteristics of mustard genotypes P13RGN-303 and RH-761. Moderate doses, particularly 20 kR, showed significant positive effects on field emergence, plant height, seed yield, and oil content, suggesting the suitability of gamma radiation for enhancing these traits. However, higher doses (25 kR) were observed to have adverse effects, underscoring the importance of optimizing irradiation doses for maximum benefit. These findings contribute valuable insights into the use of gamma irradiation for mustard breeding programs aimed at improving productivity and quality.

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Effect of different doses of gamma rays on Indian mustard (*Brassica juncea* L.) genotypes

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ABSTRACT

Mutation breeding through gamma irradiation is a widely used technique to induce genetic variability and improve crop traits. This study aimed to evaluate the effects of different doses of gamma radiation on mustard (*Brassica juncea* L.) genotypes DRMR 1165-40 and RADHIKA. The experiment was conducted in a Randomized Block Design with three replications during the *rabi* season 2023-2024 at the Field Experimentation Centre, Department of Genetics and Plant Breeding, SHUATS, Prayagraj. Mustard seeds were exposed to gamma radiation at doses of 5 kR (T1), 10 kR (T2), 15 kR (T3), 20 kR (T4), and 25 kR (T5), along with a control (T0). Results indicated that lower radiation doses (5 kR) significantly improved germination percentage, survival rate, plant height, siliqua per plant, seeds per siliqua, seed yield per plant, oil content, and test weight. In contrast, higher doses (22 kR) adversely affected these traits, leading to reduced plant vigour, delayed flowering and maturity, and lower yield attributes. The findings suggest that gamma radiation induces physiological and genetic modifications, influencing growth and reproductive traits. Lower radiation doses can be effectively utilized for trait enhancement in mustard without compromising plant health, whereas higher doses may cause detrimental effects. These results align with previous reports on mutation breeding in oilseeds and provide valuable insights for optimizing radiation doses in crop improvement programs. Further molecular and biochemical analyses are recommended to elucidate the mechanisms underlying radiation-induced variations.

Keywords: Gamma irradiation, Growth, Mustard, Mutagenesis, Yield

Indian mustard (*Brassica juncea* L.) is a vital oilseed crop in India, ranking second only to groundnut in terms of edible oil production. It belongs to the family Cruciferae (syn. Brassicaceae) and is commonly referred to as 'rai,' 'raya,' or 'laha.' The genus Brassica comprises over 159 species, with *B. juncea, B. campestris* and *B. napus* being the predominant cultivated species. India is among the leading global producers of oilseeds, with mustard playing a crucial role in the national oilseed economy. The crop contributes essential lipids for nearly 50% of the northern Indian population, underscoring its nutritional and economic significance (Gupta and Grossmann, 2014).

On a global scale, India ranks fourth in mustard seed production, accounting for approximately 14% of the total output, following the European Union (34%), China (23%), and Canada (19%). Within India, major mustard-producing states include Rajasthan, Madhya Pradesh, Uttar Pradesh, Haryana, West Bengal, and Assam, which collectively contribute 86.72% of the total cultivated area and 89.53% of the national production. Rajasthan alone accounts for nearly 45% of India's mustard output. In 2019-20, the global rapeseed-mustard cultivation area was recorded at 35.95 million hectares, with a total production of 71.49 million tonnes and an average yield of 1990 kg/h (DRMR, 2020-21).

Indian mustard is characterized by high oil content, ranging from 35% to 45%, making it a crucial source of mustard oil. The extracted oil is rich in monounsaturated and polyunsaturated fatty acids, particularly omega-3 and omega-6 fatty acids, which contribute to cardiovascular health benefits (Bhatia *et al.,* 2021). Additionally, Indian mustard contains bioactive compounds such as glucosinolates, which exhibit anticancer, antioxidant, and anti-inflammatory properties (Shukla *et al.,* 2023).

Mutagenesis plays a fundamental role in plant breeding by inducing genetic variability, a key factor for crop improvement. Natural mutations occur at a very low frequency; therefore, induced mutations through physical and chemical mutagens provide an effective strategy to enhance genetic diversity. Among physical mutagens, ionizing gamma rays have been extensively utilized in plant mutation breeding due to their high energy and strong penetrative capacity. Gamma rays, a form of electromagnetic radiation akin to X-rays but with shorter wavelengths (<0.01 Å), interact with DNA at the molecular level, leading to genetic modifications that can result in desirable morphological, physiological, and biochemical alterations (Shukla *et al.,* 2023).

The present study hypothesizes that exposure to different doses of gamma radiation will induce genetic variability in Indian mustard genotypes, leading to significant alterations in growth parameters, yield attributes and oil composition. It is anticipated that an optimal radiation dose will enhance favorable traits while mitigating deleterious effects such as

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reduced germination and abnormal plant development. Despite the extensive cultivation and economic relevance of Indian mustard, systematic studies investigating the effects of gamma irradiation on its genetic enhancement remain limited. While previous research has established the efficacy of gamma rays in inducing mutations across various crop species, their specific impact on the yield components, biochemical properties, and stress resilience of Indian mustard remains inadequately explored. A precise understanding of the dose-response relationship is imperative for optimizing mutation breeding strategies. Addressing this research gap will facilitate the development of superior mustard varieties with enhanced productivity, stress tolerance, and improved oil quality.

MATERIALS AND METHODS

The present study was conducted at the Field Experimentation Center of the Department of Genetics and Plant Breeding, Naini Agricultural Institute, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, Uttar Pradesh, during the *rabi* season of 2023-24. The objective was to assess the effects of gamma irradiation on *Brassica juncea* L. genotypes to induce beneficial mutations for crop improvement.

The mustard seeds were procured from ICAR-Directorate of Rapeseed-Mustard Research (ICAR-DRMR), Bharatpur, Rajasthan, India. The seeds were irradiated with gamma rays at the National Botanical Research Institute (CSIR-NBRI), Lucknow, using a GIC-1200 model gamma irradiator equipped with a Cobalt-60 (^60Co) radioactive source.

Two *Brassica juncea* L. varieties, i.e. DRMR 1165-40 and Radhika, were selected for the study. The experiment was arranged in a randomized block design (RBD) with six treatments, each replicated three times. The treatments included a control (T0 - un-irradiated seeds) and five gamma irradiation doses: 5 kR (T1), 10 kR (T2), 15 kR (T3), 20 kR (T4), and 25 kR (T5). Each plot had a uniform size of 2 × 2 m², with a seed rate of 5 kg per hectare. Row-to-row spacing was maintained at 30 cm, while plant-to-plant spacing was 10 cm to ensure optimal growth conditions. Standard agronomic practices, including irrigation, thinning, weeding, and pest management, were uniformly followed across all plots.

The survival rate of mustard plants under different gamma irradiation doses was assessed using the exponential survival model (FAO, 2013):

$$S(D) = S_0.e^{-kD}$$

where S(D) represents the survival rate at a given radiation dose D, S_0 is the survival rate in the control treatment, and k is the survival decline constant.

Oil content was estimated using the AOAC method, where oil was extracted using a Soxhlet apparatus with petroleum ether as the solvent. The oil percentage was calculated using the formula:

Oil Content (%) = (Weight of Extracted Oil / Weight of Seed Sample) × 100

Statistical Analysis: Data for survival rate, growth parameters, yield, and oil content were statistically analyzed using analysis of variance (ANOVA) for a randomized block design (RBD), with significance determined at a 5% probability level (P = 0.05) using the Least Significant Difference (LSD) test.

RESULTS AND DISCUSSION

The study was conducted to evaluate six treatment combinations of gamma irradiation on two mustard genotypes, DRMR 1165-40 and RADHIKA.

Gamma irradiation significantly influenced germination percentage, survival rate, and plant height. In DRMR 1165-40, the highest germination percentage (90.22%) was recorded in T1, whereas the lowest (77.25%) was observed in T5. Similarly, in RADHIKA, germination was highest in T1 (90.26%) and lowest in T5 (77.29%). These findings align with reports by Yasin and Aly (2014) and Sangsiri et al. (2005), who observed enhanced germination under low-dose irradiation. The survival rate followed a similar trend, with T1 exhibiting the highest survival percentage (87.66% in DRMR 1165-40 and 87.70% in RADHIKA), whereas T5 recorded the lowest values (74.69% and 74.73%, respectively). A decrease in survival at higher doses corroborates the findings of Voice *et al.* (2004) and Wang *et al.* (2008), who reported adverse effects of radiation on mustard.

Plant height at 90 DAS was highest in T1 (187.94 cm in DRMR 1165-40 and 191.44 cm in RADHIKA) and lowest in T5 (164.50 cm and 168.00 cm, respectively). This reduction in height at higher doses of gamma radiation was also noted by Siddiqui *et al.* (2009) and Rahimi and Bahrani (2011), indicating possible mutagenic effects that impede growth.

Gamma ray treatments influenced flowering, maturity, and yield-related traits. The earliest flowering was observed in T1 (51.33 days in DRMR 1165-40 and 53.33 days in RADHIKA), whereas T5 delayed flowering (62.33 and 64.33 days, respectively). These results align with Mishra and Singh (2014), who reported accelerated flowering at lower doses of mutagens. Days to maturity were also reduced under T1 (140.98 in DRMR 1165-40 and 138.28 in

RADHIKA), whereas the longest duration was recorded in T5 (146.87 and 144.17, respectively), consistent with findings by Thagana *et al.* (2013) and Hassan and Haleem (2014).

Yield-related traits such as siliqua per plant, seeds per siliqua and test weight exhibited dose-dependent variations. The highest number of siliqua per plant was recorded in T1 (135.89 in DRMR 1165-40 and 138.69 in RADHIKA), while T5 exhibited the lowest values (72.17 and 74.97, respectively). These findings agree with reports by Yaqoob and Ahmed (2003) and Moushree and Sabyasachi (2018), who noted similar trends under mutagenic treatments. The number of seeds per siliqua was highest in T1 (22.64 in DRMR 1165-40 and 23.10 in RADHIKA) and lowest in T5 (15.15 and 15.61, respectively). A significant reduction in seed number at higher doses was also documented by Singh and Yadav (1991) and Kumar and Mishra (1999).

Table 1 Analysis of variance on effect of different doses of gamma irradiation on Indian mustard (*Brassica juncea* L.) genotype DRMR1165-40 AND RADHIKA

Characters	Mean sum of squares		
	Treatment	Replication	Error
	DF=5	DF=2	DF=10
Germination	81.41*	330.34	171.37
Survival rate %	5.9	5.5	53.7
Plant height	3.1*	103.0	28.3
Days of 50% flowering	75.1*	191.1	86.9
Seed yield/ plant	0.1*	6.6	0.5
Silliqua/plant	72.2**	7650.1	276.9
Seeds/silliqua	9.1*	105.8	18.5
Oil content	8.3*	30.7	17.1
Days to Maturity	59.9**	272.4	105.7
Test weight (gm)	0.1*	1.1	0.5
Silliqua length	0.1**	2.6	0.5

*Indicates significant at 5% level of significance

Table 2 Mean performance of different doses of Gamma Rays in Mustard (*Brassica juncea*) genotypes

Genotypes	Traits	Treatments						SEm (+)	C.D.at 0.5%
		T0 (Control)	T1 (5kR)	T2 (10kR)	T3 (15kR)	T4 (20kR)	T5 (25kR)		
DRMR 1165-40	Germination %	82.65	90.22	86.16	80.72	79.86	77.25	2.39	7.53
	Survival %	80.09	87.66	83.60	78.16	77.30	74.69	1.30	4.13
	Plant Height	174.72	187.72	179.44	169.28	169.06	164.5	3.38	10.66
	Days to 50 % flowering	56.67	51.33	56.67	57.67	58.67	62.33	1.70	5.36
	Seed yield/plant(g)	29.76	35.08	32.12	26.87	24.58	23.57	1.30	4.10
	Silliqua/plant	98.33	135.89	101.28	88.56	78.61	72.17	0.13	0.41
	Seeds per silliqua	20.91	22.64	21.38	19.73	18.42	15.15	0.79	2.48
	Oil content %	40.98	41.51	41.32	40.78	39.45	37.79	0.76	2.38
	Days to Maturity	142.32	140.98	141.65	143.21	144.54	146.87	1.88	5.91
	Test weight (g)	3.80	3.87	3.83	3.77	3.73	3.33	0.12	0.39
	Silliqua length	3.89	4.54	4.15	3.76	3.53	3.40	0.55	1.74
RADHIKA	Germination %	82.69	90.26	86.20	80.76	79.9	77.29	2.39	7.53
	Survival %	80.13	87.7	83.64	78.20	77.34	74.73	1.30	4.13
	Plant Height	178.22	191.44	182.94	172.78	172.56	168.00	3.38	10.66
	Days to 50 % flowering	58.67	53.33	58.67	59.67	60.67	64.33	1.70	5.36
	Seed yield (g/plant)	31.11	36.43	33.47	28.21	25.92	24.91	1.30	4.10
	Silliqua/plant	101.13	138.69	104.08	91.36	81.41	74.97	0.13	0.41
	Seeds per silliqua	21.37	23.10	21.84	20.19	18.88	15.61	0.79	2.48
	Oil content %	41.18	41.71	41.52	40.98	39.65	37.99	0.76	2.38
	Days to Maturity	139.62	138.28	138.95	140.51	141.84	144.17	2.50	7.87
	Test weight (g)	3.85	3.96	3.88	3.82	3.78	3.38	0.12	0.39
	Silliqua length (cm)	3.97	4.62	4.23	3.84	3.61	3.48	0.55	1.74

Test weight showed a decreasing trend with increasing radiation dose. The highest values were observed in T1 (3.87 g in DRMR 1165-40 and 3.96 g in RADHIKA), whereas the lowest were recorded in T5 (3.33 g and 3.38 g, respectively). Similar trends were reported by Yadav *et al.* (2017), where gamma-ray-induced mustard mutants exhibited variations in seed weight and were subsequently commercialized as Binasarisha-7 and Binasarisha-8.

Gamma irradiation significantly affected oil content and seed yield per plant. The maximum oil content was recorded in T1 (41.51% in DRMR 1165-40 and 41.71% in RADHIKA), whereas the lowest was found in T5 (37.79% and 37.99%, respectively). These results indicate that lower doses of gamma radiation can enhance oil accumulation, a trend also observed by previous researchers.

Seed yield per plant followed a similar trend, with the highest yield recorded in T1 (35.08 g in DRMR 1165-40 and 36.43 g in RADHIKA), whereas T5 exhibited the lowest yield (23.57 g and 24.91 g, respectively). The observed reduction in seed yield at higher doses may be attributed to physiological and chromosomal disturbances induced by irradiation. Similar findings have been reported by various studies on the effect of mutagenic treatments on mustard yield components.

The present study revealed that the lower doses of gamma radiation (T1) consistently enhanced germination percentage, survival rate, plant height, siliqua per plant, seeds per siliqua, seed yield per plant, oil content and test weight in both DRMR 1165-40 and RADHIKA genotypes. Conversely, higher doses (T5) adversely affected these parameters, indicating a detrimental impact on plant growth and productivity. The reduction in days to flowering and days to maturity with increasing radiation exposure suggests that gamma rays influence phenological traits, potentially altering the reproductive cycle of mustard. These findings align with previous reports on mutagenic effects in Brassica species, confirming that optimal radiation doses can induce beneficial genetic variations. The observed dose-dependent responses highlight the potential of gamma irradiation as a tool for mutation breeding, with lower doses proving effective in enhancing yield-related attributes. Future studies should focus on molecular and biochemical assessments to elucidate the underlying mechanisms governing radiation-induced variations in mustard.

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GT 7: High yielding, high oil content and bold seeded variety of sesame (*Sesamum indicum* L.)

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ABSTRACT

A high yielding genotype of sesame SKT 1501 was evolved from cross between RT 334 and GT 3 at Castor-Mustard Research Station, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar and identified for release as Gujarat Til 7 (Banas Gaurav) for Gujarat state. The genotype SKT 1501 was identified owing to its superior performance in preliminary yield trial conducted at Sardarkrushinagar during *kharif* 2015. It was evaluated in multilocation trials from *kharif* 2016 to *kharif* 2020, simultaneously it was screened against various insects and diseases under field conditions at Amreli and Sardarkrushinagar. Under AICRP trial, it was tested under Initial Varietal Trial (*Kharif*) at different five centres of zone I in the year 2019. The mean seed yield of GT 7 (Banas Gaurav) variety under *kharif* season in Gujarat state has been recorded 957 kg/ha with a tune of 25.92, 18.73, 8.87, 21.49 and 18.16 per cent higher than the check varieties GT 2, GT 3, GT 4, GT 6 and GT 10, respectively. It was also found superior in quality traits *viz.,* oil content (49.06 %), linoleic acid content (44.69 %) and linolenic acid content (0.32 %). It has higher 1000 seed weight (3.48 g) than check varieties. Considering the average seed yield in *kharif* condition and quality of the genotype SKT 1501, it was proposed and identified for release as Gujarat Til 7 (Banas Gaurav) for general cultivation for sesame growing farmers of Gujarat state.

Keywords: Bold seed and Sesame, GT 7, High oil content, High seed yield

Sesame (*Sesamum indicum* L) a member of the Pedaliaceae family (Nayar, 1984) is one of the oldest oilseed crops grown throughout the tropical and sub-tropical regions of the world. Sesame is an indeterminate and primarily self-pollinating crop, with an out crossing rate ranging from 1% to 42% (Sirisha *et al.,* 2022). Cross-pollination occurs mainly through honeybee activity (Andrade *et al.,* 2014). Sesame oil is considered as the queen of high quality vegetable oil for human consumption, as it contains high levels of unsaturated fatty acids (Nupur *et al.,* 2010). Sesame seeds are nutrient-dense, comprising approximately 50% oil, 25% protein and 155 carbohydrates (Ranganatha *et al.,* 2013). The sesame oil, which is rich in oleic and linoleic acids, is widely used in cooking, salad dressing and margarine. It can be used in manufacture of soaps, paints, perfumes, insecticides and pharmaceutical products. Additionally, its high antioxidant content, particularly sesamol, enhances the shelf life of fried foods. Sesame meal a byproduct of oil extraction, serves as a high quality protein source for poultry and livestock (Wei *et al.,* 2022). India is the second largest producer of sesame in the world. It is cultivated in an area of 10.08 lakh ha in India with an annual production of 3.95 lakh tonnes and productivity of 392 kg/ha. In Gujarat, an area is 0.58 lakh ha with annual production of 0.30 lakh tonnes and productivity of 526 kg/ha (Anonymous, 2023). At present for *kharif* season, varieties GT 3, GT 4, GT 6 and GT 10 are under seed production chain and covering major area of Gujarat state [Monpara *et al.* (2008) and Monpara *et al.* (2011)]. Low productivity of sesame in India is mainly due to cultivation of varieties with poor yield potential and inconsistent yield performance under varied environmental conditions. Hence, there is a need to augment the productivity of crop through crop improvement programmes. Keeping this objective in view, breeding efforts was initiated to evolve new high yielding variety of sesame suitable in different agro-climatic conditions.

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MATERIALS AND METHODS

In order to improve sesame yield potential, hybridization programme was initiated in *kharif* 2009 at Castor-Mustard Research Station, Saradarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar. The genotype SKT 1501 was evolved from the cross between RT 334 and GT 3. The elite plants were selected from F_2 generation onwards and they were evaluated for their sustained yield ability and homozygosity through pedigree method of plant breeding. The genotype SKT 1501 was first evaluated in Preliminary Yield Trial (PYT) at station level during *kharif* 2015. The genotype was found promising, hence it was evaluated for its potentiality at various locations of Gujarat in randomized block design in different categories of state trials *viz.,* Small Scale Varietal Trial (SSVT) during *kharif* 2016 to *kharif* 2017 and Large Scale Varietal Trial (LSVT)

during *kharif* 2018 to *kharif* 2020. Besides, this genotype was evaluated in Initial Varietal Trial (*kharif*) at different five centres of zone I in the year 2019. This genotype was also screened for insects like leaf webber and mites as well as disease like phyllody, powdery mildew, cercospora leaf spot, phytopthora blight, macrophomina stem rot etc. in field condition as per standard scale. The DNA fingerprinting of SKT 1501 along with five checks (GT 2, GT 3, GT 4, GT 6 and GT 10) were performed by using 16 ISSR primers. The seed yield data was analyzed by randomized block design as suggested by Panse and Sukhatme (1985).

RESULTS AND DISCUSSION

The genotype SKT 1501 was tested in preliminary yield trial at Sardarkrushinagar during *kharif* 2015 and found promising, hence it was promoted to multilocation trials from 2016 to 2020 at different centres of Gujarat state. The genotype SKT 1501 has been tested at 17 different research stations/centres of Gujarat under *kharif* season. The mean performance of SKT 1501 for seed yield was 957 kg/ha with a tune of 25.92, 18.73, 8.87, 21.49 and 18.16 per cent higher than check varieties GT 2, GT 3, GT 4, GT 6 (local checks) and GT 10 (National check), respectively. (Table 1).

Table 1 Yield Performance of Sesame entry SKT 1501 (GT 7) in comparison with check varieties in the Gujarat State

Year/ Season	Name of Trial	Locations	Seed Yield (kg/ha)						S.Em. +	CD at 5%	CV%
			Proposed entry SKT 1501	GT 2 (LC) a	GT 3 (LC) b	GT 4 (LC) c	GT 6 (LC) d	GT 10 (NC) e			
2015	PYT	SKNagar	1477abc	553	633	784	-	-	49.00	144	9.97
		Mean	1477	553	633	784	-	-	-	-	-
		% Increase over checks		167.09	133.33	88.39	-	-	-	-	-
2016	SSVT	Amreli	457	901	1356	1429	-	999	81.34	232	16.66
		Junagadh	675abce	411	392	428	-	484	44.07	126	17.94
		Talod	1237abce	583	804	924	-	698	53.27	152	13.93
		SKNagar	1292abce	718	791	806	-	1051	46.03	132	11.04
		Dhari#	631	416	572	744	-	792	64.67	185	25.33
		Mean (4)	858	606	783	866	-	805	-	-	-
		% Increase over checks		41.58	9.58	-0.92	-	6.58	-	-	-
2017	SSVT	Amreli	519	461	523	559	-	548	35.23	102	13.87
		Junagadh	801	847	895	882	-	639	64.11	185	15.90
		SKNagar	1313ae	1070	1146	1175	-	996	65.90	190	11.79
		Dhari@	118	22	65	38	-	670	16.30	47	22.13
		Mean (3)	878	793	855	872	-	728	-	-	-
		% Increase over checks		10.72	2.69	0.69	-	20.60	-	-	-
2018	LSVT	Amreli	512	712	932	982	826	809	71.61	205	17.26
		Junagadh	2101abcde	1557	1409	1458	1198	1369	91.51	262	12.30
		Jamnagar	1233	1277	1072	1418	1068	1273	63.66	183	12.31
		Dhandhuka	642abde	425	493	625	524	541	23.56	68	08.97
		Talod	982abcde	695	347	265	776	758	45.21	130	13.67
		Targhadiya	1101e	1111	1241	1345	981	868	68.99	198	12.69
		Vallabhipur#	408	389	231	272	247	376	119.28	NS	66.91
		Mean (6)	1095	963	916	1016	896	936	-	-	-
		% Increase over checks		13.71	19.54	7.78	22.21	16.99	-	-	-
2019	LSVT	Amreli	493	473	515	552	503	574	31.23	89	11.21
		Jamnagar@	315	298	336	347	278	413	30.68	88	19.56
		Targhadiya	830bde	736	651	838	694	408	33.35	95	8.730
		Vallabhipur@	267	200	23	134	183	150	17.19	49	18.58
		Kukda@	182	177	177	220	217	148	15.15	43	16.06
		Mean (2)	662	605	583	695	599	491	-	-	-
		% Increase over checks		9.42	13.55	-4.75	10.52	34.83	-	-	-
2020	LSVT	SKNagar	604abcde	397	498	473	426	501	32.59	93	13.78
		Bhachau#	1618	612	883	1384	1669	795	190.05	540	37.97
		Targhadiya@	256	285	481	537	436	156	34.71	99	16.37
		Vallabhipur@	401	289	462	569	466	204	24.56	70	12.67
		Kukda@	468	401	465	420	445	394	20.31	58	09.94
		Mean (1)	604	397	498	473	426	501	-	-	-
		% Increase over checks		52.14	21.29	27.70	41.78	20.56	-	-	-
Overall mean (17)			957	760	806	879	-	-	-	-	-
% Increase over checks				25.92	18.73	8.87	-	-	-	-	-
Overall mean (9)			944	-	-	-	777	-	-	-	-
% Increase over GT 6				-	-	-	21.49	-	-	-	-
Overall mean (16)			924	-	-	-	-	782	-	-	-
% Increase over GT 10				-	-	-	-	18.16			
Frequency in top non-significant groups			11/17	2/17	6/17	8/17	0/9	2/16	-	-	-

Note: Figure in parenthesis indicate the number of locations a, b, c, d and e indicate significantly superior than respective check variety for seed yield
NC = National Check and LC = Local Check # Centres were not considered due to high CV%;
@The seed yield data of Dhari centre (Trial mean: 511 kg/ha) in SSVT 2017 was not considered due to below State average seed yield (State average: 605 kg/ha).
@The seed yield data of Jamnagar (Trial mean: 314 kg/ha), Vallabhipur (Trial mean: 185 kg/ha) and Kukda (Trial mean: 182 kg/ha) in LSVT 2019 were not considered due to below State average seed yield (State average: 511 kg/ha).
@The seed yield data of Targhadiya (Trial mean: 424 kg/ha), Vallabhipur (Trial mean: 387 kg/ha) and Kukda (Trial mean: 408 kg/ha) in LSVT 2020 were not considered due to below State average seed yield (State average: 426 kg/ha).

GT 7: HIGH YIELDING, HIGH OIL CONTENT AND BOLD SEEDED VARIETY OF SESAME

Table 2 Yield Performance of Sesame entry SKT 1501 (GT 7) in comparison with check varieties in the IVT AICRP Trial (Zone I)

Year/ Season	Name of Trial	Locations	Seed Yield (kg/ha)				S.Em. +	CD at 5%	CV%
			Proposed entry SKT 1501	TKG 22 (NC) a	GT 10 (NC) b	Pragati (ZC) c			
2019	Initial Varietal Trial	Amreli	697	758	794	680	42.80	122	11.77
		Hissar@	556	673	--	367	24.25	70	10.97
		Jalgaon	545	535	501	586	48.20	137	14.15
		Ludhiana	817b	783	244	917	32.32	92	10.76
		Mandor	486	771	667	599	47.20	135	15.51
		Overall Mean (4)	636	712	552	696	-	-	-
		% Increase over the checks		-10.67	15.22	-8.62			

Note: a, b and c indicate significantly superior than respective check variety for seed yield.
NC = National Check; ZC = Zonal Check
@The seed yield data of Hissar centre (Trial mean: 442 kg/ha) was not considered due to below National average yield (National average: 485 kg/ha).

In All India Co-ordinated Trials, the genotype SKT 1501 was tested in Initial varietal trial (*kharif*) under Zone I during 2019 and it recorded 636 kg/ha seed yield over four location which was 15.22 per cent higher than the National check GT 10 (Table 2).

Yield with good oil quality is also of prime importance in oilseed crops. The genotype SKT 1501 was also found superior in quality traits. It has high oil content (49.06 %) and recorded 470 kg/ha oil yield which was 28.77, 19.29, 11.11, 26.00 and 32.39 per cent higher as compared to the check varieties GT 2, GT 3, GT 4, GT 6 and GT 10, correspondingly (Table 3). Besides, it also possesses comparatively high linoleic acid content (44.69%) and linolenic acid content (0.32%) than the check varieties (Table 4).

Ancillary observations of economic attributes of SKT 1501 along with the checks are presented in Table 5. The perusal of data showed that SKT 1501 was matured in 90 days which was at par with GT 6 and early than GT 10, while late than GT 2, GT 3 and GT 4. It also recorded more number of branches per plant, number of capsules per plant, capsule length and number of seeds per capsule. It exhibited high 1000 seed weight (3.48 g) as compared to the check varieties (Table 5). Morphological characters of SKT 1501 are furnished in Table 6 and Fig. 1. This genotype possesses light purple petal with dense hairiness, tall, profuse branching, dense hairiness on capsule, four locules per capsule, long and broad oblong capsule with alternate arrangement and white colour seed.

The genotype SKT 1501 was screened for resistance to insect-pests and diseases during 2016 to 2020 under epiphytotic condition at Agricultural Research Station, JAU, Amreli and Castor-Mustard Research Station, SDAU, Sardarkrushinagar and data are presented in Table 7 and 8. It is resistant to phyllody, moderately resistant to powdery mildew and cercospora leaf spot, while moderately susceptible to phytopthora blight and macrophomina stem rot. Similarly, it is resistant to leaf webber, while moderately resistant to mite.

DNA fingerprinting of genotype SKT 1501 along with five checks (GT 2, GT 3, GT 4, GT 6 and GT 10) was performed using 16 ISSR primers. Out of 16 primers, five primers (ISSR2, ISSR3, ISSR4, ISSR8 and ISSR10) were not amplified properly across all the genotypes. Total three primers namely, ISSR14, ISSR19 and ISSR15 showed polymorphic bands between SKT 1501 and other five check varieties used in fingerprinting. Polymorphic bands were demonstrated using arrow symbol in Fig. 2.

Considering the above superior performance, SKT 1501 has been accepted by 53rd State Seed Sub-Committee Meeting held at Conference Hall, Krushi Bhavan, Gandhinagar (Gujarat) on August 27, 2021 and released as Gujarat Til 7 (Banas Gaurav) for commercial cultivation in sesame growing areas of Gujarat State. The GT 7 has been registered with national identity number (IC 638673) and conserved under long term storage at NBPGR, New Delhi. GT 7 has been notified in the Gazette of India, Ministry of Agriculture and Farmer Welfare (Department of Agriculture and Farmers Welfare), New Delhi after approval for notification from Central Sub-Committee on Crop Standards, Notification and Release of Varieties for Agricultural Crops, ICAR, Krushi Bhavan, New Delhi, with notification number S.O. 4222(E) dated September 25, 2023.

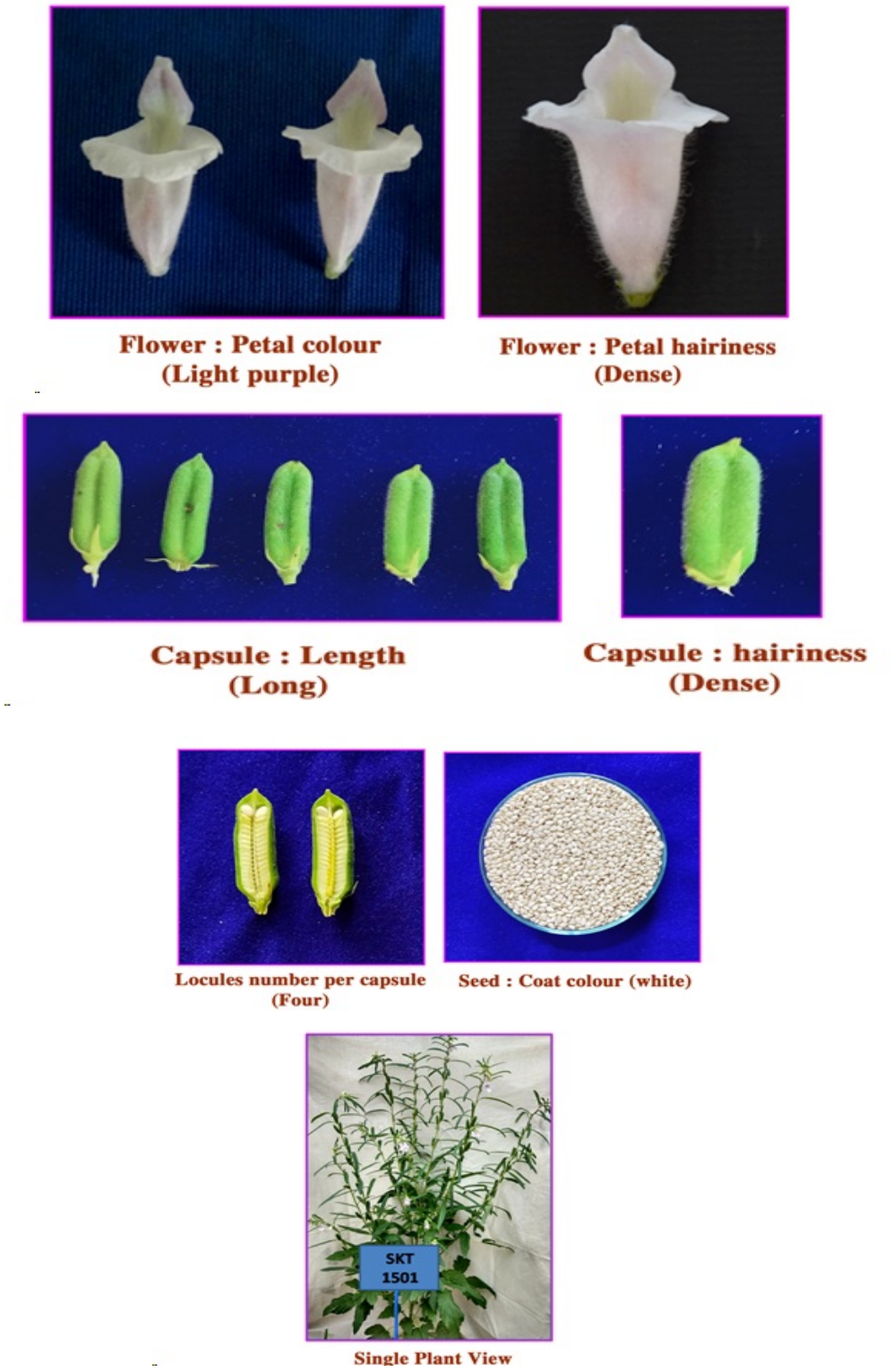

Fig. 1. Important DUS characteristics of SKT 1501 (GT 7)

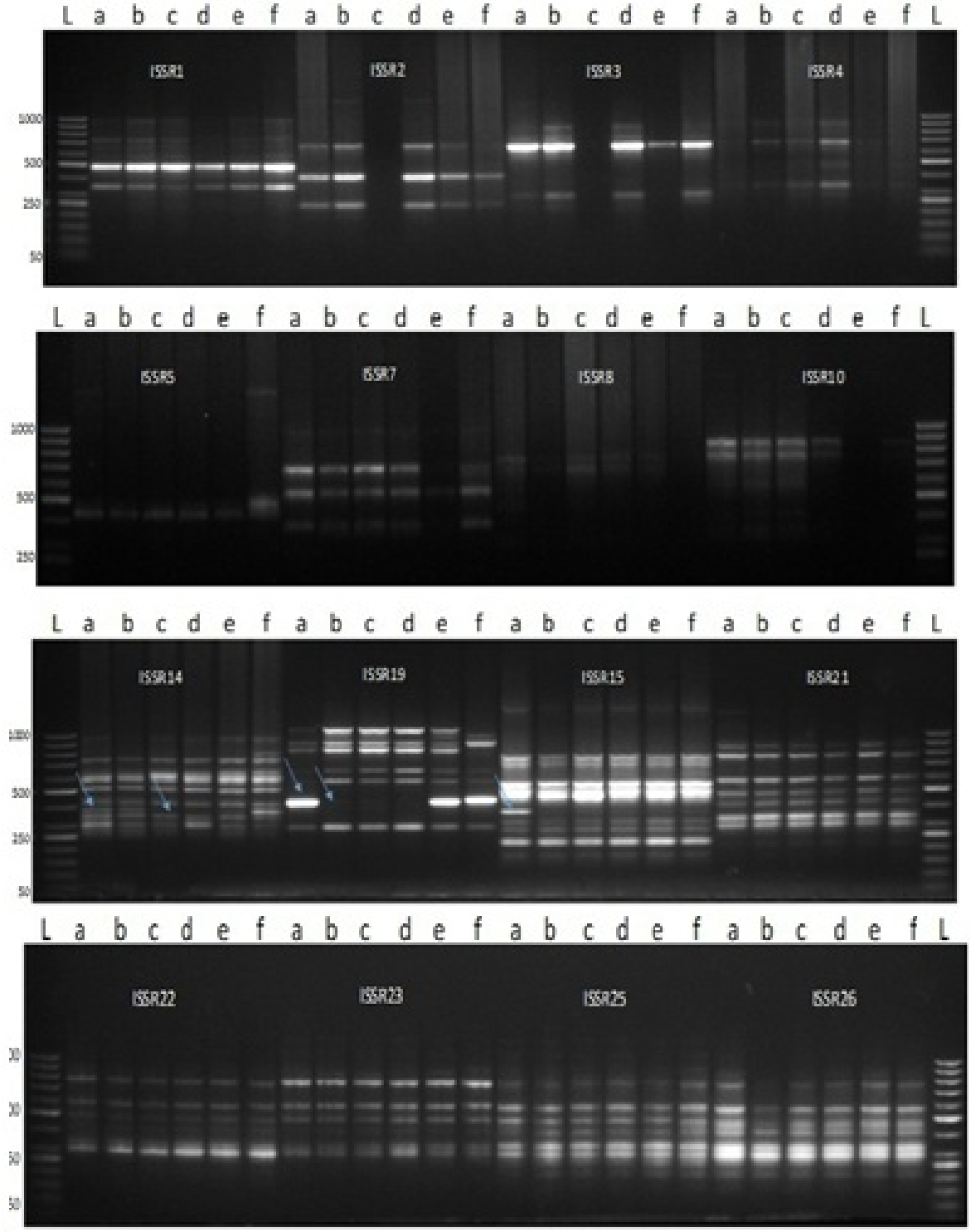

Fig. 2. DNA profiling of Sesame genotypes
L: Ladder; a: SKT 1501, b: GT 2, c: GT 3, d: GT 4, e: GT 6, f: GT 10;
ISSR number representing to ISSR markers.

Field view of proposed sesame entry SKT 1501 (GT 7)

GT 7: HIGH YIELDING, HIGH OIL CONTENT AND BOLD SEEDED VARIETY OF SESAME

Table 2 Yield Performance of Sesame entry SKT 1501 (GT 7) in comparison with check varieties in the IVT AICRP Trial (Zone I)

Year/ Season	Name of Trial	Locations	Seed Yield (kg/ha)				S.Em. +	CD at 5%	CV%
			Proposed entry SKT 1501	TKG 22 (NC) a	GT 10 (NC) b	Pragati (ZC) c			
2019	Initial Varietal Trial	Amreli	697	758	794	680	42.80	122	11.77
		Hissar@	556	673	--	367	24.25	70	10.97
		Jalgaon	545	535	501	586	48.20	137	14.15
		Ludhiana	817b	783	244	917	32.32	92	10.76
		Mandor	486	771	667	599	47.20	135	15.51
		Overall Mean (4)	636	712	552	696	-	-	-
		% Increase over the checks		-10.67	15.22	-8.62			

Note: a, b and c indicate significantly superior than respective check variety for seed yield.
NC = National Check; ZC = Zonal Check
@The seed yield data of Hissar centre (Trial mean: 442 kg/ha) was not considered due to below National average yield (National average: 485 kg/ha).

Table 3 Oil yield (kg/ha) of proposed entry SKT 1501 (GT 7) in comparison with check varieties

Entry/Varieties	Mean seed yield (kg/ha)	Oil content (%)	Oil yield (kg/ha)	% Oil yield increase over checks
SKT 1501	957	49.06	470	
GT 2 (LC)	760	48.02	365	28.77
GT 3 (LC)	806	48.84	394	19.29
GT 4 (LC)	879	48.17	423	11.11
GT 6 (LC)	777	47.98	373	26.00
GT 10 (NC)	782	45.42	355	32.39

Table 4 Oil content and fatty acid profile of proposed entry along with checks

Character		Proposed entry SKT 1501	(LC)				(NC)
			GT 2	GT 3	GT 4	GT 6	GT 10
Oil content (%)	Mean	49.06	48.02	48.84	48.17	47.98	45.42
	Range	48.55-49.82	47.60-48.45	47.80-49.08	47.45-48.60	47.47-48.51	43.57-46.34
Palmitic acid (%)	Mean	9.26	11.17	11.20	10.71	10.52	10.69
	Range	8.97-9.36	10.99-11.30	11.14-11.28	10.55-10.83	10.21-10.79	10.45-10.85
Stearic acid (%)	Mean	5.24	4.82	5.05	4.97	4.48	5.38
	Range	5.03-5.32	4.57-5.00	4.80-5.12	4.81-5.08	4.38-4.60	5.33-5.47
Oleic acid (%)	Mean	40.49	42.43	41.10	40.18	43.08	40.32
	Range	40.40-40.60	42.35-42.54	40.72-41.42	40.10-40.34	42.89-43.27	40.00-40.46
Linoleic acid (%)	Mean	44.69	41.34	42.45	43.94	41.73	43.36
	Range	44.58-44.82	41.25-41.40	42.29-42.61	43.68-44.21	41.39-41.98	43.22-43.53
Linolenic acid (%)	Mean	0.32	0.23	0.22	0.20	0.19	0.24
	Range	0.29-0.36	0.21-0.28	0.13-0.27	0.17-0.24	0.11-0.24	0.19-0.31

Table 5 Ancillary observation of economic attributes of proposed entry along with checks

Character		Proposed entry	(LC)				(NC)
		SKT 1501	GT 2	GT 3	GT 4	GT 6	GT 10
Days to flowering	Mean	43	36	36	37	37	44
	Range	38-43	33-41	34-39	34-40	35-39	42-46
Days to maturity	Mean	90	84	85	83	90	95
	Range	88-94	81-89	80-89	76-87	88-92	88-97
Plant height (cm)	Mean	130	114	111	102	116	125
	Range	125-149	109-130	106-130	95-120	106-135	116-139
Number of branches per plant	Mean	4.40	2.98	3.45	3.48	2.00	4.10
	Range	3.84-6.00	2.54-3.90	2.67-5.80	2.71-4.90	1.56-2.44	3.33-6.68
Number of capsules per plant	Mean	81	59	56	55	38	70
	Range	74-85	45-80	37-71	36-78	35-41	51-83
Capsule length (cm)	Mean	2.7	2.5	2.6	2.5	2.6	2.3
	Range	2.5-2.8	2.4-2.6	2.3-2.8	2.4-2.7	2.4-2.8	2.2-2.4
Number of seeds per capsule	Mean	72	68	70	70	66	61
	Range	64-76	60-72	62-74	64-74	61-72	52-68
1000 seed weight (g)	Mean	3.48	2.98	3.25	2.86	3.21	3.02
	Range	3.19-3.50	2.51-3.00	3.16-3.34	2.59-2.91	3.11-3.46	2.54-3.10

Table 6 Morphological characters of SKT 1501 along with checks (As per DUS Guidelines)

Descriptors/Characters	SKT 1501	(LC)				(NC)
		GT 2	GT 3	GT 4	GT 6	GT 10
Time of flowering: Days to 50% flowering (Early/Medium/Late)	Medium	Medium	Medium	Medium	Medium	Late
Flower: Petal colour (White/Light purple/Dark purple)	Light purple	Light purple	Light purple	Light purple	Light purple	Light purple
Flower: Petal hairiness (Absent/Sparse/Dense)	Dense	Sparse	Sparse	Sparse	Sparse	Sparse
Plant: Height of stem (cm) (Short/Medium/Tall)	Tall (125-149)	Medium (109-130)	Medium (106-130)	Medium (95-120)	Medium (106-135)	Medium (116-139)
Plant: Branching (Absent/Few/Medium/ Profuse)	Profuse	Medium	Medium	Medium	Few	Profuse
Plant : Branching pattern (Basal/Top branching)	Basal branching	Basal branching	Basal branching	Basal branching	Basal branching	Basal branching
Stem: Hairiness (Absent/Sparse/Dense)	Absent	Sparse	Absent	Absent	Absent	Absent
Leaf lobes (Slightly lobed/Deeply lobed)	Slightly lobed	Slightly lobed	Slightly lobed	Slightly lobed	Slightly lobed	Slightly lobed
Leaf: Size (Small/Medium/Large)	Large	Large	Large	Large	Medium	Medium
Leaf: Serration of margin (Weak/Strong)	Weak	Weak	Weak	Weak	Weak	Weak
Capsule: Hairiness (Absent/Sparse/Dense)	Dense	Sparse	Absent	Absent	Absent	Absent
Capsule: Locule number/capsule (Four/Six/Eight)	Four	Four	Four	Four	Four	Four
Capsule: Shape (Taperd/Narrow/Oblong/Broad oblong/Square)	Broad Oblong	Narrow oblong	Broad Oblong	Narrow oblong	Narrow oblong	Tapperd
Capsule: Number /leaf axil (one/more than one)	One	More than one	One	More than one	More than one	More than one
Capsule: Arrangement (Alternate/Opposite/ Cluster)	Alternate	Multi Opposite	Opposite	Cluster	Opposite	Alternate
Capsule: Length (cm) (Short/Medium/Long)	Long (2.5-2.8)	Medium (2.4-2.6)	Long (2.3-2.8)	Medium (2.4-2.7)	Long (2.4-2.8)	Medium (2.2-2.4)
Maturity: Days to maturity (Early/Medium/Late)	Late	Medium	Medium	Medium	Late	Late
Seed: Coat colour (White/Grey/Light brown/Dark brown/Black)	White	White	White	White	White	Black
Seed: 1000 seeds weight (g) (Low/Medium/High/ Very high)	High	Medium	High	Medium	High	Medium
Seed Oil: Content (Low/Medium/High/ Very high)	Medium	Medium	Medium	Medium	Medium	Medium

Table 7 Rating of incidence of diseases

(1) Phyllody incidence (%)

Disease	Location	Year and season	Name of trial	Varieties					
				SKT 1501	(LC)				(NC)
					GT 2	GT 3	GT 4	GT 6	GT 10
Phyllody incidence (%)	Amreli	2016	SSVT	5.04	2.83	2.50	2.24	-	2.52
		2017	SSVT	0.50	1.87	0.94	1.96	-	0.50
		2018	LSVT	0.00	0.00	0.00	0.00	0.00	0.00
		2019	LSVT	1.75	3.95	1.56	2.22	3.74	1.68
		2020	LSVT	0.00	0.00	0.00	0.00	0.00	0.00
	SKNagar	2020	LSVT	11.13	15.60	14.18	20.15	10.86	6.47
	Mean			3.07 (R)	4.04 (R)	3.20 (R)	4.43 (R)	3.65 (R)	1.86 (R)
	Range			0.00-11.13	0.00-15.60	0.00-14.18	0.00-20.15	0.00-10.86	0.00-6.47

R = 0-10%, MR = >10-25%, MS = >25-50%, S = >50-70%, HS = >70%

(2) Powdery mildew severity (%)

Disease	Location	Year and season	Name of trial	Varieties					
				SKT 1501	(LC)				(NC)
					GT 2	GT 3	GT 4	GT 6	GT 10
Powdery mildew severity (%)	Amreli	2016	SSVT	55.52	42.33	51.50	51.06	-	0.50
		2017	SSVT	8.32	8.16	3.95	11.88	-	0.50
		2018	LSVT	12.93	17.65	17.56	36.99	11.96	0.50
		2019	LSVT	0.00	0.00	0.00	0.00	0.00	0.00
		2020	LSVT	0.00	0.00	0.00	0.00	0.00	0.00
	SKNagar	2020	LSVT	17.50	17.50	18.75	20.00	13.75	0.00
	Mean			15.71 (MR)	14.27 (MR)	15.29 (MR)	19.99 (MR)	6.43 (R)	0.25 (R)
	Range			0.00-55.52	0.00-42.33	0.00-51.50	0.00-51.06	0.00-13.75	0.00-0.50

R = 0-10%, MR = >10-20%, MS = >20-30%, S = >30-50%, HS = >50%

(3) Cercospora leaf spot severity (%)

Disease	Location	Year and season	Name of trial	Varieties					
				SKT 1501	(LC)				(NC)
					GT 2	GT 3	GT 4	GT 6	GT 10
Cercospora leaf spot severity (%)	Amreli	2016	SSVT	03.86	04.97	04.40	04.81	-	04.97
		2017	SSVT	03.89	04.96	04.77	05.40	-	09.37
		2018	LSVT	02.93	02.93	04.47	03.45	03.89	04.96
		2019	LSVT	15.81	19.38	17.78	20.39	17.86	36.97
		2020	LSVT	46.00	56.00	54.00	68.00	36.00	58.00
	SKNagar	2020	LSVT	07.50	07.75	07.00	08.75	06.00	08.50
	Mean			13.33 (MR)	16.00 (MR)	15.40 (MR)	18.47 (MR)	15.94 (MR)	20.46 (MS)
	Range			02.93-46.00	02.93-56.00	04.40-54.00	03.45-68.00	03.89-36.00	04.96-58.00

R = 0-10 %, MR = >10-20 %, MS = >20-30 %, S = >30-50 %, HS = >50 %

(4) Phytopthora blight severity (%)

Disease	Location	Year and season	Name of trial	Varieties					
				SKT 1501	(LC)				(NC)
					GT 2	GT 3	GT 4	GT 6	GT 10
Phytopthora blight severity (%)	Amreli	2016	SSVT	31.91	26.66	33.40	30.48	-	9.98
		2017	SSVT	12.34	30.89	30.68	39.99	-	4.35
		2018	LSVT	0.00	0.00	0.00	0.00	0.00	0.00
		2019	LSVT	40.99	49.48	43.97	49.99	44.98	6.40
		2020	LSVT	86.00	88.00	84.00	86.00	88.00	16.00
	SKNagar	2020	LSVT	0.00	0.00	0.00	0.00	0.00	0.00
	Mean			28.54 (MS)	32.51 (S)	32.01 (S)	34.41 (S)	33.25 (S)	6.12 (R)
	Range			0.00-86.00	0.00-88.00	0.00-84.00	0.00-86.00	0.00-88.00	0.00-16.00

R = 0-10 %, MR = >10-20 %, MS = >20-30 %, S = >30-50 %, HS = >50 %

(5) Macrophomina stem rot incidence (%)

Disease	Location	Year and season	Name of trial	Varieties					
				SKT 1501	(LC)				(NC)
					GT 2	GT 3	GT 4	GT 6	GT 10
Macrophomina stem rot incidence (%)	Amreli	2016	SSVT	74.34	17.02	32.89	27.63	-	19.75
		2017	SSVT	11.26	17.03	12.96	13.85	-	22.89
		2018	LSVT	47.06	39.24	27.37	29.72	41.83	27.79
		2019	LSVT	19.30	15.08	20.12	28.18	21.34	20.41
		2020	LSVT	17.39	05.26	20.00	07.41	12.00	35.71
	SKNagar	2020	LSVT	0.00	0.00	0.00	0.00	0.00	0.00
	Mean			28.23 (MS)	15.61 (MR)	18.89 (MR)	17.80 (MR)	18.79 (MR)	21.09 (MR)
	Range			0.00-74.34	0.00-39.24	0.00-32.89	0.00-29.72	0.00-41.83	0.00-35.71

R = 0-10 %, MR = >10-25 %, MS = >25-50 %, S = >50-70 %, HS = >70 %
R=Resistant; MR=Moderately Resistant; MS=Moderately Susceptible; S=Susceptible and HS=Highly Susceptible

Table 8 Rating of incidence of insect-pests

(1) Capsule damage by Leaf webber (%)

Insect pest	Location	Year and season	Name of trial	Varieties					
				SKT 1501	(LC)				(NC)
					GT 2	GT 3	GT 4	GT 6	GT 10
Capsule damage by Leaf webber (%)	Amreli	2016	SSVT	1.24	0.64	0.75	0.88	-	0.96
		2017	SSVT	1.38	1.30	3.04	1.95	-	1.47
		2018	LSVT	1.29	1.52	1.30	2.35	3.47	1.46
		2019	LSVT	1.14	2.66	3.77	1.61	1.88	1.20
		2020	LSVT	0.00	0.00	0.00	0.00	0.00	0.00
	SKNagar	2020	LSVT	1.13	1.25	1.50	0.88	1.75	0.75
	Mean			1.03 (R)	1.23 (R)	1.73 (R)	1.28 (R)	1.78 (R)	0.97 (R)
	Range			0.00-1.38	0.00-2.66	0.00-3.77	0.00-2.35	0.00-3.47	0.00-1.47

R = < 5 %, MR = >5-10 %, MS = >10-15 %, S = >15-25 %, HS = >25 %

(2) Plant damage by Mite (%)

Insect pest	Location	Year and season	Name of trial	Varieties					
				SKT 1501	(LC)				(NC)
					GT 2	GT 3	GT 4	GT 6	GT 10
Plant damage by Mite (%)	Amreli	2016	SSVT	31.47	35.62	22.82	24.26	-	6.71
		2017	SSVT	25.17	33.09	35.24	40.00	-	2.95
		2018	LSVT	4.01	7.60	4.87	7.19	6.08	0.68
		2019	LSVT	0.00	0.00	0.00	0.00	0.00	0.00
		2020	LSVT	91.30	78.60	83.30	88.90	84.00	14.30
	SKNagar	2020	LSVT	06.25	06.75	05.50	07.50	06.50	04.75
	Mean			26.37 (MR)	26.94 (MR)	25.29 (MR)	27.98 (MR)	24.15 (MR)	4.90 (R)
	Range			0.00-91.30	0.00-78.60	0.00-83.30	0.00-88.90	0.00-84.00	0.00-14.30

R = 0-20%, MR = >20-30%, S = > 30%
R=Resistant; MR=Moderately resistant; MS=Moderately susceptible; S=Susceptible and HS=Highly susceptible

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Unravelling genetic diversity in linseed using hierarchical cluster and principal component analysis

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ABSTRACT

The present investigation was carried out with 178 linseed accessions including two checks (PKVNL-260 & TL-99) at AICRP on Linseed and Mustard farm, College of Agriculture, Nagpur to assess the extent of genetic diversity and to select the potential diverse parents for the improvement of yield and yield attributing traits in linseed. The accessions were divided into 13 divergent groups using the Wards clustering approach. Cluster III, was largest and consists of 28 accessions, shows a fair connection of genetic variation. Out of 11 PC, PC1 to PC4 with more than one Eigen values account for 65.23 percent of the variability. The accessions viz., EC0718827, EC0718829, EC22813, EC0041764, EC0041598, EC0022813-B, EC0718848, EC0000526, EC41659, EC0541202, EC0000541-A and EC0001403 showed genetic diversity for seed yield per plant, number of capsules per plant and resistance to bud fly infestation. The information obtained from this study can be used to plan crosses and maximize the use of genetic diversity and expression of heterosis.

Keywords: Genetic diversity, Hierarchical cluster analysis, Linseed, PCA, Seed yield

Linseed (*Linum usitatissimum* L) also known as flax or alsi or tisi originated in the Middle East and expands throughout to Asia and Europe. The world's leading producers of linseed were Europe, China, Argentina, the United States, and Canada (Lidefelt, 2007). Linseed is a multipurpose crop known for its seed oil, fiber (flax), probiotic, and nutraceutical properties (Kaur *et al.,* 2017). It is one of the major *rabi* oilseed crops that is cultivated next to mustard and rapeseed in terms of both area and productivity in India (Kumar and Kumar, 2021; Dash *et al.,* 2017). It is self-pollinating crop with high unsaturated fatty acids like Linolenic acid, omega-3 fatty acids (57%) and omega-6 fatty acids (8%) and rich source of protein (20.0 - 27.8%), calcium (170 mg/100 grains) and phosphorus (370 mg/100 grains) and magnesium (Satish *et al.,* 2017, Maddock *et al.,* 2005). Linseed has an important position in the world market because of its oil, which is used for manufacturing paints, varnishes, and other products in the industries (Gill, 1987). The residue cake, obtained after oil extraction, contains about 9.7% oil, 32% carbohydrate, and 32% protein and rich proteinaceous feed for animals and livestock (Singh *et al.,* 2011).

In India, Madhya Pradesh, Uttar Pradesh, Maharashtra, Chhattisgarh, Bihar, and Orissa are the major linseed growing states and occupy an area of 0.19 million hectares with 0.14 million tonnes production and 690 kg/ha productivity (Ministry of Agriculture, GOI, 2022-2023, Annual Report AICRP Linseed, 2022-23). India holds fifth position in area after Kazakhstan, Russian Federation, Canada and China but ranks sixth in production after Kazakhstan, Canada, Russian Federation, China and USA (FAO Stat., 2019). Increase in the productivity of linseed is anticipated through improved breeding techniques, which call for accurate information on the nature and degree of genetic variability present in linseed accessions (Tiwari *et al.,* 2020). The goal of plant breeders is to achieve high-yielding, early maturity, and disease-resistant varieties that are superior to those of existing varieties (Patial *et al.,* 2019). Among various multivariate techniques, Ward's clustering and Principal Component Analysis (PCA) were best models useful in the selection of diverse parents in core collection and their utilization in crop improvement. Hence, the main scope of this study was to evaluate the potential genetic diversity by using Ward's clustering and PCA.

MATERIALS AND METHODS

The experimental material consisting of 178 linseed accessions including two checks *viz.,* PKVNL-260 and TL-99 were sown in Augmented Block Design during Rabi 2021-22 at AICRP on Linseed and Mustard farm, College of Agriculture, Nagpur. Each accession was sown in a single row with 30 cm x 5 cm spacing. Data was recorded on eleven traits *viz.,* days to 50 percent flowering, plant height (cm), number of primary branches per plant, flower size (mm), number of capsules per plant, days to maturity, seed yield per plant (g), 1000 seed weight (g), bud fly infestation (%,) Alternaria blight infestation and powdery mildew infestation (score). The recorded data was subjected to assess genetic divergence by hierarchical cluster analysis and PCA in JMP 17.0 statistical software (SAS Institute Inc., Cary, NC, USA) for yield and other important traits.

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RESULTS AND DISCUSSION

The results indicate enormous genetic diversity among the 178 linseed accessions, as indicated by highly significant mean squares of all characters studied shown in Table 1. Dhirhi *et al.* (2016), Paul *et al.* (2017) and Singh and Tewari (2016) found similar results for yield and yield-attributing traits in linseed. Using the Wards clustering method, hierarchical cluster analysis was carried out in 178 linseed accessions on all the characters. Cluster XIII had the highest cluster mean for the number of branches per plant (15.50) and days to 50 percent flowering (70.33). When it comes to days to maturity (107) and bud fly infestation % (43.0), cluster X had the highest cluster mean. In contrast, seed yield per plant (5.83) and number of capsules per plant (186.70) were recorded as highest for cluster XII. The highest cluster means for plant height (77.17), flower size (25.88), 100 seed weight (10.30), Alternaria blight infestation % (9.50), and powdery mildew infestation (score) (40.67) were also recorded by the clusters IX, VIII, IV, V, and XI (Table 2). Similar, findings are found in according to Nizar *et al.* (2015) and Upadhyay *et al.* (2019) for genetic variability among linseed accessions. There is a good chance to hybridize accessions from different clusters to incorporate them into a breeding program for yield enhancement.

The linseed accessions are grouped into 13 divergent clusters (Table 3). The cluster III (28) with maximum accessions followed by cluster XIII (23), cluster V (19), cluster VII (18), cluster I and II (17), cluster XI (15), cluster IV, VIII and IX (9), cluster VI (6), cluster X, VI and XII (4). Similar, results were reported by Jay (1995) and Sathish *et al.* (2022). These results indicate that there are some fascinating possibilities to improve crop productivity as some accessions show significant differences when compared to the checks. Thus, the development of accessions with desirable qualities arises from crossing the linseed accessions with early extra descendants from one cluster with another cluster that has a high seed yield, more capsules per plant, and disease resistance. Rather than relying on geographic diversity, which might not be a helpful activity for the identification of useful parents (Begum *et al.,* 2007), parental selection must be carried out from the clusters with higher mean performance. This will assist in obtaining transgressive segregants having stable heritability (Hussain *et al.,* 2022) or heterotic cross, which aid in increasing the seed yield. The hierarchical cluster analysis revealed that the greater genetic distance among the linseed accessions aids in improving the hybridization program.

Table 1 Analysis of variance for yield and yield attributing traits in linseed accessions

Source of variation	d.f	Mean sum of squares										
		DFF (days)	DM (days)	PH (cm)	NBPP	FS (mm)	NCPP	1000 SW (g)	SYPP (g)	BFI %	AI %	PMI (Score)
Blocks (Ignoring treatments)	7	269.18**	229.86**	84.78**	14.05**	522.04**	4918.16**	3.40**	4.15**	465.83**	20.03**	76.40**
Treatments (eliminating blocks)	177	15.04**	18.11**	52.77**	3.56**	4.50**	860.74**	0.91**	0.79**	41.11**	2.85**	47.34**
Checks	1	25.00**	36.30*	100.00**	14.06**	1.00	2332.90**	7.43**	3.61**	13.51**	0.90	49.00**
Checks + Var vs. Var	176	14.98**	18.01**	52.50**	3.50**	4.52**	852.37**	0.87**	0.78**	41.27**	2.86**	47.33**
Error	7	0.28	3.052	1.98	1.06	1.14	88.29	0.04	0.21	0.16	0.20	7.86
Block (eliminating Check +Var)	7	9.00**	26.45**	17.12**	0.99	2.11	26.68	0.05	0.30	1.2**	2.08**	6.72
Entries (Ignoring blocks)	177	25.32**	26.16**	55.45**	4.07**	7.37**	1054.19**	1.04**	0.95**	59.48**	3.56**	50.09**
Checks	1	25.00**	36.30*	100.00**	14.06**	1.00	2332.90**	7.43**	3.61**	13.51**	0.90	49.00*
Varieties	175	24.24**	26.02**	50.61**	4.01**	7.44**	1026.33**	0.85**	0.93**	54.94**	3.42**	46.4**
Checks vs. Varieties	1	216.18**	40.26**	856.95**	5.41	1.05	4650.06**	27.64**	1.19*	900.52**	30.14**	697.13**
Error	7	0.28	3.05	1.98	1.06	1.14	88.29	0.04	0.21	0.16	0.20	7.86

* Significant at 5% level, ** Significant at 1% level

Note: DFF - Days to 50 percent flowering (days), DM - Days to Maturity (days), PH - Plant height (cm), NBPP - No. of Branches per plant, FS - Flower size (mm), NCPP - No. of Capsules per plant,1000 SW - 1000 seed weight (g), SYPP - Seed yield per plant(g), BFI % - Bud fly Infestation %, AI %- Alternaria Infestation %, PMI % - Powdery Mildew infestation (Score).

Table 2 Cluster means for yield and yield attributing traits in linseed accessions

Cluster	Days to 50 percent flowering	Days to maturity	Plant height (cm)	No. of branches per plant	Flower size (mm)	No. of capsules per plant	1000 seed weight (g)	Seed yield per plant (g)	Bud fly infestation %	Alternaria infestation %	Powdery mildew infection (score)
1	57.98	105.99	57.20	5.98	21.00	68.08	8.67	1.61	16.09	3.44	16.87
2	59.51	106.05	58.34	7.55	20.69	102.43	9.37	3.21	23.56	5.97	18.25
3	65.69	106.39	61.88	7.64	15.59	117.30	8.32	1.72	21.65	4.29	23.00
4	51.67	106.00	76.33	4.93	20.88	125.33	10.30	1.87	4.67	1.40	13.33
5	55.00	106.00	62.83	6.00	21.88	79.60	8.53	1.85	15.83	9.50	14.67
6	59.00	105.00	53.50	4.00	22.88	69.07	8.83	2.28	22.02	9.17	14.67
7	66.00	106.33	69.33	4.80	20.38	49.37	9.40	1.02	19.23	6.17	9.33
8	50.00	103.33	48.17	4.50	25.88	55.00	10.20	3.08	15.73	1.60	10.67
9	61.00	106.67	77.17	6.57	19.38	128.17	9.60	1.52	35.17	8.87	9.63
10	62.00	107.00	52.33	8.17	21.88	75.67	9.40	3.17	43.00	2.37	30.67
11	61.33	106.67	53.83	7.00	22.88	38.13	9.80	2.90	20.67	3.17	40.67
12	62.00	106.03	62.50	6.93	19.38	186.70	8.40	5.83	25.17	5.20	14.67
13	70.33	106.00	39.50	15.50	19.88	63.83	7.40	1.13	19.00	7.70	26.67

Note: Bold figures indicate maximum and minimum values in each character

Table 3 Grouping of genotypes into different clusters

Cluster	Number of genotypes	Name of the genotypes
I	17	EC1066, EC0041601-A, EC0541203, EC0041213, EC0541207, EC0041469, EC0041621 – B, EC0041687, EC0022872, EC0541194, EC0541198, EC5410194, EC541196, EC0541226, EC0041774 – A, EC0041478, EC0718852.
II	17	EC0001419, EC0001403, EC0011748, EC0041601 – A, EC0041755, EC0000538, EC0000541 – A, EC041667, EC0041735, EC0041650, EC0041653, EC00414678 – B, EC0115148, EC054119, EC0001388, EC0000522, EC0541210.
III	28	EC1386, EC1474, EC0541202, EC0001432, EC0110474, EC0399084, EC0001437, EC0041723, EC0041700, EC0041562, EC41466, EC41659, EC41741, EC98994, EC1588, EC0541213, EC0541226, EC0541215, EC0541220, EC0000531 – A, EC0001005 – B, EC0009827, EC0520246, EC0001550 –B, EC0041621, EC0541196, EC0000526, EC0115174.
IV	9	EC1424, EC0022388, EC0718850, EC054214, EC0041622, EC0718824, EC0541216, EC0041495 – 1, EC0541213.
V	19	EC1628, EC1645, EC99001, EC0041734, EC45890, EC0012538, EC0041528, EC0118743, EC0041646, EC0041762, EC0041467, EC0001475, EC0001476, EC0041607– 2, EC80490, EC51904, EC0399086, EC0041720, EC0041400.
VI	6	EC0541218, EC0541210, PKV-NL 260, EC0541223, EC0399082, TL 99.
VII	18	EC14539, EC0718847, EC0718831, EC41623, EC0718826, EC0718842, EC0023208, EC0041579, EC718830, EC0718843, EC0001457, EC0455084, EC0041649, EC0041647, EC0001395–1, EC0001433, EC0541219, EC0041615.
VIII	9	EC0541205, EC0080490, EC0001396, EC0718851, EC0718827, EC0041765, EC22648, EC0041582, EC22813.
IX	9	EC0541227, EC0001459, EC0001443, EC000545, EC0041672 – A, EC0541215, EC0041753, EC0006160, EC0520247.
X	4	EC0718828, EC0541208, EC0718829, EC0041619.
XI	15	EC0041687–A, EC0541195, EC0541212, EC0041672, EC0041737, EC0041547–A, EC0001395, EC0001451, EC0041764, EC0041598, EC0718823, EC718846, EC0541224, EC541225, EC004181.
XII	4	EC0000543, EC0718845, EC0001465, EC0041726.
XIII	23	EC0001551, EC0718834, EC718835, EC0041768, EC244634, EC0041758, EC0541204, EC0718825, EC0158985, EC00411623, EC0041644, EC0041535, EC541206, EC041643, EC0541211, EC0541217, EC0110289, EC0002711, EC000564, EC0399085, EC0022813 – B, EC0541201, EC0718848.

PCA is a reliable method that shows the importance of the major contributors to the total variation at each axis of differentiation. While the variables with smaller values closer to zero have a reduced effect, those with high magnitudes have a considerable impact on the clustering (Mohammadi, 2002). Out of 11 principal components, PC1, PC2, PC3 & PC4 with more than one eigen values account for 65.23% of the variability (Table 4). The PC1 (24.03%), PC2 (17.85%), PC3 (14.30%), and PC4 (9.05%) revealed genetic variability across the 178 linseed accessions for yield-related traits. The remaining 34.77% of genetic variability was attributable to the other seven principal components. The combination of plant height, number of capsules per plant, number of branches per plant, bud fly

infestation (%), powdery mildew infestation (%), days to maturity, and 1000 seed weight contributing to PC1 (24.03%) led the most to diversity among accessions. Positive load from the number of capsules per plant, number of primary branches per plant, Alternaria infestation%, bud fly infestation (%), days to maturity, 1000 seed weight, days to 50 percent flowering, seed yield per plant, and flower size were found in the PC2, which accounted for 17.85% of the genetic diversity. Positive loadings from plant height, number of capsules per plant, Alternaria infestation %, and bud fly infestation % accounted for of the overall variation in the PC3 (14.30%). Plant height, number of capsules per plant, powdery mildew infestation (score), 1000 seed weight, days to 50 percent flowering, and seed yield per plant were the variables that explained PC4 (9.05%). The findings are conformity with Rizvi *et al.* (2018), and Kumar and Paul (2016). The obtained PCA results are consistent with the findings of Hussain *et al.* (2022) and Kumar and Kumar (2021). Based on the earlier information, it can be concluded that the first four principal components were related to various traits in linseed mostly associated with high seed yielding accessions and also these traits can identify the diverse accessions which could be employed in hybridization program for improvement of linseed.

Based on the present study, it was concluded that the linseed accessions under investigation exhibited an appropriate genetic diversity. The Hierarchical cluster analysis and PCA revealed that the linseed accessions EC0718827, EC0718829, EC22813, EC0041764, EC0041598, EC0022813-B, EC0718848, EC0000526, EC41659, EC0541202, EC0000541-A, and EC0001403 exhibited genetic diversity for capsules per plant and seed yield per plant with resistance to bud fly infestation. Hence, we can use these accessions in hybridization programs and select the best segregants for segregating generations for further linseed improvement programmes.

Table 4 Factor loading of eleven different traits with respect to different principal factor in linseed accessions

Character	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11
Days to 50 percent flowering	-0.41	0.11	-0.05	0.19	-0.27	0.62	0.10	-0.48	-0.15	0.16	-0.19
Days to maturity	0.42	0.13	-0.18	-0.19	-0.28	-0.07	0.65	-0.28	0.18	-0.33	-0.15
Plant height (cm)	0.16	-0.21	0.58	0.19	0.10	0.03	0.50	0.15	-0.07	0.50	-0.13
Number of branches per plant	0.05	0.51	-0.38	-0.14	0.12	-0.20	-0.02	-0.01	0.14	0.65	-0.27
Flower size(mm)	-0.49	0.11	-0.01	-0.13	0.16	0.11	0.37	0.15	0.55	0.01	0.48
Number of capsules per plant	0.13	0.45	0.36	0.24	0.12	-0.28	-0.09	-0.54	-0.07	-0.02	0.44
1000 seed weight(g)	0.43	0.01	-0.10	0.15	-0.55	0.29	-0.18	0.21	0.18	0.27	0.45
Seed yield per plant (g)	-0.10	0.39	-0.06	0.71	-0.08	-0.08	0.12	0.42	0.03	-0.27	-0.21
Bud fly infestation %	0.30	0.28	0.35	-0.14	0.31	0.49	-0.26	0.04	0.41	-0.19	-0.29
Alternaria infestation %	-0.09	0.47	0.22	-0.47	-0.18	0.13	0.11	0.36	-0.54	-0.09	0.12
Powdery mildew infestation (Score)	0.29	-0.02	-0.41	0.16	0.59	0.36	0.21	0.03	-0.35	-0.01	0.28
Eigen values	2.64	1.96	1.57	1.00	0.83	0.72	0.60	0.53	0.42	0.39	0.32
Proportion variance %	24.03	17.85	14.30	9.05	7.63	6.59	5.41	4.87	3.79	3.58	2.90
Cumulative variance %	24.03	41.88	56.18	65.23	72.86	79.45	84.86	89.73	93.52	97.1	100

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Soil nutrient dynamics and microbial activity as influenced by fertility levels and liquid biofertilizers in gobhi sarson (*Brassica napus* L.)

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ABSTRACT

The experiment was conducted during winter (*rabi*) season of 2022-23 at CSKHPKV Shivalik Agricultural Research and Extension Centre (SAREC), Kangra, Himachal Pradesh to study the effect of fertility levels and liquid biofertilizers on soil nutrient status and microbial activity in gobhi sarson (*Brassica napus* L.). The experiment was laid out in a split plot design with three fertility levels (control, 75% of recommended dose of fertilizers and 100% RDF) in main plots and six liquid biofertilizers [*Azotobacter*, phosphate solubilizing microorganism (PSMO), potassium mobilizing biofertilizer (KMB), NPK consortia + zinc solubilizing biofertilizer (ZSB), ZSB and control (no biofertilizer)] in sub plots. The results demonstrated that application of 100% RDF recorded higher available N (277 kg/ha), P (20.8 kg/ha) and soil microbial population of bacteria, fungi and actinomycetes. However, 75% RDF recorded higher available K (225.1 kg/ha) and higher DTPA extractable Zn (2.76 mg/ha) were recorded in control treatment. Among liquid biofertilizers, higher available nitrogen, phosphorus, potassium and DTPA extractable zinc in soil was recorded with *Azotobacter,* PSMO, KMB and ZSB treatments, respectively. Similarly, higher microbial population was recorded with *Azotobacter.* However, the effect of fertility levels and liquid biofertilizers on soil organic carbon was not significant.

Keywords: *Azotobacter,* Biofertilizers, Gobhi sarson, Microbial activity, Nutrient dynamics

Gobhi Sarson (*Brassica napus* L.) a key oilseed crop, belongs to the Brassicaceae family. Globally, India stands as the third largest producer of rapeseed-mustard followed by Canada and China. In India, Rapeseed-mustard ranks first in total oilseed production with 13.16 milllion tonnes, occuping 33.24% of total oilseeds production (Anonymous, 2024). Rapeseed contains approximately 36-42% oil, commonly used as a condiment in pickles and for flavoring curries and vegetables. The oil cake serves as cattle feed and manure, containing 5.2% nitrogen (N), 1.0% phosphorus (P_2O_5) and 1.4% potassium (K_2O).

The significance of soil health and fertility in crop production under Indian conditions is becoming increasingly important. The continuous decline in soil fertility is recognized as one of the serious challenge in the post Green Revolution era (Dwivedi and Meena, 2015). Excessive nutrient removal by crops combined with the use of imbalanced and insufficient fertilizers has led to widespread multi-nutrient deficiencies in both soils and plants. Nitrogen is commonly deficient in most Indian soils plays a crucial role in the growth of Brassica crops. It is a fundamental component of amino acids, nucleic acids, proteins and enzymes (Singh and Meena, 2004). Similarly, phosphorus is an essential part of key cellular components such as nucleotides, nucleic acids, phospholipids and it significantly enhances root development in crops. Hence, balanced application of NPK is crucial for mustard-rapeseed cultivation as it stimulates metabolic activities, energy transformation and chlorophyll synthesis, leading to optimal growth and yield attributes (Jat *et al.,* 2020). It enhances mustard-rapeseed productivity and profitability while maintaining soil health. Liquid biofertilizers are aqueous solution comprising 10-40% microbial cells along with dispersants, surfactants and a liquid carrier such as water or oil. Compared to solid inoculants, they offer notable advantages including an extended shelf life of 1.5-2 years, higher microbial density, ease of handling and compatibility with modern agricultural equipment. Additionally, they tolerate temperatures up to 45°C and can be applied to both seeds and soil. An ideal carrier should be cost-effective, readily available, non-toxic and possess optimal pH, high water-holding capacity and physicochemical uniformity. However, proper storage under cool conditions is crucial to maintaining microbial viability and functionality (Allouzi *et al.,* 2022). They contribute significantly by improving soil fertility, boosting crop productivity and increasing agricultural output as they are environmentally eco-friendly. *Azotobacter* is free living nitrogen-fixing bacteria, secretes plant growth-promoting substances such as auxins, gibberellic acid and cytokinins. Meanwhile, phosphate-solubilizing bacteria (PSB) enhance phosphorus availability by releasing it from inaccessible organic sources

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through the production of phosphatase enzymes (Achari *et al.,* 2023). Potassium-mobilizing bacteria (KMB) enhance the mobilization of potassium from soil minerals, making it more accessible to plants (Olaniyan *et al.,* 2022). NPK consortia contains rhizobium, *azotobacter,* PSB and KMB bacterial culture which increase the availability for primary nutrients (IFFCO). Indian soils exhibit zinc deficiency while in some cases, zinc is present but remains inaccessible to plants. Zinc-solubilizing bacteria (ZSB) enhance zinc availability through multiple mechanisms like chelation, proton extrusion and organic acid secretion (Masood *et al.,* 2022).

Application of biofertilizers results in increased mineral and water uptake, root development, vegetative growth and nitrogen-fixation (Solanki *et al.,* 2018). Thus, the present experiment was conducted to study the effect of chemical fertilizers and liquid biofertilizers on soil nutrient status and microbial activity in gobhi sarson under North Western Himalaya region.

MATERIALS AND METHODS

A field experiment was conducted during *rabi* 2022-23 at the experimental farm of Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya (CSK HPKV), Shivalik Agricultural Research and Extension Centre (SAREC), Kangra, Himachal Pradesh, India. Geographically, the experimental farm is situated at 32° 09° N latitude, 76° 22° E longitude and 700 meter above the mean sea level. The soil of the field experimentation was clay loam in texture having pH 5.61. The soil sample was taken prior to experiment was low in available nitrogen (275.7 kg/ha), medium in available phosphorus (18.3 kg/ha) and available potassium (227.4 kg/ha). The study also examined initial soil microbial population of bacteria [24.3×10^5 Colony-forming unit (CFU) g/soil)], fungi (16.2×10^3 CFU g/soil) and actinomycetes (8.7×10^3 CFU g/soil).

The experiment was conducted using a split-plot design with three fertility levels assigned to the main plots *viz.,* control (no fertilizer), 75% of the recommended dose of fertilizers (RDF) and 100% RDF. Six liquid biofertilizer treatments were allocated to the sub-plots *viz., Azotobacter,* phosphate solubilizing microorganisms (PSMO), potassium mobilizing biofertilizer (KMB), zinc solubilizing biofertilizer (ZSB), NPK consortia + ZSB and control (no biofertilizer). Each treatment was replicated three times. Seed inoculation with liquid biofertilizers was carried out by soaking seeds in the biofertilizer solution for 30 minutes, followed by shade drying for half an hour prior to sowing. The field plots measured 11.76 m² each. Nitrogen was supplied using fertilizers from the Indian Farmers Fertilizers Cooperative Limited (IFFCO) 12:32:16 and urea, while potassium was provided as muriate of potash (MOP). According to the main plot treatments, a full dose of phosphorus and potassium along with one-third of the nitrogen dose was applied as a basal treatment. The remaining nitrogen were applied in two equal splits using urea at the vegetative and flowering stages. The recommended fertilizer dose was 120 kg N, 60 kg P_2O_5 and 40 kg K_2O per hectare. To study the response of gobhi sarson to different treatments, the analysis has been done in respect to soil chemical and biological properties. The soil samples were taken from 0-15 cm soil, composited, air dried under shade, grind and stored for further analysis. Initial soil pH was determined by using method given by Jackson (1973). Soil organic carbon content was determined by rapid titration method of Walkley and Black (1934). Soil available nitrogen, phosphorous, potassium and DTPA extractable zinc was determined by using alkaline potassium permanganate method (Subbiah and Asija, 1956), 0.5 M sodium bicarbonate method (Olsen *et al.,* 1954), 1 N ammonium acetate extraction method (Jackson, 1973) and Atomic Absorption Spectrophotometric (AAS) method (Lindsay and Norvell, 1978), respectively. Soil microbial population *viz.,* Bacteria, fungi and actinomycetes were quantified using the serial dilution spread plate technique, employing Nutrient Agar (NA), Potato Dextrose Agar (PDA) and actinomycetes isolation agar for each respective microorganism group, respectively (Standard plate count technique by Wollum, 1982).

The statistical analysis for split plot design was conducted by adopting the ANOVA (Analysis of variance) techniques as explained by Cocharan and Cox (1957). The critical difference (CD) values among treatments were worked out at 5% level of probability wherever F values were found significant. Data analysis was undertaken in Operational Statistics (OPSTAT) software (http://14.139.232.166/opstat).

RESULTS AND DISCUSSION

Soil chemical properties

The available nitrogen, phosphorus, potassium and DTPA-extractable zinc at harvest were significantly affected by varying fertility levels (Table 1). The available N content was ranged from 254.4 to 277 kg/ha. The highest available N was found in 100% RDF that was 1.3 kg/ha more than initial value (275.7 kg/ha) followed by 75% RDF and control. Similarly, the highest available P in soil was observed in 100% RDF followed by 75% RDF. Whereas, higher available K (225.1 kg/ha) was recorded with 75% RDF that was statistically at par with 100% RDF. In contrast, the highest DTPA-extractable zinc was recorded in the control (2.76 mg/ha) comparable to 75% RDF. The enhancement in nutrient availability may be ascribed to the systematic application of graded fertilizer doses, which

facilitate the development of a fertility gradient. The statement is in line with the findings by Madhuri *et al.* (2020) and Mahapatra *et al.* (2021).

Different liquid biofertilizers showed significant effect on available NPK and DTPA extractable Zn. Seed inoculation with *Azotobacter* recorded significantly higher available nitrogen (277.5 kg/ha) being at par with NPK consortia + ZSB and PSMO over control (no biofertilizer). However, significantly higher available phosphorus (20.5 kg/ha), potassium (231.9 kg/ha) and DTPA extractable zinc (2.89 mg/ha) was recorded with PSMO, KMB and ZSB treatments, respectively and all remained at par with NPK consortia + ZSB. Availability of all the nutrients were positively affected by application of biofertilizers either single inoculation or coinoculation. Biofertilizers may increase soil fertility through atmospheric nitrogen fixation, dissolution of insoluble phosphates and release of growth-promoting compounds into the plant's rhizosphere (Mazid and Khan, 2015). Availability of potassium from structural K and exchangeable pools through solubilization, acidolysis and chelation by KSM has also been reported by Uroz *et al.* (2009). ZSB increase the availability of Zn by converting the insoluble form of Zn into available form by chealation (Batool *et al.,* 2021). The effect of fertility levels and biofertilizers on soil organic carbon was not significant.

Table 1 Effect of fertility levels and microbial consortia on soil chemical and microbial properties after harvest of gobhi sarson (*Rabi*, 2022-23)

Treatment		Available N (kg/ha)	Available P (kg/ha)	Available K (kg/ha)	DTPA Extractable Zn (mg/ha)	Organic carbon (g/kg)	Bacteria ($\times 10^5$ CFU g/soil)	Fungi ($\times 10^3$ CFU g/soil)	Actinomycetes ($\times 10^3$ CFU g/soil)
Fertility levels									
F1	Control (no fertilizer)	254.4	15.7	211.8	2.76	6.36	25.4	15.5	8.5
F2	75% RDF	270.2	18.0	225.1	2.73	6.41	28.1	17.5	9.1
F3	100% RDF	277.0	20.8	220.2	2.65	6.43	29.3	21.4	9.9
	SEm±	1.1	0.1	1.5	0.01	0.05	0.5	0.3	0.1
	CD (P=0.05)	4.3	0.5	6.1	0.04	NS	2.0	1.3	0.5
Microbial consortia									
T1	Azotobacter	277.5	18.1	212.8	2.62	6.50	30.9	20.5	9.8
T2	PSMO	270.6	20.5	215.0	2.65	6.47	29.7	19.2	9.6
T3	KMB	263.6	17.6	231.9	2.70	6.32	25.7	16.8	8.9
T4	ZSB	260.9	16.9	218.7	2.89	6.38	26.4	17.6	9.0
T5	NPK consortia + ZSB	275.7	19.9	227.6	2.85	6.46	28.4	18.2	9.2
T6	Control (no biofertilizer)	255.0	16.0	208.3	2.58	6.29	23.9	15.7	8.5
	SEm±	3.1	0.2	2.9	0.04	0.07	0.5	0.3	0.1
	CD (P=0.05)	9.1	0.7	8.5	0.12	NS	1.6	0.9	0.3

RDF: Recommended dose of fertilizer; PSMO: Phosphate solubilizing microorganism; KMB: Potassium mobilizing biofertilizer; ZSB: Zinc solubilizing biofertilizer; CFU: Colony-forming unit

Soil microbial properties

Microbial population is a crucial biological factor that positively impact soil health and crop yield. The presence of soil microbes plays a vital for enhancing crop productivity by facilitating nutrient cycling, suppressing diseases and improving soil structure. The data showed that the main effect of fertility levels and biofertilizers significantly varied the microbial population. Application of 100% RDF recorded significantly more fungal and actinomycetes population over 75% RDF and control while higher bacterial population with 100% RDF being at par with 75% RDF. Adequate amount of nutrients in soil (N, P and K) promote the crop growth and better root activity stimulating secretion of the root exudates which promotes higher bacterial growth in soil. Similar findings were reported by Ratanoo (2020).

Seed inoculation with different liquid biofertilizers showed significantly higher microbial population over control. Maximum bacterial and actinomycetes population were recorded in *Azotobacter* inoculation being at par with PSMO treatment. Significantly higher fungal population was observed in *Azotobacter* treatment. These results were supported by findings of Zainuddin *et al.* (2022) who concluded that application of biofertilizers that utilized beneficial microorganisms can augment the microbial

processes which enhance nutrient availability and boost beneficial microorganism survivability.

Application of 100% RDF resulted in higher availability of N and P along with an increased microbial population (bacteria, fungi and actinomycetes). In contrast, potassium availability was maximized under 75% RDF while the highest DTPA-extractable zinc content was observed in the control treatment. Seed inoculation with liquid biofertilizers significantly enhanced the availability of N, P, K and Zn through treatments with *Azotobacter,* phosphate-solubilizing microorganism (PSMO), potassium-mobilizing bacteria (KMB) and zinc-solubilizing bacteria (ZSB), respectively while also promoting microbial population, particularly with *Azotobacter.* Overall, biofertilizer treatments exhibited a significantly positive impact on soil chemical and biological properties.

Conflict of Interest: The authors declare no conflict of interest.

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Effect of irrigation scheduling and thiourea on productivity of Indian Mustard [*Brassica juncea* (L.) Czern & Coss]

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ABSTRACT

Effect of Irrigation Scheduling and Thiourea on Productivity of Indian Mustard [*Brassica juncea* (L.) Zern & Coss] was evaluated at Agronomy Farm, S.K.N. College of Agriculture, Jobner during Rabi season 2019-20. The experiment consisted of five irrigation scheduling *viz.,* surface irrigation, sprinkler, drip irrigation at 0.4 IW/CPE ratio, 0.6 IW/CPW ratio and 0.8 IW/CPE ratio, respectively and three levels of foliar application of thiourea (control, thiourea @ 500 ppm and thiourea @ 750 ppm). The experiment was laid out in split plot design with three replications. The results revealed that scheduling of irrigation at drip irrigation at 0.6 IW/CPE ratio, was at par with drip irrigation at 0.8 IW/CPE ratio and recorded significantly higher plant height, dry matter accumulation, siliquae per plant, seeds per siliqua, seed yield (2.12 t/ha), straw yield (6.62 t/ha), biological yield (8.74 t/ha), net returns (₹ 70,661/ha) and B:C ratio (3.14) as compared to surface irrigation and at 0.4 IW/CPE ratio. Foliar application of thiourea @ 500 ppm, was found at par with foliar application of thiourea @ 750 ppm, leading to higher plant height, dry matter accumulation, siliquae per plant, seeds per siliqua, seed yield (1.98 t/ha), straw yield (6.48 t/ha) and biological yield (8.50 t/ha), net returns (₹ 66,016/ha) and B:C ratio (3.14) as compared to control. Based on the above findings, drip irrigation at 0.6 IW/CPE ratio with foliar application of thiourea @ 500 ppm could be recommended in mustard crops Rajasthan.

Keywords: Irrigation Scheduling, Indian Mustard, Rajasthan, Thiourea

Oilseed crops are main source of energy in the diet of Indians. Though, India has become self-reliant with respect to food grains but still lagging behind in the production of oilseeds. Rapeseed-mustard is an important group of oilseed crop in the world. Total area of mustard in India is 62.3 lakh hectares with annual production of about 93.4 lakh tonnes and an average productivity of about 1499 kg/ha. In Rajasthan, it occupies 25 lakh hectares with annual production of 41.96 lakh tonnes (Anonymous, 2018-19). Indian mustard (*Brassica juncea* L.) is a major Rabi oilseed crop of the country and occupies a prominent place being next in importance to soybean and groundnut, both in area and production. Despite of significant contribution of mustard in human nutrition and national economy, productivity of this crop in the state has almost been stagnant which is a matter of great concern. There is no scope of increasing the area under this crop at the cost of food grain crops. Thus, the only way to increase the production and its productivity is through new crop production technology.

Drip and micro sprinklers are important compare of micro irrigation system able to manage high water potential continuously, thus minimizing fluctuation in soil water contents in the effective root zone and hold the promise of increased crop yield and quality with the ability to provide small but frequent water application have been found superior in terms of water savings, high irrigation efficiency and crop yield. Micro irrigation refers to a variety of irrigation methods in which water is delivered directly to small areas through emitters or applicators placed along a water delivery line (typically a polyethylene hose). Flow rates per "dripper" emission device are typically small (1.5-8 lakh/liter), although some micro spray systems have such large flow rates (40-60 lakh/liter) that they might also be classified as small flow rate, permanent, solid set sprinklers. Because drip/micro irrigation systems are "solid set," they have the potential for automation. However, the majority of these systems are operated manually, with a large percentage having automatic filter back flush operations.

In IW/CPE approach, known amount of irrigation water is applied when cumulative pan evaporation reaches predetermined level. For practical purpose irrigation should be started when allowable depletion of available moisture in the root zone. The proper irrigation scheduling can play a major role in increasing the water use efficiency and the productivity of crops by applying the required amount of water when it is needed. On the other hand, the poor irrigation scheduling can lead to the development of crop water deficit and result in a reduced yield due to water and nutrient deficiency.

Abiotic stress is the common feature and deterrent to crop production especially in rain fed crops. Some

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agro-chemicals have proved effective to mitigate the environmental stresses. In this regard thiourea plays a vital role in the physiology of plants both as sulphydryl compound and as an amino compound like urea. The foliar spray of thiourea increase the plant photosynthetic efficiency and canopy photosynthesis due to presence of S-H group as an integral constituent of these thiols. Its beneficial effect appears to be due to delayed senescence of both vegetative and reproductive organs as thiourea has cytokine in like activity, particularly delaying senescence (Halmann, 1980). Thiourea is also known to increase photosynthetically active leaf surface during grain filling period in cereals (Sahu *et al*., 1993). Foliar spray of thiourea has been reported not only to improve growth and development of plants, but also the dry matter partitioning for increased grain yield (Arora, 2004). Present investigation was carried out to study the impact of irrigation scheduling and thiourea on growth and productivity of mustard.

MATERIALS AND METHODS

The experiment was conducted at Agronomy Farm, S.K.N. College of Agriculture, Jobner, Jaipur (Rajasthan). Geographically, Jobner is situated 45 km away from Jaipur in western side at 75°28' East longitude and 26°05' North latitude and with an altitude of 427 meters above mean sea level in Jaipur district of Rajasthan. The region fall under agro climatic zone IIIA of Rajasthan state named as semi-arid eastern plains. The climate of this region is a typically semi-arid, specially characterized by extremes of temperature during both summer and winters. The average annual rainfall of this region varies from 350 to 400 mm, most of which is contributed by the south-west monsoon during the month of July to September. The soil of the experimental plot was loamy sand in texture (82.9% sand, 9.8% silt and 7.3% clay) and low in fertility status (0.19% O.C, 130.2 kg available N/ha, 17.5 kg available P/ha and 7.89 kg available S mg/kg) and neutral condition in nature (pH=7.92).

The seed of mustard variety RH-406 @ 5 kg/ha was used for sowing in the experiment. The sowing was done in rows at 30 cm apart behind the plough. The experimental mustard crop was fertilized uniformly with 45 kg N/ha and 30 kg P_2O_5/ha through urea and DAP, respectively. Half dose of the nitrogen along with full dose of phosphorous was applied at the time of sowing as basal. The remaining dose of nitrogen was top dressed in two equal splits after irrigation at 30 and 40 days after sowing of the crop.

The experiment was laid out in split plot design with three replications. The main plot constituted of five irrigation scheduling treatments i.e., surface irrigation, sprinkler irrigation, drip irrigation at 0.4 IW/CPE ratio, drip irrigation at 0.6 IW/CPE ratio and drip irrigation at 0.8 IW/CPE ratio. Sub plot basement consisted of treatment *viz.,* control, thiourea @ 500 ppm and thiourea @ 750 ppm. Thiourea is a sulphydral compound and its empirical formula is CH_4N_2S. It is applied as foliar spray at pre flowering and pod formation stage.

Statistical analysis: The experimental data recorded were subjected to statistical analysis using analysis of variance technique suggested by Panse and Sukhatme (1985). The critical difference (CD) was worked out to assess the significance of treatments mean wherever the 'F' test was found significant at 5 per cent level of probability. To elucidate the nature and magnitude of treatments effects, summary tables along with SEm± and CD at 5 per cent are given. All these statistical estimates were computed by standard statistical procedures (Panse and Sukhatme, 1985).

RESULTS AND DISCUSSION

Growth attributes

Plant population: Plant stand per metre row length of mustard crop was not affected significantly by irrigation scheduling and application of thiourea at 20 DAS and at harvest (Table 1).

Plant height: Irrigation scheduling through drip significantly increased plant height over surface irrigation. Among irrigation scheduling, drip irrigation at 0.6 IW/CPE ratio, being at par with drip irrigation at 0.8 IW/CPE ratio (210.2 cm), recorded significantly higher plant height over surface irrigation, sprinkler and drip irrigation at 0.4 IW/CPE ratio (Table 1).

Foliar application of thiourea significantly increased the plant height at harvest. Thiourea @ 500 ppm, being at par with thiourea @ 750 ppm, recorded significantly higher plant height over control.

Dry matter accumulation: Scheduling drip irrigation at 0.6 IW/CPE ratio (182 g) recorded significantly higher dry matter accumulation at harvest over surface irrigation, sprinkler and drip irrigation at 0.4 IW/CPE ratio (167 g) and remained statistically at par with drip irrigation at 0.8 IW/CPE ratio (184 g) (Table 1). The drip irrigation at 0.6 IW/CPE ratio significantly increased the dry matter accumulation by 19.62, 7.43 and 8.90 per cent at harvest as compared to surface irrigation, sprinkler and drip irrigation at 0.4 IW/CPE ratio, respectively. It is well established fact that where sufficient soil moisture for continued growth is maintained by providing drip irrigation it leads to greater development of green tissue area and results in a higher photosynthetic assimilation (Bharti *et al*., 2007). As a result, plant growth improves leading to higher accumulation of the total dry matter.

Table 1 Effect of irrigation scheduling and thiourea on plant population of mustard at different growth stages

Treatments	Plant stand/meter row length		Plant height (cm) at harvest	Dry matter accumulation/ meter row length (g) at harvest
	20 DAS	At harvest		
Irrigation methods				
Surface irrigation	9.94	9.13	170.1	152
Sprinkler	10.01	9.30	195.6	169
Drip irrigation at 0.4 IW/CPE ratio	9.98	9.26	184.2	167
Drip irrigation at 0.6 IW/CPE ratio	10.08	9.40	208.3	182
Drip irrigation at 0.8 IW/CPE ratio	10.11	9.44	210.2	184
SEm+	0.23	0.24	6.4	4.4
CD (P=0.05)	NS	NS	20.8	14.3
CV (%)	8.97	10.06	11.4	8.9
Thiourea application				
Control	10.12	9.15	182.7	154
Thiourea @ 500 ppm	10.03	9.35	195.7	176
Thiourea @ 750 ppm	9.91	9.41	202.6	184
SEm+	0.16	0.18	4.1	3.2
CD (P=0.05)	NS	NS	12.2	9.5

Foliar application of thiourea significantly improved the dry matter accumulation at harvest. Foliar spray of thiourea @ 750 ppm (184 g), recorded significantly higher dry matter accumulation over control, which was at par with thiourea @ 500 ppm (176 g). Foliar application of thiourea @ 500 ppm increased dry matter accumulation by 10.86 per cent at harvest over control. Higher cytokine in like activity of thiourea and vigorous vegetative growth of the crop having higher chlorophyll content of leaves might have helped to persist the photosynthesis activity for longer period (Premaradhya *et al.,* 2018). The observed improvement in overall vegetative growth of the crop with the foliar application of thiourea in the present investigation is in conformity with those of Jat (2007).

Yield attributes and yield

Number of siliquae per plant: Scheduling drip irrigation at 0.6 IW/CPE ratio recorded significantly higher siliquae/plant as compared to surface irrigation, sprinkler and drip irrigation at 0.4 IW/CPE ratio and the per cent increase was 27.7, 11.4 and 15.7, respectively (Fig 1). However, drip irrigation at 0.6 IW/CPE ratio remained at par with drip irrigation at 0.8 IW/CPE ratio.

Foliar application of thiourea significantly enhanced the number of siliqua/plant of mustard. Foliar application of thiourea @ 500 ppm (310), being at par with thiourea @ 750 ppm (322), recorded significantly higher number of siliqua per plant over control (268). The foliar spray of thiourea @ 500 ppm increased siliquae per plant by 15.51 per cent over control.

Number of seeds per siliqua: Drip irrigation at 0.6 IW/CPE ratio recorded significantly increased number of seeds per siliqua over surface irrigation, sprinkler and drip irrigation at 0.4 IW/CPE ratio and remained at par with drip irrigation at 0.8 IW/CPE ratio (Table 2). Drip irrigation at 0.6 IW/CPE ratio produced 24.6, 11.3 and 13.2 per cent higher number of seeds per siliqua as compared to surface irrigation, sprinkler and drip irrigation at 0.4 IW/CPE ratio, respectively.

Foliar application of thiourea observed significantly higher the number of seeds per siliqua of mustard. Foliar spray of thiouea @ 750 ppm recorded significantly highest seeds per siliqua of mustard over control and foliar spray of thiourea @ 500 ppm. Application of thiourea @ 750 ppm increased number of seeds per siliqua by 17.8 and 7.5 per cent over control and foliar spray of thiourea @ 500 ppm, respectively.

Test weight: Drip irrigation at 0.8 IW/CPE ratio (4.60 g), recorded significantly higher test weight over surface irrigation (3.28 g), sprinkler (3.83 g) and drip irrigation at 0.4 IW/CPE ratio (3.75 g) (Table 2). The drip irrigation at 0.6 IW/CPE ratio increased the test weight by 35.37, 15.93 and 18.40 per cent over surface irrigation, sprinkler and drip irrigation at 0.4 IW/CPE ratio, respectively.

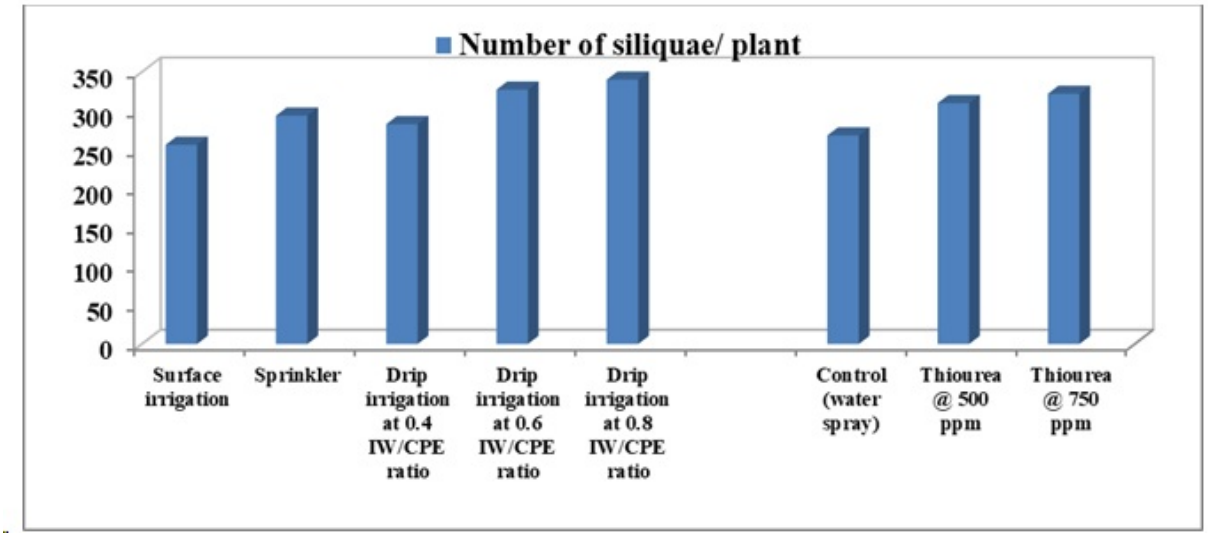

Fig. 1. Impact of irrigation scheduling and thiourea on number of siliquae/plant

Table 2 Effect of irrigation scheduling and thiourea on number of siliquae per plant, number of seeds per siliqua and test weight of mustard

Treatments	Number of siliquae/ plant	Number of seeds/ siliqua	Test weight (g)
Irrigation scheduling			
Surface irrigation	256	13.2	3.28
Sprinkler	294	14.8	3.83
Drip irrigation at 0.4 IW/CPE ratio	283	14.6	3.75
Drip irrigation at 0.6 IW/CPE ratio	327	16.5	4.44
Drip irrigation at 0.8 IW/CPE ratio	340	16.9	4.60
SEm+	8.8	0.4	0.10
CD (P=0.05)	28.7	1.4	0.34
CV (%)	10.2	9.8	8.97
Thiourea			
Control	268	13.2	3.48
Thiourea @ 500 ppm	310	15.6	4.15
Thiourea @ 750 ppm	322	16.8	4.31
SEm+	6.1	0.3	0.07
CD (P=0.05)	17.8	0.8	0.21

A perusal of data presented in Table 4.4 and Fig. 4.2 also revealed that the foliar application of thiourea significantly increased the test weight of mustard over control. Foliar spray of thiourea @ 750 ppm (4.31 g), recorded significantly enhanced test weight of mustard over control (3.48 g). The foliar spray of thiourea @ 500 ppm increased test weight by 19.3 per cent over control.

Seed yield: Scheduling drip irrigation at 0.6 IW/CPE ratio obtained significantly higher seed yield (2.12 q/ha) of mustard as compared to surface irrigation (1.61 q/ha), sprinkler (1.86 q/ha) and drip irrigation at 0.4 IW/CPE ratio (1.84 t/ha), and it was statistically at par with drip irrigation at 0.8 IW/CPE ratio (2.19 t/ha) (Fig 2). Drip irrigation at 0.6 IW/CPE ratio improved the seed yield of

mustard by 31.3, 14.1 and 15.0 per cent over surface irrigation, sprinkler and drip irrigation at 0.4 IW/CPE ratio, respectively. The yield increase in drip irrigation with greater IW/CPE ratio was higher due to frequency of water application through drip irrigation resulting in favourable micro climate and maintains constant soil moisture near to field capacity which helps in increasing the yield (Piri *et al*., 2020).

Table 3 Effect of irrigation scheduling and thiourea on seed, straw, biological yield and harvest index of mustard

Treatments	Seed yield (t/ha)	Straw yield (t/ha)	Biological yield (t/ha)	Harvest index (%)
Irrigation scheduling				
Surface irrigation	1.61	5.30	6.91	23.4
Sprinkler	1.86	5.95	7.80	23.9
Drip irrigation at 0.4 IW/CPE ratio	1.84	5.94	7.78	23.7
Drip irrigation at 0.6 IW/CPE ratio	2.12	6.62	8.74	24.3
Drip irrigation at 0.8 IW/CPE ratio	2.19	6.82	9.00	24.3
SEm+	0.07	0.18	0.21	0.6
CD (P=0.05)	0.21	0.58	0.70	NS
CV (%)	12.0	10.1	9.2	8.9
Thiourea				
Control	1.69	5.08	6.77	24.9
Thiourea @ 500 ppm	1.98	6.50	8.50	23.3
Thiourea @ 750 ppm	2.10	6.81	8.91	23.5
SEm+	0.05	0.12	0.16	0.5
CD (P=0.05)	0.13	0.36	0.47	NS

Table 4 Effect of irrigation scheduling and thiourea on net returns and B:C ratio of mustard

Treatments	Net returns (₹/ha)	B:C ratio
Irrigation scheduling		
Surface irrigation	53,966	3.15
Sprinkler	61,235	3.06
Drip irrigation at 0.4 IW/CPE ratio	57,567	2.76
Drip irrigation at 0.6 IW/CPE ratio	70,661	3.14
Drip irrigation at 0.8 IW/CPE ratio	73,667	3.21
Thiourea		
Control	52,771	2.77
Thiourea @ 500 ppm	66,016	3.14
Thiourea @ 750 ppm	71,470	3.28

Foliar application of thiourea significantly increased the seed yield of mustard over control. Foliar application of thiourea @ 500 ppm (1.98 t/ha), which was at par with foliar spray of thiourea @ 750 ppm (2.10 t/ha), recorded significantly enhanced seed yield of mustard over control (1.69 t/ha). The foliar spray of thiourea @ 500 ppm increased seed yield by 17.0 per cent over control.The increase in yield attributes and yield obtained with thiourea application was most probably due to increased crop photosynthesis favored by both improved efficiency and source to sink relationship (Premaradhya *et al.,* 2018).

Straw yield: Drip irrigation recorded significantly higher straw yield of mustard over surface irrigation (Table 3). The

drip irrigation at 0.6 IW/CPE ratio (6.62 t/ha), being at par with drip irrigation at 0.8 IW/CPE ratio (6.82 t/ha), recorded significantly higher straw yield of mustard as compared to surface irrigation (5.30 t/ha), sprinkler (5.95 t/ha) and drip irrigation at 0.4 IW/CPE ratio (5.94 t/ha). The drip irrigation at 0.6 IW/CPE ratio increased straw yield of mustard by 25.1, 11.4 and 11.5 per cent over surface irrigation, sprinkler and drip irrigation at 0.4 IW/CPE ratio, respectively.

Foliar application of thiourea significantly increased the straw yield of mustard over control. Foliar application of thiourea @ 500 ppm (6.50 t/ha), being at par with foliar spray of thiourea @ 750 ppm (6.81 t/ha), recorded significantly enhanced straw yield of mustard over control (5.08 t/ha). The foliar spray of thiourea @ 500 ppm increased straw yield by 27.7 per cent over control.

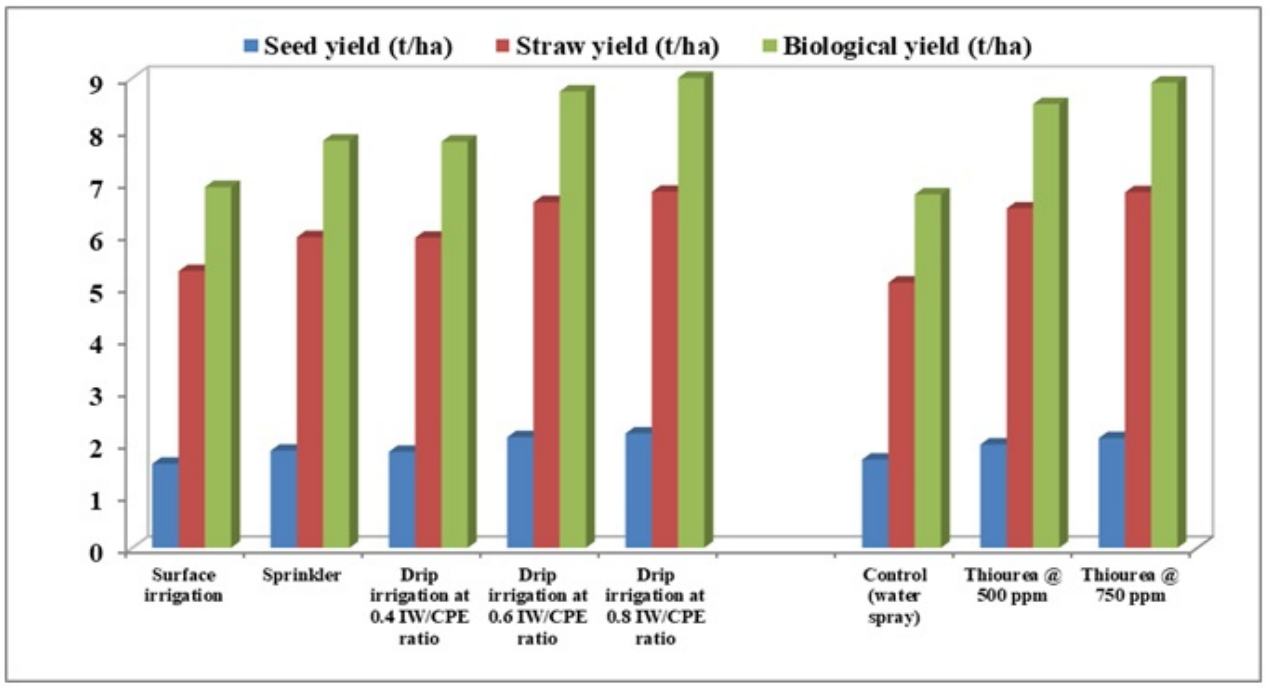

Fig. 2. Impact of irrigation scheduling and thiourea on productivity of mustard

Biological yield: Drip irrigation at 0.6 IW/CPE ratio obtained significantly higher biological yield (8.74 t/ha) of mustard as compared to surface irrigation (6.91 t/ha), sprinkler (7.80 t/ha) and drip irrigation at 0.4 IW/CPE ratio (7.78 t/ha) (Table 3). But, it remained statistically at par with drip irrigation at 0.8 IW/CPE ratio (9.00 t/ha). Drip irrigation at 0.6 IW/CPE ratio increased the biological yield significantly by 26.5, 12.0 and 12.4 per cent over surface irrigation, sprinkler and drip irrigation at 0.4 IW/CPE ratio, respectively.

A further reference to data (Table 4.5 and Fig 4.3) also indicated that the foliar application of thiourea significantly increased the biological yield of mustard crop. Foliar application of thiourea @ 500 ppm (8.50 t/ha), being at par with foliar spray of thiourea @ 750 ppm (8.91 t/ha), recorded significantly enhanced biological yield of mustard over control (6.77 t/ha). The foliar spray of thiourea @ 500 ppm increased biological yield by 25.0 per cent over control.

Economics: Irrigation scheduled at drip irrigation at 0.6 IW/CPE ratio recorded significantly higher net returns (Rs 70,661/ha) as compared to surface irrigation (₹ 53,966/ha), sprinkler (₹ 61,235/ha) and drip irrigation at 0.4 IW/CPE ratio (₹ 57,567/ha) and remained statistically at par with 0.8 IW/CPE ratio (₹ 73667/ha) (Table 4). The drip irrigation at 0.6 IW/CPE ratio significantly increased net returns by 6.7, 5.9 and 18.5 per cent as compared to surface irrigation, sprinkler and drip irrigation at 0.4 IW/CPE ratio. Irrigation scheduled drip irrigation at 0.8 IW/CPE ratio obtained maximum B:C ratio (3.21) which was higher as compared to drip irrigation at 0.4 IW/CPE ratio (2.76) and remained statistically at par with surface, sprinkler and drip irrigation at 0.6 IW/CPE ratio. The irrigation scheduled at drip irrigation at 0.8 IW/CPE ratio significantly increased B:C ratio to the tune of 16.3 per cent over drip irrigation 0.4 IW/CPE ratio.

Foliar spray of thiourea @ 750 ppm recorded significantly highest net returns (₹ 71,470/ha) over control

(₹52,771/ha) and application of thiourea @ 500 ppm (₹66016/ha). The application of thiourea @ 750 ppm increased net returns by 35.4 and 8.3 per cent over control and thiourea @ 500 ppm, respectively. Further data showed that thiourea @ 500 ppm recorded significantly higher B:C ratio (3.14) in mustard crop over control (2.77) and remained at par with thiourea @ 750 ppm (3.28). The thiourea @ 500 ppm increased B:C ratio by 13.3 per cent over control.These results are in conformity with Devi *et al*. (2015); Rank (2007).

The studies on irrigation scheduling and thiourea application the scope for improvement in mustard productivity with the help of agronomic interventions particularly in Rajasthan where moisture deficit and abiotic stress conditions prevail. It may be inferred that scheduling of irrigation at 0.6 IW/CPE ratio along with foliar application of thiourea @ 500 ppm was found most suitable for obtaining higher seed yield, net returns and B:C ratio in mustard.

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Effect of zinc and iron fertilization on yield potential, soil fertility status and economics of mustard (*Brassica juncea* L.) in sandy loam soil

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ABSTRACT

A field experiment was conducted on Indian mustard during *rabi* season of 2021-22 at Sardarkrushinagar, Gujarat in loamy sand soil. Total eight treatments were laid out in randomized block design with four replications. Gujarat Dantiwada Mustard 4 was sown at a distance of 45 cm × 15 cm. Seed yield (2324 kg/ha) and stover yield (5381 kg/ha) were recorded significantly maximum with the application of treatment RDF + 4.0 kg $ZnSO_4$/ha and 7.5 kg $FeSO_4$/ha soil application as basal + 0.5% $ZnSO_4$ and 0.5% $FeSO_4$ foliar spray at 30 and 45 DAS (T8). Nutrient status in soil after harvest *viz.,* available nitrogen, phosphorus and potash were not affected significantly but sulphur (12.7 ppm), zinc (0.448 ppm) and iron (4.52 ppm) status were found significantly higher with the application of RDF + 8.0 kg $ZnSO_4$/ha and 15.0 kg $FeSO_4$/ha soil application as basal (T2). Similarly, maximum benefit cost ratio (3.85) was also found with application of RDF + 8.0 kg $ZnSO_4$/ha and 15.0 kg $FeSO_4$/ha soil application as basal (T2).

Keywords: Economics, Iron, Mustard, Yield and Zinc

Brassica juncea, commonly known as Indian mustard, brown mustard, leaf mustard, oriental mustard and vegetable mustard, is a species of mustard plant. Mustard plants belong to the genera Brassica. Currently the area, production and productivity of rapeseed-mustard in Gujarat was 2.14 lakh ha, 4.24 lakh tonnes and 1976 kg/ha, respectively (GOI, 2021). Micronutrient (Zn, Fe) fertilization has led to the improvement of growth, seed yield and nutritional quality of Indian mustard. Various methods including soil, foliar and seed treatment with Zn and Fe application to crops have been reported for alleviating their deficiency. Foliar feeding of micronutrients is usually cheap, more effective with greater nutrient use efficiency and considerably reduce environmental pollution by reducing the quantity of fertilizers added. In addition, foliar fertilization has been evidenced to promote root growth, leading to an increased uptake of nutrients by crops. Recent research has shown that a small amount of nutrients, particularly Zn and Fe supplied through foliar spray, have resulted in significant increases in the yield of crops. It is well-known that oilseed crops require adequate N with optimum amount of micronutrients (Zn, Fe) for the production of a sustainable yield. Thus, the supply of nutrients Zn, Fe and N through fertilizers in adequate amounts is essential for getting higher yield and quality of oilseed crops. Therefore, the present study was performed to determine the influence of foliar-applied Zn, Fe and urea on yield, nutrient concentration and uptake, as well as nutrient use efficiencies in Indian mustard.

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MATERIALS AND METHODS

A field experiment was conducted at Agronomy Instructional Farm, Chimanbhai Patel College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat. Geographically, Sardarkrushinagar is situated at 24°19' North latitude and 72°19' East longitude with an elevation of 154.52 meter above the mean sea level and situated in the North Gujarat Agro-climatic region. Climate of this region is sub-tropical monsoon type and falls under semi-arid region. In general, the monsoon is warm and moderately humid, winter is fairly cold and dry, while summer is largely hot and dry.The soil of experimental field was loamy sand in texture, low in organic carbon (0.27%), electrical conductivity (0.13 dS/m) and low available nitrogen (135.3 kg/ha), medium in available phosphorus (40.52 kg P_2O_5/ha), medium in available potash (272.4 kg K_2O/ha), low in available sulphur (9.91 ppm), DTPA- extractable Zn (0.31 mg/kg) and DTPA extractable Fe (3.55 mg/kg) having pH value of 7.3. Total eight treatments were laid out in randomized block design with four replications. Gujarat Dantiwada Mustard 4 was sown at a distance of 45 cm × 15 cm. The recommended doses 50 kg nitrogen/ha, 50 kg phosphorus/ ha, 40 kg sulphur/ha were applied uniformly to all the plots and zinc sulphate and iron sulphate applied as per treatments. Full dose of phosphorus, sulphur and half dose of nitrogen were applied at the time of sowing and the remaining half dose of nitrogen was applied at 30-35 DAS. The sources for nitrogen, phosphorous and sulphur are urea, Di ammonium phosphate, bentonite sulphur. The net return was worked out by subtracting the total cost of cultivation

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from the gross return and benefit: cost ratio (BCR) was calculated on the basis of the following formula:

$$\text{Benefit : cost ratio} = \frac{\text{Gross realization (₹/ha)}}{\text{Total cost of cultivation (₹/ha)}}$$

Available nutrient in soil at initial and after harvest of crop were analysed by standard analytical methods (Table 1).

Table 1 Standard analytical methods for analysis of available nutrients status in soil

Nutrients	Method	Reference
Available N	Alkaline permanganate	Subbiah and Asija, 1956
Available P_2O_5	Olsen's method	Olsen, 1954
Available K_2O	Flame photometric method	Jackson, 1973
Available S	Turbidimetric method	Chaudhary and Cornfield, 1966
Available Zn	DTPA method	Lindsay and Norvell, 1978
Available Fe	DTPA method	Lindsay and Norvell, 1978

RESULTS AND DISCUSSION

Yield: Application of RDF + 4.0 kg $ZnSO_4$/ha and 7.5 kg $FeSO_4$/ha as soil application at basal + 0.5% $ZnSO_4$ and 0.5% $FeSO_4$ foliar spray at 30 and 45 DAS produced significantly higher seed yield (2324 kg/ha) and stover yield (5381 kg/ha) but its statistically comparable to treatments T2 (2289 and 5300 kg/ha), T7 (2271 and 5277 kg/ha), T6 (2181 and 5207 kg/ha) and T5 (2060 and 4971 kg/ha) (Table 2). Nevertheless, application of RDF alone resulted in a considerably lower seed (1946 kg/ha) and stover yield (4471 kg/ha). These significant differences were observed because of the application of $ZnSO_4$ and $FeSO_4$ as they increased availability of Fe and Zn in soil (Table 3). So, plant can easily uptake these micronutrients from the soil and resulted into enhanced growth of plant and increase yield of the mustard. The findings were confirmed to those reported by Singh *et al.* (1993); Malewar *et al.* (2001) and Adkine *et al.* (2017).

Soil fertility status after harvest: Zinc and iron fertilization greatly improved the available pool of Fe, Zn, and S status, but it had no distinct effect on the available phosphorus, nitrogen and potash status in soil after harvest of crop (Table 3). Available sulphur (12.7 ppm) in soil was found significantly higher with application of RDF + 8.0 kg $ZnSO_4$/ha and 15.0 kg $FeSO_4$/ha soil application at basal. Because of the low sulphur status at the experimental site, the application of sulphur may have increased the amount of plant available sulphur in the soil at harvest (Damor, 2019). Zinc (0.448 ppm) and iron (4.52 ppm) content in soil after harvest was found significantly higher with application of RDF + 8.0 kg $ZnSO_4$/ha and 15.0 kg $FeSO_4$/ha soil application at basal. The higher solubility, diffusion and mobility of the applied inorganic Zn and Fe fertilizer might be the reason for increased Fe and Zn status in soil. The results were confirmed by Ranparia (2001) and Patel *et al.* (2007).

Table 2 Effect of zinc and iron fertilization on yield and economics of mustard

Treatment	Yield (kg/ha)		Net realization (₹/ha)	BCR
	Seed	Stover		
T_1: RDF (50-50-00-40 kg N-P_2O_5-K_2O-S/ha)	1947	4471	87401	3.40
T_2: RDF+ 8.0 kg $ZnSO_4$/ha and 15.0 kg $FeSO_4$/ha soil application at basal	2289	5300	110263	3.85
T_3: RDF+ 0.5% $ZnSO_4$ and 0.5% $FeSO_4$ foliar spray at 30 DAS	1963	4560	89521	3.38
T_4: RDF+ 0.5% $ZnSO_4$ and 0.5% $FeSO_4$ foliar spray at 45 DAS	2008	4711	92521	3.46
T_5: RDF+ 0.5% $ZnSO_4$ and 0.5% $FeSO_4$ foliar spray at 30 and 45 DAS	2060	4971	94832	3.44
T_6: RDF+ 4.0 kg $ZnSO_4$/ha and 7.5 kg $FeSO_4$/ha soil application at basal + 0.5% $ZnSO_4$ and 0.5% $FeSO_4$ foliar spray at 30 DAS	2181	5207	103327	3.69
T_7: RDF+ 4.0 kg $ZnSO_4$/ha and 7.50 kg $FeSO_4$/ha soil application at basal + 0.5% $ZnSO_4$ and 0.5% $FeSO_4$ foliar spray at 45 DAS	2271	5277	109211	3.84
T_8: RDF+ 4.0 kg $ZnSO_4$/ha and 7.5 kg $FeSO_4$/ha soil application at basal + 0.5% $ZnSO_4$ and 0.5% $FeSO_4$ foliar spray at 30 and 45 DAS	2324	5381	111509	3.81
SEm±	95.31	228.6	-	-
CD at 5%	280	672	-	-
CV %	8.95	9.17	-	-

Table 3 Effect of zinc and iron fertilization on soil fertility status after harvest of mustard

Treatment	Available nutrients					
	N (kg/ha)	P (kg/ha)	K(kg/ha)	S (ppm)	Zn (ppm)	Fe (ppm)
T_1: RDF (50-50-00-40 kg N-P_2O_5-K_2O-S/ha)	138.4	43.6	257.1	10.1	0.300	3.48
T_2: RDF+ 8.0 kg $ZnSO_4$/ha and 15.0 kg $FeSO_4$/ha soil application at basal	143.5	44.7	263.8	12.7	0.448	4.52
T_3: RDF+ 0.5% $ZnSO_4$ and 0.5% $FeSO_4$ foliar spray at 30 DAS	139.1	43.6	258.6	10.6	0.303	3.45
T_4: RDF+ 0.5% $ZnSO_4$ and 0.5% $FeSO_4$ foliar spray at 45 DAS	139.9	43.7	259.1	10.7	0.305	3.46
T_5: RDF+ 0.5% $ZnSO_4$ and 0.5% $FeSO_4$ foliar spray at 30 and 45 DAS	140.5	43.8	260.4	10.9	0.309	3.51
T_6: RDF+ 4.0 kg $ZnSO_4$/ha and 7.5 kg $FeSO_4$/ha soil application at basal + 0.5% $ZnSO_4$ and 0.5% $FeSO_4$ foliar spray at 30 DAS	142.1	44.2	262.3	11.3	0.375	4.05
T_7: RDF+ 4.0 kg $ZnSO_4$/ha and 7.50 kg $FeSO_4$/ha soil application at basal + 0.5% $ZnSO_4$ and 0.5% $FeSO_4$ foliar spray at 45 DAS	142.9	44.3	263.5	11.7	0.380	4.07
T_8: RDF+ 4.0 kg $ZnSO_4$/ha and 7.5 kg $FeSO_4$/ha soil application at basal + 0.5% $ZnSO_4$ and 0.5% $FeSO_4$ foliar spray at 30 and 45 DAS	143.8	44.9	264.1	12.4	0.381	4.11
SEm±	6.31	1.91	6.89	0.49	0.017	0.19
CD at 5%	NS	NS	NS	1.5	0.05	0.55
CV %	8.93	8.66	5.28	8.74	9.81	9.72

Economics: The highest net realization of ₹1,11,509/ha was obtained due to application of RDF + 4.0 kg $ZnSO_4$/ha and 7.5 kg $FeSO_4$/ha as soil application at basal + 0.5% $ZnSO_4$ and 0.5% $FeSO_4$ foliar spray at 30 and 45 DAS (T8) followed by net realization of ₹110263/ha which was obtained under application of RDF + 8.0 kg $ZnSO_4$/ha and 15.0 kg $FeSO_4$/ha as soil application at basal (T2). The lower value of net realization of ₹89,332/ha was obtained under application RDF alone (Table 1). While considering BCR value, the significantly maximum BCR value of 3.85 was incurred with the application of RDF + 8.0 kg $ZnSO_4$/ha and 15.0 kg $FeSO_4$/ha as soil application at basal (T2) followed by application of RDF + 4.0 kg $ZnSO_4$/ha and 7.5 kg $FeSO_4$/ha as soil application at basal + 0.5% $ZnSO_4$ and 0.5% $FeSO_4$ foliar spray at 30 and 45 DAS having BCR value of 3.81.

It is concluded that mustard crop should be fertilized with RDF along with 8.0 kg $ZnSO_4.7H_2O$/ha and 15.0 kg $FeSO_4$.7H2O/ha as soil application at basal for getting higher yield and net returns in loamy sand soil.

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Effect of mulching practices and foliar nutrition on groundnut (*Arachis hypogaea* l.) productivity under raised bed method of cultivation

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ABSTRACT

A field experiment was conducted at ARS, Kawadimatti, UAS Raichur during *kharif,* 2022 to study the effect of mulching practices and foliar nutrition on groundnut under raised bed method of cultivation. The experiment was laid out in a split plot design with three main plots consisting of different mulching practices i.e., no mulch, paddy straw mulch and plastic mulch and four sub plots i.e., foliar spray of water, $FeSO_4$ @ 0.5%, $ZnSO_4$ @ 0.5% and KCl @ 0.5% at 40 DAS each replicated thrice. Results revealed that the plastic mulch recorded the highest pod yield (2320 kg/ha), haulm yield (4164 kg/ha), harvest index (0.36), gross returns (₹ 1,44,057/ha), net returns (₹ 78,879/ha) and B:C (2.21). Foliar spray of $ZnSO_4$ @ 0.5 % recorded higher pod yield (2252 kg/ha), haulm yield (4124 kg/ha), gross returns (₹1,40,005/ha), net returns (₹75,675/ha). Among interaction effects plastic mulch combined with $ZnSO_4$ spray recorded higher pod yield (2471 kg/ha), haulm yield (4354 kg/ha), harvest index (0.36), gross returns (₹ 1,53,281/ha), net returns (₹86,368/ha) and B:C (2.29). Lower weed density and dry weight were recorded in plastic mulch practice. Thus, spraying of $ZnSO_4$ @ 0.5 % at 40 DAS in plastic mulch may be recommended in groundnut cultivation.

Keywords: Economics, Foliar nutrition, Groundnut, Plastic mulch, Paddy straw mulch, Yield

The widespread preference for peanuts, both as a source of nutrition and as cattle fodder, has led to a significant expansion in peanut cultivation in India. India as the global leader in groundnut cultivation. Groundnuts are recognized for their high oil and protein content, making them the second most prominent oilseed crop worldwide after soybeans. In the context of global agriculture, groundnuts are grown over 30.63 m ha, yielding approximately 51.85 mt annually, with a productivity rate of 1690 kg/ha. In the Indian subcontinent, groundnut cultivation spans 5.70 m ha, producing 10.13 m t, with a productivity of 1777 kg//ha. (Anonymous, 2022). Among the states, Karnataka stands out as a major groundnut producer, followed by Gujarat, Rajasthan, Madhya Pradesh and Tamil Nadu. However, Karnataka's productivity is comparatively low at 846 kg/ha, highlighting the need to address various factors contributing to this underwhelming performance, with poor soil nutrition being a significant concern.

Groundnut cultivation involves unique characteristics, given that the pods grow underground, necessitating loose and well-aerated soil for peg penetration and pod development. One method to enhance crop yield is the raised bed technique, particularly suitable for regions with poor drainage or limited rainfall. Additionally, mulching is a practice of covering the soil surface with organic material, significantly impacts soil temperature, moisture retention and weed suppression. Moreover, optimizing mineral nutrition is vital for high groundnut production due to the crop's high nutrient requirements and addressing deficiencies is key to achieving substantial yields. Micronutrient deficiencies, particularly zinc (Zn) and iron (Fe), have become a pressing concern, partly due to the introduction of high-yield varieties, increased cropping intensity, and the limited use of organic fertilizers. Foliar nutrition, a method of directly applying nutrients to the plant, is crucial in situations where immediate nutrient correction is needed, circumventing the nutrient's movement through the soil.

In light of these considerations, an investigation was undertaken to explore the impact of mulching practices and foliar nutrition on groundnut cultivation under the raised bed method. This research seeks to provide valuable insights into optimizing groundnut production, a critical endeavour for ensuring food security and sustainable agriculture in India.

MATERIALS AND METHODS

A field experiment was carried out at Agricultural Research Station, Kawadimatti, University of Agricultural Sciences, Raichur, during *kharif* 2022 in sandy clay loam soil. Soil of the experimental site was slightly alkaline in reaction (pH 8.56), low in organic carbon (0.48%), available N (271 kg/ha), K (260 kg/ha), high in available P (46 kg/ha) and available Zinc (1.12 mg/kg). There were three main plots (mulching practices) *viz*., M1 - No mulch, M2 - Paddy straw mulch and M3 - Plastic mulch and four sub

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plots (foliar nutrition) *viz.,* S1 - Water Spray, S2 - Fe (0.5 % foliar spray at 40 DAS), S3 - Zn (0.5 % foliar spray at 40 DAS) and S4 - K (0.5 % foliar spray at 40 DAS) comprising totally twelve treatment combinations.

The experimental plot was ploughed and harrowed to bring the soil to fine tilth. Uniform 1.0 m width raised beds were prepared. Recommended dose of FYM was applied on beds @ 3.0 t/ha to all the plots and incorporated well into soil two weeks prior to sowing. A uniform dose of RDF (25:50:25:25:25:500 N : P_2O_5 : K_2O : $ZnSO_4$: $FeSO_4$: $CaSO_4$. $2H_2O$ kg/ha) was applied prior to sowing. Variety Phule Warna (KDG 128) was sown on 10th August 2022 with a seed rate of 35-40 kg/ha and harvested on 7th December 2022. The soil was covered with paddy straw (@3.0 t/ha) and plastic mulch as per treatment. Foliar spray of $ZnSO_4$, $FeSO_4$ and KCl was given at 40 DAS as per the treatments respectively. The mean annual rainfall received was 933 mm in 50 rainy days. Rainfall during crop growth period was 518 mm from August to November, 2022. The average minimum and maximum air temperature during crop growth period (August, 2022 to November, 2022) ranged from 20 to 31°C and 19 to 30°C, respectively. Observations on yield and yield parameters were recorded as per standard procedures. The cost of cultivation was computed taking into consideration the cost of various operations and inputs used for raising the crop. The gross returns computed using the prevailing market price for the produce. The net returns were computed by deducting the cost of cultivation from the gross returns. The benefit cost ratio was worked out by dividing the gross returns by cost of cultivation. The density and dry weight of the weed flora was recorded by placing 50 cm x 50 cm quadrate thrice per plot for evaluating the relative efficacy of the different mulches and the data were presented on number and g per square meter basis. The experimental data recorded were analysed statistically as per Analysis of Variance (ANOVA) technique.

RESULTS AND DISCUSSION

Mulching significantly increased the yield parameters of groundnut compared to no mulch. Among the different mulches, plastic mulch (M3) recorded significantly higher number of pods/plant (34.59), kernel yield/plant (29.29 g), hundred seed weight (42.41 g), pod yield (2320 kg/ha), haulm yield (4164 kg/ha) and harvest index (0.36), but it was on par with paddy straw mulch (M2). This might be due to soil covered with plastic mulch keeps more water, which helps plants use water better and also affects how nutrients become available to plants. This plastic covering also makes plants work better like how they sweat (transpiration), and how they manage things inside (osmotic potential and proline content). Similar findings were reported by Pradhan *et al.* (2018).

Foliar application of $ZnSO_4$ @ 0.5 % at 40 DAS (S3) recorded significantly higher number of pods/plant (32.52), kernel yield/plant (27.26 g), hundred seed weight (42.81 g), pod yield (2252 kg/ha) and haulm yield (4124 kg/ha). Harvest index did not differ significantly among foliar nutrition. This might be attributed to the reason that foliar application of micronutrients in balanced proportion during the crop growth period enhanced the number of nodules per plant, cell division, cell elongation process and photosynthetic activity leading to production and accumulation of more carbohydrates and auxins which favours the retention of more flowers ultimately leading to higher number of reproductive parts per plant, pod setting and pod weight. The similar results were recorded by Sabra *et al.* (2019) and Nayak *et al.* (2023)

Among interaction, plastic mulch along with foliar application of $ZnSO_4$ @ 0.5 % at 40 DAS (M3S3) recorded significantly higher number of pods/plant (37.25), kernel yield/plant (33.01 g), hundred seed weight (45.23 g), pod yield (2471 kg/ha), haulm yield (4354 kg/ha) and harvest index (0.36), but was found on par with treatments receiving mulching and other foliar sprays. Plastic mulch had a significant influence on enhancing the physiological parameters *viz.,* transpiration, osmotic potential, proline content, chlorophyll content, total dry matter production/plant and yield/plant and finally resulting in increased pod and haulm yield. Foliar application increased pod yield and haulm yield due to higher total dry matter accumulation as well as Zn, Fe uptake and their translocation to the reproductive parts resulted in improvement of yield attributes like number of pods/plant, kernel yield/plant and hundred seed weight. These results were in conformity with the findings of Thakur *et al.* (2010) and Nakum *et al.* (2019). Similar results in Soybean yield parameters were enhanced with Zinc sulphate application along with biofertilizers as indicated by Achari *et al.* (2023)

Among the different mulches, plastic mulch (M3) recorded significantly higher gross returns (₹1,44,057/ha), net returns (₹ 78,879/ha) and B-C ratio (2.21) which was on par with paddy straw mulch (M2). Foliar application of $ZnSO_4$ @ 0.5 % at 40 DAS (S3) recorded significantly higher gross returns (₹1,40,005/ha) and net returns (₹75,675/ha), but B-C ratio did not differ significantly among foliar nutrition. Among interaction, plastic mulch along with foliar application of $ZnSO_4$ @ 0.5 % at 40 DAS (M3S3) recorded significantly higher gross returns (₹1,53,281/ha), net returns (₹ 86,368/ha) and B-C ratio (2.29). An increase in pod yield and haulm yield of groundnut, increased the gross and net returns which was a result of increased growth and yield attributes by the creation of a favourable microclimate

and suppression of weeds. Similar results were also reported by Ravisankar *et al.* (2014) and Jain *et al.* (2018). Foliar nutrition recorded higher gross and net returns even with higher cost of cultivation which was because of higher productivity. Meresa *et al.* (2020) and Patil *et al.* (2020) reported similar findings and confirmed that net returns can be increased by foliar nutrition.

The data on weed density and dry weight of only main plots were presented and subplots data were non-significant (Table 4). Plastic mulch recorded lower weed density and weed dry weight at 30 and 60 DAS. Higher weed density (4.63 and 7.087 m^{-2}) and dry weight of weeds (3.83 and 4.41 gm^{-2}) were observed in no mulch treatment compared to paddy straw mulch treatment. Weed flora associated with groundnut comprises diverse species, which reduce the yield upto 81% groundnut pod yield (Jat *et al.,* 2011).

Application of plastic mulch on raised bed and $ZnSO_4$ spray treatment registered significantly higher yield and yield parameters but the combination of paddy straw mulch and $ZnSO_4$ spray were found statistically comparable. Economic analysis also revealed that, higher net returns and benefit cost ratio were obtained with the treatment of plastic mulch on raised bed and $ZnSO_4$ spray.

Table 1 Yield and Yield attributes of groundnut as influenced by mulching practices and foliar nutrition under raised bed method of cultivation

Treatment	Number of pods per plant	Kernel yield per plant (g)	Hundred seed weight (g)	Pod yield (kg/ha)	Haulm yield (kg/ha)	Harvest index
Main plot: Mulching practices (M)						
M1	19.71b	17.51c	38.64b	1829b	3636b	0.33b
M2	31.33a	24.77b	41.17ab	2199a	3979a	0.36a
M3	34.59a	29.29a	42.41a	2320a	4164a	0.36a
S.Em±	0.43	0.38	0.32	62	32	0.004
CD (5%)	1.30	1.12	0.95	185	95	0.012
Sub plot: Foliar nutrition (S)						
S1	24.63c	19.03c	39.69b	2007b	3784c	0.34a
S2	29.65ab	25.68ab	40.46b	2116ab	3953b	0.35a
S3	32.52a	27.26a	42.81a	2252a	4124a	0.35a
S4	27.38bc	23.45b	40.01b	2088b	3844bc	0.35a
S.Em±	0.89	0.61	0.31	46	29	0.005
CD (5%)	2.64	1.85	0.92	137	88	0.014
Interactions (M×S)						
M1 S1	14.06f	13.17f	37.85f	1719e	3530g	0.33b
M1 S2	22.96e	20.58de	38.94d-f	1859de	3679fg	0.34ab
M1 S3	23.97e	18.53e	39.26d-f	1891de	3770ef	0.33ab
M1 S4	17.85f	17.78e	38.52ef	1846de	3565g	0.34ab
M2 S1	26.85de	19.94de	39.96c-e	2038cd	3805ef	0.35ab
M2 S2	31.96bc	25.94c	40.67b-d	2215a-c	3963cd	0.36a
M2 S3	35.03ab	30.25ab	43.94a	2394ab	4248a	0.36a
M2 S4	29.26cd	22.95cd	40.12b-e	2150bc	3899de	0.35ab
M3 S1	32.99a-c	23.99c	41.27bc	2266a-c	4018cd	0.36a
M3 S2	34.02a-c	30.53ab	41.75b	2275a-c	4216ab	0.35ab
M3 S3	37.25a	33.01a	45.23a	2471a	4354a	0.36a
M3 S4	36.35ab	29.63b	41.38bc	2269a-c	4067bc	0.36a
S.Em±	1.54	1.05	0.53	79	50	0.01
CD (5%)	4.61	3.14	1.57	236	149	0.03

Note: Means with same alphabets do not differ significantly as per DMRT

Table 2 Economics of groundnut as influenced by mulching practices and foliar nutrition under raised bed method of cultivation

Treatment	Cost of cultivation (₹/ha)	Gross returns (₹/ha)	Net returns (₹/ha)	B:C
Main plot: Mulching practices (M)				
M1	59179	114247b	55068b	1.93b
M2	63429	136604ab	73175a	2.15a
M3	65179	144057a	78879a	2.21a
S.Em±	-	3705	3705	0.05
CD (5%)	-	11114	11114	0.14
Sub plot: Foliar nutrition (S)				
S1	60830	125006b	64177b	2.05a
S2	64330	131690ab	67360ab	2.04a
S3	64330	140005a	75675a	2.17a
S4	60892	129843ab	68951b	2.13a
S.Em±	-	2693	2693	0.04
CD (5%)	-	8075	8075	0.12
Interactions (M×S)				
M1 S1	57413	107629e	50216d	1.87d
M1 S2	60913	116086de	55173d	1.91cd
M1 S3	60913	118170de	57257cd	1.94cd
M1 S4	57476	115104de	57628cd	2.0b-d
M2 S1	61663	126813cd	65150b-d	2.06a-d
M2 S2	65163	137484bc	72321a-c	2.11a-d
M2 S3	65163	148564ab	83401a	2.28a
M2 S4	61726	133554bc	71828a-c	2.16a-c
M3 S1	63413	140577a-c	77164ab	2.22ab
M3 S2	66913	141500a-c	74587ab	2.11a-d
M3 S3	66913	153281a	86368a	2.29a
M3 S4	63476	140870a-c	77395ab	2.22ab
S.Em±	-	4664	4664	0.08
CD (5%)	-	13988	13988	0.23

Table 3 Weed density and dry weight as influenced by mulching practices in groundnut under raised bed method of cultivation

Treatment	Weed density per m^2 at 30 DAS	Weed density per m^2 at 60 DAS	Weed Dry weight per m^2 at 30 DAS	Weed dry weight per m^2 at 60 DAS
Main plot: Mulching practices (M)				
M1- No mulch	4.63(20.4)a	7.08(49.2)a	3.83(13.7)a	4.41(18.4)a
M2 – Paddy straw mulch	3.42(10.8)a	5.29 (27.1)b	2.66(6.1)b	2.94(7.7)b
M3 – Plastic mulch	1.00(0.0)b	1.00(0.0)c	1.00(0.0)c	1.00(0.0)c
S.Em±	0.18	0.03	0.04	0.02
CD (5%)	0.54	0.09	0.12	0.06

Note: Values in parenthesis are original

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Status of insect pests of safflower and their natural enemies through survey in Bidar district of Karnataka, India

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ABSTRACT

In the present paper, the pest status of aphids and lepidopteran pests and their natural enemies on safflower in Bidar district of Karnataka were recorded through survey. During the survey, one aphid (*Uroleucon compositae* (Theobald)) and five lepidopteran pests *viz.,* safflower leaf eating caterpillar (*Condica capensis* (Guénee)), tobacco cutworm (*Spodoptera litura* (Fabricius)), beet armyworm (*Spodoptera exigua* (Hübner)), capsule borer (*Helicoverpa armigera* (Hübner)) and bordered straw moth/capsule borer (*Heliothis peltigera* (Denis and Schiffmüller)) were recorded. Among the lepidopteran pests, *C. capensis* and *S. litura* were predominant at vegetative stage. While *S. exigua* was also observed at vegetative stage, but the population was meagre. The capsule borers, *H. armigera* and *H. peltigera* were predominant at reproductive stage. Further, 12 natural enemies were recorded on aphid and lepidopteran pests. Of which, four genera of spiders (*Araneus* sp., *Runcinia* sp., *Cheiracanthium* sp. and *Neoscona* sp.) were recorded for the first time on aphid and lepidopteran pests. Two species of hymenopteran (*Cotesia rificrus* (Haliday), *Rogas aligarhensis* Qadri) and a dipteran (*Megaselia scalaris* (Loew)) were recorded on lepidopteran pests. Besides, the natural occurrence of two fungal pathogens (*Beauveria bassiana* and *Metarhizium rileyi*) on *H. peltigera* was also recorded.

Keywords: Aphid, Fungal Pathogens, Lepidopteran Pest, Natural Enemies, Safflower

Safflower cultivation is under threat due to various insect pest attacks, which considerably reduces the yield (Singh *et al.,* 1999). The crop is known to be attacked by 101 insect pest species belonging to different orders (Patil and Halolli, 2005). Of these, hemipterans particularly aphids and lepidopterans like defoliators and capsule borers are causing huge economic loss (Singh *et al.,* 1996). In India, six aphid species *viz., Uroleucon carthami* (Hille Ris Lambers), *Uroleucon orientalis* Kulkarni, *Uroleucon jaceae* (Linnaeus), *Macrosiphum compositae* (Theobald), *Macrosiphum* spp. and *U. compositae* are reported on safflower (Esfahani *et al.,* 2012). Among these, *U. compositae* and *U. carthami* causes severe crop loss throughout the country (Hanumantharaya *et al.,* 2007b). Among these two species, *U. compositae* is the most destructive and causes 35 to 72 per cent yield loss during heavy infestation (Hanumantharaya *et al.,* 2007a).

Five lepidopteran pest species *viz., C. capensis, H. armigera, H. peltigera, C. conducta* and *S. exigua* reported as pests on safflower from India (Rajsekhar and Rai, 1989; Mallapur *et al.,* 1997; Balikai, 2000; Hanumantharaya *et al.,* 2007a, 2009; Akashe *et al.,* 2013). Among the lepidopteran pest species, *C. capensis* and *H. armigera* commonly known as safflower leaf eating caterpillar and capsule borer, respectively, are considered as economically important pests of safflower and cause huge crop loss. The *C. capensis* alone causes 62.20 to 100 per cent yield loss due to extensive foliage feeding by large number of larvae (Rajsekhar and Rai, 1989). While capsule borer causes up to 50 per cent yield loss by directly inflicting damage to flower buds, ovaries and developing seeds (Lewin *et al.,* 1973). The natural enemies of safflower pests are also recorded across India by Akashe *et al.* (2013), Hanumantharaya *et al.* (2007a), Mallapur *et al.* (1997), Rajsekhar and Rai (1989) and Pawar *et al.* (1985). Totally, 16 natural enemies belonging to Hymenoptera, Coleoptera and Diptera are reported from India.

In Karnataka, a few researchers like Mallapur *et al.* (1997) reported *C. conducta* on safflower through surveys in six major safflower growing districts of Northern dry-tract of Karnataka. In another study, Balikai (2000) recorded *H. armigera* and *P. capensis* on safflower from two northern districts of Karnataka through roving survey. Hanumantharaya *et al.* (2009) also reported capsule borer, *H. armigera* and leaf eating caterpillar, *P. capensis* are the major pests of safflower in eight districts of northern parts of Karnataka through roving survey.

The natural enemies of safflower pests are also recorded in northern parts of Karnataka by Mallapur *et al.* (1997), who reported predators like coccinellids and chrysopids and a parasitoid, *Pseudendaphis* sp. on *U. compositae.* Balikai (2000) also reported predators like coccinellids (*Menochilus sexmaculatus* (Fabricius) and *Coccinella* spp.) and parasitoid like *Pseudendaphis* on aphid. Hanumantharaya *et al.* (2007a) reported only predators like *Chrysoperla*

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carnea (Stephens) and coccinellids. Despite these works, no attempt has been made to document the pests occurring on safflower in Bidar district of Karnataka. Bidar is the major safflower growing area in Karnataka, with an area of 10.01 thousand hectare and production of 8,806 metric tonnes (NABARD-PLP; Agriculture Department, 2020-21). Secondly, the climatic condition of Bidar is quite different from other parts of northern Karnataka. In this context, the present study was undertaken to record the status of aphids and lepidopteran pests on safflower and their natural enemies in Bidar district of Karnataka.

MATERIALS AND METHODS

Fixed plot survey methodology was adopted in major safflower growing talukas *viz.,* Bidar (17.91°N, 77.51°E), Bhalki (18.05°N, 77.21°E), Humnabad (17.76°N, 77.13°E), Aurad (18.24°N, 77.41°E) and Basavakalyan (17.87°N, 76.94°E) of Bidar district during *rabi* season 2020-21. Sampling was carried-out thrice during the cropping period at monthly intervals (First survey during December, 2020, second and third surveys during January, 2021 and February, 2021, respectively) in 25 fields distributed across five taluks. The pests and natural enemy population were recorded following standard procedures (Araujo *et al.,* 2019). The aphid population was recorded from 5 cm length of two apical twigs per plant on ten randomly selected plants. The capsule borers and leaf eating caterpillar population were recorded as number per plant on randomly selected ten plants. Similarly, on ten randomly selected plants, the number of predators per plant was recorded. The data on pest and natural enemy populations recorded across different locations were subjected to analysis of variance (ANOVA) to assess the significance of differences among means. The critical difference (CD) at 5% probability level was used to separate the means wherever the F-test was found significant. Standard error of mean (SEm±) was also calculated to indicate variability within the data.

To document the parasitoids of lepidopteran pests, the larvae were collected from the plants in the field and were kept for parasitoid emergence in the laboratory. The emerged parasitoids were preserved in 90 per cent alcohol for identification and documentation purposes. To know the per cent parasitization of safflower leaf eating caterpillar, *C. capensis* by *C. ruficrus* and *R. aligarhensis* hundred larvae were collected from the safflower field and were reared in the laboratory until pupal stage. During rearing, the number of larvae parasitized were recorded and per cent parasitization was calculated.

RESULTS AND DISCUSSION

During the survey, an aphid (*U. compositae*) and five lepidopteran pests *viz.,* safflower leaf eating caterpillar (*C. capensis*), tobacco cutworm (*S. litura*), beet armyworm (*S. exigua*), capsule borer (*H. armigera*) and bordered straw moth/capsule borer (*H. peltigera*) were recorded on safflower (Table 1, Figs. 1a-6b). In the current study, *H. peltigera* and *S. litura* were recorded for the first time on safflower from Karnataka and India, respectively.

Safflower aphid, U. compositae (Hemiptera: Aphididae)

The *U. compositae* was observed on safflower at vegetative stage and continued till harvest of the crop. The population range of *U. compositae* during the cropping period was 10.98 to 16.21 per 5 cm of two apical twigs of the plant (Table 2).

Based on the mean population level it clearly indicates that, the *U. compositae* population was more at Wagalagaon (Bhalki), followed by Soldapka (Basavakalyan) and Markhal (Bidar) as compared to other areas, but these variations were non-significant, because all location belongs to the same agroclimatic zone. The safflower aphid population was more during December in all the surveyed locations as compared to January and February (Table 2). It can be attributed to weather conditions. In general, the aphid population will be more during the cooler season of the year, particularly in the months of November and December which is quite congenial for the rapid multiplication of aphids.

Safflower leaf eating caterpillar, *C. capensis* (Lepidoptera: Noctuidae)

The *C. capensis* was noticed on safflower at early stage and continued till harvest. The population range of *C. capensis* during the cropping period was 0.90 to 1.39 larvae per plant (Table 2). Based on the mean population level it clearly indicates that the *C. capensis* population was higher in Wagalagaon (Bhalki), followed by Soldapka (Basavakalyan) and Markhal (Bidar) as compared to other areas (Table 2) but these variations were non-significant, because all location belongs to same agroclimatic zone. Further, the population was more in Dharwad district as compared to other districts in Northern Karnataka

Safflower leaf eating caterpillar population was more during December in all the surveyed locations as compared to January and February (Table 2). It can be attributed to stage of the crop. Usually, the *C. capensis* occurs on safflower crop at early vegetative stage so it gets sufficient foliage for its survival. At later stage, the crop may stop yielding foliage leading to scarcity of food for better survival.

Table 1. Aphids and lepidopteran pests on safflower in Bidar district of Karnataka

Pest (Common name)	Scientific name	Family	Order
Safflower aphid	*Uroleucon compositae* (Theobald)	Aphididae	Hemiptera
Safflower leaf eating caterpillar	*Condica capensis* (Guenee)	Noctuidae	Lepidoptera
Tobacco cutworm	*Spodoptera litura* (Fabricius)	Noctuidae	Lepidoptera
Beet armyworm	*Spodoptera exigua* (Hubner)	Noctuidae	Lepidoptera
Safflower capsule borer	*Helicoverpa armigera* (Hubner)	Noctuidae	Lepidoptera
Bordered straw moth / capsule borer	*Heliothis peltigera* (Schiffmuller)	Noctuidae	Lepidoptera

Table 2. Aphids and lepidopteran pests population on safflower in Bidar district of Karnataka

Taluka	Villages	Aphid, *U. compositae* (No. of aphids on 5 cm two apical twigs/plant)			Mean	Safflower leaf eating caterpillar, *C. capensis* (No. of larvae/plant)			Mean	Tobacco cutworm, *S. litura* (No. of larvae/plant)			Mean	Beet armyworm, *S. exigua* (No. of larvae/plant)			Mean	Capsule borers, *H. armigera* & *H. peltigera* (No. of larvae/plant)			
		Dec	Jan	Feb		Dec	Jan	Feb		Dec	Jan	Feb		Dec	Jan	Feb		Dec	Jan	Feb	Mean
Bidar	Chitta	20.50	19.10	2.55	14.05 ± 9.98	2.25	1.30	0.00	1.18 ± 1.13	2.25	1.10	0.00	1.12 ± 1.13	0.25	0.00	0.00	0.08 ± 0.14	0.20	1.85	0.00	0.68 ± 1.02
	Markhal	19.40	24.23	1.20	14.94 ± 12.15	2.30	1.37	0.30	1.32 ± 1.00	1.80	1.00	0.00	0.93 ± 0.82	0.18	0.00	0.00	0.06 ± 0.10	0.40	2.13	0.00	0.84 ± 1.02
Humnabad	Mangalgi	23.50	14.65	1.40	13.18 ± 11.01	1.50	1.30	0.00	0.93 ± 0.81	1.05	0.85	0.00	0.63 ± 0.90	0.22	0.00	0.00	0.07 ± 0.13	0.35	2.15	0.00	0.83 ± 1.13
	Nimbura	28.43	14.30	0.83	14.52 ± 13.80	1.83	1.53	0.00	1.12 ± 0.98	1.93	1.03	0.40	1.12 ± 0.53	0.10	0.07	0.00	0.06 ± 0.05	0.43	2.30	0.00	0.91 ± 1.22
Bhalki	Walsang	18.50	11.20	3.25	10.98 ± 7.46	2.15	1.15	0.00	1.10 ± 1.08	2.05	0.50	0.15	0.90 ± 0.47	0.40	0.25	0.00	0.22 ± 0.20	0.55	2.05	0.00	0.87 ± 1.06
	Wagalagaon	20.37	23.10	5.17	16.21 ± 9.66	2.23	1.60	0.33	1.39 ± 0.97	1.57	1.17	0.00	0.91 ± 0.65	0.15	0.00	0.00	0.05 ± 0.09	0.63	1.97	0.00	0.87 ± 1.00
Aurad	Koudgaon	19.80	16.65	1.70	12.72 ± 9.67	1.75	1.40	0.00	1.05 ± 0.93	1.40	0.60	0.30	0.77 ± 0.82	0.13	0.00	0.00	0.04 ± 0.08	0.40	2.55	0.00	0.98 ± 1.37
	Shambelli	20.30	18.70	1.43	13.48 ± 10.46	1.63	1.07	0.00	0.90 ± 0.83	1.02	1.20	0.00	0.74 ± 0.98	0.26	0.09	0.00	0.12 ± 0.13	0.67	1.90	0.00	0.86 ± 0.96
Basavakalyan	Soldapka	26.55	18.30	1.90	15.58 ± 12.55	2.65	1.35	0.00	1.33 ± 1.33	1.95	0.80	0.10	0.95 ± 1.03	0.23	0.13	0.00	0.13 ± 0.14	0.15	1.75	0.00	0.63 ± 0.97
	Hulsoor	26.53	14.97	1.43	14.31 ± 12.56	1.83	1.40	0.00	1.08 ± 0.96	1.33	0.93	0.00	0.75 ± 0.63	0.34	0.00	0.00	0.11 ± 0.20	0.50	2.03	0.00	0.84 ± 1.06
	CD				NS				NS				NS				NS				NS
	SEm±				6.40				0.58				0.47				0.08				0.64

Note: Each value is an average of 10 plants; Dec - December; Jan - January; Feb - February; S.Em+ - Standard Error Mean; CD - Critical Difference; NS - Non-significant

Tobacco cutworm, *S. litura* (Lepidoptera: Noctuidae)

During the survey, the *S. litura* was found feeding on safflower at early stage and continued till harvest, but the population was meager. The population range of S. litura during the cropping period was 0.63 to 1.12 larvae per plant. Based on the mean population level it clearly indicates that variations in the S. litura population were non-significant, because all location belongs to same agroclimatic zone (Table 2). The tobacco cutworm population was more during December as compared to January and February (Table 2). It can be attributed to the stage of the crop. Usually, the S. litura occurs on safflower crop at early vegetative stage so it gets adequate foliage for its survival. At later stage, the crop may stop yielding foliage leading to a dearth of food for its survival.

Beet armyworm, *S. exigua* (Lepidoptera: Noctuidae)

Like *S. litura,* the *S. exigua* was also found feeding on safflower. It was noticed in the early stage and continued up to the flowering stage, but the population was negligible. The mean *S. exigua* population during the cropping period was 0.04 to 0.22 larvae per plant (Table 2). Based on the mean population level it clearly indicates that the *S. exigua* population was higher in Walsang (Bhalki), followed by Soldapka (Basavakalyan) and Shambelli (Aurad) as compared to other areas, but these variations were non-significant, because all location belongs to same agroclimatic zone.

The *S. exigua* population was higher during December compared to January. While, in February the population was zero in all the surveyed locations (Table 2). Usually, the S. litura occurs on safflower crop at early vegetative stage so it gets adequate foliage for its survival. At later stage, the crop may stop yielding foliage leading to a dearth of food for its survival.

Safflower capsule borers, *H. armigera* and *H. peltigera* (Lepidoptera: Noctuidae)

In the present study, two lepidopteran species, *H. armigera* and *H. peltigera* were found feeding on safflower. These two species attacks both vegetative and reproductive stages of the crop. Initially, larvae feed on the leaves. Later, they attack the reproductive parts of the plant. While feeding on capsule, larvae keep half of the body inside and remaining half outside (Fig. 4a). The population range of capsule borers was 0.63 to 0.98 larvae per plant. Their population was higher at Koudgaon (Aurad), followed by Nimbura (Humnabad) and Walsang (Bhalki) as compared to other areas (Table 2), but these variations were non-significant, because all location belongs to same agroclimatic zone.

In the present study, two species of lepidopteran capsule borers namely: *H. armigera* and *H. peltigera* were recorded. The capsule borers population were more during January in all the surveyed locations as compared to December. While in February, population was zero in all the locations (Table 2). From the above results, it is clearly indicated that among lepidopteran pests, *C. capensis* is observed as predominant pest species on safflower, followed by capsule borers *viz., H. armigera* and *H. peltigera.*

The present findings are corroborated with findings of Selim (1977), Mallapur *et al.* (1997), Pawar *et al.* (1985), Balikai (2000), Hanumantharaya *et al.* (2007a and 2009) and Akashe *et al.* (2013) who reported an aphid, *U. compositae* and lepidopteran pests *viz., C. capensis, H. armigera* and *H. peltigera* on safflower in various regions of India.

Natural enemies on safflower pests

During the survey, 12 natural enemies were recorded on safflower pests. Of these, nine were predators and three were parasitoids (Table 3 and Figs. 7-22).

Predators

Among nine predators recorded, four species were coccinellids (*Cheilomenes sexmaculata* (Fabricius), *Coccinella transversalis* (Fabricius), *Harmonia octomaculata* (Fabricius) and *Hippodamia variegata* (Goeze)), a species of green lace wing (*Chrysoperla zastrowi* (Esben-Petersen)) and four unidentified species of spiders (*Araneus* sp., *Runcinia* sp. *Cheiracanthium* sp. and *Neoscona* sp.) (Figs. 7-15). Among predators, coccinellids were predominant.

Coccinellids (Coleoptera: Coccinellidae)

Grubs and adults of coccinellids were found feeding on U. *compositae.* The population range of coccinellids was 0.22 to 0.88 per plant (Table 4). Based on the mean population level it clearly indicates that, the coccinellids population was higher in Markhal (Bidar), followed by Shambelli (Aurad) and Chitta (Bidar) as compared to other areas (Table 4). During the survey, four species of coccinellids were recorded *viz., C. sexmaculata, C. transversalis, H. octomaculata* and *H. variegata.* Their population was more during December as compared to January and February (Table 4). It can be attributed to the density of the pest population. The density of the pest population was more during the first survey as compared to remaining two surveys. Hence, it can be concluded that the higher density of pest population harbors the higher coccinellids population.

Green lace wing, *C. zastrowi* (Neuroptera: Chrysopidae)

Like coccinellids, the grubs of *C. zastrowi* were also found feeding on *U. compositae.* The population range of *C. zastrowi* in three surveys was 0.42 to 0.77 per plant. Based on the mean population level it clearly indicates that the *C. zastrowi* population was higher in Chitta (Bidar), followed by Soladapka (Basavakalyan) and Walsang (Bhalki) as compared to other areas (Table 4). The *C. zastrowi* population was higher during December as compared to January and February (Table 4). It can be attributed to the density of the pest population. The density of the pest population was more during the first survey as compared to remaining two surveys. Hence, it can be concluded that the higher density of pest population harbors the higher *C. zastrowi* population.

Spiders (Araneae)

The spiders are general predators which kill the prey by piercing the venom through chelicerae in the body of prey. In the present study, four unidentified species of spiders such as *Araneus* sp. (Araneidae), *Runcinia* sp. (Thomisidae), *Cheiracanthium* sp. (Cheiracanthiidae) and *Neoscona* sp. (Araneidae) were found feeding on *U. compositae, C. capensis, H. armigera* and *H. peltigera.* The population range of spiders was 0.52 to 0.72 per plant (Table 4).

Based on the mean population level it clearly indicates that the spider population was more in Chitta (Bidar), followed by Mangalgi (Humnabad) and Koudgaon (Aurad) as compared to other areas (Table 4). It can be attributed to the density of the pest population. The density of the pest population was higher during the first survey as compared to remaining two surveys. Hence, the high density of pest population harbors the higher spider's population. Spider population was more during December as compared to January and February (Table 4). In the current study, four genera of spiders (*Araneus, Runcinia, Cheiracanthium* and Neoscona) were recorded for the first time on safflower pests.

The current findings are in line with the findings of Balikai (2000), Hanumantharaya *et al.* (2009) and Akashe *et al.* (2013) who recorded the coccinellids and a green lace wing were found feeding on aphids in Dharwad and Solapur regions of Karnataka and Maharashtra, respectively. In another study, Esfahani *et al.* (2012) who also recorded the lady bird beetles and a green lacewing preying on safflower pests in Mosul regions of northern Iran.

Parasitoids

Among four parasitoids recorded on lepidopteran pests in the present study, two were hymenopteran (*C. ruficrus, Rogas* and *aligarhensis*) and one was a dipteran parasitoid, *M. scalaris* (Table 3 and Figs. 16-22). Among the parasitoids, hymenopterans were the major parasitoids on lepidopteran pests. The *C. ruficrus* (Braconidae) was major larval-endo parasitoid on *C. capensis* and *H. armigera.* While another Broconid, *R. aligarhensis* was found parasitizing the larval stages of the *C. capensis.* Further, *C. ruficrus* recorded 61 per cent of parasitization of *C. capensis,* followed by *R. aligarhensis* (21 per cent). In the previous studies, Paliwal and Jakhmola (1981) reported *A. ruficrus* and *R. percurrens* were the most potential parasitoids of *C. capensis.* Similarly, Rajsekhar and Rai (1989) reported *A. ruficrus* and *Rogas* sp. were predominant in parasitizing the *C. Conducta.* The present results align with Kamath and Hugar (2001), who also reported that *C. ruficrus* and *R. percurrens* were potential larval parasitoids of *C. capensis.* Likewise, Deshmukh *et al.* (2021) and Tang *et al.* (2021) reported *M. scalaris* found parasitizing *Spodoptera frugiperda* (J. E. Smith).

In the present study, a dipteran parasitoid, *M. scalaris* (Phoridae) was observed for the first time as a larval and pupal parasitoid of *S. litura.* During the present survey, no parasitoids were recorded on aphid. In the current study, *H. peltigera* was naturally infected by the two fungal pathogens like *B. bassiana* (Fig. 26) and *M. rileyi* (Fig. 27). To our knowledge, this is the first record of the natural occurrence of fungal pathogens, *B. bassiana* and *M. rileyi* on *H. peltigera* on safflower.

Table 3. Natural enemies of aphids and lepidopteran pests on safflower in Bidar district of Karnataka

Natural enemy	Scientific name	Family	Order	Predator/Parasitoids	Host's
Predators					
Coccinellids	*Cheilomenes sexmaculata* (F.)	Coccinellidae	Coleoptera	Insect predator	*U. compositae*
	Coccinella transversalis (F.)	Coccinellidae	Coleoptera	Insect predator	
	Harmonia octomaculata (F.)	Coccinellidae	Coleoptera	Insect predator	
	Hippodamia variegate (G.)	Coccinellidae	Coleoptera	Insect predator	
Green lacewing	*Chrysoperla zastrowii* (E.)	Chrysopidae	Neuroptera	Insect predator	
Spiders	*Araneus* sp.	Araneidae	Araneae	Non-insect predator	*U. compositae, C. capensis, H. armigera* and *H. peltigera*
	Cheiracanthium sp.	Cheiracanthiidae	Araneae	Non-insect predator	
	Neoscona sp.	Araneidae	Araneae	Non-insect predator	
	Runcinia sp.	Thomisidae	Araneae	Non-insect predator	
Parasitoids					
Braconid wasp	*Cotesia ruficrus* (H.)	Braconidae	Hymenoptera	Larval parasitoid	*C. capensis* and *H. armigera*
Braconid wasp	*Rogas aligarhensis* (Q.)	Braconidae	Hymenoptera	Larval parasitoid	*C. capensis*
Humpbacked fly	*Megaselia scalaris* (L.)	Phoridae	Diptera	Larval and pupal parasitoid	*S. litura*

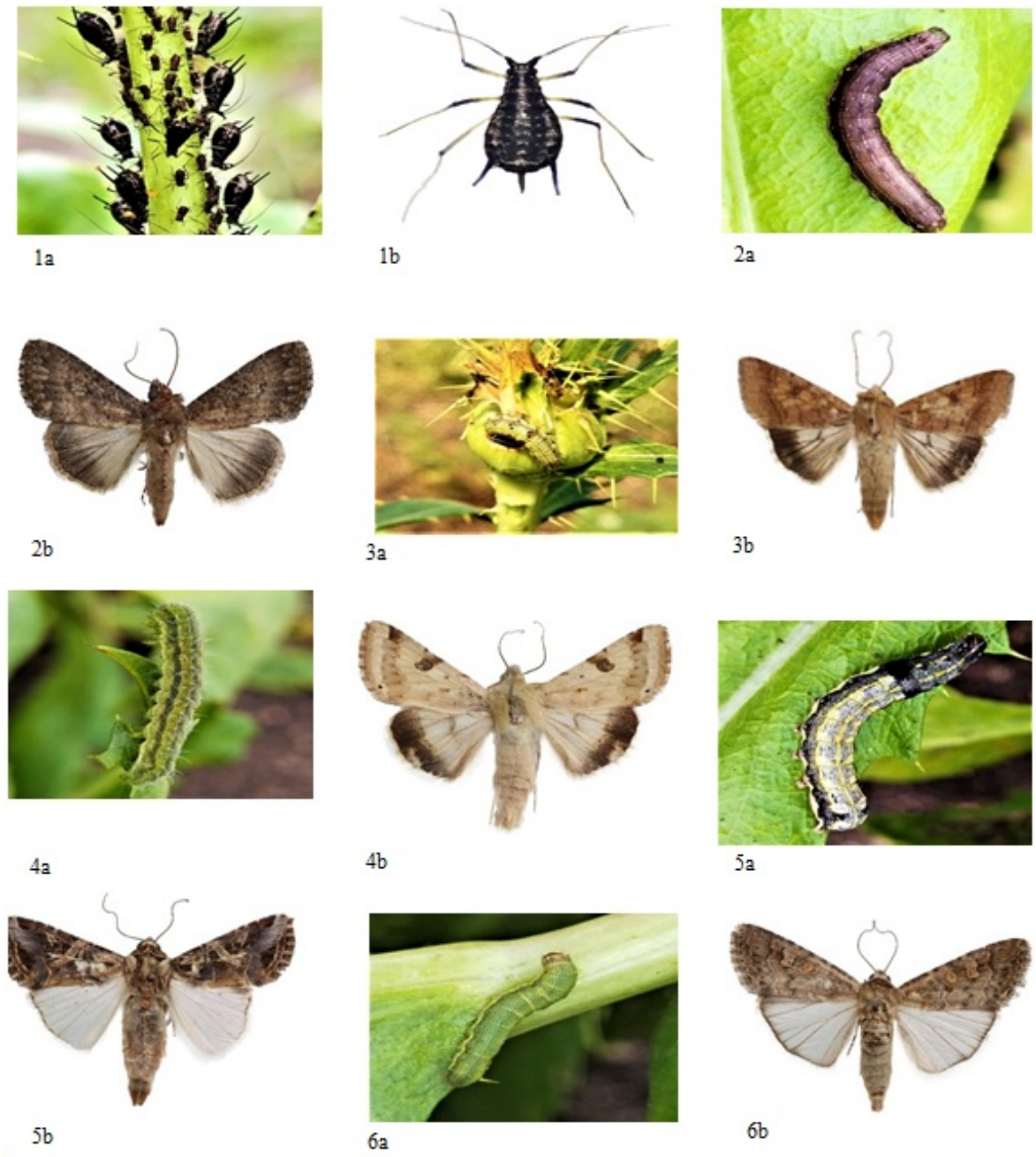

Fig. 1a-6b. Aphids and lepidopteran pests associated with safflower.
1 a & b. *U. compositae,* 1a. *Nymphs and* adults, 1b. Adult. 2 a & b. *C. capensis,*
2a. Larva, 2b. Adult. 3 a & b. *H. armigera,* 3a. Larva, 3b. Adult. 4 a & b. *H. peltigera,*
4a. Larva, 4b. Adult. 5 a & b. *S. litura,* 5a. Larva, 5b. Adult. 6 a & b. *S. exigua,* 6a. Larva, 6b. Adult.

Fig. 7-15. Insect and non-insect predators associated with safflower pests.
7. *C. sexmaculata.* 8. *C. transversalis.* 9. *H. octomaculata.* 10. *H. variegata.*
11. *C. zastrowi.* 12. *Araneus* sp. 13. *Cheiracanthium* sp. 14. *Neoscona* sp. 15. *Runcinia* sp.

Table 4. Natural enemies (predators) population on aphids and lepidopteran pests of safflower in Bidar district of Karnataka

Taluka	Villages	Coccinellids (No. of coccinellids/ plant)			Mean	Green lace wing (No. of green lace wing grubs/plant)			Mean	Spiders (No. of spiders/plant)			Mean
		Dec	Jan	Feb		Dec	Jan	Feb		Dec	Jan	Feb	
Bidar	Chitta	0.55	0.45	0	0.33 ± 0.29	1.55	0.75	0	0.77 ± 0.78	1.35	0.80	0	0.72 ± 0.68
	Markhal	1.67	0.97	0	0.88 ± 0.84	1.07	0.87	0	0.65 ± 0.57	1.27	0.63	0	0.63 ± 0.64
Humnabad	Mangalgi	0.35	0.30	0	0.22 ± 0.19	1.10	0.65	0	0.58 ± 0.55	1.25	0.85	0	0.70 ± 0.64
	Nimbura	0.57	0.27	0	0.28 ± 0.29	1.07	0.83	0	0.63 ± 0.56	1.07	0.50	0	0.52 ± 0.54
Bhalki	Walsang	0.85	0.05	0	0.30 ± 0.48	1.55	0.50	0	0.68 ± 0.79	1.00	0.65	0	0.55 ± 0.51
	Wagalagaon	0.60	0.30	0	0.30 ± 0.30	1.03	0.77	0	0.60 ± 0.54	1.40	0.53	0	0.64 ± 0.71
Aurad	Kaudgaon	0.45	0.45	0	0.30 ± 0.26	1.00	0.35	0	0.45 ± 0.51	1.30	0.75	0	0.68 ± 0.65
	Shambelli	0.63	0.50	0	0.38 ± 0.33	1.00	0.80	0	0.60 ± 0.53	1.03	0.87	0	0.63 ± 0.55
Basavakalyan	Soldapka	0.60	0.25	0	0.28 ± 0.30	1.70	0.40	0	0.70 ± 0.89	1.10	0.50	0	0.53 ± 0.55
	Hulsoor	0.53	0.40	0	0.31 ± 0.28	0.93	0.33	0	0.42±0.47	1.47	0.73	0	0.53 ± 0.74

Note: Each value is an average of 10 plants; Dec - December; Jan - January; Feb - February

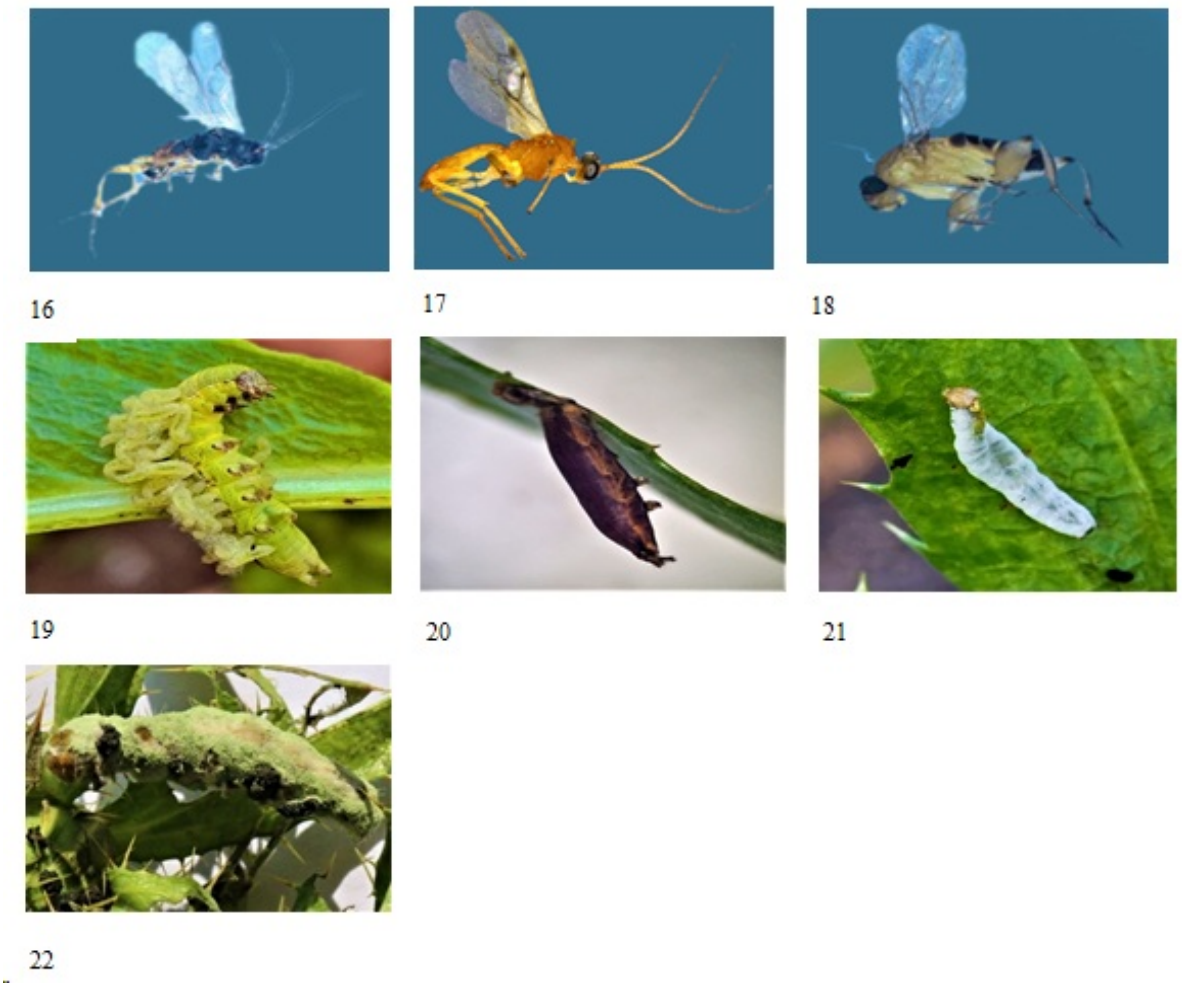

Fig. 16-22. Parasitoids associated with major lepidopteran pests of safflower.
16. *C. ruficrus.* 17. *R. aligarhenesis.* 18. *M. scalaris.* 19. *C. capensis* parasitized by *C. ruficrus.*
20. *C. capensis* parasitized by *R. aligarhenesis.* 21. *H. peltigera* infected by *B. bassiana.*
22. *H. peltigera* infected by *M. rileyi*

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Co-integration and causality analysis of castor markets in India

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ABSTRACT

This study analyzed long-run spatial integration of castor prices across five major markets in India i.e., Kurnool, Bhabhar, Ramanagara, Sumerpur, and Narayanpet-using econometric tools such as Johansen's multivariate cointegration approach, Augmented Dickey-Fuller (ADF) test, Granger causality test, and Vector Error Correction Model (VECM). The findings confirmed the presence of cointegration, indicating a six-year price relationship among these markets. The results of the Granger causality test to explore the direction and extent of price transmission between states revealed unidirectional causality between the following market pairs Kurnool-Narayanpet, Kurnool-Ramanagara, Narayanpet-Ramanagara, and Sumerpur-Ramanagara. Additionally, the analysis showed that the Ramanagara market significantly influenced prices in three other major markets-Kurnool, Sumerpur, and Narayanpet. These results highlight strong inter-market linkages, with Ramanagara playing a pivotal role in driving price movements across the castor markets.

Keywords: Cointegration, Castor, Granger Causality, Market Integration, VECM

The Indian castor market plays a crucial role in the global castor oil industry. Understanding the price dynamics and relationships among different castor markets within India is vital for policy makers, traders, and farmers. This study investigates the co-integration and causality among major castor markets in India to provide insights into price transmission mechanisms.

Castor is one of the oldest cultivated crops and it contributes to only 0.15% of the vegetable oil produced in the world. The oil produced from this crop is considered to be of importance to the global specialty chemical industry because it is the only commercial source of a hydroxylate fatty acid. Castor plant is grown in arid and semi-arid regions.

Area under castor reported during 2024-25 was 8.199 lakh ha (20.260 lakh acres) as against 9.500 lakh ha (23.475 lakh acres) during the same period in 2023-24. Among states, Gujarat is leading with 5.991 lakh ha (14.804 lakh acres) under castor followed by Rajasthan 1.700 lakh ha (4.201 lakh acres), Andhra Pradesh 0.356 lakh ha (0.880 lakh acres), Karnataka 0.039 lakh ha (0.096 lakh acres) and Odisha 0.039 lakh ha (0.096 lakh acres). According to Central Government 1st advance estimates, all India castor production in 2024-25 is at 15.53 lakh tonnes. In India, major castor producing states are Gujarat (12.72 lakh tonnes), Rajasthan (2.61 lakh tonnes), Andhra Pradesh (0.11 lakh tonnes), Jharkhand (0.02 lakh tonnes) and Karnataka (0.02 lakh tonnes).

An indirect means of analysing market efficiency is to test for market integration. Three types of market integration are identified: inter-temporal, vertical and spatial. Inter temporal market integration relates to the arbitrage process across periods. Vertical market integration is concerned with stages in marketing and processing channels. Spatial integration is concerned with the integration of spatially distinct markets i.e., if price changes in one market are fully reflected in alternative markets then these markets are said to be spatially integrated. The concept of market integration has normally been applied in studies involving spatial market inter-relatedness. Market integration is a central issue in many contemporary debates concerning the issues of market liberalization. Market integration is perceived as a precondition for effective market reform in developing countries. The high degree of market integration means the markets are quite competitive and provide little justification for extensive and costly government intervention designed to improve competitiveness and enhance market efficiency. Markets that are not integrated may convey inaccurate picture about price information that might distort production decisions and contribute to inefficiencies in markets, harm the ultimate consumer and lead to low production and sluggish growth.

Goletti and Babu (1994) studied the extent of market integration of maize markets in Malawi in order to understand how it had been affected by market liberalization. Several measures of integration were used to analyze both the co movement of prices and the price adjustment process over time using monthly retail prices of maize at eight main locations over the period between January 1984 to December 1991. The study concluded that liberalization increased market integration. Afolami (2001) investigated the degree of cowpea market integration in Uganda using such measures as bivariate correlation coefficients, co-integration and Granger-Causality.

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Campiche *et al.* (2007) studied the relation between crude oil prices and variation of agricultural commodities using a vector error correction model. Cointegration results showed that corn and soybean prices were cointegrated with crude oil price during 2006-2007. Awal *et al.* (2009) examined the pricing efficiency of exportable fresh vegetables markets in Bangladesh and its export markets by using Engle-Granger (EG) test, Cointegration Regression for Durbin Watson (CRDW) test and Error Correction Methods (ECM). Zhang *et al.* (2010) used VEC model and Granger test on the monthly data from 1989 to 2008 and reported that there was no long run and short-run causality between the fuel (oil, gasoline and ethanol) and agricultural commodity (corn, soybeans, wheat, sugar and rice) prices. Nazlioglu *et al.* (2013) investigated the relationship between the world oil prices and the agricultural commodity prices by using the monthly data from 1980 to 2010 and the panel co-integration and the Granger causality techniques. The results of their study showed that the change in oil prices and the weak dollar have a strong impact on many agricultural commodity prices. Esposti and Listorti (2013) investigating on national and international markets observed that trade policy regime had an important role in price transmission mechanisms and they put forward a trade policy intervention to mitigate the impact of price exuberance. The authors analyzed agricultural price transmission during price bubbles, in particular, considering Italian and international weekly spot (cash) price data over years 2006-2010. Kumari *et al.* (2019) examined cointegration of major redgram markets and price movement in major markets in Telangana using econometric tools like Augmented Dickey-Fuller (ADF), Johansen's cointegration test, Granger causality test and Vector Error Correction Model (VECM). Kumari et al. (2022) studied Co-integration and causality analysis of castor markets in Telangana state, the results showed that Devarakadra market influenced the prices in the other three major markets i.e., Badepalli, Gadwal and Narayanpet.

MATERIALS AND METHODS

For price integration, simple bivariate correlation coefficients measure price movements of a commodity in different markets. This is the simplest way to measure the spatial price relationships between two markets. However, this method clearly has some limitations, as it cannot measure the direction of price integration between two markets. The co-integration procedure measures the degree of price integration and takes into account the direction of price integration. This econometric technique provides more information than the correlation procedure does, as it allows for the identification of both the integration process and its direction between two markets.

Market Integration Test

Market integration is tested using the co-integration method, which requires that (i) Two variables, say P_{it} and P_{jt} are non-stationary in levels but stationary in first differences i.e. $P_{it} \sim I(1)$ and $P_{jt} \sim I(1)$.

There exists a linear combination between these two series, which is stationary i.e. ν_{it} (=P_{it} $\hat{\alpha}$ $\hat{\beta} P_{it}$) ~I (0).

So the first step is to test whether each of the univariate series is stationary. If they are both I (1) then we may go to the second step to test cointegration. The Engle and Granger (1987) procedure is the Common way to test cointegration.

Unit root test

The regression analysis of non-stationary time series produces spurious results, which can be misleading (Ghafoor, *et al.,* 2009). The most appropriate method to deal with non-stationary time series for estimating long-run equilibrium relationships is cointegration, which necessitates that time series should be integrated of the same order. Augmented Dickey-Fuller (ADF) and Phillips-Perron test (PP) are used to verify the order of integration for each individual series. The ADF test tests the null hypothesis of unit root for each individual time series. The rejection of the null hypothesis indicates that the series is non-stationary and vice-versa (Dickey and Fuller, 1981). The number of the appropriate lag for ADF is chosen for the absence of serial correlation using Akaike Information Criterion (AIC). The ADF test is based on the Ordinary Least Squares (OLS) method and requires estimating the following model.

$$\Delta lnP_t = \alpha_0 + \delta_1 t + \gamma lnP_{t-1} + \sum_{j=1}^{q} \vartheta_j \Delta lnP_{t-j} + \varepsilon_t$$

Where, P the price in each market, Δ is the difference parameters (i.e., $\Delta P1 = P_t - P_{t-1}$, $P_{t-1} = P_{t-1} - P_{t-2}$ and $P_{n-1} = P_{n-1} - P_{n-2}$) and so on, α_0 is the constant or drift, t is the time or trend variable, q is the number of lags length and ε_t is a pure white noise error term.

Johansen Cointegration

If two series are potentially co-integrated, at least one co-integration relationship exists. Co-integration may be affected by some factors, such as transportation cost, tariffs, and so on. The two tests, i.e., trace and max Eigen statistics of Johansen's approach based on the vector autoregressive

model (VAR) were put into the application to analyze the co-integrating vectors between the selected Castor markets.

The maximum likelihood (ML) method of cointegration is applied to check long-run wholesale prices relation between the selected markets of Telangana (Johansen, 1988; Johansen and Juselius, 1992). The starting point of the ML method is vector autoregressive model of order (k) and may be written as:

$$P_t = \sum_{i=1}^{k} A_t P_{t-1} + \mu + \beta_t + \varepsilon_t; \ (t=1, 2, 3 \ldots T)$$

Where, (n*1) denotes the vector of non-stationary or integrated at order one, i.e., I (1) prices series. The procedure for estimating the cointegration vectors is based on the Vector error correction model (VECM) representation given by:

$$\Delta P_t = \prod P_{t-1} + \sum_{i=1}^{k-1} \Gamma_i \, \Delta P_{t-i} + + \beta \mu_t + \varepsilon_t$$

Where,

Γ_i= -(I-Π_i-…..,T); i=1,2,….k-1

Π = – (I-Π, –……Π_k)

Both Γi and Π i are the n*n matrices of the coefficient conveying the short and long run information respectively, μ is a constant term, t is a trend, and ε_t is the n-dimensional vector of the residuals that is identical and independent distributed. The vector ΔPt is stationary P_t is integrated at order one I (1) which will make unbalance relation as long as Π matrix has a full rank of k. In this respect, the equation can be solved by inversing the matrix Π^{-1} for P_t and as a linear combination of stationary variable (Kirchgässner *et al.,* 2012). The stationary linear combination of the P_t determines by the rank of Π matrix. If the rank r of the matrix Π r=0 the matrix is the null and the series underlying is stationary. If the rank of the matrix Π is such that 0 < rank of (Π) = r < n then there are n × r cointegrating vectors. The central point of the Johansen's procedure is simply to decompose Π into two n × r matrices such that Π=αβ'. The decomposition of Π implies that the β'P_t are r stationary linear combination.

Johansen and Juselius, (1990) proposed two likelihood ratio test statistics (Trace and Max Eigen test statistics) to determine the number of cointegrating vectors as follows:

$$J_{trace} = -T \sum_{i=r+1}^{N} \ln (1 - \hat{\lambda}_1)$$

$$\lambda_{max} = -T ln (1 - \hat{\lambda}_{r+1})$$

Where, r is the number cointegrated vector, $\hat{\lambda}_t$ is the eigen value and $\hat{\lambda}_{r+1}$ is the $(r + 1)^{th}$ largest squared eigen value obtained from the matrix Π and the T is the effective number of observation. The trace statistics tested the null hypothesis of r cointegrating vector(s) against the alternative hypothesis of n cointegrating relations. The Max Eigen statistic tested the null hypothesis (r =0) against the alternative $(r + 1)$.

Vector Error Correction Model (VECM)

If price series are *I* (1), then one could run regressions in their first differences. However, by taking first differences, we lose the long-run relationship that is stored in the data. This implies that one needs to use variables in levels as well. Advantage of the vector error correction model (ECM) is that it incorporates variables both in their levels and first differences. By doing this, VECM captures the short-run disequilibrium situations as well as the long-run equilibrium adjustments between prices. Even if one demonstrates market integration through cointegration, there could be disequilibrium in the short-run i.e., price adjustment across markets may not happen instantaneously. It may take some time for the spatial price adjustments. VECM can incorporate such short-run and long-run changes in the price movements.

A VECM formulation, which describes both the short-run and long-run behaviors of prices, can be formulated as:

$$\Delta P_{it} = \gamma_1 + \gamma_2 \Delta P_{jt} - \pi \hat{v}_{it-1} + v_{it}, \qquad (4)$$

In this model, is the impact multiplier (the short-run effect) that measures the immediate impact that a change in P_{jt} will have on a change in P_{it}. On the other hand, π is the feedback effect or the adjustment effect that shows how much of the disequilibrium is being corrected, that is the extent to which any disequilibrium in the previous period effects any adjustment in the P_{it} period of course $\hat{v}_{t-1} = P_{it-1} - \hat{p}_1 - \hat{p}_2 P_{ji-1}$ and therefore from this equation we also have p_2 being the long-run response.

Granger Causality Test

If a pair of series is cointegrated then there must be Granger causality in at least one direction, which reflects the direction of influence between series (in our case prices). Theoretically, if the current or lagged terms of a time-series variable, say P_{jt}, determine another time-series variable, say P_{it}, then there exists a Granger causality relationship between P_{jt} and P_{it}, in which is Granger caused by P_{jt}. Bessler and Brandt (1982) first introduced this test into research on market integration to determine the leading market.

From the above analysis, the model is specified as follows:

$$\Delta P_{it} = \theta_{11}\Delta P_{it-1} + \ldots + \theta_{1n}\Delta P_{it-n} + \theta_{21}\Delta P_{jt-1} + \ldots \theta_{2n}\Delta P_{jt-n} - \gamma_1(P_{it-1} - \alpha P_{jt-1} - \delta) + \varepsilon_{1t}$$

$$\Delta P_{jt} = \theta_{31}\Delta P_{jt-1} + \ldots + \theta_{3n}\Delta P_{it-n} + \theta_{41}\Delta P_{it-1} + \ldots \theta_{4n}\Delta P_{it-n} - \gamma_2(P_{it-1} - \alpha P_{jt-1} - \delta) + \varepsilon_{2t}$$

The following two assumptions are tested using the above two models to determine the Granger causality relationship between prices.

$$\theta_{21} = \ldots = \theta_{2n} = \cdots = \gamma_1 = 0 \text{ (No causality from } P_{jt} \text{ to } P_{it})$$

$$\theta_{41} = \ldots = \theta_{4n} = \cdots = \gamma_2 = 0 \text{ (No causality from } P_{it} \text{ to } P_{jt})$$

E Views software was used for the analysis.

RESULTS AND DISCUSSION

The price data used for the analysis consist of monthly modal prices of Castor (₹/qtl) in five major markets *viz.,* Kurnool (Andhra Pradesh), Bhabhar (Gujarat), Ramanagara (Karnataka), Sumerpur (Rajasthan) and Narayanpet (Telangana) of the India over the period six years from January 2019 to December 2024. The data was taken from the official website https://agmarknet.gov.in/. The Castor modal price trend of all the selected markets is presented in Fig. 1, which shows the symmetric behavior in the movement of prices in all the selected markets. The maximum modal price of ₹7393/q was prevailed in Bhabhar while the minimum price was found in Ramanagara ₹3077/q followed by Kurnool ₹ 3088 /q.

Descriptive Statistics

Summary statistics result showed that the price of Castor remained most volatile in Ramanagara followed by Bhabhar as measured by coefficient of variation. Bhabhar is the biggest Castor market in India and the prices are dependent upon the demand of the other markets. The highest average prices of Castor were found in Bhabhar market, while lowest average prices were in Kurnool (Table 1).

Order of the Integration

In order to check the stationarity of price series of castor, the standard ADF and PP unit root tests, were applied to determine the order of integration. The unit root test regression implies that regressing the first difference of a series with its one period lag and several lags (as suggested by the various lag length criterion) of the first differenced series. The null hypothesis of ADF and PP tests is accepted or rejected based on the critical value and corresponding probability value. The results of the ADF and PP test values were below the critical value at 5% level of significance indicating the non existence of unit root test. This implied that the Castor price series are non stationary at level in all the major markets in India i.e., Kurnool, Bhabhar, Ramanagara, Sumerpur and Narayanpet. All the major markets i.e., Kurnool, Bhabhar, Ramanagara, Sumerpur and Narayanpet were stationary at first difference I (1).

Table 1 Summary Statistics of the monthly modal Prices for Castor in major markets for India from the period January 2019 to December 2024 (in ₹/q)

	Kurnool	Bhabhar	Ramanagara	Sumerpur	Narayanpet
Mean	5039	5568	5122	5323	5140
Median	5208	5684	5253	5490	5327
Maximum	6894	7393	7024	7053	6967
Minimum	3088	3482	3077	3466	3284
Std. Dev.	983	1095	1044	987	985
CV	19.50	19.67	20.39	18.54	19.16

Co-integration Analysis

Johansen's Co-integration test for selected Castor markets for the long-run co-integration was performed. The results of Johansen's maximum likelihood tests (maximum Eigen-value and trace test) are presented in Table 3. The first null hypothesis of maximum eigen-value and trace test, tests the no co-integration ($r = 0$) against the alternative hypothesis ($r \geq 1$) of at least one co-integrated equation prevailed in the VAR system. Both, the maximum Eigen-value and trace test reject the null hypothesis of no co-integration. The rejection/acceptance of the null hypothesis is decided by the trace max- Eigen test statistic values against their critical value and corresponding probability value which is less than test statistic in the first null hypothesis. Similarly, the null hypotheses from $r \leq 1$ to $r \leq 3$ and $r \leq 4$ for both the statistics were rejected against their alternative hypotheses from the $r \geq 1$ to $r \geq 4$ and $r=5$ as their critical values were less than the test statistics and the corresponding probability values were also less than 0.05. This implied that there were five co-integrating relationships in the joint co-integration analysis of all five Castor markets.

Granger causality test

After confirming the integration of price series, pair-wise Granger causality test was performed for five major Castor markets to comprehend causal relation between them. The results of the Granger causality analysis presented in Table 4 explicate no presence of bidirectional causality market pair. The unidirectional causality markets pairs were Kurnool - Narayanpet, Kurnool - Ramanagara, Narayanpet - Ramanagara and Sumerpur - Ramanagara. It means that a price change in the former market in each pair Granger cause the price formation in the latter market. The remaining markets did not show causality. It meant that the price change in the latter market did not feed back into the price in the former market.

Short run and long run behavior of market prices

Since the Johansen's multiple co-integration test results showed that the selected Castor markets were having long run equilibrium relationship and presence of co-integration between them, the Vector Error Correction model (VECM) among the selected markets of Castor was employed to know the speed of adjustments for the prices of Castor among selected markets, for short run and long run equilibrium of prices. The results of VECM are presented in Table 5.

The estimate of VECM revealed that one month lag price of Kurnool market was affecting current prices of Ramanagara and Narayanpet markets. Two months lag price of Kurnool market was affecting current prices of Narayanpet market. One month lag price of Bhabhar market was affecting current prices of itself. One month lag price of Ramanagara market was affecting current prices of itself and Kurnool market. Two months lag price of Ramanagara market was affecting current prices of itself and Kurnool market. One month lag price of Sumerpur market was affecting current prices of itself. One month lag price of Narayanpet market was affecting current prices of itself. Two months lag price of Narayanpet market was affecting current prices of itself.

This study examined the spatial market integration and price behavior of Castor markets in India through co-integration analysis, using monthly price data from January 2019 to December 2024. The ADF unit root test revealed that Castor price series were non-stationary at the level across all major markets but became stationary at the first difference (I (1)). Johansen's co-integration test confirmed that the price series were co-integrated, indicating long-term market relationships. Granger causality analysis found no bidirectional causality among market pairs. However, unidirectional causality was observed between the following pairs: Kurnool-Narayanpet, Kurnool-Ramanagara, Narayanpet-Ramanagara, and Sumerpur-Ramanagara. The Vector Error Correction Model (VECM) highlighted the influence of lagged prices: Kurnool's one-month lag affected current prices in Ramanagara and Narayanpet, while its two-month lag influenced Narayanpet. Bhabhar's one-month lag influenced its own prices. Ramanagara's one-month lag impacted its own prices and those of Kurnool, while its two-month lag affected both as well. Sumerpur and Narayanpet markets exhibited self-influence at both one and two-month lags. The findings suggest strong market integration with varying lagged price effects across regions useful for forecast policy analysis and strategic making.

Table 2 ADF and PP Tests for Unit Root in the modal prices of Castor

Augmented Dickey-Fuller test results at level				Phillips-Perron test results at level		
	t-Statistic	Prob.*	Remarks	t-Statistic	Prob.*	Remarks
Kurnool	-1.47	0.54	Non-stationary	-1.33	0.60	Non-stationary
Bhabhar	-1.16	0.68	Non-stationary	-1.22	0.65	Non-stationary
Ramanagara	-1.09	0.71	Non-stationary	-2.22	0.20	Non-stationary
Sumerpur	-1.49	0.52	Non-stationary	-1.45	0.54	Non-stationary
Narayanpet	-1.26	0.64	Non-stationary	-1.29	0.62	Non-stationary
Augmented Dickey-Fuller test results after differencing				Phillips-Perron test results after differencing		
ΔKurnool	-8.43*	0.00	Stationary	-8.50*	0.00	Stationary
ΔBhabhar	-7.93*	0.00	Stationary	-7.93*	0.00	Stationary
ΔRamanagara	-14.91*	0.00	Stationary	-9.45*	0.00	Stationary
ΔSumerpur	-8.95*	0.00	Stationary	-8.94*	0.00	Stationary
ΔNarayanpet	-8.08*	0.00	Stationary	-8.06*	0.00	Stationary

Notes: * denote significance at 1% levels of significance and ? denote the first difference of the time series

Table 3 Johansen's Co-Integration Test Results of five major Castor Market prices in India

Hypothesized No. of CE(s)	H0	H1	Eigen value	Trace Statistics results			Max-Eigen Statistics results		
				Trace Statistics	0.05 Critical Value	P-Value	Max-Eigen Statistic	0.05 Critical Value	P-Value
None *	r=0	r≥1	0.50	171.38	69.82	0.000	47.17	33.88	0.000
At most 1*	r≤1	r≥2	0.47	124.21	47.86	0.000	43.35	27.58	0.000
At most 2 *	r≤2	r≥3	0.42	80.86	29.80	0.000	36.84	21.13	0.000
At most 3 *	r≤3	r≥4	0.32	44.03	15.49	0.000	26.68	14.26	0.000
At most 4 *	r≤4	r=5	0.23	17.35	3.84	0.000	17.35	3.84	0.000

Notes: * denote the rejection of null hypothesis at 5% level of significance

Tables 4 Market pair wise results of the Granger Casualty test

Lagged Periods	Markets Pairs	F-Statistic	P-Value	Decision of null hypothesis	Remarks
1	Kurnool - Bhabhar	0.78	0.46	Reject	No causality
	Bhabhar - Kurnool	2.26	0.11	Reject	No causality
2	Narayanpet- Bhabhar	0.24	0.79	Reject	No causality
	Bhabhar- Narayanpet	2.13	0.13	Reject	No causality
3	Ramanagara - Bhabhar	0.94	0.40	Reject	No causality
	Bhabhar - Ramanagara	2.87	0.06	Reject	No causality
4	Sumerpur - Bhabhar	0.02	0.98	Reject	No causality
	Bhabhar - Sumerpur	1.54	0.22	Reject	No causality
5	Narayanpet - Kurnool	1.31	0.28	Reject	No causality
	Kurnool – Narayanpet*	4.85	0.01	Do not reject	Unidirectional
6	Ramanagara - Kurnool	0.69	0.51	Reject	No causality
	Kurnool – Ramanagara*	5.61	0.01	Do not reject	Unidirectional
7	Sumerpur- Kurnool	1.07	0.35	Reject	No causality
	Kurnool - Sumerpur	1.98	0.15	Reject	No causality
8	Ramanagara - Narayanpet	0.00	1.00	Reject	No causality
	Narayanpet – Ramanagara*	4.90	0.01	Do not reject	Unidirectional
9	Sumerpur - Narayanpet	1.40	0.25	Reject	No causality
	Narayanpet - Sumerpur	0.65	0.52	Reject	No causality
10	Sumerpur – Ramanagara*	3.51	0.04	Do not reject	Unidirectional
	Ramanagara - Sumerpur	0.63	0.54	Reject	No causality

Note: * represents the level of significance at 5% level

Table 5 Vector Error Correction Model for Castor prices for Major five selected markets in India

	Kurnool	Bhabhar	Ramanagara	Sumerpur	Narayanpet
C	[0.01474]	[-0.20146]	[-0.42945]	[-0.23972]	[-0.05417]
Kurnool (-1)	[1.69697]	[1.43320]	[-2.46012]	[0.02891]	[2.49641]
Kurnool (-2)	[1.51651]	[1.53426]	[-0.80481]	[0.38277]	[2.83788]
Bhabhar (-1)	[-1.10576]	[-2.39998]	[0.00316]	[0.83042]	[-0.34141]
Bhabhar (-2)	[-1.50475]	[-1.29329]	[-0.78333]	[-0.11510]	[-0.74103]
Ramanagara (-1)	[-3.44196]	[0.18411]	[-4.23231]	[0.58131]	[-0.91531]
Ramanagara (-2)	[-2.51179]	[-0.70746]	[-4.17778]	[-0.59454]	[-1.15153]
Sumerpur (-1)	[-1.27865]	[0.23548]	[1.47673]	[-3.61138]	[0.24621]
Sumerpur (-2)	[0.40414]	[0.79923]	[1.26336]	[-1.14577]	[0.85870]
Narayanpet (-1)	[0.84155]	[-0.85690]	[0.51664]	[-0.62600]	[-4.50292]
Narayanpet (-2)	[0.11610]	[-1.24337]	[0.62093]	[-0.09875]	[-3.26454]

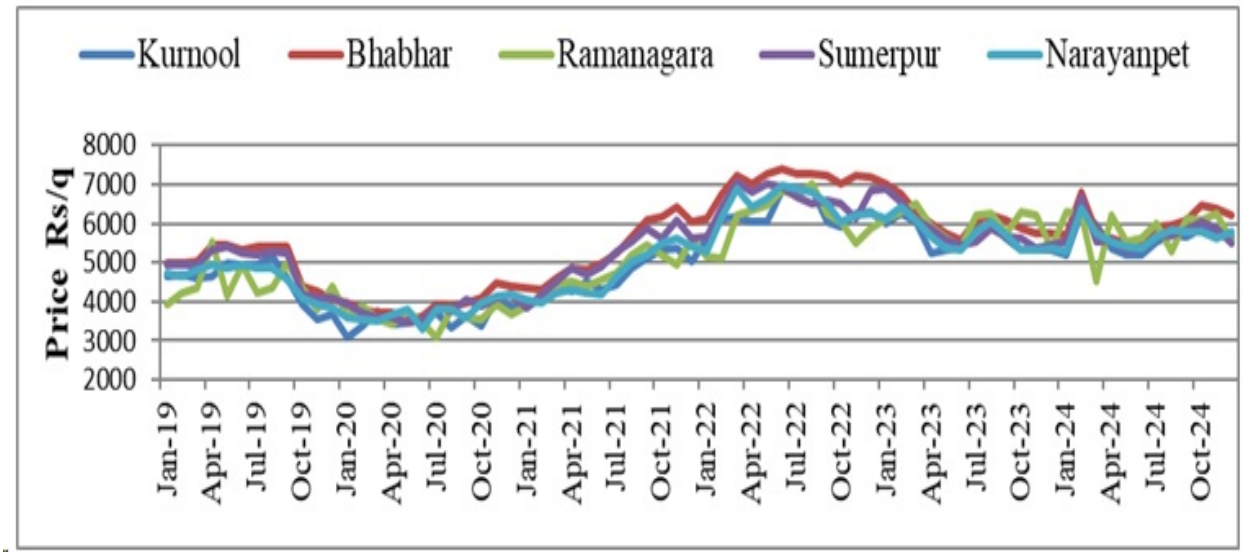

Fig. 1. Price behavior (`Rs/quintal) of Castor crop in major selected markets in India

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INDIAN SOCIETY OF OILSEEDS RESEARCH

Instructions to Authors for Preparation of Manuscript for Journal of Oilseeds Research

Prospective author(s) are advised to consult **Issue No. 27(1) June, 2010 of the Journal of Oilseeds Research** and get acquainted with the minor details of the format and style of the Journal. Meticulous compliance with the instructions given below will help quick handling of the manuscript by the reviewers, editor and printers. **Manuscripts are considered for publication in the Journal only from members of the ISOR.**

General

Full-length articles, short communications, book reviews and review articles are published in the Journal. Review articles and book reviews are published usually by invitation. Full length articles and short communications should report results of original investigations in oilseeds, oil bearing plants and relevant fields of science. Choice of submitting the paper(s) either as full length paper or short communication rests with the authors. The Editor(s) or Reviewer(s) will examine their suitability or otherwise only in that specific category. Each article should be written in English correctly, clearly, objectively and concisely. All the statements made in the manuscript should be clear, unambiguous, and to the point. Plagiarism is a crime and therefore, no part of the previously published material can be reproduced exactly without prior permission from the original publisher or author(s) as deemed essential and the responsibility of this solely rests on the authors. Also, authors shall be solely responsible for the authenticity of the results published as well as the inferences drawn thereof. Telegraphic languages should be avoided. The data should be reported in a coherent sequence. Use active voice. Active voice is clear, unambiguous and takes less space. Use past tense while reporting results. Do not repeat ideas in different forms of sentences. Avoid superfluous sentences such as `it is interesting to note that', `it is evident from the table that' or `it may be concluded that' etc. Use % for percent, %age for percentage, / for per, @ for at the rate of hr for hours, sec for seconds. Indicate date as 21 January 2010 (no commas anywhere). Spell out the standard abbreviations when first mentioned eg. Net assimilation rate (NAR), general combining ability (GCA), genetic advance (GA), total bright leaf equivalents (TBLE), mean sum of squares (MSS).

Manuscript

The language of the Journal is English. Generally, the length of an article should not exceed 3,000 words in the case of the full-length article and 750 words in the case of short communication. However, completeness of information is more important. Each half-page table or illustration should be taken as equivalent to 200 words. It is desirable to submit the manuscript online https://epubs.icar.org.in/index.php/JOR/about/submissions (preferred because of ease of handling during the review process) or as an e-mail attachment (in MS Word document; double line space; Times New Roman; font size 12) to editorisor@gmail.com. Every page (of the manuscript), including the title page, references, tables, etc. should be numbered. Punctuation marks help to show the meanings of words by grouping them into sentences, clauses, phrases, and other ways. These marks should be used properly if the reader of a paper is to understand exactly the intended meaning. Receipt of the manuscript (received online or as an email attachment) will be acknowledged by the editorial office of the Society, giving a manuscript number which should be quoted in all subsequent correspondence regarding that particular article.

Full-length Articles

Organization of the Manuscript

Before reading the instructions given below, the author(s) would better have a close look at the latest issue of the Journal.

Full-length article comprises the following sections.

(a) Short title
(b) Title
(c) Author/Authors
(d) Institution and Address with PIN (postal) code
(e) Abstract (along with key words)
(f) Introduction
(g) Materials and Methods
(h) Results and Discussion
(i) Acknowledgments (if any)
(j) References
(k) Tables and figures (if any)

Guidelines for each section are as follows:

All these headings or matter thereof should start from left hand side of the margin, without any indent.

Short Title

A shortened title (approximately of 30 characters) set in capital letters should convey the main theme of the paper.

Title

Except for prepositions, conjunctions, pronouns and articles, the first letter of each word should be in capital letter. The title should be short and should contain key words and phrases to indicate the contents of the paper and be attractive. Jargons and telegraphic words should be avoided. In many cases, actual reading of the paper may depend on the attractiveness of the title.

Author/Authors

The name(s) of author(s) should be typed in capital letters a little below the title, starting from the left margin. Put an asterisk on the name of the corresponding author. **Give the Email ID of the corresponding author** as a footnote.

Institution and Address

This matter will come below the name(s) of the author(s). Name of the Laboratory/Department, followed by the name of the Institution/Organization/University where the work reported in the paper was carried out shall come below the name(s) of author(s). Complete postal address, which should include city/town, district, and state, followed by PIN (postal) code is to be furnished. In case any author has left the above address, this should be indicated as a footnote.

Abstract

The paragraph should start with the word Abstract (in bold font). The abstract should comprise brief and factual summary or salient points of the contents and the conclusions of the investigation reported in the paper and should refer to any new information therein. As the abstract is an independent entity, it should be able to convey the gist of the paper in a concise manner. It will be seen by many more people than will read the paper. The abstract, as concise as possible, should not exceed 250 words in length. Everything that is important in the paper must be reflected in the abstract. It should provide to the reader very briefly the rationale, objectives or hypothesis, methods, results and conclusions of the study described in the paper. In the abstract, do not deflect the reader with promises such as 'will be discussed' or 'will be explained'. Also do not include reference, figure or table citation. At first mention in the abstract, give complete scientific name for plants and other organisms, the full names of chemicals and the description of soil order/series. Any such names or descriptions from the abstract need not be repeated in the text. It must be remembered that the abstracting journals place a great emphasis on the abstract in the selection of papers for abstracting. If properly prepared, they may reproduce it verbatim.

"Key words" should, follow separately after the last sentence of the abstract. "Key words" indicate the most important materials, operations, or ideas covered in the paper. Key words are used in indexing the articles.

Introduction (To be typed as side-heading, starting from the left-hand margin, a few spaces below the key words)

This section is meant to introduce the subject of the paper. Introduction should be short, concise and indicate the objectives and scope of the investigation. To orient readers, give a brief reference to previous concepts and research. Limit literature references to essential information. When new references are available, do not use old references unless it is of historical importance or a landmark in that field. Emphasis should be given among other things on citing the literature on work done under Indian conditions. Introduction must include: (a) a brief statement of the problem, justifying the need for doing the work or the hypothesis on which the work is based, (b) the findings of others that will be further developed or challenged, and (c) an explanation of the approach to be followed and the objectives of the research described in the paper. If the methods employed in the paper are new, it must be indicated in the introduction section.

Materials and methods (To be typed as side-heading, starting from the left-hand margin, a few spaces below the introduction)

This part of the text should comprise the materials used in the investigation, methods of experiment and analysis adopted. This portion should be self-explanatory and have the requisite information needed for understanding and assessing the results reported subsequently. Enough details should be provided in this section to allow a competent scientist to repeat the experiments, mentally or in fact. The geographical position of soil site or soils used in the experiment or site of field trial should be identified clearly with the help of coordinates (latitude & longitude) and invariably proper classification according to Soil Taxonomy (USDA), must be indicated to the level of Great-group, Suborder or Order as far as possible. Specify the period during which the experiment(s) was conducted. Send the article after completion of the experiment(s) not after a gap of 5 years. Instead of kharif and rabi use rainy and winter season respectively. Please give invariably the botanical names for local crop names like raya, bajra moong, cholam etc. Botanical and zoological names should confirm to the international rules. Give authorities. Go through some of our recent issues and find out the correct names. Give latest correct names from authentic source. For materials, give the appropriate technical specifications and quantities and source or method of preparation. Should a product be identified by trade name, add the name and location of the manufacturer or a major distributor in parenthesis after the first mention of the product. For the name of plant protection chemicals, give popular scientific names (first letter small), not trade names (When trade name is given in addition, capitalize the first letter of the name). Known methods of analysis should be indicated by referring to the original source, avoiding detailed description. Any new technique developed and followed should be described in fair detail. When some specially procured or proprietary materials are used, give their pertinent chemical and physical properties. References for the methods used in the study should be cited. If the techniques are widely familiar, use only their names in that case.

Results and Discussion (To be typed as a side-heading, a few spaces below the matter on "Materials and Methods")

This section should discuss the salient points of observation and critical interpretation thereof in past tense. This should not be descriptive and mere recital of the data presented in the tables and diagrams. Unnecessary details must be avoided but at the same time significant findings and special features should be highlighted. For systematic discussion, this section may be divided into sub-sections under side-heading and/or paragraph side heading. Relate the results to your objectives. While discussing the results, give particular attention to the problem, question or hypothesis presented in the introduction. Explain the principles, relationships, and generalizations that can be supported by the results. Point out any exceptions. Explain how the results relate to previous findings, support, contradict or simply add as data. Use the Discussion section to focus on the meaning of your findings rather than recapitulating them. Scientific speculation is encouraged but it should be reasonable and firmly founded in observations. When results differ from previous results, possible explanations should be given. Controversial issues should be discussed clearly. References to published work should be cited in the text by the name(s) of author(s) as follows: Mukherjee and Mitra (1942) have shown or It has been shown (Mukherjee and Mitra, 1942)..... If there are more than two authors, this should be indicated by et al. after the surname of the first author, e.g., Mukherjee et al. (1938).

Always conclude the article by clearly crystallizing the summary of the results obtained along with their implications in solution of the practical problems or contribution to the advancement of the scientific knowledge.

Acknowledgments (To be typed as given above, as a side-heading, well below the concluding portion of Conclusions)

The author(s) may place on record the help, and cooperation, or financial help received from any source, person or organization. This should be very brief, and omitted, if not necessary.

References (To be typed as above, as side heading below Acknowledgement)

The list of references must include all published work referred to in the text. Type with double line spacing. Do not cite anonymous as author; instead cite the name of the institute, publisher, or editor. References should be arranged alphabetically according to the surnames of the individual authors or first authors. Two or more references by the same author are to be cited chronologically; two or more in the same year by the letters a, b, c, etc. All individually authored articles precede those in which the individual is the first or joint author. Every reference cited in the article should be included in the list of References. This needs rigorous checking of each reference. Names of authors should not be capitalized.

The reference citation should follow the order: author(s), year of publication, title of the paper, periodical (title in full, no abbreviations, italics or underlined), volume (bold or double underlining), starting and ending pages of the paper. Reference to a book includes authors(s), year, title (first letter of each word except preposition, conjunction, and pronouns in capitals and underlined), the edition (if other than first), the publisher, city of publication. If necessary, particular page numbers should be mentioned in the last. Year of publication cited in the text should be checked with that given under References. Year, volume number and page number of each periodical cited under "References" must be checked with the original source. The list of references should be typed as follows:

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Reference to unpublished work should normally be avoided and if unavoidable it may be mentioned only in the text.

Short Communication

Conceptually short communication is a first report on new concept, ideas and methodology which the author(s) would wish to share with the scientific community and that the detailed paper would follow. Short Communication is akin to an advance booking for the report on the findings. Short communications may include short but trend-setting reports of field or laboratory observation(s), preliminary results of long-term projects, or new techniques or those matters on which enough information to warrant its publication as a full length article has still not been generated but the results need to be shared immediately with the scientific community. The style is less formal as compared with the "full-length" article. In the short communications, the sections on abstract, materials and methods, results and discussion, and conclusion are omitted; but the material is put concisely in the same sequence but without formal sections. The other instructions are the same as in the case of the full-length articles.

Tables

Tables should not form more than 20% of the text. Each table should be typed on separate sheet and should have on the top a table number (in Arabic numerals *viz*. 1, 2, 3 etc.) and a caption or title which should be short, but sufficiently explanatory of the data included in the table. Information in the table should never duplicate that in the text and vice versa. Symbols (asterisks, daggers, etc. or small letters, *viz*., a, b, etc.) should be used to indicate footnotes to tables. Maximum size of table acceptable is what can be conveniently composed within one full printed page of the journal. Over-sized tables will be rejected out-right. Such tables may be suitably split into two or more small tables.

The data in tables should be corrected to minimum place of decimal so as to make it more meaningful. Do not use full stop with CD, SEm $\pm$, NS (not C.D., S.E.m $\pm$, N.S.). Do not put cross-rules inside the table. Tables should be numbered consecutively and their approximate positions indicated in the margin of the manuscript. Tables should not be inserted in the body of the text. Type each table on a separate sheet. Do not use capital letters for the tabular headings, do not underline the words and do not use a full-stop at the end of the heading. All the tables should be tagged with the main body of the text i.e. after references.

Figures

Figures include diagrams and photographs. Laser print outs of line diagrams are acceptable while dot-matrix print outs will be rejected. Alternatively, each illustration can be drawn on white art card or tracing cloth/ paper, using proper stencil. The lines should be bold and of uniform thickness. The numbers and letterings must be stenciled; free-hand drawing will not be accepted. Size of the illustrations as well as numbers, and letterings should be sufficiently large to stand suitable reduction in size. Overall size of the illustrations should be such that on reduction, the size will be the width of single or double column of the printed page of the Journal. Legends, if any, should be included within the illustration. Each illustration should have a number followed by a caption typed/ typeset well below the illustration.

Title of the article and name(s) of the author(s) should be written sufficiently below the caption. The photographs (black and white) should have a glossy finish with sharp contrast between the light and the dark areas. Colour photographs/ figures are not normally accepted. One set of

the original figures must be submitted along with the manuscript, while the second set can be photocopy. The illustrations should be numbered consecutively in the order in which they are mentioned in the text. The position of each figure should be indicated in the margin of the text. The photographs should be securely enclosed with the manuscript after placing them in hard board pouches so that there may not be any crack or fold. Photographs should preferably be 8.5 cm or 17 cm wide or double the size. The captions for all the illustrations (including photographs) should be typed on a separate sheet of paper and placed after the tables.

Expression of Plant Nutrients on Elemental Basis

The amounts and proportions of nutrient elements must be expressed in elemental forms e.g. for ion uptake or in other ways as needed for theoretical purposes. In expressing doses of nitrogen, phosphatic, and potassic fertilizers also these should be in the form of N, P and K, respectively. While these should be expressed in terms of kg/ha for field experiments, for pot culture studies the unit should be in mg/kg soil.

SI Units and Symbols

SI Units (System International d 'Unities or International System of Units) should be used. The SI contains three classes of units: (i) base units, (ii) derived units, and (iii) supplementary units. To denote multiples and sub-multiples of units, standard abbreviations are to be used. Clark's Tables: Science Data Book by Orient Longman, New Delhi (1982) may be consulted.

Some of these units along with the corresponding symbols are reproduced for the sake of convenience.

Names and Symbols of SI Units

Physical quantity | Symbol for SI Unit | Remarks | Physical quantity | Symbol for SI Unit | Remarks

Primary Units

length	l		time	t	
metre	m		second	s	
mass	m		electric current	I	
kilogram	kg		ampere	A	

Secondary Units

plane angle	radian	rad	Solid angle	steradian	sr

Unit Symbols

centimetre	cm	microgram	µg
cubic centimetre	cm^3	micron	µm
cubic metre	m^3	micronmol	µmol
day	d	milligram	mg
decisiemens	dS	millilitre	mL
degree-Celsium	°C [=(F-32)x0.556]	minute	min
gram	g	nanometre	nm
hectare	ha	newton	N
hour	h	pascal	Pa
joule J	(=10^7 erg or 4.19 cal.)	second	s
kelvin	K (=°C+273)	square centimetre	cm^2
kilogram	kg	square kilometre	km^2

kilometre	km	tonne	t
litre	L	watt	W
megagram	Mg		

Some applications along with symbols

adsorption energy	J/mol (=cal/molx4.19)	leaf area	m^2/kg
cation exchange capacity	cmol (p+)/kg (=m.e./100 g)	nutrient content in plants (drymatter basis)	µg/g, mg/g or g/kg
Electrolytic conductivity	dS/m (=mmhos/cm)	root density or root length density	m/m^3
evapotranspiration rate	$m^3/m^2/s$ or m/s	soil bulk density	Mg/m^3 (=g/cm^3)
heat flux	W/m^2	specific heat	J/kg/K
gas diffusion	$g/m^2/s$ or $m^3/m^2/s$ or m/s	specific surface area of soil	m^2/kg
water flow	$kg/m^2/s$ (or) m^3m^2s (or) m/s	thermal conductivity	W/m/K
gas diffusivity	m^2/s	transpiration rate	$mg/m^2/s$
hydraulic conductivity ion uptake	m/s	water content of soil	kg/kg or m^3/m^3
(Per kg of dry plant material)	mol/kg	water tension	kPa (or) MPa

While giving the SI units the first letter should not be in capital i.e cm, not Cm; kg not Kg. There should not be a full stop at the end of the abbreviation: cm, not cm. kg, not kg.; ha, not ha.

In reporting the data, dimensional units, viz., M (mass), L (length), and T (time) should be used as shown under some applications above. Some examples are: 120 kg N/ha; 5 t/ha; 4 dS/m etc.

Special Instructions

I. In a series or range of measurements, mention the unit only at the end, e.g. 2 to 6 cm2, 3, 6, and 9 cm, etc. Similarly use cm2, cm3 instead of sq cm and cu m.

II. Any unfamiliar abbreviation must be identified fully (in parenthesis).

III. A sentence should not begin with an abbreviation.

IV. Numeral should be used whenever it is followed by a unit measure or its abbreviations, e.g., 1 g, 3 m, 5 h, 6 months, etc. Otherwise, words should be used for numbers one to nine and numerals for larger ones except in a series of numbers when numerals should be used for all in the series.

V. Do not abbreviate litre to` l' or tonne to `t'. Instead, spell out.

VI. Before the paper is sent, check carefully all data and text for factual, grammatical and typographical errors.

VII. Do not forget to attach the original signed copy of `Article Certificate' (without any alteration, overwriting or pasting) signed by all authors.

VIII. On revision, please answer all the referees' comments point-wise, indicating the modifications made by you on a separate sheet in duplicate.

IX. If you do not agree with some comments of the referee, modify the article to the extent possible. Give reasons (2 copies on a separate sheet) for your disagreement, with full justification (the article would be examined again).

X. Rupees should be given as per the new symbol approved by Govt. of India.

Details of the peer review process

Manuscripts are received mainly through e-mails and in rare cases, where the authors do not have internet access, hard copies of the manuscripts may be received and processed. Only after the peer review the manuscripts are accepted for publication. So there is no assured publication on submission. The major steps followed during the peer review process are provided below.

Step 1. ***Receipt of manuscript and acknowledgement***: Once the manuscript is received, the contents will be reviewed by the editor/associate editors to assess the scope of the article for publishing in JOR. If found within the scope of the journal, a Manuscript (MS) number is assigned and the same will be intimated to the authors. If the MS is not within the scope and mandate of JOR, then the article will be rejected and the same is communicated to the authors.

Step 2. ***Assigning and sending MS to referees***: Suitable referees will be selected from the panel of experts and the MS (soft copy) will be sent to them for their comments - a standard format of evaluation is provided to the referees for evaluation along with the standard format of the journal articles and the referees will be given 4-5 week time to give their comments. If the comments are not received, reminders will be sent to the referees for expediting the reviewing process and in case there is still no response, the MS will be sent to alternate referees.

Step 3. ***Communication of referee comments to authors for revision***: Once the referee comments and MS (with suggestions/ corrections) are received from the referees, depending on the suggestions, the same will be communicated to the authors with a request to attend to the comments. Authors will be given stipulated time to respond and based on their request, additional time will be given for attending to all the changes as suggested by referees. If the referees suggest no changes and recommend the MS for publication, then the same will be communicated to the authors and the MS will be taken up for editing purpose for publishing. In case the referees suggest that the article cannot be accepted for JOR, then the same will be communicated to the authors with proper rationale and logic as opined by the referees as well as by the editors.

Step 4. ***Sending the revised MS to referees***: Once the authors send the revised version of the articles, depending on the case (like if major revisions were suggested by referees) the corrected MS will be sent to the referees (who had reviewed the article in the first instance) for their comments and further suggestions regarding the acceptability of publication. If only minor revisions had been suggested by referees, then the editors would look into the issues and decide take a call.

Step 5. ***Sending the MS to authors for further revision***: In case referees suggest further modifications, then the same will be communicated to the authors with a request to incorporate the suggested changes. If the referees suggest acceptance of the MS for publication, then the MS will be accepted for publication in the journal and the same will be communicated to the authors. Rarely, at this stage also MS would be rejected if the referees are not satisfied with the modifications and the reasoning provided by the authors.

Step 6. ***Second time revised articles received from authors and decision taken***: In case the second time revised article satisfies all the queries raised by referees, then the MS will be accepted and if not satisfied the article will be rejected. The accepted MS will be taken for editing process where emphasis will be given to the language, content flow and format of the article.

Then the journal issue will be slated for printing and also the pdf version of the journal issue will be hosted on journal webpage.

Important Instructions

- Data on field experiments have to be at least for a period of 2-3 years
- Papers on pot experiments will be considered for publication only as short communications
- Giving coefficient of variation in the case of field experiments Standard error in the case of laboratory determination is mandatory. For rigorous statistical treatment, journals like Journal of Agricultural Science Cambridge, Experimental Agriculture and Soil Use and Management should serve as eye openers.

SPECIAL ANNOUNCEMENT

In a recently conducted Executive Committee meeting of the Indian Society of Oilseeds Research, it was decided to increase the scope of the Journal of Oilseeds Research by accommodating vibrant aspects of scientific communication. It has been felt that, the horizon of scientific reporting could be expanded by including the following types of articles in addition to the Research Articles, Shor Communications and Review Articles that are being published in the journal as of now.

Research accounts (not exceeding 4000 words, with cited references preferably limited to about 40-50 in number): These are the articles that provide an overview of the research work carried out in the author(s)' laboratory, and be based on a body of their published work. The articles must provide appropriate background to the area in a brief introduction so that it could place the author(s)' work in a proper perspective. This could be published from persons who have pursued a research area for a substantial period dotted with publications and thus research account will provide an overall idea of the progress that has been witnessed in the chosen area of research. In this account, author(s) could also narrate the work of others if that had influenced the course of work in authors' lab.

Correspondence (not exceeding 600 words): This includes letters and technical comments that are of general interest to scientists, on the articles or communications published in Journal of Oilseeds Research within the previous four issues. These letters may be reviewed and edited by the editorial committee before publishing.

Technical notes (less than 1500 words and one or two display items): This type of communication may include technical advances such as new methods, protocols or modifications of the existing methods that help in better output or advances in instrumentation.

News (not exceeding 750 words): This type of communication can cover important scientific events or any other news of interest to scientists in general and vegetable oil research in particular.

Meeting reports (less than 1500 words): It can deal with highlights/technical contents of a conference/ symposium/discussion-meeting, etc. conveying to readers the significance of important advances. Reports must

Meeting reports should avoid merely listing brief accounts of topics discussed, and must convey to readers the significance of an important advance. It could also include the major recommendations or strategic plans worked out.

Research News (not exceeding 2000 words and 3 display items): These should provide a semi-technical account of recently published advances or important findings that could be adopted in vegetable oil research.

Opinion (less than 1200 words): These articles may present views on issues related to science and scientific activity.

Commentary (less than 2000 words): This type of articles are expected to be expository essays on issues related directly or indirectly to research and other stake holders involved in vegetable oil sector.

Book reviews (not exceeding 1500 words): Books that provide a clear in depth knowledge on oilseeds or oil yielding plants, production, processing, marketing, etc. may be reviewed critically and the utility of such books could be highlighted.

Historical commentary/notes (limited to about 3000 words): These articles may inform readers about interesting aspects of personalities or institutions of science or about watershed events in the history/development of science. Illustrations and photographs are welcome. Brief items will also be considered.

Education point (limited to about 2000 words): Such articles could highlight the material(s) available in oilseeds to explain different concepts of genetics, plant breeding and modern agriculture practices.

Note that the references and all other formats of reporting shall remain same as it is for the regular articles and as given in Instructions to Authors

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