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Genomic regions linked to reniform nematode (*Rotylenchulus reniformis*) resistance in castor

POORNIMA KUMARI[1], P GIRIBABU[2], MANMODE DARPAN MOHANRAO AND S SENTHILVEL[*]

ICAR-Indian Institute of Oilseeds Research, Rajendranagar, Hyderabad-500 030, Telangana

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ABSTRACT

Reniform nematode (*Rotylenchulus reniformis*) infects castor and makes it vulnerable to vascular wilt and root rot diseases. In this study, a population consisting of 92 recombinant inbred lines (RILs) derived from the cross between reniform nematode resistant line JC-12 and susceptible line 48-1 was used to identify the genomic regions linked to reniform nematode resistance. The parents, F_1 and RILs were screened against reniform nematode in pot culture with artificial inoculation of nematodes. The scoring for nematode resistance was done on the basis of number of nematodes extracted from the soil at 60 days after inoculation. The mean nematode count in 48-1 and JC-12 was 215.0±9.1 and 77.8±4.8 nematodes/ml of soil wash, respectively. The F_1 reaction (217.3±13.2 nematodes/ml of soil wash) was similar to the susceptible parent indicating that nematode resistance in JC-12 is recessive in nature. The nematode count in RILs ranged from 43.8 to 327.3. QTL mapping using a linkage map consisting of 1,090 SNP markers resulted in the identification one QTL each on chromosome-6 and chromosome-8, linked to resistance. This is the first report on mapping of genomic regions linked to reniform nematode resistance in castor, which form the basis for furthering the research on genetic and molecular biology of nematode resistance in castor.

Keywords: Castor, Genome mapping, Inheritance, Reniform nematode resistance

Castor is an important non-edible oilseeds crop having multifarious industrial applications. Castor seed oil and its derivatives are used in manufacturing of several industrial products including paints, coatings, inks and lubricants (Ogunniyi *et al.*, 2006). Castor is a good candidate for biodiesel production owing to its ability to grow as annual crop in marginal soils and short growing duration compared to other non-edible oilseeds like Jatropha (Shrirame *et al.*, 2011). In India, castor is cultivated in an area of 0.75 million hectares, which accounts for 65% of the world's castor acreage. India produces 1.2 million tonnes of castor seed per annum contributing to more than 85% of the world's castor production (FAOSTAT, 2019).

Castor is known to be infected by many nematode species among which, reniform nematode (*Rotylenchulus reniformis*) is considered as an economically important pest (Seshadri and Shivakumar, 1963). *Rotylenchulus reniformis* is an obligate, sedentary semi-endoparasite. The vermiform juvenile females penetrate the host root system where they establish a feeding site. It has a wide host range affecting more than 300 plant species including important crops like cotton, cowpea, grapes, papaya etc. Symptoms are not specific and in general not very apparent. Die-back, stunting and growth reduction have been reported in castor fields heavily infested with reiniform nematodes (Seshadri and Sivakumar, 1963; Verma and Prasad, 1969). In addition, leaf shedding, early flowering, malformed and discoloured seeds, decreased yield and inferior quality of oil have also been reported as the consequences of reniform nematode infestation in castor (Sivakumar and Seshadri, 1971). The estimated yield loss due to reniform nematode was 13.9% (Jain *et al.*, 2007).

Moreover, reinform nematodes were found to be associated with diseases like Fusarium wilt and Macrophomina root rot in castor (Chattopadhyay and Reddy, 1995). In a study on interaction between *Rotylenchulus reniformis* and *Fusarium oxysporum* f. sp. *ricini* in castor, *Rotylenchulus reniformis* alone and in combination with *F. oxysporum* f. sp. *ricini* found to reduce plant growth of both wilt susceptible and resistant hybrids. Fusarium wilt - Reniform nematode interaction was found to be synergistic when the two pathogens were inoculated together and wilt resistant cv. GCH-4 became susceptible in the presence of reniform nematode and wilt disease appeared earlier in different combinations of nematode and fungus, than fungus alone (Patel *et al.*, 2000a). In a study on interaction between reniform nematode and *Macrophomina*, early appearance of disease and high mortality was observed when the plants were inoculated with *Rotylenchulus reniformis* and *Macrophomina phaseolina* together compared to inoculation of *Macrophomina* alone or *Macrophomina* followed by the nematode (Patel *et al.*, 2000b).

[1]Osmania University, Hyderabad-500007; [2]Present Address: ICAR-National Research Centre for Banana, Thayanur Post, Tiruchirapalli-620 102, Tamil Nadu; [*]Corresponding author's E-mail: senthilvel.senapathy@icar.gov.in

Though soil solarisation and application of carbofuran at the rate of 2 kg a.i/ha were found to reduce losses from reniform nematode infection, the use of genetic resistance is a better and environmental friendly option to counter the reniform nematode infection (Schrimsher *et al.,* 2014). The cultivation of resistant cultivars helps in bringing down the nematode population in the soil over years, which might have greater impact on other more susceptible crops such as cotton and cowpea. Many castor cultivars and hybrids were identified as moderately resistant to reniform nematodes (Barre *et al.,* 2013). A castor inbred line JC-12 was found to harbour less reniform nematodes in the repeated screenings at ICAR-Indian Institute of Oilseeds Research (ICAR-IIOR), Hyderabad, India. The present study was aimed at genetic characterization and QTL mapping of reniform nematode resistance in castor inbred line 'JC-12'.

MATERIALS AND METHODS

Plant materials: JC-12 is an elite inbred line developed by Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur, India. It was found to be resistant in the in-house screenings (Fig 1). JC-12 was crossed with a susceptible line 48-1. 48-1 (Jwala) is a notified commercial variety developed by ICAR-IIOR. JC-12, 48-1, (JC-12 × 48-1) F_1 and a sub-set (92 lines) of recombinant inbred line (RIL) population derived from the cross JC-12 × 48-1 were used in this study.

Screening against reniform nematode: The seeds of parents, F_1 and RILs were sown in plastic pots containing 250 g of sterilized sand and soil mixture (1:1). Single plant was maintained per pot and 10 pots were maintained for each line. The culture of *Rotylenchulus reniformis* maintained on susceptible castor plants at ICAR-IIOR was used for screening. Ten days after sowing, 500 reniform nematodes (mixed life stages) suspended in 1 ml water were added to the soil in each pot. Pots were placed in a glass house at a constant temperature of ~30°C. Pots were watered time to time so as to maintain the soil moisture (Fig 2). Sixty days after inoculations, nematodes were extracted from the soil following the modified Cobb's sieving and decanting technique (Christie and Perry, 1951). The nematodes were counted under stereo-bionocular microscope and the count was expressed as 'number of nematodes/ml of soil suspension'.

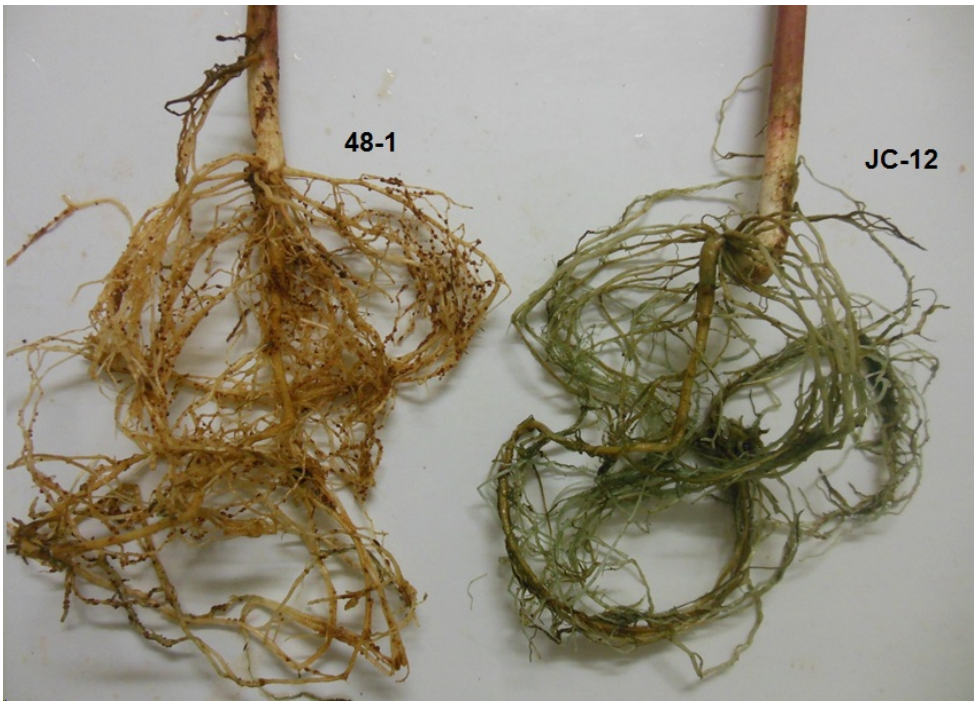

Fig. 1. Egg masses of reniform nematode on the roots of castor inbred lines 48-1 and JC-12

QTL mapping: The genotypic data for 1,090 SNP loci generated earlier was used for constructing the linkage map. Linkage map construction and QTL mapping were carried out using the QTL IciMapping software version 4.1.0.0 (Meng *et al.,* 2015). Anchor markers with known chromosomal locations as per Xu *et al.* (2021) were used for

identification of chromosomes. The markers were grouped into linkage groups representing 10 castor chromosomes at the logarithm of odds (LOD) threshold of >3. The markers within the linkage group were ordered using 'nnTwoOpt' algorithm. Map distances between markers were calculated using the Haldane mapping function. Refinement of marker order within each chromosome was done with rippling function 'SARF' along with window size of '5'. The QTL mapping was carried using inclusive interval mapping algorithm for additive effects (Li *et al.,* 2007). LOD threshold to identify significant QTL was set at >2.5.

Fig. 2. Screening of JC-12 × 48-1 RILs for reniform nematode resistance in glasshouse

RESULTS AND DISCUSSION

The present study was aimed at genetic characterization of reniform nematode resistance in an elite castor inbred line JC-12. The screening against the reniform nematodes through artificial inoculation has confirmed that JC-12 is resistant. The mean number of nematodes harboured by JC-12 was 77.75/ml of soil wash, which was significantly lesser compared to 48-1, which harboured 215.0/ml of soil wash (Table 1). The hybrid plants of JC-12 × 48-1 recorded on an average 217.2 nematodes/ml of soil wash similar to the susceptible parent. Therefore, it was inferred that resistance to reniform nematode in castor is recessive as that of soybean (Harville *et al.,* 1985). The recombinant inbred line (RILs) population from the cross JC-12 × 48-1 showed wide variation for nematode count. The mean nematode count/g of soil ranged from 43.75 to 309.75 (Fig 3). Positive and negative transgressive segregations were detected in the RIL population suggesting that alleles from both parental lines might have contributed to the resistance. Six RILs (P3-96, P3-108-NSp, P3-109NSp, P3-131Sp, P3-131NSp & P3-241) were found to harbour lesser nematodes than the susceptible parent JC-12. These RILs being homozygous lines can be directly used for breeding for resistance to reniform nematode in castor.

Linkage analysis yielded 10 linkage groups representing the haploid chromosome number of castor. Inclusive composite interval mapping using SNP genotypic data and mean nematode count for the RILs identified one significant QTL (LOD >2.5) each on chromosome-6 and chromosome -8 (Fig 4). The details of QTL are given in Table 2. Previously, genomic regions linked to reniform nematodes have been identified in crops like *Gossypium arboreum* (Erpelding and Stetina, 2018), *Gossypium barbadense* (Gutiérrez *et al.,* 2011) and soybean (Ha *et al.,* 2007; Wilkes *et al.,* 2020). However, the present study is the first report on mapping of QTLs for reniform nematode resistance in castor to the best of our knowledge. This study has laid the foundation for further validation of putative regions associated with reniform nematode resistance and its use in marker assisted selection for developing reniform nematode resistance inbred lines in castor.

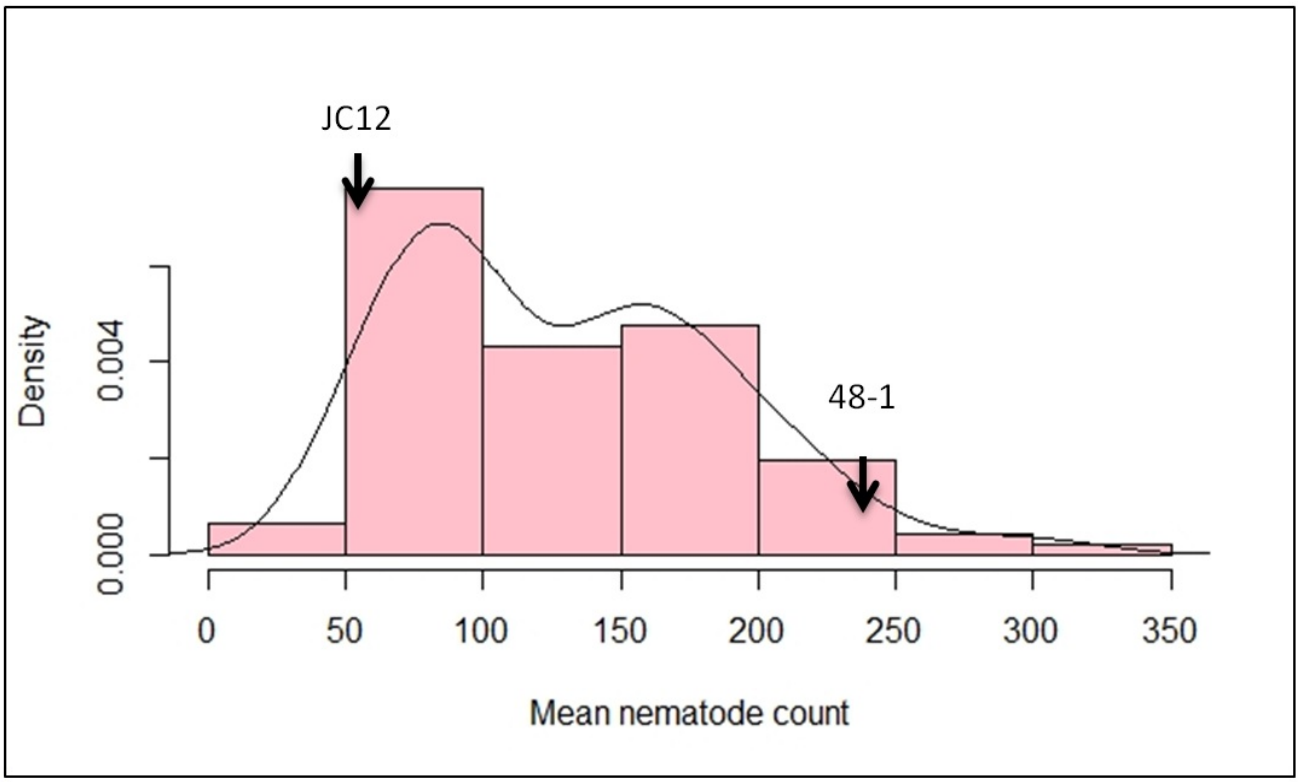

Fig. 3. Frequency distribution of nematode count in RIL population

Table 1 Reniform nematode count in JC-12, 48-1, F_1 and RILs

Particulars	JC-12	48-1	(JC-12 × 48-1)F_1	(JC-12 × 48-1)RILs
Mean	77.8	215	217.2	131.5
Range	69-87	199-237	189-251	44-327
Sd	9.6	18.3	26.4	61. 2
Se	4.8	9.1	13.2	6.3

Table 2 QTLs linked to reniform nematode resistance in JC-12 × 48-1 RIL population

Chromosome	Position	Left marker	Right marker	LOD	R^2	LOD support interval	Additive effect	Allele source
6	79	Rc_29666-381712	Rc_29666-471509	5.04	17.91	76.5 – 81.5	-26.13	JC-12
8	66	Rc_28037-33296	Rc_28151-12413	3.62	13.23	64.5 – 67.5	-22.34	JC-12

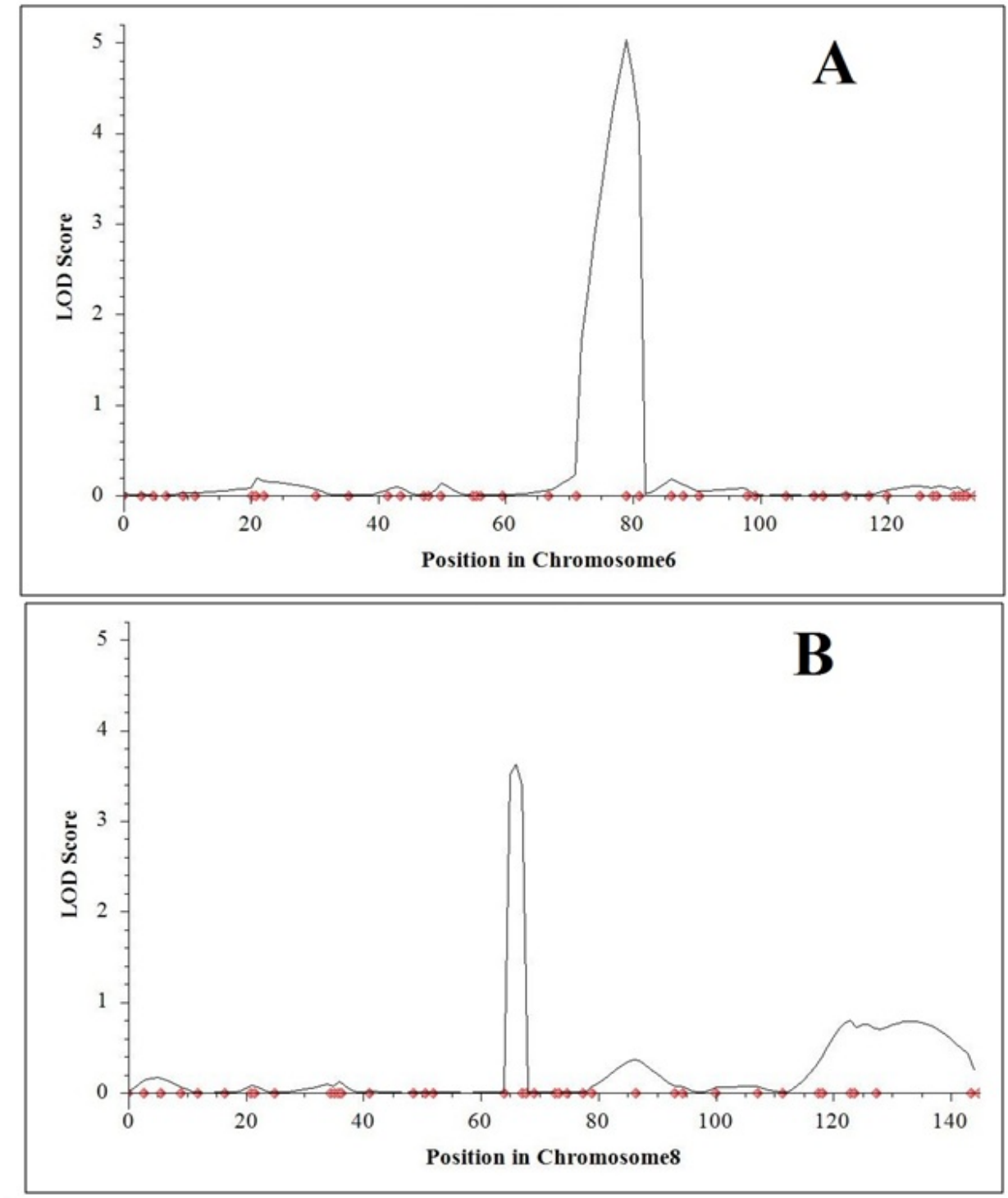

Fig. 4. QTL positions on chromosome 6 (A) and chromosome 8 (B)

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Lack of transcripts' locus of origin data limits the study of gene-level diversity in triacylglycerol biosynthesis pathway

S GEETHANJALI[1*], VELU MANI SELVARAJ[2], PALCHAMY KADIRVEL[3], S K LOKESH[1] AND G VAISHNAVI[1]

Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu

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ABSTRACT

Triacylglycerol (TAG) biosynthesis in plants is complex and involves several genes with specific roles in the Kennedy pathway. Analysis of the evolutionary pattern and diversity of these genes can help to improve understanding of TAG biosynthesis in oilseed crops. In this study, an attempt was made to explore the diversity of genes: *DGAT1, DGAT2, GPAT9* and *LPAT2* across 13 oilseed crops using the sequence information of the model species, *Arabidopsis thaliana*. A total of 213 protein sequences corresponding to these genes were retrieved from the NCBI database by BLAST, multiple sequence alignment was performed and a phylogenetic tree was constructed. *DGAT1*, *DGAT2* and *GPAT9* sequences produced distinct species-wise clusters with several distinct sub-clusters, indicating monophyletic and highly divergent nature with specialized roles in different species. *LPAT2* sequences did not produce species-wise distinct clusters, indicating their polyphyletic nature. Diverse candidate gene sequences and phylogenetic relationships presented in this study would help to study TAG biosynthesis through genome-wide analysis in oilseed crops.

Keywords: Candidate genes, Diversity, Kennedy pathway, Phylogeny, Triacylglycerol

In India, vegetable oil is produced from different oilseed crops including annuals (Rapeseed & Mustard, Soybean, Groundnut, Sesame, Castor bean, Sunflower, Safflower, Linseed and Niger) as well as perennials (Coconut and Oil palm). India is the fourth major producer of vegetable oil in the world after USA, China, and Brazil. In India, the annual oilseed crops are grown in an area of 260 lakh hectares with a production of 320 lakh tonnes (agricoop.nic.in). Among the monocot oilseed crops, coconut is grown in an area of about 22 lakh ha (https://www.coconutboard.gov.in/Statistics.aspx) and oil palm is grown in an area of about three lakh ha (https://nmoop.gov.in/Publication/Status_Paper_OilPalm.pdf). Oilseed crops exhibit a high level of variability in their oil content and fatty acid composition, which determines their nutritional and industrial value (Jain, 2020). Genetic and genomic strategies are widely applied in oilseed crops to breed cultivars with improved oil content and quality, thus enhancing their commercial value (Murphy, 2014). Therefore, understanding the genetic and molecular basis of oil biosynthesis is essential for developing crop breeding strategies for improvement of oil quality as desired by consumers and industry.

Vegetable oil is, biochemically, triacylglycerol (TAG), and is commonly referred to as triglyceride. Oilseed crops synthesize and store TAG in seeds, which serves as a source of carbon and energy to support seedling development. TAG synthesis in plants is a complex biochemical process and differs from that of other eukaryotes. In plants, fatty acids synthesized in the plastids are transported to the endoplasmic reticulum where they are assembled onto the glycerol backbone to produce triacylglycerol. TAG synthesis in plants involves the acyl CoA dependent pathway (Bates *et al.*, 2013) and the acyl CoA independent pathway (Banas *et al.*, 2000; Dahlqvist *et al.*, 2000). The acyl CoA dependent pathway, also known as the Kennedy pathway, is the common pathway for TAG biosynthesis in most of the oilseed crops (Bates *et al.*, 2013). The Kennedy pathway comprises of three key acyl CoA dependent acyl transferases, namely glycerol-3-phosphate acyltransferase (GPAT), lysophosphatidic acid acyltransferase (LPAT), and diacyl glycerol acyltransferase (DGAT), which are involved in a stepwise reaction. Nine *GPAT* genes (*GPAT1* to *GPAT9*), five *LPAT* genes (*LPAT1* to *LPAT5*), and two *DGAT* genes (*DGAT1* and *DGAT2*) have been identified and characterized in *Arabidopsis thaliana* (Yang *et al.*, 2010). Of these, *GPAT9, LPAT2, DGAT1*, and *DGAT2* from *A. thaliana* have been demonstrated to be involved in TAG accumulation using transgenic approaches and knockout mutants. In addition to *A. thaliana*, the role of these genes in increasing the oil content has also been elucidated in other oilseed crops. For instance, *LPAT2* of groundnut is known to enhance oil content in *A. thaliana* (Chen *et al.*, 2015); *LPAT2, LPAT3B*, and *LPATB* increase ricinoleic acid content in castor (Kim *et al.*, 2020). *GPAT9* is known to be involved in high oil accumulation in groundnut seeds (Lv *et al.*, 2020). Diverse GPAT genes and their homologues with variation in gene structure and function have been reported

[1]Department of Genetics & Plant Breeding, Coimbatore, Tamil Nadu
[2]RVS Agricultural College, Thanjavur, Tamil Nadu
[3]ICAR-Indian Institute of Oilseeds Research, Rajendranagar, Hyderabad-500 030, Telangana
*Corresponding author's E-mail: geethanjalitnau@yahoo.com

in a wide range of plant species (Waschburger *et al.*, 2018). The diversity in these genes and the distribution of their isoforms may affect the substrate selectivity and therefore the fatty acid composition and TAG accumulation in oilseeds (Kim *et al.,* 2020). Analysis of the evolutionary pattern and diversity of these genes can therefore help to improve our understanding of TAG biosynthesis in oilseed crops. With this aim, the present study was undertaken to explore the diversity of genes involved in Kennedy pathway across oilseed crops by using the sequence information of the model species, *A. thaliana*.

Recent significant improvements in DNA sequencing methodology have resulted in the accumulation of redundant sequence information for a single locus in biological information databases such as NCBI. There is no defined methodology available to ensure the genes that are obtained from the protein database for each of the target species are distinct and are paralogs, not protein isoforms of the same gene, to conduct appropriate diversity analysis. Efforts were made to counter the presence of multiple sequences of the same gene-origin by using several filters. The results were analysed and further improvements that need to be adopted to improve the analysis are discussed.

MATERIALS AND METHODS

Selection of oilseed crops: Thirteen species of oilseed crops belonging to different botanical families were considered for the present study (Table 1). An algal species, *Chlamydomonas reinhardtii* was included as an out-group.

Retrieval of protein sequences of the selected genes involved in the Kennedy pathway: Protein sequences of *AtDGAT1, AtDGAT2, AtGPAT9* and *AtLPAT2* in *A. thaliana* were downloaded in fasta format from https://www.arabidopsis.org.

Basic local alignment search: The non-redundant protein sequence database limited only to the listed species (Table 1) was queried independently with each of the above mentioned proteins using the blastp suite of the Basic Local Alignment Search Tool (BLAST) available at NCBI database (https://blast.ncbi.nlm.nih.gov). The search was performed using default settings.

Data reduction by filtering: Considering the large number of hits obtained for each of the query proteins, four successive filtering steps were carried out. The first-level filtering with an E-value cut-off of 10^{-9} was used to restrict the number of hits to a manageable number for the preliminary analysis. The second level of filtering involved selection of hits that were $\pm$10% of the query length. The third-level filtering required retaining only one of the many isoforms of a hit by manual curation. The fourth-level filtering involved detecting and retaining only one of the identical hits by using distance scores from pair-wise alignment of the hits. The entries retained in the final filtered list were considered for multiple sequence alignment.

Multiple sequence alignment and construction of phylogenetic tree: The sequences were multiple-aligned using the CLUSTALW algorithm with default settings available within MEGA X [Molecular Evolutionary Genetics Analysis] (Kumar *et al*., 2018) which included a gap opening penalty of 10 and gap extension penalty of 0.1 for pairwise alignment and a gap opening penalty of 10 and gap extension penalty of 0.2 for multiple sequence alignment. Pairwise distance was computed and the phylogram was constructed using the Neighbour-Joining method using default settings.

RESULTS AND DISCUSSION

A total of 214 protein sequences corresponding to the candidate genes *DGAT1* (62), *DGAT2* (41), *GPAT9* (28) and *LPAT2* (83) were retrieved from the database after filtering. The majority of the sequences in the analysis corresponded to rapeseed, soybean, and groundnut. In the final analysis, protein sequences from safflower for all the genes studied were lacking. However, it should be noted that the sequences found in this study are not exhaustive and some of the sequences could have been removed due to the filtering parameters used. For instance, there were two hits for *DGAT2* protein sequences from flax, but they were removed during the second level of filtering. Though stringent parameters were used to retrieve only the gene specific sequences, it is possible that the retrieved sequences may overlap with other genes. For example, *DGAT1* and *DGAT2* are paralogs, and all the selected genes are acyl transferases and therefore share structural homology. The number of protein sequences retrieved species-wise for each of the genes is provided in Table 2.

The phylogram of *DGAT1* consisted of five clusters (Fig. 1). Clusters I to IV comprised of dicot oilseed crops. Cluster I included oilseed crops of the Fabaceae family, namely groundnut and soybean forming distinct sub-clusters. Cluster II contained mostly Brassicaceae family members, such as mustard and rapeseed, as well as castor bean and linseed, which formed a separate sub-clade. Cluster III comprised of sesame belonging to the Pedaliaceae family. Cluster IV included sunflower of the Asteraceae family. Cluster V included the monocots, namely coconut and oil palm, belonging to the Palmaceae family.

The phylogram of *DGAT2* consisted of four clusters (Fig. 2). Clusters I, II, and III comprised of dicot oilseed crops, while cluster IV included the outgroup *C. reinhardtii.* Cluster I included oilseed crops of the Fabaceae family,

namely groundnut and soybean, similar to those observed in the phylogenetic tree of *DGAT1*. However, castor, which belongs to the Euphorbiaceae family, was grouped with sunflower and sesame in Cluster II, while mustard and rapeseed, belonging to the Brassicaceae family, were grouped separately in Cluster III. The phylogram did not consist of monocots as no hits of *DGAT2* for coconut or oil palm were obtained during the blast search.

The phylogram of *GPAT9* consisted of five clusters (Fig. 3). Cluster I included all the Brassicaceae oilseed crops. Cluster II included three oilseed crops, namely sunflower, sesame, and castor. Cluster III included oilseed crops of the Fabaceae family, namely groundnut and soybean. Cluster IV included coconut and oil palm belonging to the Palmaceae family. *C. reinhardtii* stood out distinctly as an out-group in Cluster V. The phylogram generated based on *DGAT2* and *GPAT9* showed a similar clustering pattern of the species studied.

The phylogram of *LPAT2* consisted of three major clusters (Fig. 4). Oil palm served as an out group. Cluster I indicated a mixed grouping of dicots along with a monocot and comprised of several sub-clusters of *Brassica* sp., sesame, castor, sunflower, and soybean. Cluster II was divided into three sub-clusters: sub-cluster I included members of the Fabaceae family, sub-cluster II included sunflower, sesame, and castor, and sub-cluster III included only *Brassica* spp. The polyphyletic oil palm accession evokes intrigue and requires further investigation.

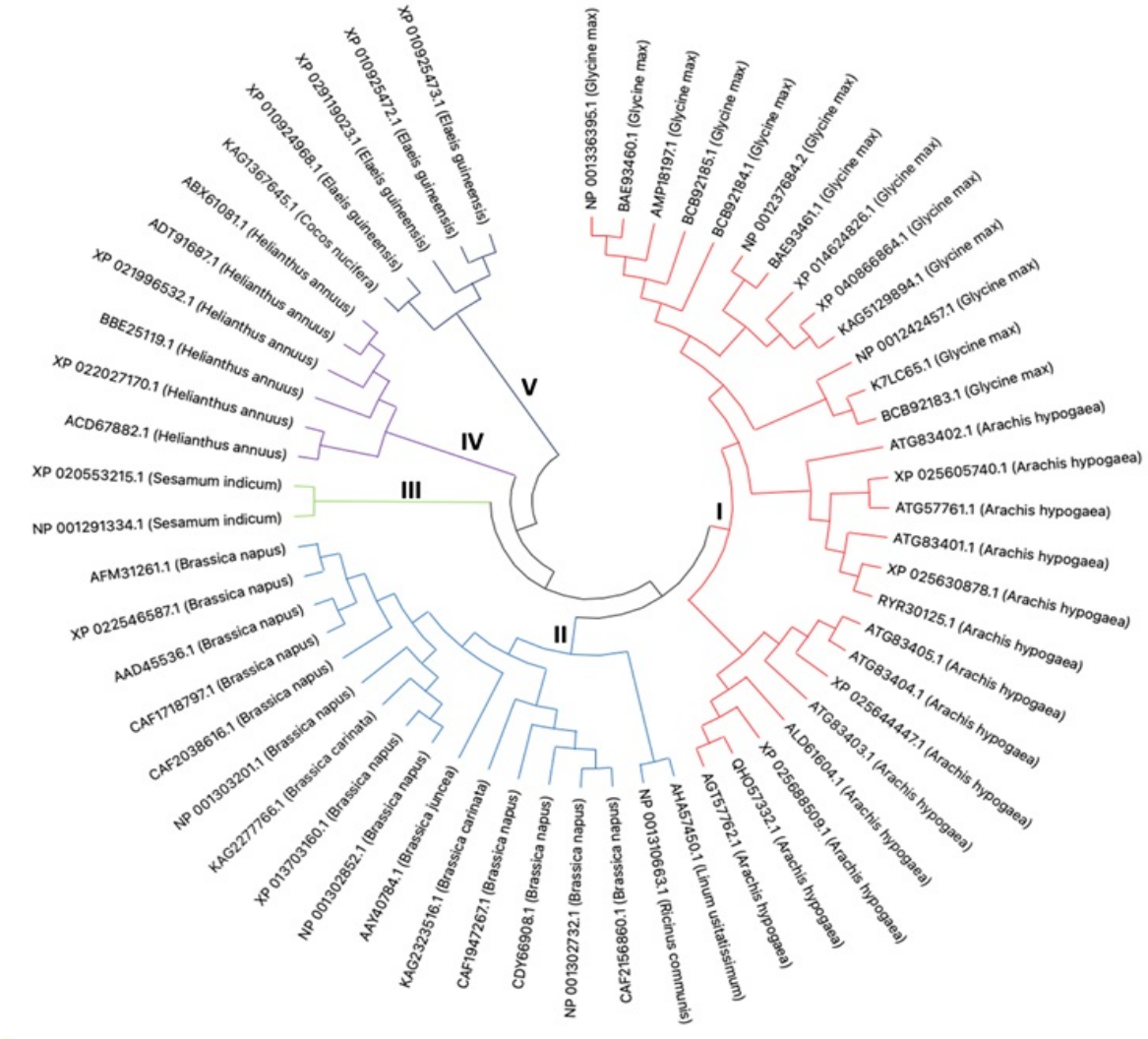

Fig. 1. Phylogram of *DGAT1* sequences in oilseed crops

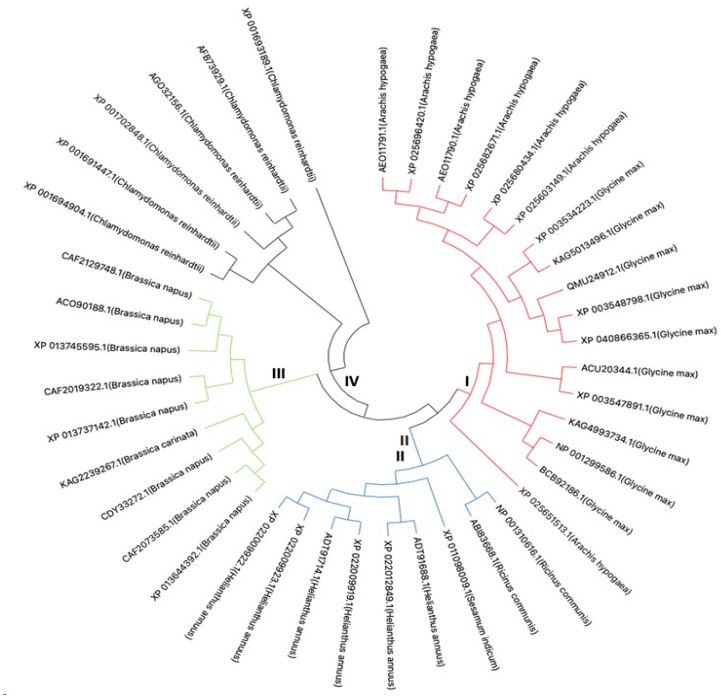

Fig. 2. Phylogram of *DGAT2* sequences in oilseed crops

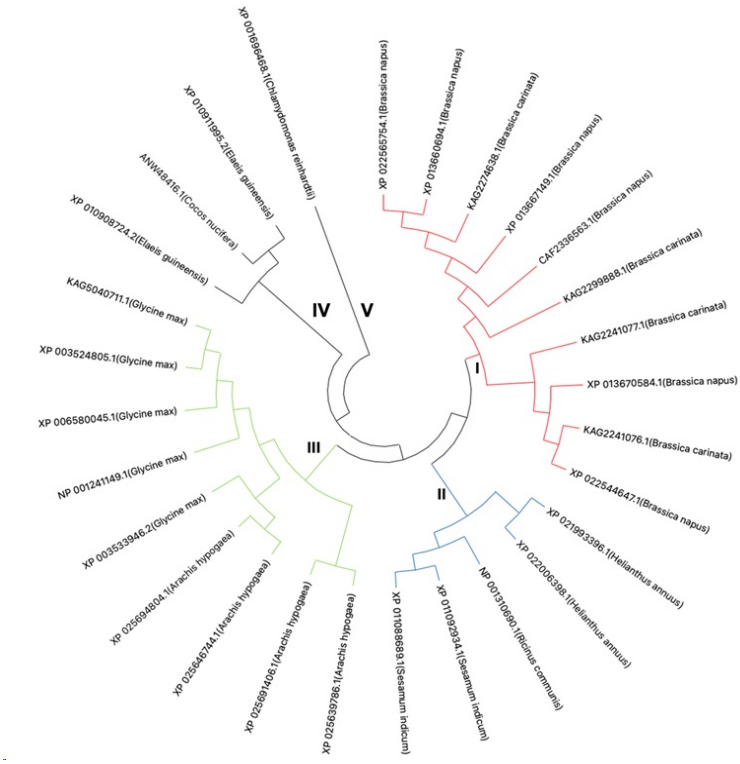

Fig. 3. Phylogram of *GPAT9* sequences in oilseed crops

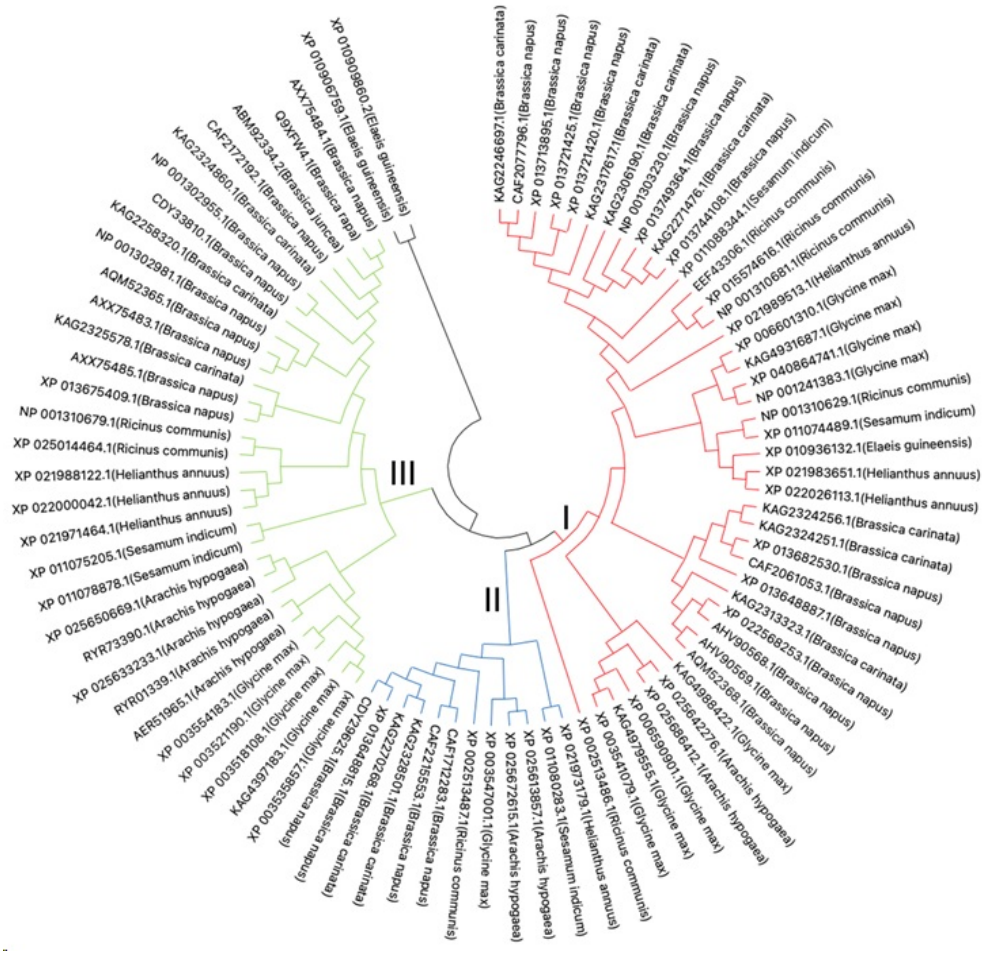

Fig. 4. Phylogram of *LPAT2* sequences in oilseed crops

Table 1 Details of oilseed crops included for the comparative sequence analysis of genes involved in triglyceride synthesis

Crop	Species	Family	Kingdom
Groundnut	*Arachis hypogaea L.*	Fabaceae	Dicots
Soybean	*Glycine max* L.	Fabaceae	Dicots
Sunflower	*Helianthus annuus* L.	Asteraceae	Dicots
Sesame	*Sesamum indicum* L.	Pedaliaceae	Dicots
Ethiopian mustard	*Brassica carinata* L.	Brassicaceae	Dicots
Indian mustard	*Brassica juncea* L.	Brassicaceae	Dicots
Rapeseed	*Brassica napus* L.	Brassicaceae	Dicots
Field mustard	*Brassica rapa* L.	Brassicaceae	Dicots
Castor bean	*Ricinus communis* L.	Euphorbiaceae	Dicots
Safflower	*Carthamus tinctorius* L.	Asteraceae	Dicots
Linseed	*Linum usitatissimum* L.	Linaceae	Dicots
Coconut	*Cocos nucifera* L.	Palmaceae	Monocots
Oil palm	*Elaeis guineensis* (Jacq)	Palmaceae	Monocots

Table 2 Number of protein sequences (filtered) of candidate genes retrieved from the database

Species	Genes			
	DGAT1	*DGAT2*	*GPAT9*	*LPAT2*
A. hypogaea	14	7	4	9
G. max	13	10	5	14
H. annuus	6	6	2	7
S. indicum	2	1	2	5
B. carinata	2	1	4	10
B. juncea	1	0	0	1
B. napus	12	8	6	25
B. rapa	0	0	0	1
R. communis	1	2	1	8
C. tinctorius	0	0	0	0
L. usitatissimum	1	0	0	0
C. nucifera	6	0	1	0
E. guineensis	4	0	2	3
C. reinhardtii	0	6	1	0
Total	62	41	28	83

Phylogenetic analysis revealed the evolutionary pattern and diversity of the genes involved in the Kennedy pathway across oilseed crops. The *DGAT1, DGAT2* and *GPAT9* sequences produced distinct species wise clusters with several distinct sub-clusters. However, the *LPAT2* sequences were distinct between dicots and monocots, but within dicots, no species-wise distinctness could be found. These results suggest that *DGAT1, DGAT2* and *GPAT9* were monophyletic, while *LPAT2* was polyphyletic. A monophyletic clade is a group of organisms/sequences with its descendants sharing the most common ancestor, while a polyphyletic clade is a group of unrelated organisms/sequences lacking a common ancestor. The monophyletic clades of *DGAT1, DGAT2* and *GPAT9* suggested the possibilities of: (1) the presence of multiple genes or copies or isoforms within each species, indicating the divergence and specificity of genes involved in oil biosynthesis in different species; and (2) gene duplication of *DGAT1, DGAT2* and *GPAT9* would have occurred after speciation. The polyphyletic clades of *LPAT2* indicated that gene duplication events could have occurred before the species had diverged. These hypotheses can be validated by mapping these sequence accessions onto chromosomes and by comparing the accessions in the paralog database. Phylogenetic analysis has been used to understand the diversity and evolutionary pattern of genes involved in the Kennedy pathway. Turchetto-Zolet *et al*. (2016) performed a phylogenetic analysis of *DGAT1, DGAT2, DGAT3* and *WS/DGAT* across 22 species, which revealed that these genes were very divergent and possibly had distinct origin in plants. Similarly, Waschburger *et al.* (2018) studied the phylogeny of GPATs across 39 species, including plants and algae and found that plant GPATs grouped into distinct clades, which were further supported by variations in gene structure and function. However, research on the characterization of genes involved in oil biosynthetic pathways in plants is limited, particularly in minor oilseed crops like safflower, linseed and niger. Developments in plant genomics provide opportunities for genome-wide discovery of diverse candidate genes and alleles of oil biosynthetic pathways. For instance, Brown *et al*. (2012) identified new candidate genes for increasing ricinoleic acid content in castor through tissue-specific transcriptome sequencing. Lv *et al*. (2020) studied allelic polymorphisms of *AhGPAT9* and reported elite haplotypes associated with high oil content in groundnut. With the availability of next generation sequencing technologies and reference genome sequences for oilseed crops (Wu *et al.,* 2021), novel alleles of diverse genes involved in the Kennedy pathway can be identified, which can be exploited for genetic improvement of oil content and quality in crops through molecular breeding approaches.

Our study has been limited in its sensitivity to capturing different protein paralogs of each gene in the species of interest. This is because of the lack of explicit information pertaining to the locus of origin of the protein sequence. The series of filters that we imposed to restrict protein isoforms might have led to the loss of true paralogs from the comparison. One way to overcome this limitation would be to manually curate the locus of origin of each protein sequence obtained as a blast hit. Efforts need to be put in to glean the locus of origin of those sequences for which the information is not explicitly provided. Analysis with such curated protein sequences would provide a better picture of

the specialization of genes involved in vegetative oil biosynthesis in oilseed crops. Nevertheless, our results do indicate lineage-level diversification of proteins involved in triglyceride biosynthesis in plants.

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Heterosis response and combining ability (variance and effect) analysis for seed yield and its components in Indian mustard (*Brassica juncea* L.) under timely sown condition

JAGDISH PRASAD CHAURASIYA*, MAHAK SINGH AND NAGESHWAR

Chandra Shekhar Azad University of Agriculture and Technology, Kanpur-208 002, Uttar Pradesh

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ABSTRACT

Diallel analysis excluding reciprocal crosses comprising of seven parents and their 21 specific hybrids was carried out to identify high heterotic combinations and their relationship in terms of general and specific combining ability (GCA and SCA) in Indian mustard. Highest economic heterosis for yield was observed in crosses namely; NRCHB-101 x Pusa M-21, Urvashi x Pusa Bold, NRCDR-2 x Urvashi, Maya x Pusa Bold, and Maya x NRCDR-2. The economic parents Urvashi, Pusa Bold, NRCHB-101, Pusa M-21, NRCDR-2, Maya and RGN-73 were the best parents for majority of the characters with Urvashi and Pusa Bold being the best economic parents for seed yield/plant and its contributing characters as their GCA and *per se* performances were high. Analysis of variance for GCA and SCA signified differences for majority of the characters. Ratio of GCA and SCA variances was below unity for majority of the characters except Days to 50% flowering and Days to maturity, which suggested superior role of non-additive genetic variance in the inheritance of these traits. The crosses namely Maya x NRCDR-2, Maya x Pusa Bold, NRCDR-2 x Urvashi, NRCHB-101 x Pusa M-21 and Urvashi x Pusa Bold showed high *per se* performance as well as SCA effects for seed yield and its contributing characters. The above best parents and best crosses could be used in hybridization and heterosis breeding respectively.

Keywords: *Brassica juncea,* Combining ability, Diallel, Indian mustard, GCA, Heterosis, SCA

Indian mustard (*Brassica juncea*) is a naturally autogamous species, and depending on the environmental condition and frequency of pollinating insect (bees) 5-30% out crossing is observed. Based on ploidy level, Indian mustard is an amphidiploid (2n=36), derived from interspecific cross between two diploid species i.e., *Brassica rapa* (2n=20) and *Brassica nigra* (2n=16) followed by natural chromosome doubling. Among the various oilseed crops grown globally, the estimated area, production and yield of rapeseed-mustard was 36.68 m.ha, 72.42 mt and1974 kg/ha, respectively, during 2017-18. Globally India accounts for 19.8% and 9.8% of the total acreage and production. In India the area, production, and productivity of rape seed and mustard was 6.07 m.ha, 7.92 mt and 1304 kg/ha during 2017-18 (Anonymous, 2018). During the same time, in Uttar Pradesh, the area, production and yield was 0.66 m.ha, 0.84 mt and 1080 kg/ha, respectively (Anonymous, 2017). The demand of edible oils is increasing very rapidly with increasing population and has been estimated to be 28.40 million tones for the year 2030 and 41.6 million tone for the year 2050 (Arvind Kumar, 2017; Chauhan *et al*., 2021). Seed yield, seed quality and other yield related parameters of brassica oilseed crop are the main focus of improvement (Singh, 2003; Saini, 2015; Kumar, 2017; Prajapati *et al.,* 2021; Durgeshwari *et al.,* 2021).

*Corresponding author's E-mail: jagdish4925@gmail.com

Diallel mating design has been extensively used in both self and cross pollinated species to understand the nature of gene action involved in the expression of quantitative traits. Several reports in past have appeared which indicate that diallel analysis is the quickest method to understand the genetic nature of quantitatively inherited traits and to ascertain the prepotency of parents. For the study of inheritance of quantitative characters and evaluation of various possible breeding procedures in heterosis phenomena, comprehensive study of combining ability is essential (Allard, 1960). In *Brassica*, combining ability studies emphasise the preponderance effect of GCA on yield and most of the yield components, indicating the importance of additive gene action (Singh, 2017). On the other hand, Saini, (2015) reviewed evidences for the presence of significant SCA effects for yield and yield components, indicating the importance of non-additive gene action. Singh *et al.* (2005) reported that non-additive genetic effects in addition to additive effects accounted for yield heterosis. Kumar *et al.* (2017) observed that high heterosis is the result of high sca effects. Therefore, this paper deals with estimation of relative importance of GCA and SCA variances and effects for yield and its components in Indian mustard.

MATERIALS AND METHODS

Seven morphologically diverse genotypes/varieties *viz.,* Maya, NRCDR-2, NRCHB-101, RGN-73, Pusa M-21,

Urvashi and Pusa Bold were crossed in half diallel mating design (without reciprocals). Evaluation of parents and F_1 (7 parents and 21 F_1s) was conducted in randomized complete block design with three replications and with the spacing of 45 cm between rows and 20 cm between plants at Oilseed Research Farm, Kalyanpur, C.S. Azad University of Agriculture and Technology, Kanpur (UP) during *rabi* 2016-2017. All the recommended agronomic practices were adopted for raising the crop. These genotypes/varieties were chosen on the basis of their contrast for days to 50% flowering, days to maturity, plant height (cm), number of primary branches/plant, number of secondary branches/plant, number of siliquae/plant, number of seeds/siliqua, 1000-seed weight (g), biological yield/plant (g), harvest index (%), oil content (%) and seed yield/plant (g). The mean data of each plot was used for statistical analysis. Oil content was estimated by NMR method. The combining ability analysis was done as per the procedure for Method 2, Model I as suggested by Griffing (1956).

RESULTS AND DISCUSSION

The analysis of variance (Table 1) revealed the presence of significant variance among the treatments for all the characters namely, days to 50% flowering, days to maturity, plant height, number of primary branches/plant, number of secondary branches/plant, number of siliquae/plant, number of seeds/siliquae, 1000-seed weight, biological yield/plant, harvest index, oil content, and seed yield/plant. Among the parents significant differences were observed for all the characters except biological yield while among the F_1s it was significant for all the traits except for number of secondary branches/plant, number of seed/siliqua and biological yield/plant. The differences were significant for all the characters revealed when parents and F_1s were compared. Similar observations have been reported by others in Indian mustard (Patel *et al.,* 2012; Arifullah, 2013).

Estimation of economic heterotic response: Economic heterosis observed among 21 F_1 crosses for twelve characters over respective economic parents has been summarized in Table 2. This indicated that no single cross was good for all the characters studied. One of the most important breeding objectives in Indian mustard was to breed early maturity varieties with lower values of days to flowering desirable. The expression of heterosis for days to 50% flowering ranged from -0.88 (NRCDR-2 x NRCHB-101) to -6.17 (RGN-73 x Urvashi). Out of twenty one crosses all cross combination showed negative and significant heterosis, except the crosses NRCDR-2 x NRCHB-101.Top five best cross combinations namely, RGN-73 x Urvashi, RGN-73 x Pusa Bold, Maya x Urvashi, Maya x Pusa M-21 and Maya x RGN-73. Parmar *et al*. (2004) also reported significant negative heterosis for days to 50 per cent flowering. The range of heterosis for days to maturity varied from -0.50 (NRCDR-2 x Pusa M-21) to -2.48 (NRCHB-101 x Urvashi), out of 21 crosses, 14 crosses showed negative and significant heterosis. Top five best cross combinations for this trait were NRCHB-101 x Urvashi, NRCHB-101 x RGN-73, Urvashi x Pusa Bold, RGN-73 x Urvashi and Maya x RGN-73.The same findings were also reported by Turi *et al.* (2006) and Dar *et al.* (2012). The range of heterosis for plant height varied from -0.92 (NRCDR-2 x Pusa M-21) to -4.07cm (Maya x NRCHB-101). Significant and negative heterosis for plant height is preferred. Out of the 21 crosses all were showing significant negative heterosis except crosses, NRCDR-2 x Pusa M-21. Top five best cross combinations were Maya x NRCHB-101, Maya x RGN-73, Maya x Pusa Bold, Maya x Urvashi, and NRCDR-2 x NRCHB-101. The same findings were also reported by Tyagi *et al.* (2000). The heterosis ranged for no. of primary branches/plant from 0.00 (Pusa M-21 x Urvashi) to 8.33 (Pusa M-21 x Pusa Bold). Out of 21 crosses none of the crosses showed positive and significant heterosis. (Table 2). The heterosis range for number of secondary branches/plant was from 0.00 (Maya x RGN-73) to 5.56 (Pusa -21 x Pusa Bold). The top best five cross combinations Maya x NRCDR-2, Maya x Pusa M-21, NRCHB-101 x Pusa M-21, RGN-73 x Pusa M-21 and Pusa M-21 x Pusa Bold showed positive and significant heterosis. Similar results were also reported by Singh *et al.* (2007) and Aher *et al.* (2009). The heterosis ranged for number of siliqua/plant from -0.48 (Maya x NRCHB-101) to 0.68 (Maya x NRCDR-2). Among the crosses none showed significant and positive heterosis for number of siliqua per plant. The heterosis ranged for number of seeds per siliqua from 0.00 (Maya x RGN-73) to 7.32 (RGN-73 x Pusa M-21). The best five cross combinations for the trait were RGN-73 x Pusa M- 21, RGN-73 x Pusa Bold, Pusa M-21 x Pusa Bold, NRCDR-2 x Pusa M-21 and NRCDR-2 x NRCHB-101. Similar results were also reported by Mahto and Haider (2004). The heterosis for 1000-seed weight (g) ranged from -3.10 (NRCDR-2 x RGN-73) to 13.33g (NRCHB-101 x Pusa Bold). Crosses NRCHB-101 x Pusa Bold, NRCDR-2 x Pusa Bold, Maya x Pusa Bold, RGN-73 x Pusa Bold and Urvashi x Pusa Bold exhibited positive and significant heterosis in F_1 generation over economic parent. Similar results were also reported by Meena *et al.* (2014) and Kulbe *et al.* (1998). The heterosis for biological yield per plant (g) ranged from -0.90 (RGN-73 x Urvashi) to 4.38 (Maya xNRCDR-2) g. Out of 21 crosses the 11 crosses were showed significant and positive heterosis and the top five crosses were Maya x NRCDR-2, Maya x NRCHB-101,

Maya x RGN-73, Maya x Pusa Bold and NRCDR-2 x Urvashi. Similar e findings were also reported by Tyagi et al. (2000). The heterosis range for harvest index (%) was -1.24 (NRCHB-101 x Urvashi) to 6.28% (Urvashi x Pusa Bold). Out of 21 crosses only four crosses namely, Urvashi x Pusa Bold, NRCDR-2 x Urvashi, Pusa M-21 x Pusa Bold and Maya x Urvashi exhibited positive and significant heterosis for harvest index. Similar findings have been reported earlier by Dholu *et al.* (2014). The heterosis range for oil content (%) was -0.37 (NRCDR-2 x Pusa Bold) to 4.72 % (Urvashi x Pusa Bold). Out of 21 crosses, 11 crosses exhibited significant and positive response for this trait. The top best five crosses for the trait were Urvashi x Pusa Bold, NRCDR-2 x NRCHB-101, Pusa M-21 x Urvashi, Maya x Pusa M-21 and RGN-73x Urvashi for these characters (Table 2). The same findings were also reported by Kazymanski *et al.* (1993) and Singh *et al.* (1996). The heterosis for seed yield per plant (g) ranged from -0.40 (Maya x NRCHB-101) to 9.56 (NRCHB-101 x Pusa M-21). Ten crosses exhibited desirable and significant heterosis while top best five crosses were NRCHB-101 x Pusa M-21, Urvashi x Pusa Bold, NRCDR-2 x Urvashi, Maya x Pusa Bold and Maya xNRCDR-2. Similar findings were also reported by Ranjeet *et al.* (2007) and Singh *et al.* (2009b).

Analysis of variance for combining ability: The analysis of variance for combing ability (Table 2) indicated that variance due to general combining ability (GCA) and specific combining ability (SCA) were significant. General combining ability (GCA) showed significant variability for all the characters as reported earlier by Vaghela *et al.* (2011) and specific combining ability showed highly significant differences among majority of characters except days to maturity, plant height, and biological yield/plant. The variance due to GCA was higher than that for SCA for characters namely days to 50% flowering, days to maturity, plant height, number of siliquae/plant and 1000 Seed weight, which meant that there was preponderance of additive gene action and therefore, progeny selection might be effective for the genetic improvement of such characters. The variance due to SCA was higher than the GCA variances for characters namely number of secondary branches/plant, number of seeds/siliquae, biological yield/plant, harvest index, oil content and seed yield/plant, which meant that there was preponderance of non-additive gene action (dominance and epistasis) and therefore, heterosis breeding may be rewarding for such characters. The results were in agreement with the studies of Rao and Gulati (2001) and Patel *et al.* (1993). The GCA and SCA variances were of equal magnitude for the character namely number of primary branches/plant; inferring additive and non-additive genes equally contributing for the expression of this character. In such conditions reciprocal recurrent selection may be resorted to for population improvement.

Estimation of General combining ability (GCA): The estimates GCA of the parents revealed (Table 3) that no single parent was the best general combiner for all the characters under study. The promising combiners based on *per se* performances and significant GCA effects were RGN-73 and Urvashi for Days to 50% flowering; Urvashi, RGN-73 and NRCHB 101 for days to maturity; Maya and NRCHB-101 for Plant height (cm); Pusa Bold and Pusa M-21 for number of primary branches/plant, Pusa M-21 for number of secondary branches/plant, Maya and NRCHB-101 for number of siliquae/plant, RGN-73 and Pusa M-21 for number of seeds/siliqua, Pusa Bold, NRCHB-101 and NRCDR-2 for 1000-seed weight, Maya for biological yield, Urvashi and Pusa Bold for Harvest index, Urvashi, Pusa M-21 and Maya for oil content and Urvashi and Pusa Bold for higher seed yield/plant. These results are in accordance with Singh *et al.* (2005), Sadanand *et al.* (2009), Patel *et al.* (2012) and Gami and Chauhan (2013). Urvashi and Pusa Bold appeared to be the best general combiners for majority of the characters. The parents discussed above had high general combining ability and fixable component of gene action (additive and additive x additive) type of epistasis, these could be successfully exploited for developing homozygous lines for each character through pedigree selection which later can be used as parents to produce heterotic hybrid. These parental lines can be utilized for producing the inter-mating population in order to get desirable recombinants in Indian mustard.

Estimation of specific combining ability (SCA): The results of SCA effects of hybrids revealed that none of the hybrids were consistently superior for all the characters. Analysis of specific combining ability is an important parameter for judging the specific combinations for exploiting it though heterosis breeding programme. The best specific cross combinations were selected based on their SCA effects. The specific combining ability effects and per se performance obtained from the analysis are presented in Table 4. A perusal of the results revealed that the F_1 crosses, Maya x Pusa M-21 and NRCHB-101 x Urvashi for days to 50% flowering, RGN-73 x Pusa Bold for number of primary branches/plant, Maya x NRCDR-2 and NRCDR-2 x NRCHB-101 for number of siliquae/plant, Maya x Pusa M-21 and NRCDR-2 x NRCHB-101 for Number of seeds/siliqua, Urvashi x Pusa Bold, Pusa M-21 x Pusa Bold, RGN-73 x Pusa Bold, NRCHB-101 x Pusa Bold, NRCDR-2 x Pusa Bold and Maya x Pusa Bold for1000- seed weight, Urvashi x Pusa Bold and NRCDR-2 x NRCHB-101 for oil content and Maya x NRCDR-2, Maya x Urvashi, Maya x

Pusa bold, NRCDR-2 x Urvashi, NRCHB-101 x Pusa M 21,Urvashi x Pusa Bold for seed yield/plant were the superior/best specific combiners. The findings are supported by the reports of earlier researchers (Dixit *et al.,* 2007; Yadav *et al.,* 2009; Vaghela *et al.,* 2011; Maurya *et al.,* 2012).

Table 1 ANOVA of Parent's vs F_1's for 12 characters in a 7 x 7 parental diallel cross of Indian mustard: mean sum of squares

Sources of variance	d.f.	Days to 50% flowering	Days to maturity	Plant height (cm)	No. of primary branches/ plant	No. of secondary branches/ plant	No. of siliquae/ plant	No. of seeds/ siliqua	1000-seed weight(g)	Biological yield/plant (g)	Harvest index (%)	Oil content (%)	Seed yield/ plant (g)
Replication	2	0.04	0.74	4.30	0.96	0.87	8.05	0.75	0.01	1.92	2.37	0.44	0.38
Treatments	27	4.08**	2.38**	9.27**	6.82**	2.06**	487.92**	1.87**	1.28**	7.56**	3.88**	3.52**	1.81**
Parents	6	5.41**	2.32*	19.21**	7.94**	2.98**	925.21**	2.41**	1.06**	6.38	2.48*	1.97**	0.71*
F_1s	20	2.88**	1.92*	6.13**	4.06**	0.45	303.87 **	0.94	1.17**	2.00	3.06**	2.01**	0.91**
Parents vs F_1s	1	20.00**	12.00**	12.44**	55.25**	28.67**	1545.14**	17.29**	4.96**	125.83**	28.91**	43.21**	26.38**
Error	54	0.79	0.92	2.67	0.82	0.82	35.78	0.58	0.02	3.77	0.82	0.43	0.16
Total	83	1.84	1.39	4.85	2.77	1.22	182.19	1.00	0.43	4.96	1.86	1.44	0.70

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

Table 2 ANOVA for combining ability and related statistics of 12 characters in a 7 x 7 parental diallel cross of F_1's in Indian mustard

Sources of variances	d.f.	Days to 50% flowering	Days to maturity	Plant height (cm)	No. of primary branches /plant	No. of secondary branches/ plant	No. of siliquae/ plant	No. of seeds/ siliqua	1000-seed weight (g)	Biological yield/plant (g)	Harvest index (%)	Oil content (%)	Seed yield/ plant (g)
GCA	6	4.22**	2.41**	12.38*	6.76**	1.02**	504.24**	1.27**	1.45**	3.79**	3.46**	1.86**	0.65*
SCA	21	0.54*	0.33	0.44	0.99**	0.59*	65.04**	0.44**	0.14**	2.16	0.68**	0.98**	0.59**
Error	54	0.26	0.31	0.89	0.27	0.27	11.93	0.19	0.01	1.26	0.27	0.14	0.05
σ^2gca		0.44	0.23	1.28	0.72	0.08	54.70	0.12	0.16	0.28	0.35	0.19	0.07
σ^2sca		0.28	0.02	-0.45	0.72	0.32	53.11	0.25	0.13	0.90	0.40	0.84	0.53
GPR		1.57	11.5	-2.44	1.00	0.25	1.02	0.48	1.23	0.31	0.87	0.22	0.13

*, ** Significant at 5% and 1% level, respectively; GCA = General combining ability, SCA = Specific combining ability,
GPR = Ratio of General and specific combining abilities

Table 3 Estimates of gca effects for 7 parents along with their mean performance for 12 characters

Parents	Days to 50% flowering		Days to maturity		Plant height (cm)		No. of primary branches/plant		No. of secondary branches/plant		No. of siliquae/plant	
	gca effect	Mean	gca effect	Mean	gca effect	Mean	gca effect	Mean	gca effect	Mean	gca effect	Mean
Maya	-0.02	73.33	0.15	133.33	-1.68**	172.00	-0.32*	9.00	0.30	17.66	12.33**	343.66
NRCDR-2	1.13**	75.66	0.56**	134.00	0.46	176.66	-0.14	9.33	0.00	17.00	-11.47**	288.00
NRCHB-101	0.58**	75.00	-0.55**	132.66	-0.72*	174.33	-0.77**	8.00	-0.32*	15.33	5.33**	331.00
RGN-73	-0.68**	72.33	-0.51**	132.33	-0.24	175.33	-0.99**	7.00	-0.03	17.00	-1.36	322.33
Pusa M-21	-0.20	73.00	0.67**	134.33	2.12**	180.33	0.45**	10.33	0.45**	18.00	-2.14*	320.00
Urvashi	-0.83**	72.00	-0.47**	132.00	0.23	176.33	0.19	10.00	-0.51	15.66	1.26	326.33
Pusa Bold	0.02	73.66	0.15	133.66	-0.16	176.00	1.59**	12.00	0.11	17.33	-3.95**	310.00
$\bar{X}^p$		73.56		133.81		175.85		9.38		16.85		320.18
SE (gi) ±	0.15		0.10		0.29		0.16		0.16		1.06	
SE (gi - gj) ±	0.24		0.26		0.44		0.24		0.24		1.62	

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

Table 3 (contd...)

No. of seeds / siliqua		1000-seed weight (g)		Biological yield / plant (g)		Harvest index (%)		Oil content (%)		Seed yield/plant (g)	
gca effect	Mean	gca effect	Mean	gca effect	Mean	gca effect	Mean	gca effect	Mean	gca effect	Mean
-0.46**	11.00	-0.17**	4.91	1.03**	55.66	-0.35*	26.08	0.25*	39.38	0.05	14.50
0.16	13.00	0.26**	5.63	0.67	55.00	-0.02	26.26	-0.31*	37.69	0.12	14.45
-0.28*	12.00	0.12**	5.40	0.00	53.43	-0.79**	25.97	-0.12	38.16	-0.28**	13.91
0.27*	13.33	-0.24**	4.81	-0.44	52.00	-0.23	26.78	-0.74**	37.65	-0.28**	13.92
0.60**	13.66	-0.41**	4.26	-0.70*	51.99	-0.27	26.28	-0.38**	39.36	-0.23**	13.65
-0.28*	12.33	-0.28**	4.86	-0.55	52.46	0.91**	28.29	0.59**	39.49	-0.28**	14.69
-0.02	12.66	0.73**	6.02	-0.01	53.96	0.76**	27.78	-0.05	38.82	0.35**	14.99
	12.56		5.12		53.50		26.77		38.65		14.30
0.13		0.02		0.34		0.16		0.11		0.07	
0.20		0.03		0.52		0.24		0.17		0.10	

Table 4 Estimate of sca effects and mean performance for 12 characters of 21 F_1's

Hybrid combinations	Days to 50 % flowering		Days to maturity		Plant height (cm)		No. of primary branches/plant		No. of secondary branches/plant		No. of siliquae/plant	
	sca effect	Mean	sca effect	Mean	sca effect	Mean	sca effect	Mean	sca effect	Mean	sca effect	Mean
Maya x NRCDR-2	0.16	74.00	-0.25	133.00	0.04	174.00	0.02	10.33	0.49	18.66	17.52**	346.00
Maya x NRCHB-101	-0.29	73.00	-0.14	132.00	0.22	173.00	0.31	10.00	0.49	18.33	-3.30	342.00
Maya x RGN-73	-0.03	72.00	-0.51	131.66	0.41	173.66	0.20	9.66	-0.14	18.00	-0.26	338.33
Maya x Pusa M-21	-1.18*	71.33	-0.36	133.00	-0.63	175.00	0.43	11.33	0.05	18.66	0.19	338.00
Maya x Urvashi	-0.21	71.66	0.12	132.33	-0.07	173.66	0.35	11.00	0.68	18.33	-0.89	340.33
Maya x Pusa Bold	0.27	73.00	0.16	133.00	-0.33	173.00	0.94*	13.00	0.05	18.33	4.00	340.00
NRCDR-2X NRCHB-101	0.56	75.00	-0.21	132.33	-0.59	174.33	0.46	10.33	0.45	18.00	18.52**	340.00
NRCDR-2x RGN-73	-0.84	72.33	-0.58*	132.00	-0.41	175.00	0.69*	10.33	0.16	18.00	1.89	316.66
NRCDR-2 x Pusa M-21	-0.01	73.66	-0.10	133.66	0.89*	178.66	0.57	11.66	0.01	18.33	-2.33	311.66
NRCDR-2 x Urvashi	-0.69	72.33	0.05	132.66	-0.56	175.33	0.17	11.00	0.64	18.00	-0.74	316.66
NRCDR-2x Pusa Bold	-0.55	73.33	0.42	133.66	-0.48	175.00	0.43	12.66	0.01	18.00	-1.52	310.66
NRCHB-101 x RGN-73	-0.29	72.33	-0.47	131.00	-0.22	174.00	0.31	9.33	0.82*	18.33	-1.93	329.66
NRCHB-101xPusa M-21	-0.44	72.66	-0.32	132.33	-0.59	176.00	0.54	11.00	0.68	18.66	3.19	334.00
NRCHB-101 x Urvashi	-1.14*	71.33	-0.51	131.00	0.30	175.00	0.13	10.33	0.64	17.66	-3.89	330.33
NRCHB-101xPusaBold	-0.66	72.66	-0.81*	131.33	-0.30	174.00	0.72*	12.33	0.68	18.33	2.00	331.00
RGN73 x PusaM-21	0.16	72.00	-0.03	132.66	-0.41	176.66	0.43	10.66	0.38	18.66	0.89	325.00
RGN-73 x Urvashi	-0.21	71.00	0.12	131.66	-0.85*	174.33	0.69*	10.66	0.34	17.66	1.81	329.33
RGN-73 x Pusa Bold	-0.73	71.33	-0.18	132.00	0.22	175.00	1.28*	12.66	0.05	18.00	2.70	325.00
Pusa M-21 x Urvashi	0.31	72.00	0.27	133.00	0.11	177.66	0.57	12.00	-0.14	17.66	1.93	328.66
Pusa M-21 xPusa Bold	-0.21	72.33	-0.36	133.00	-1.15*	176.00	0.17	13.00	0.56	19.00	2.81	324.33
Urvashi x Pusa Bold	0.08	72.00	-0.88*	131.33	-0.26	175.00	0.43	13.00	0.19	17.66	9.41**	334.33
$\bar{x}$		72.44		132.31		174.96		11.25		18.20		330.09
SE (sij) ±	0.46		0.49		0.84		0.46		0.46		3.09	
SE (sij - sik) ±	0.68		0.73		1.25		0.69		0.69		4.60	

Table 4 (contd...)

No. of seeds per siliqua		1000-seed weight (g)		Biological yield / plant (g)		Harvest index (%)		Oil content (%)		Seed yield / plant (g)	
sca effect	Mean	sca effect	Mean	sca effect	Mean	sca effect	Mean	sca effect	Mean	sca effect	Mean
-0.06	13.00	-0.01	5.63	0.77	58.10	1.12*	28.53	0.90**	40.73	0.75**	16.19
0.72*	13.33	0.12	5.62	0.74	57.40	-0.32	26.32	0.23	40.25	-0.11	14.93
0.50	13.66	-0.07	5.05	0.99	57.20	-0.24	26.97	0.38	39.78	0.02	15.06
0.83*	14.33	0.13	5.09	0.91	56.86	0.03	27.19	0.46	40.99	-0.09	15.01
0.39	13.00	-0.13	4.96	0.41	56.50	0.64	28.98	0.15	40.89	0.58*	16.18
0.46	13.33	0.51**	6.62	0.23	56.88	0.77	28.97	-0.08	40.00	0.61**	16.29
1.09*	14.33	-0.18*	5.76	-0.01	56.23	0.36	27.34	1.86**	41.32	0.00	15.11
-0.13	13.66	0.27**	5.84	0.82	56.67	0.15	27.68	-0.54	38.30	0.21	15.31
0.20	14.33	0.42**	5.82	0.61	56.20	0.01	27.50	0.44	40.41	0.10	15.26
0.43	13.66	0.19*	5.72	1.25	56.98	0.88	29.56	0.69	40.86	0.62**	16.29
-0.17	13.33	0.19*	6.74	0.51	56.79	0.45	28.99	-0.18	39.34	0.44*	16.18
-0.35	13.00	0.16*	5.59	1.13	56.31	0.31	27.08	-0.15	38.87	0.10	14.80
-0.02	13.66	0.17*	5.43	1.16	56.08	0.29	27.02	0.47	40.63	1.67**	16.42
0.20	13.00	0.11	5.50	0.79	55.86	0.03	27.94	0.08	40.45	0.13	15.40
-0.06	13.00	0.42**	6.83	0.60	55.21	-0.20	27.57	0.51	40.22	-0.20	15.13
0.43	14.66	-0.01	4.88	1.32	55.80	0.25	27.54	0.47	40.01	0.34	15.09
-0.02	13.33	-0.12	4.89	0.55	55.16	0.00	28.47	1.19**	40.94	0.54*	15.80
0.72	14.33	0.28**	6.32	0.66	55.82	0.62	28.95	0.15	39.24	0.35	15.68
-0.02	13.66	-0.08	4.77	0.14	54.50	0.50	28.93	0.16	41.04	-0.04	15.28
0.39	14.33	0.30**	6.17	0.31	55.20	0.86	29.15	0.61	40.83	0.32	15.71
-0.06	13.00	0.26**	6.26	0.96	56.01	0.60	30.07	0.92**	41.35	0.44*	16.34
	13.61		5.69		56.32		28.13		41.30		15.59
0.39		0.07		1.00		0.46		0.33		0.20	
0.58		0.10		1.49		0.69		0.50		0.30	

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

Table 5 Top ranking five economic crosses for seed yield/plant and its contributing characters

Cross combinations	sca effect	*per se* performance	Magnitude of gca effect	
			P1	P2
NRCHB-101 x Pusa M-21	1.67**	16.42	-0.28**	-0.23**
Urvashi x Pusa Bold	0.44**	16.34	-0.28**	0.35**
NRCDR-2 x Urvashi	0.62**	16.30	0.12**	-0.28**
Maya x Pusa Bold	0.61**	16.29	0.05**	0.35**
Maya x NRCDR-2	0.75**	16.19	0.05**	0.12**

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

Table 6 Estimate of heterosis over economic parent for 12 characters in 21 F_1's

Hybrid combinations	Days to 50 % flowering	Days to maturity	Plant height (cm)	No. of primary branches/plant	No. of secondary branches/plant	No. of siliquae per plant	No. of seeds/ siliqua	1000-seed weight (g)	Biological yield/plant (g)	Harvest index (%)	Oil content (%)	Seed yield/ plant (g)
Maya x NRCDR-2	-2.20*	-0.99	-3.51**	-13.89*	3.70**	0.68	-4.88**	-6.53**	4.38**	0.85	3.14*	8.03**
Maya x NRCHB-101	-3.52**	-1.74**	-4.07**	-16.67**	1.85*	-0.48	-2.44**	-6.64**	3.11**	-6.97**	1.92	-0.40
Maya x RGN-73	-4.85**	-1.99**	-3.70**	-19.44**	0.00	-1.55	0.00	-16.10**	2.77**	-4.69*	0.73	0.47
Maya x Pusa M-21	-5.73**	-0.99	-2.96**	-5.56	3.70**	-1.65	4.88**	-15.49**	2.15**	-3.89*	3.80**	0.11
Maya x Urvashi	-5.29**	-1.49*	-3.70**	-8.33	1.85*	-0.97	-4.88**	-17.70**	1.51*	2.44	3.54**	7.96**
Maya x Pusa Bold	-3.52**	-0.99	-4.07**	8.33	1.85*	-1.07	-2.44**	9.85**	2.18**	2.39	1.30	8.65**
NRCDR-2 x NRCHB-101	-0.88	-1.49*	-3.33**	-13.89*	0.00	-1.07	4.88**	-4.37**	1.13*	-3.38*	4.63**	0.80
NRCDR-2 x RGN-73	-4.41**	-1.74**	-2.96**	-13.89*	0.00	-7.86**	0.00	-3.10*	1.81*	-2.16	-3.02*	2.16
NRCDR-2 x Pusa M-21	-2.64**	-0.50	-0.92	-2.78	1.85*	-9.31**	4.88**	-3.43*	0.96	-2.79	2.33	1.82
NRCDR-2 x Urvashi	-4.41**	-1.24*	-2.77**	-8.33	0.00	-7.86**	0.00	-5.09**	2.37**	4.46*	3.48**	8.69**
NRCDR-2 x Pusa Bold	-3.08**	-0.50	-2.96**	5.56	0.00	-9.60**	-2.44**	11.84**	2.02**	2.47	-0.37	7.91**
NRCHB-101 x RGN-73	-4.41**	-2.48**	-3.51**	-22.22**	1.85*	-4.07**	-4.88**	-7.19**	1.16*	-4.29*	-1.56	-1.27
NRCHB-101 x Pusa M-21	-3.96**	-1.49*	-2.40**	-8.33	3.70**	-2.81	0.00	-9.90**	0.75	-4.51*	2.88*	9.56**
NRCHB-101 x Urvashi	-5.73**	-2.48**	-2.96**	-13.89*	-1.85*	-3.88**	-4.88**	-8.74**	0.35	-1.24	2.42	2.71
NRCHB-101 x Pusa Bold	-3.96**	-2.23**	-3.51**	2.78	1.85*	-3.69*	-4.88**	13.33**	0.98	-2.54	1.85	0.96
RGN-73 x Pusa M-21	-4.85**	-1.24*	-2.03**	-11.11	3.70**	-5.43**	7.32**	-18.97**	0.24	2.66	1.31	0.67
RGN-73 x Urvashi	-6.17**	-1.99**	-3.33**	-11.11	-1.85	-4.17**	-2.44*	-18.75**	-0.9s0	0.62	3.66**	5.40*
RGN-73 x Pusa Bold	-5.73**	-1.74**	-2.96**	5.56	0.00	-5.43**	4.88**	4.87**	0.29	2.33	-0.63	4.62*
Pusa M-21 x Urvashi	-4.85**	-0.99	-1.48**	0.00	-1.85*	-4.36**	0.00	-20.80**	-2.10**	2.24	3.92**	1.96
Pusa M-21 x Pusa Bold	-4.41	-0.99	-2.40**	8.33	5.56**	-5.36**	4.88**	2.38*	0.80	3.03*	3.39*	4.80*
Urvashi x Pusa Bold	-4.85	-2.23**	-2.96**	8.33	-1.85*	-2.72	-4.88**	3.87*	0.62	6.28*	4.72**	9.03**
SE(EP)=	0.72	0.78	1.33	0.73	0.73	4.88	0.62	0.11	1.58	0.74	0.53	0.32

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

A close examination of heterotic response of the crosses revealed that none of the crosses was consistently good for all the characters. The top ranking five economic crosses for seed yield/plant were NRCHB-101 x Pusa M-21, Urvashi x Pusa Bold, NRCDR-2 x Urvashi, Maya x Pusa Bold and Maya x NRCDR-2 which showed positive and significant economic heterosis for seed yield/plant. GCA effects revealed that the Urvashi and Pusa Bold having significant and positive GCA effects were found to be the best combiner for majority of the seed yield contributing characters, while SCA effects revealed that the top five specific combiners (Table 5) namely Maya x NRCDR-2, Maya x Urvashi, Maya x Pusa Bold, NRCDR-2 x Urvashi, NRCHB-101 x Pusa M 21, Urvashi x Pusa Bold were identified as superior specific combiners for majority of the seed yield contributing characters. These crosses can be exploited at commercial level.

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Response to spacing and weed management on sunflower (*Helianthus annuus* L.) productivity

N MEENA*, M MOHAMED YASSIN[1] AND M MOHAMED AMANULLAH[1]

AICRP on Sorghum, KVK, Chamarajanagar-571 127, UAS, Bengaluru, Karnataka

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ABSTRACT

A field trial was conducted to study the response of spacing and weed management practices on yield and yield attributes of sunflower during *kharif* seasons of 2016 and 2017 at Eastern block farm of Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu. Two years of field trial, revealed that yield attributes *viz*., head diameter, seed weight per head, number of seeds per head, 100 seed weight, volume weight and seed yield were higher under the spacing of 75 cm × 25cm. Hand weeding at 15 and 30 DAS recorded higher yield among the weed management practices.

Keywords: Spacing, Sunflower, Yield, Weed management

Sunflower is popularly known as "Surajmukhi" and it is one of the fastest growing oilseed crops in India. In the world, sunflower is the fourth largest oilseed crop after soybean, rapeseed and mustard. It is a photo and thermo-insensitive, short-duration, deep-rooted, drought-resistant, widely adaptable crop that offers assurance for its cultivation for boosting oilseed production (Sarkar *et al*., 2005; Langhi *et al.,* 2021). The per capita consumption of edible oil recommended by Indian Council of Medical Research (ICMR) is 20 g per day per head. By 2030 AD, India's cooking oil imports expected to grow at 3.4% per annum. It is essential to enhance the productivity of important oilseed crops through appropriate production technologies to meet the increasing future demand. There is increased domestic demand for vegetable oils and fats, at the rate of 6 per cent per annum, but our domestic production has been rising at just about 2 per cent per annum (Anonymous, 2016; Chauhan *et al.,* 2021). In India, the average yield of most of the oilseeds is extremely low compared to other countries of the world. In India, 90 per cent of requirement of sunflower oil is imported and only 10 per cent of the requirement is met by the domestic production (Anonymous, 2016). Under this economic pressure, there is a supreme need to increase the domestic production either by improving production through improved technologies or by introducing new hybrids with higher yield potential.

The most important factors causing the reduction in sunflower yield are heavy weed infestation and improper row spacing (Mohapatra *et al.,* 2020) Wide row spacing permits the weeds to exploit available resources judiciously. Row spacing plays an important role in determining yield and yield components. To sustain the productivity of sunflower, it is prime need to practice high density planting, systems with narrow and ultra-narrow spacing which will cover the soil canopy as early as possible compared to the conventional row widths. It helps in shading out weeds and reduces their competition with the crop and permit to operate the mechanical weeder in the rows due to the change in the row spacing. The study was designed with the objective to optimize the spacing to facilitate mechanised weeding in sunflower.

[1]Professor, TNAU, Coimbatore-641 003, Tamil Nadu
*Corresponding author's E-mail: meena3n@gmail.com

MATERIALS AND METHODS

A field trial was conducted during *kharif* seasons of 2016 and 2017 to study the response of spacing and weed management practices on yield and yield components of sunflower at Eastern block farm of Tamil Nadu Agricultural University, Coimbatore. The experiment was laid out with strip plot design and replicated thrice. The treatment comprised of five horizontal factors as plant spacing *viz.,* S1: 60cm×30cm (55,555 plants/ha), S2: 75cm×25cm (53,333 plants/ha), S3: 75cm×20cm (66,666 plants/ha), S4: 90cm×20cm (55,555 plants/ha) and S5: 90cm×15cm (74, 074 plants/ha). Five vertical factors as weed management practices like W1: (Pre-emergence herbicide pendimethalin at 1.0 kg/ha followed by hand weeding at 30DAS), W2: (Pre- emergence herbicide pendimethalin at 1.0 kg/ha followed by weeder used at 30DAS), W3: (two times weeder used at 15DAS and 30DAS), W4: (two times hand weeding at 15DAS and 30DAS) and W5: (weedy check). The soil of the experimental field was sandy clay loam in the texture. The sunflower hybrid, CO-2 was used as test genotype. Weed management treatment were imposed as par the schedule. The recommended fertilizer dose followed for sunflower was 60: 90: 60 kg NPK/ha. Half of the dose of N and K and full dose of P were applied as basal to all the treatments. The remaining N and K were top dressed at 30

DAS and earthing-up operation was done at 30 DAS. The crop was harvested at maturity stage observations on head diameter (cm), seed weight/head (g), number of seeds/head, 100 seed weight (g), volume weight (g/100 ml) and seed yield/net plot (kg) of each treatment was recorded. The computed data were subjected to statistical analysis as per the procedures given by (Gomez and Gomez, 1984). The treatment differences were worked out at five per cent probability level.

RESULTS AND DISCUSSION

Increase in yield attributes like head diameter (25.1cm and 25.7cm), seed weight/head (87.9g and 88.3g), number of seeds/head (1357 and 1365), 100 seed weight (5.80g and 5.87g) and seed volume weight (34.8g and 37.1g) was recorded with spacing 75cm × 25cm which was on par with the spacing of 60cm × 30cm (Tables 1 & 2) during both the years of *kharif* 2016-17 and 2017-18. Decreasing trend was observed in yield attributes with the spacing of 90cm×15cm. Significant increase in the yield components with spacing of 75cm × 25cm might be due to relatively less interplant competition due to wider space and better plant growth in terms of stem girth and root volume.

Adequate supply of nutrients, more growth, higher Dry Matter Production (DMP)/plant enabled more translocation of photosynthates for development of sink under wider spacing (Sandhi *et al*., 2014). Lower head diameter recorded in 90cm × 15cm spacing. This might be due to poor availability of nutrients for growth and more competition for moisture, light, nutrients and space. Our results are in agreement with Goksoy *et al.* (2002), who reported reduced head diameter with increasing plant population.

The production of flowers, the subsequent development into seeds and their retention in the plants are controlled by spacing. Different spacing had pronounced significant effect on seed weight per head in *kharif* seasons of both the years. The spacing of 75cm × 25cm and 60cm x 30cm recorded significantly increased seed weight/head among all the spacings. This is attributed to the competition free growing environment which enabled more production of food reserve which was effectively translocated to the seeds. It is also attributed to the availability of more sun light and higher light interception by the actively spreading and horizontally developed plant, which could produce higher photosynthates. This result is in conformity with the findings of Soomro *et al*. (2015).

A significant influence due to spacing was noticed with regard to number of seeds/head. During all the seasons of the experimentation, higher number of seeds per head was observed under 75cm × 25cm and 60cm x 30cm spacings. This might be due to higher nutrient availability and more light interception under wider spacing, which lead to higher number of seeds/head. Effective transfer of photosynthates from source to sink at wider spacing also might have increased number of seeds per headas reported by Yasin *et al*. (2013).

A favorable effect due to different spacings was also observed on 100 seed weight. Among the spacing, 75cm × 25cm and 60cm × 30cm registered higher 100 seed weight during both the seasons. This might be due to beneficial effect of optimum plant spacing that would have enhanced the growth due to less competition and more availability of nutrients and these nutrients might have translocated efficiently from source to sink, which led to increased seed weight (Bindra and Kharwara, 1992).

Significant variation in seed volume weight was observed due to different spacings in both the seasons of experimentation. The spacing 75cm × 25cm and 60cm x 30cm recorded higher seed volume weight compared to other spacings. High seed weight also favoured high seed volume weight. Similar view was expressed by Vahid *et al.* (2014).

Lesser weed competition during critical period of crop growth showed positive response with yield attributing characters. Hand weeding at 15 and 30 DAS recorded higher head diameter (26.7cm and 27.2cm), seed weight per head (87.2g and 87.6g) , number of seeds/head (1383 and 1395), 100 seed weight (5.70g and 5.76g) and seed volume weight (33.6g and 36.4g) and it was on par with pre emergence application of pendimethalin at 1.0 kg/ha + power weeder weeding at 30 DAS and pre emergence application of pendimethalin at 1.0 kg/ha + hand weeding at 30 DAS during the *kharif* seasons of 2016-17 and 2017-18. Inuganti *et al.* (2021) reported that the number of seeds/head is typically the most affected yield component by various biotic and abiotic stresses, which ultimately reflects in lower sunflower yield. Superior yield attributes might be due to favourable growing environment throughout the crop growth by proper weed check and lowering down the competition due to weeds which might have helped the sunflower crop to utilize the available resources to the maximum extent, which reflected on higher growth and yield attributing characters. Similar findings were reported by Sumathi *et al.* (2010) in sunflower. Increased yield attributes were recorded due to high conversion of source to sink due to increased translocation due to optimum growing condition.

Different spacings exerted significant influence on the seed yield of sunflower. The seed yield was significantly higher under spacing 75cm × 25cm and the increase in the seed yield over 60 cm x 30 cm was higher during both the seasons. Even though the 60 cm x 30 cm registered higher values for most of the growth and yield attributing characters, because of lesser plant population/unit area it could not compensate the seed yield obtained in 75cm x 25cm (S2) under increased row spacing.

Table 1 Effect of spacing and weed management practices on seed yield (kg/ha) of sunflower

Treatment	2016					
	W1	W2	W3	W4	W5	Mean
S1	1958	1328	1103	2072	969	1486
S2	1895	1922	1721	1997	909	1689
S3	1805	1842	1650	1886	932	1623
S4	1456	1487	1348	1551	736	1316
S5	1648	1669	1538	1728	803	1477
Mean	1752	1650	1472	1847	870	
	S	W	S at W	W at S		
S. Ed (±)	50	53	86	88		
CD(P=0.05)	122	129	191	196		
Treatment	2017					
	W1	W2	W3	W4	W5	Mean
S1	2201	1488	1272	2225	981	1633
S2	2134	2145	1878	2170	927	1851
S3	2014	2021	1801	2076	953	1773
S4	1633	1668	1513	1699	767	1456
S5	1829	1846	1683	1861	820	1608
Mean	1962	1834	1629	2006	890	
	S	W	S at W	W at S		
SEd (±)	55	58	95	97		
CD (P=0.05)	134	141	211	215		

Table 2 Effect of spacing and weed management practices on yield attributes of sunflower

Treatment	Head diameter (cm)		Seed number per head		Seed weight per head (g)		100 seed weight (g)		Seed volume weight (g/100ml	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
S1	24.2	24.7	1315	1324	82.1	82.4	5.36	5.41	32.8	35.1
S2	25.1	25.7	1357	1365	87.9	88.3	5.80	5.87	34.8	37.1
S3	22.1	22.4	1190	1220	69.1	69.5	4.80	4.84	30.2	32.1
S4	23.0	23.3	1238	1255	74.6	74.9	5.20	5.25	31.1	33.2
S5	21.1	21.4	1050	1062	57.9	58.2	4.50	4.54	27.4	29.2
S. Ed (±)	0.7	0.7	39	40	2.4	2.5	0.16	0.16	1.0	1.1
C.D (P=0.05)	1.8	1.8	96	98	6.0	6.0	0.39	0.40	2.4	2.6
W1	24.3	24.7	1336	1363	78.0	78.4	5.04	5.09	31.6	34.1
W2	25.5	25.9	1355	1378	82.2	82.6	5.32	5.37	32.5	35.2
W3	21.5	21.9	1213	1235	73.2	73.5	5.02	5.07	31.0	33.3
W4	26.7	27.2	1383	1395	87.2	87.6	5.70	5.76	33.6	36.4
W5	17.6	17.8	864	856	51.0	51.3	4.58	4.61	27.7	27.7
SEd (±)	0.8	0.8	41	42	2.5	2.5	0.17	0.18	1.0	1.1
CD (P=0.05)	1.9	1.9	101	102	6.1	6.1	0.43	0.43	2.6	2.7

The increased yield in 75cm × 25cm might be due to increased row spacing of 75cm which is also optimum spacing for sunflower which contributed to increased head diameter and more number of seeds/head and seed volume weight as compared to 60 cm x 30 cm. Sunflower planted under intra-row spacings between 25cm and 30cm recorded higher seed yield as revealed by Ali *et al.* (2011) and Rauf *et al*. (2012) .The lowest yield was recorded under 90 x 20 cm which might be due to narrow intra row plant spacing that resulted in competition between plants for available resources like light, moisture and nutrient which led in the reduction of sunflower yield.

Weed management practices exerted significant influence on the seed yield of sunflower. The level of weed infestation directly affects the intensity of competitive relationship between sunflower crop and weeds. Lower seed yield of 870 and 890 kg/ha was obtained under weedy check due to heavy infestation of weeds during *kharif* seasons of 2016-17 and 2017-18. This resulted in 52.89 and 55.63 per cent reduction, respectively in seed yield during both the seasons, when compared to hand weeding at 15 and 30 DAS and per cent reduction of 50.34 and 54.63 during *kharif* seasons of 2016-17 and 2017-18, compared to pendimethalin at 1.0 kg/ha as pre emergence + HW on 30 DAS. Weeds compete with crop for light, nutrients and water. So, the crop under weedy check could not obtain the above in optimum quantity thereby there was a reduction in leaf area, dry matter production and number of leaves. This would have reflected in poor yield attributes and finally poor yield under weedy check. The findings are in line with the results of Bhondve *et al*. (2009) who had reported that presence of weeds throughout the growing season caused poor crop growth and yield reduction in weedy check.

Favourable crop growth environment with a minimum disturbance due to biotic factors like lesser weed competition influence the crop yield by increasing the yield attributes. Among the weed management practices, hand weeding at 15 and 30 DAS recorded higher seed yield (1847 and 2006 kg/ha during the *kharif* seasons of 2016-17 and 2017-18). The competition free environment increased the capacity of source and sink in turn producing good growth and physiological attributes coupled with yield attributes like head diameter, number of seeds/head, 100-seed weight as a result of lower weed density and dry weight resulting for higher WCE might be the reason for increased productivity. Choudhary *et al.* (2012) reported that weed free plot recorded 68.7 % higher seed yield. Similarly Yadav *et al.* (2011) stated that weed free check gave maximum seed yield of 1840 kg /ha as against 518 kg/ha under un-weeded control due to weed free competition free growing condition. The next best treatment was pendimethalin at 1.0 kg/ha as pre emergence + HW on 30 DAS with the seed yields of 1752 and 1962 kg/ha during *kharif* seasons of 2016-17 and 2017-18. These results are in accordance with the findings of Choudhary *et al.* (2012) who reported that application of pendimethalin at 1.5 lit/ha and one hand weeding at 25 DAS increase the crop yield due to weed free environment.

Interaction between spacing and weed management was positive. The treatment combination of spacing 60cm × 30cm coupled with hand weeding twice at 15 and 30 DAS (S1W4) recorded higher seed yield during both the seasons of experimentation. These results are in conformity with the finding of Asaduzzaman *et al.* (2010) in black gram that spacing 30cm × 10cm along with hand weeding twice at 25 and 40 DAS recorded higher yield. Lower seed yield was reported under the combination of spacing 90cm × 20cm coupled with weedy check (S4W5) due to wider row spacing which increased the weed density which caused less availability of resources for growth and yield.

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Photosynthetic characteristics in wild, cultivated species and interspecific inbred lines of safflower

KAMMILI ANJANI, PASALA RATNAKUMAR* AND S N SUDHAKARA BABU

ICAR-Indian Institute of Oilseeds Research, Rajendranagar, Hyderabad-500 030, Telangana

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ABSTRACT

Safflower (*Carthamus tinctorius* L.), a multipurpose oilseed crop is the only cultivated species in the Carthamus genus. *Carthamus* spp. have been explored for resistance to biotic and abiotic stresses but not for physiological efficiency. Photosynthetic traits of ten wild and ten cultivated species and six interspecific inbred lines were studied to understand the expression of the photosynthetic traits among them, and for trait introgression from wild species in interspecific derivatives. Relations among some physiological traits in interspecific inbred lines differed from those observed in wild and cultivated species. The high photosynthesis (Pn), low transpiration (E) and high intrinsic water use efficiency (iWUE) could be introgressed from the wild species, *C. lanatus* and *C. turkesthanicus* into safflower cultivars. Inheritance of photosynthetic traits from wild to cultivated species indicated that wild species are exploitable for safflower improvement. This study suggests the utilization of wild species for their high Pn, low E and iWUE characteristics for developing abiotic stress-tolerant safflower cultivars.

Keywords: Photosynthesis, Safflower, Wild species, intrinsic Water Use Efficiency

Safflower (*Carthamus tinctorius* L.) is the only cultivated species in the genus Carthamus. Kazakhstan, Russian Federation, the USA, Mexico, Turkey, India and China are the other major safflower producing countries (http://faostat3.fao.org as of 15 March 2019). Safflower is used for many purposes such as edible oil, biofuel, bird feed, pharmaceutical applications, dye-making, food colouring and flavouring, etc (Neelima *et al.*, 2021). Crop improvement in safflower has been targeted for incorporating quantitative agronomic traits and has met with incremental success. This approach is reaching saturation in terms of exploitable genetic variability. Assessing the basic physiological process of photosynthetic parameters is a grey area with huge opportunities for carbon fixation ultimately resulting in crop's productivity improvement. Further increase in crop yield will depend largely on increasing photosynthesis. Elevated CO_2 research has shown a remarkably close relation between yield and photosynthesis (Long *et al.*, 2006). Literature suggests that the plant with lower canopy temperature (CT) gives higher yield as reported in other studies (Beebe *et al.*, 2013; Pandey *et al.*, 2021; Sravanthi *et al.,* 2021) and leaf temperature is considered as an important trait for selection of high yielding lines (Ainsworth and Rogers, 2007). Cooler canopy temperatures are the consequence of transpiration (Ratnakumar *et al.*, 2009) and varies depending on soil water availability. The importance of evaporative cooling is a major component of the leaf energy balance, and leaf temperature can be used as an indicator for the rates of water loss or stomatal opening. Also, a lowered CT and increased stomatal conductance ensure higher CO_2 inside the leaf and in turn higher carbon fixation efficiency (Pandey *et al.*, 2021; Sravanthi *et al.*, 2021). Similar to this, water use efficiency (WUE) is defined as the amount of carbon assimilated as biomass or grain produced per unit of water used by the crop and at the leaf level it is directly related to the physiological processes controlling the gradients of CO_2 and H_2O. The effect of increasing CO_2 on photosynthetic rate (Pn) and WUE is normally positive as the difference between the ambient air and the intercellular spaces is increased and under light, CO_2 within the leaf is rapidly converted to carbohydrates.

Wild species of crops are the sources of novel genes and are widely used for improving crop species. Approximately 25 species have been reported in the *Carthamus* genus. The wild species grow naturally from northwestern India westwards to and around the Mediterranean Sea (Knowles, 1980). Natural and artificial hybridization of safflower with wild species has been reported (Ashri and Knowles, 1960; Schank and Knowles, 1964; Ashri and Rudich, 1965; Heaton and Klisiewicz, 1981; Mayerhofer *et al.,* 2011). Living materials of only 13-15 *Carthamus* species are available in various gene banks. Six wild Carthamus spp. *viz., C. oxyacantha, C. palaestinus, C. glaucus, C. creticus, C. lanatus* and *C. turkesthanicus* are maintained at ICAR-Indian Institute of Oilseeds Research (ICAR-IIOR), Hyderabad, India. The wild species, *C. oxyacantha* was collected through explorations in India (Anjani *et al.,* 1999) and the remaining species were introduced from the USDA, USA through ICAR-National Bureau of Plant Genetic Resources, New Delhi, India. The wild species were

*Corresponding author's E-mail: ratna.pasala@icar.gov.in

self-pollinated for several years to bring genetic uniformity within each species. Some of the Carthamus wild species were identified as the source of resistance to biotic and abiotic stresses of safflower (Bassiri and Sionit, 1975; Pallavi *et al.,* 2007; Prasad and Anjani, 2008; Sabzalian *et al.,* 2010; Majidi *et al.,* 2011) and were used for improving safflower for disease resistance (Anjani *et al.,* 2007; Pallavi *et al.,* 2007, Prasad and Anjani, 2008). One of the wild species, *C. oxyacantha* was the donor of sterile cytoplasm for developing cytoplasmic-genic male sterility system in safflower (Anjani, 2008). Wild species have been also widely utilized in several crop species for improving resistance to biotic and abiotic stresses as well as quality and yield traits. They have also been identified as potential sources of physiological traits for crop improvement (Masumoto *et al.,* 2004; Rajesh *et al.,* 2016). With no information on physiological characteristics of safflower wild species, the present study was undertaken to assess the photosynthetic traits in wild and cultivated species and interspecific inbred lines; and their inheritance into cultivated species were also assessed.

MATERIALS AND METHODS

Plant material and experimental set up: Ten accessions of six wild species *viz., C. oxyacantha* (2n=24), *C. palaestinus* (2n=24), *C. glaucus* (2n=20), *C. creticus* (2n=64), *C. lanatus* (2n=44) and *C. turkesthanicus* (2n=64), three cultivated varieties *viz.,* A1, 'Nira', and 'Manjira' and seven genotypes namely, 96-506-1-21, SFS-9990, JSI-116, PI 537598, EC 543318, 1831-P8 and GMU-2484 belonging to cultivated species, *C. tinctorius* (2n=24) and six inbred lines derived from crosses between *C. tinctorius* (A1, 'Manjira' and 'Nira') and four wild species *viz., C. oxyacantha, C. palaestinus, C. glaucus* and *C. turkesthanicus* were planted at research farm of ICAR-Indian Institute of Oilseeds Research (ICAR-IIOR), at ICRISAT, Patancheru, Hyderabad, India (17.366°N and 78.478°E) during the post rainy winter season (October) in the year 2010. The experimental materials were grouped into cultivated species, wild species and interspecific inbred lines for convenience of comparison of results between the groups. Each experimental material was grown in two rows of 5 m length in six replications following the complete randomized block design (CRBD). The spacing between rows was 45 cm and between plants was 20 cm. The soil of experimental plot was Vertisols; fertilizers were applied as per the recommendation and need based plant protection measures were adopted. The crop was raised under receding soil moisture as normal practice and no irrigation was provided. The maximum temperature during crop period (October 2010- February 2011) has ranged from 28-35.6°C and the minimum was between 11.2-22.6°C. The relative humidity was between 91 and 97%, and the total rainfall received during the crop period was 204 mm.

The hybrid nature of the six interspecific inbred lines used in the present study was primarily confirmed through examining the morphological traits in F_1 generation. These traits were intermediate to both wild and cultivated species confirming introgression of genome from both parents in F_1 background. The hybrid nature of F_1 of each cross was further confirmed through genotyping of wild and cultivated species parents and F_1 using SSR markers (Anjani *et al*., 2011; Anjani *et al*., 2018).

Gas exchange parameters: Physiological parameters such as net photosynthesis (Pn, μ mol/m^2/s), stomatal conductance (gs, mol/m^2/s), leaf intercellular CO_2 concentration (Ci, μ mol CO_2/mol), transpiration (E, m mol/m^2/s) were recorded at full flowering stage using portable IRGA i.e., photosynthesis system (LICOR-6400-40 Fluorometer) and intrinsic water use efficiency (iWUE, μ mol/m^2/s/m mol/m^2/s^1) was calculated as ratio of Pn to E and carboxylation efficiency was calculated as ratio of Pn to Ci. Gas exchange measurements were made between 11:00 and 13:00h of IST on cloud free sunny days using six replicates for each test material at 45 days after planting. For measuring Pn, gs, Ci and E, photosynthetic leaf chamber (2 inch) was clipped onto fully expanded leaf, which had been exposed to sunlight. The chamber was held in such an angle that the enclosed leaf surface faced the sun, to avoid the shading inside the curette. Photosynthetic active radiation (PARi) at the upper surface of the leaf chamber was measured by calibrated sensor mounted on the same surface of the leaf chamber. It varied between 1150 and 1350 μ mol/m^2/s during the most Pn measurements. Atmospheric CO_2 was 400ppm, ambient temperature was 34 to 36°C and RH was 55 to 60% recorded during gas exchange measurements.

Statistical analysis: The aggregate mean values were subjected to statistical analysis. The essential statistics like variance and coefficient of variation were calculated using the method described by Panse and Sukhatme (1964). The analysis of variance was analysed using complete randomized block design (CRBD) to understand the significance levels. The Pearson correlation coefficients in Carthamus wild species, cultivated species (*C. tinctorius*), and interspecific inbred lines was also estimated. The diagonal histogram and scatter plots of bivariate correlation were developed using R statistical programming language (R-3.5.2 win) in an integrated development environment (RStudio-1.0.143). The simple graphical representation of mean values was developed using MS Office Excel 2007 programme (www.microsoft.com) where Carthamus wild species (W), cultivated species, *C. tinctorius* (C) was taken

on X-axis and photosynthetic traits such as Pn: net photosynthesis; gs: stomatal conductance; Ci: leaf intracellular CO_2 concentration; E: transpiration; iWUE: intrinsic water use efficiency; Pn/Ci: carboxylation efficiency were taken on Y-axis.

RESULTS AND DISCUSSION

Gas exchange traits: The *Carthamus* wild species, cultivated species (*C. tinctorius*), and interspecific inbred lines were evaluated for photosynthetic traits and analysis of variance indicated significant variation for gs, Pn, and Pn/Ci (Table 1). The mean data on various photosynthetic parameters observed in wild and cultivated species and interspecific inbred lines are illustrated in Table 2 and in Figure 1. The Pn ranged from 55.68±0.75 to 65.48±1.55 µ mol/m^2/s^1 among wild species with a mean of 62.17 µ mol/m^2/s^1 and 39±0.63 to 51.17±0.13 µ mol/m^2/s^1 among cultivated species genotypes with a mean of 46.08 µ mol/m^2/s^1. Among interspecific inbred lines, Pn was between 47.50±0.49 and 60.44±0.62 µ mol/m^2/s^1 with a mean of 54.09 µ mol/m^2/s1. The per se performance of Pn was higher in wild species followed by interspecific lines, indicating that the trait might have been inherited from wild in these interspecific lines. Furthermore, stomatal conductance (gs) was between 0.12±0.02 and 0.38±0.09 mol/m^2/s^1 among wild species with a mean of 0.25 mol/m^2/s^1 and between 0.08±0.01 and 0.18±0.03 mol/m^2/s^1 among cultivated species with a mean of 0.13 mol/m^2/s^1. In case of interspecific inbred lines, gs ranged from 0.04±0.01 to 0.18±0.04 mol/m^2/s^1 with a mean 0.12 mol/m^2/s^1. The Transpiration rate (E) ranged from 5.68±0.56 to 12.81±1.42 µ mol/m^2/s^1 among wild species and 4.89±0.58 to 12.03±1.03 µ mol/m^2/s^1 among cultivated species genotypes while it was between 2.71±0.56 and 8.46±1.53 µ mol/m^2/s^1 among interspecific inbred lines. The mean E in interspecific inbred lines group was 6.61 m mol/m^2/s^1 while it was 9.43 m mol/m^2/s^1 and 7.07 m mol/m^2/s^1 in wild and cultivated species groups, respectively. Similar to Pn, a higher gs and E were observed among the wild species; however in cultivated and interspecific lines it was almost similar suggesting that gs and E were not inherited characters from wild in the interspecific lines. Another important photosynthesis related trait, leaf intracellular CO_2 was found higher in wild (321.56 µ mol/m^2/s^1) and cultivated (339.71µ mol/m^2/s^1) groups as compared to that in interspecific inbred lines (168.9 µ mol/m^2/s^1), indicating that wild species maintained a higher intracellular CO_2 which was due to higher gs and E. Together by maintaining a higher gs, E and intracellular CO_2, wild species ensured higher Pn rates compared to interspecific lines and cultivated species. On the other hand, lower gs among the cultivated species might be due to stomatal limitation. The mean carboxylation efficiency of interspecific inbred lines (0.38) was higher than that of wild (0.20) and cultivated species (0.14). The iWUE ranged from 4.90±0.62 to 10.26±1.2 µ mol/m^2/s^1/m mol/m^2/s^1 among wild species with a mean of 6.87 µ mol/m^2/s^1/ m mol/m^2/s^1; whereas from 4.19±0.40 and 9.62±1.07 µ mol/m^2/s^1 /m mol/m^2/s^1 among cultivated species genotypes with a mean of 7.05 µ mol/m^2/s^1 /m mol/m^2/s^1. The mean iWUE of interspecific inbred lines group was 8.46 µ mol/m^2/s1 /m mol/m^2/s^1 with a range of 6.45±1.19 to 25.95±5.0 µ mol/m^2/s^1 /m mol/m^2/s^1. The interspecific lines showed a higher iWUE which attributed to lower E rates indicating their adaptation to warm and dry conditions.

Among wild species, the lowest gs (0.12±0.02 m mol/m^2/s^1) and E (5.68±0.56 m mol/m^2/s^1) and the highest iWUE (10.26±1.2 µ mol/m^2/s^1 /m mol/m^2/s^1) were observed in *C. lanatus* accession, CART 57/83. The interspecific inbred line, A1-57/83, derived from (A1 x CART 57/83) cross showed high iWUE (11.04±0.79 µ mol/m^2/s^1 /m mol/m^2/s^1). The highest gs (0.42±0.08 mol/m^2/s^1) and E (12.81±1.42 m mol/m^2/s^1) and the lowest iWUE (4.9±0.62 µ mol/m^2/s^1 /m mol/m^2/s^1) were observed in *C. creticus* accession, CART 10/79.

Among the interspecific inbred lines, the highest Pn (62.13±0.39 µmol$m^{-2}s^{-1}$) and iWUE (25.95±5.0 µ mol/m^2/s^1 /m mol/m^2/s^1) and the lowest gs (0.04±0.01 mol/ m^2/s) and E (2.71±0.56 m mol/m^2/s^1) were observed in the interspecific inbred line, A1-63/79 derived from (A1 x CART 63/79) cross. The interspecific inbred line, Man-63/79 derived from (Manjira x CART 63/79) cross also recorded higher Pn (53±0.67 µ mol/m^2/s^1) and iWUE (10.38 ±1.6 µ mol/m^2/s^1 /m mol/m^2/s^1) as compared to its cultivated species parent. The wild species parent (*C. turkesthanicus* accession, CART 63/79) of A-63/79 and Man-63/79 had very high Pn and iWUE compared to cultivated species parents, A1 and 'Manjira'.

Correlations among traits: Correlations among physiological traits were studied separately for wild species, cultivated species and interspecific inbred lines (Fig.2). Pn had negative association with Ci and iWUE and positive relation with gs and E in wild and cultivated species while it had significantly strong positive association with Ci and iWUE and negative relation with gs and E in interspecific inbred lines. The stomatal conductance (gs) showed significantly strong positive relation with E and negative relation with Ci and iWUE in all three categories of experimental material i.e. wild and cultivated species and interspecific inbred lines. Ci had negative association with E and positive association with iWUE while E had significantly strong negative association with iWUE in all three groups.

Previous studies suggested that the wild species could act as potential donors of physiological traits in rice and other crops (Zhao *et al.,* 2008; Dutra *et al.,* 2011). Investigating physiological parameters in wild species and information on inheritance of physiological traits from wild

to cultivated species would help in improving resource-use efficiency in modern cultivars. Clear differences in physiological traits have been reported earlier among many crop species and their wild relatives as well as among the progenies derived from crosses between cultivated and wild species (Dornhoff and Shibles, 1970; Criswell and Shibles, 1971; Zhao *et al.,* 2008; Zhao *et al.,* 2010; Haritha *et al.,* 2019).

Table 1 Analysis of variance of among wild, cultivated species and interspecific inbred lines for photosynthetic traits

Traits	DF	SS	MSS	F value	Pr(>F)
Pn	5	338.4	338.4	7.225	0.0129*
gs	5	361.6	361.6	6.041	0.0216*
Ci	5	5.6	5.59	0.075	0.787
E	5	225.4	225.4	3.44	0.076
iWUE	5	66.6	66.55	0.923	0.346
Pn/Ci	5	627.8	627.8	12.88	0.00148**

*0.05 and **0.01 significance level. Pn: Net photosynthesis, gs: stomatal conductance, Ci: leaf intracellular CO2, E: transpiration, iWUE: intrinsic water use efficiency and Pn/Ci: carboxylation efficiency

Table 2 Net photosynthesis (Pn), stomatal conductance (gs), leaf intracellular CO_2 (Ci), transpiration (E), intrinsic water use efficiency (iWUE) and carboxylation efficiency (Pn/Ci) in wild and cultivated species and interspecific inbred lines

Genotype	Identity code/name	Pn ($\mu molm^{-2}s^{-1}$)	gs ($molm^{-2}s^{-1}$)	Ci ($\mu molCO_2mol^{-1}$)	E ($mmolm^{-2}s^{-1}$)	iWUE ($\mu molm^{-2}s^{-1}/mmolm^{-2}s^{-1}$)	Pn/ Ci
Wild species							
C. lanatus	CART 7/77	62.13±0.70	0.25±0.02	321.58±0.84	9.57±0.36	6.49±0.23	0.19
	CART 65/76	64.92±0.65	0.38±0.09	317.55±0.93	11.05±0.87	5.88±0.48	0.20
	CART 57/83	55.68±0.75	0.12±0.02	331.67±1.02	5.68±0.56	10.26±1.2	0.17
C. creticus	CART 10/79	62.72±1.66	0.42±0.08	319.30±2.26	12.81±1.42	4.90±0.62	0.20
C. glaucus	CART 43/76	63.10±1.47	0.19±0.03	320.31±2.00	8.95±0.85	7.05±0.55	0.20
C. oxyacantha	2563-p9-p2	65.30±1.18	0.23±0.03	317.30±1.50	9.76±0.89	6.69±0.84	0.21
	IP-16	62.30±0.35	0.14±0.01	320.66±0.96	7.10±0.24	8.81±0.31	0.19
C. turkesthanicus	CART 63/79	63.50±1.10	0.18±0.04	320.30±1.77	7.80±1.25	8.14±2.36	0.20
	CART 66/76	60.28±1.66	0.26±0.07	324.47±2.49	9.90±1.69	6.09±0.97	0.19
C. palaestinus	PI 235663		0.19±0.04	318.12±2.40	7.83±1.50	8.36±3.59	0.21
Cultivated species							
C. tinctorius	A1	40.27±1.92	0.18±0.03	350.34±1.93	7.06±1.33	5.70±0.87	0.11
	'Manjira'	42.63±0.90	0.09±0.01	340.36±1.19	5.43±0.60	7.94±0.83	0.14
	'Nira'	49.37±1.70	0.17±0.04	334.98±3.36	8.86±1.00	5.80±0.85	0.16
	96-506-1-21	39.00±0.63	0.17±0.03	350.61±0.80	7.02±1.04	5.78±0.76	0.11
	SFS-9990	42.77±0.62	0.10±0.01	347.05±0.80	5.22±0.34	8.26±0.56	0.12
	JSI-116	48.77±0.66	0.08±0.01	338.63±0.86	5.39±0.43	9.16±0.67	0.14
	PI 537598	45.80±0.46	0.08±0.01	342.83±0.76	4.89±0.58	9.62±1.07	0.13
	EC 543318	51.17±0.13	0.16±0.01	334.23±0.14	8.82±0.13	5.80±0.10	0.15
	1831-P8	50.50±0.74	0.18±0.02	323.08±1.22	12.03±1.03	4.19±0.40	0.16
	GMU-2484	50.53±0.43	0.09±0.02	336.15±0.89	6.06±0.89	8.64±1.03	0.15
Interspecific inbred lines							
A1 x CART 63/79	A1-63/79	62.13±0.39	0.04±0.01	308.10±4.94	2.71±0.56	25.95±5.0	0.20
A1 x CART 57/83	A1-57/83	60.44±0.62	0.10±0.01	251.46±6.30	5.60±0.44	11.04±0.7	0.24
Manjira x CART 63/79	Man-63/79	53.42±0.67	0.11±0.03	163.65±4.30	6.09±1.23	10.38±1.6	0.20
Nira x IP16	Nira-IP16	51.80±0.26	0.14±0.03	122.00±3.01	6.96±0.91	7.82±0.74	0.42
A1 x CART 43/76	A1-43/76	49.26±0.19	0.13±0.02	100.13±2.02	6.81±0.70	7.54±0.76	0.49
Manjira x CART 43/76	Man-43/76	47.50±0.49	0.18±0.04	68.07±1.65	8.46±1.53	6.45±1.19	0.70

±: standard error of mean

The present investigation showed within and between species variation for photosynthetic traits. Safflower wild species recorded higher Pn than the cultivated species. Higher photosynthesis in wild species than the modern cultivars was reported in other crop species also (Evans and Dunstone, 1970; Pradhan and Panda, 2018; Masumoto *et al.,* 2004; Zhao *et al.,* 2010). The high net photosynthesis ability of wild species is encouraging to exploit wild species for improving cultivated safflower for photosynthetic efficiency. Enhancing photosynthesis has a vital role in enhancing yield potential of crops (Long *et al.,* 2015). Recent progress in breeding for seed yields in cereal crops was mostly associated with photosynthetic parameters (Fischer and Edmeades, 2010). There were reports of introgression of high photosynthesis efficiency of wild species into cultivated rice (Zhao *et al.,* 2003; Haritha *et al.,* 2017).

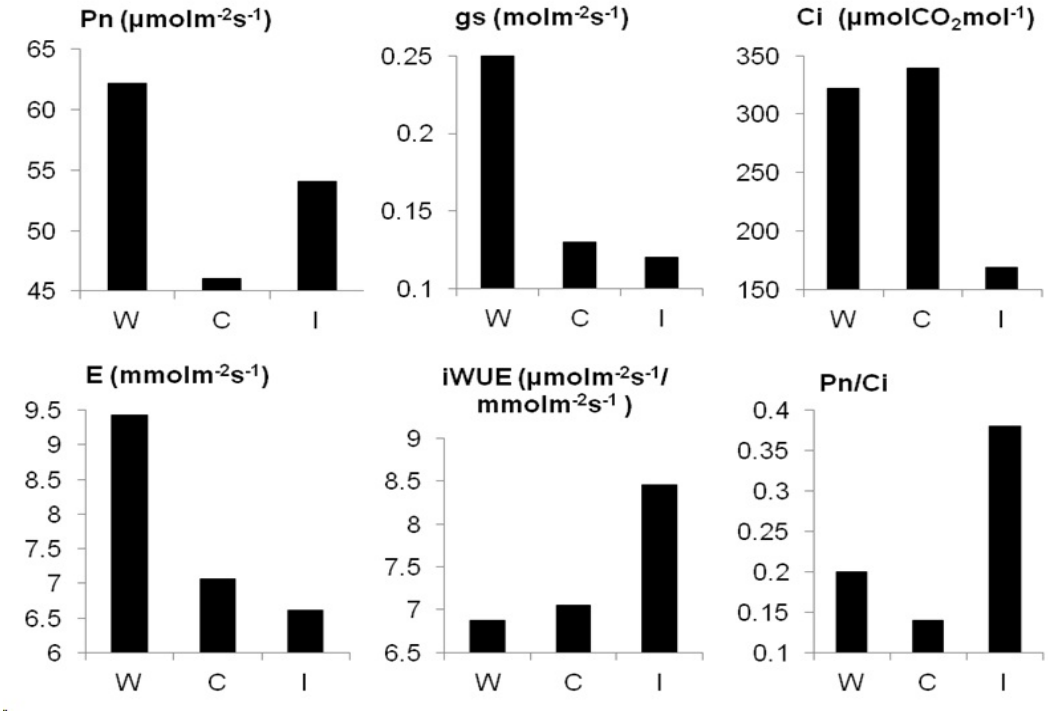

Fig. 1. Mean values of physiological traits in *Carthamus* wild species (W), cultivated species, *C. tinctorius* (C) and interspecific inbred lines (I). Pn: net photosynthesis; gs: stomatal conductance; Ci: leaf intracellular CO_2 concentration; E: transpiration; iWUE: intrinsic water use efficiency; Pn/Ci: carboxylation efficiency

Many studies reported positive linear correlations among Pn, gs and E (Evans and Loreto, 2000; Sergiu *et al.,* 2014; Pushp, 2015). Similar positive correlations were observed in wild and cultivated species but in interspecific inbred lines Pn was negatively related to gs and E whereas gs and E were positively related. The negative relation between Pn and E was because of positive relation between gs and E.

The strong positive association of Pn with iWUE and Ci in interspecific inbred lines differed from those observed in wild and cultivated species where the relations were negative. iWUE showed strong negative relationship with gs and E, which indicated regulatory role of stomatal conductance in maintaining the water use efficiency. The association of Pn in interspecific inbred lines was strongly negative with E and gs and positive with iWUE. The negative relation of Pn with gs and E and its positive relation with iWUE and Ci in interspecific inbred lines hint at the possibility of developing high yielding drought tolerant varieties through wide hybridization in safflower.

The highest iWUE (25.95±5.01 µ mol/m^2/s^1/m mol/m^2/s^1) observed in the interspecific inbred line, A1-63/79 derived from (A1 x CART 63/79) cross was due to high Pn (62.13±0.39 µ mol/m^2/s^1), low gs (0.04±0.01) and low E (2.71±0.56 m mol/m^2/s^1). Similar results were also reported in tomato species and interspecific lines (Zeist *et al.,* 2018). The interspecific inbred lines *viz.,* Man-63/79 and A1-57/83 also had high iWUE and low E. The low E in these inbred lines might be due to low stomatal conductance

because the relation of E with gs was strongly positive. High Pn associated with low gs was observed in water stress tolerant plants which had higher iWUE (Pazzagli *et al.,* 2016). Borba *et al.* (2017) reported high Pn and low gs and E, and better water use efficiency in F_2BC1 population of *Solanum pennellii* x *S. lycopersicum* cross. Both A1-63/79 and Man-63/79 were better than their respective parents with respect to iWUE. They were better than their cultivated species parents and close to their wild species parent (*C. turkesthanicus*, accession CART 63/79) with respect to net photosynthesis. The low E, high iWUE and high net photosynthesis capability of *C. lanatus* accession, CART 57/83 have been introgressed into the interspecific inbred line, A1-57/83 derived from (A1 x CART 57/83) cross. The high Pn, low E and high iWUE make the inbred lines capable of tolerating water stress. High Pn, low gs and low E were found to be promising in selecting water stress tolerant tomato genotypes (Borba *et al.,* 2017, Zeist *et al.,* 2018). Selection for increased photosynthetic efficiency *per se* was mostly successful in maize (Crosbie *et al.,* 1977).

The results of our experiment indicate the opportunity that exists for safflower improvement for gas exchange traits and iWUE through exploiting the gene pools of wild relatives. Inclusion of wild species in breeding may enhance genetic variation and help in selecting progenies with better recombinants for desirable physiological traits. The results have demonstrated introgression of high Pn, low E and high iWUE characteristics of the wild species, *C. lanatus* and *C. turkesthanicus* into cultivated species, and occurrence of better recombinants for these traits in interspecific inbred lines derived from crosses between these species and cultivated one. High Pn and low E will have apparent positive effects on iWUE improvement. E and iWUE directly influence yield, any increase in E would have a negative effect in crop growth rate, and this negative impact can be reduced by selecting for high Pn. So it is necessary to select genotypes where interdependence between iWUE and E is lower. Among the several adoptive strategies, improving the efficiency of water use for biomass production along with photosynthetic rate is perhaps the most relevant mechanism for achieving optimum productivity under drought. This study suggests utilization of *C. lanatus* and *C. turkesthanicus* for developing drought tolerant safflower cultivars.

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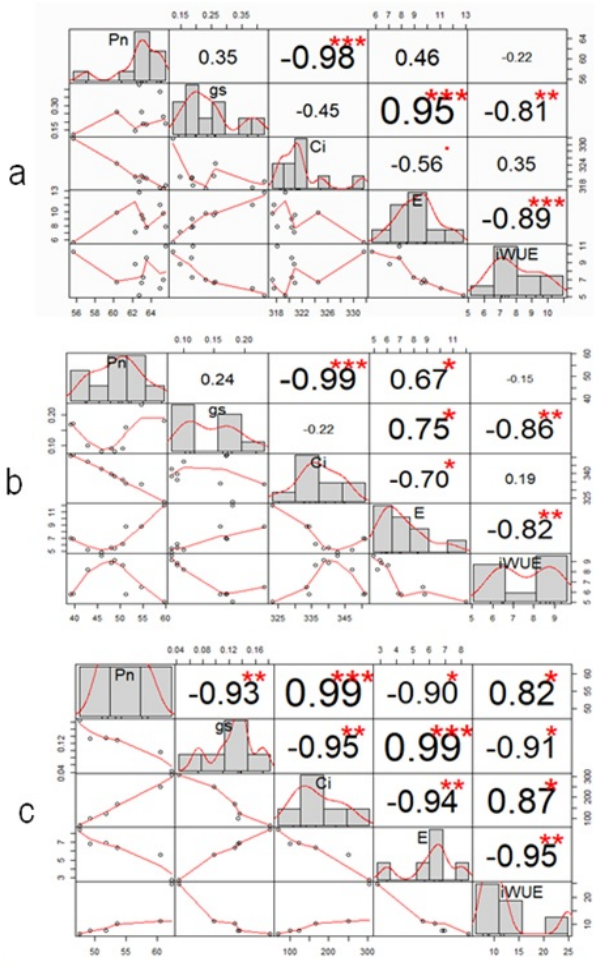

Fig. 2. Pearson correlation coefficients in a) *Carthamus* wild species, b) cultivated species (*C. tinctorius)*, and c) interspecific inbred lines. Significance of correlations among pairs of physiological traits are indicated above the diagonal. The size of asterisk is proportional to significance level. Histograms of each trait are shown on the diagonal, and scatter plots of bivariate correlation among pairs of traits are shown below the diagonal histograms

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Isolation of lignocellulolytic bacteria from oil palm mesocarp waste

P VEERA LAKSHMI AND K PARANI*

Sri Parasakthi College for Women, Manonmaniam Sundaranar University, Courtallam-627 802, Tamil Nadu

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ABSTRACT

In the present investigation, the bacterial strains were isolated from oil palm mesocarp waste dumped soil by serial dilution and pour plate method. The highest lignocellulolytic index was produced by bacterial isolate OPMW 6 (1.5 mm) followed by OPMW 5 (1.2 mm), OPMW 1 (0.83 mm), OPMW3 and OPMW 4 (0.71 mm). Changes in methylene blue color supplemented in nutrient broth from blue to light green or yellow were observed by three bacterial isolates (OPMW1, OPMW 3 and OPMW5), and rest of the bacterial isolates recorded less decolourization as the cultures were incubated between 24 and 48 hours respectively with highest percentage of dye decolourization produced by bacterial isolate i.e. OPMW5 (83.3%) and lowest rate of decolourization by bacterial isolate OPMW 6 (13.54%) as inoculated in nutrient broth supplemented with methylene blue dye. Utilization of gallic acid as supplemented in minimal salt media recorded positive reaction for 4 bacterial isolates (OPMW 2, OPMW 5, OPMW 6 and OPMW 8) by the formation of dark colouration and addition of α-naphthol in MSM inoculated broth cultures showed formation of purple blue colour due to production of laccase activity. Among the 4 best isolates having the highest indices, two isolates of bacteria (OPMW5 and OPMW6) had the best lignocellulolytic activity. Based on enzyme biosynthesis ability, the OPMW5 and OPMW6 were selected as the best isolates as it can synthesie cellulase and ligninase enzymes. These enzymes are known to accelerate the degradation of oil palm mesocarp fibre substrate.

Keywords: Bacterial isolates, Laccase, Lignocellulolytic index, Oil palm mesocarp waste

Palm oil industry is one of the major types of agro industries that produce lignocellulosic material. A palm oil mill industry produces waste after extraction of the crude palm oil, over 6.0×10^2 million tons of harvestable palm oil biomass is produced annually worldwide (Kumar *et al.*, 2011) but only about 10% of these are used as finished product. The remaining by-product, consisting of mesocarp fibre, empty fruit bunches, fronds, trunks, kernels, palm oil mill effluent, are discarded as waste. These wastes can be converted to a valuable product, but it is usually avoided because of the expected low valuable product yield when converted. The primary challenge of converting those wastes using biological method is due to the presence of ligno cellulose contents: cellulose, hemicelluloses and lignin. The presence of these components bring about low yield due to low accessibility of micro crystalline cellulose fibres and presence of lignin and hemi cellulose on the surface of the cellulose, which prevents cellulose from assessing the substrate efficiently (Zhany *et al.*, 2007). Suriadikarta *et al.* (2006) reported that some types of bacteria such as *Pseudomonas* sp., *Bacillus* sp., *Streptomyces* sp., *Flavobacterium* sp., *Clostridium* sp., *Thermonospora* sp. are capable of decomposition of lignocellulosic materials. Keeping in view, the present study was focused to isolate and screen the potential lignocellulolytic bacteria in oil palm mesocarp waste dumped soil and to check the growth of bacteria on selective media for their enzyme production in solid and liquid cultures.

*Corresponding author's E-mail: paranik@sriparasakthicollege.edu.in

MATERIALS AND METHODS

Oil palm waste dumped soil was collected from the oil palm industry, Yeroor Estate, Palode (Dt.), Kerala, India. Further, the soil was used for the isolation of lignocellulolytic bacteria by serial dilution and pour plate method with the selective media. Isolated colonies were further purified by streaking on nutrient agar plates and the pure cultures were maintained in NA slants and stored in refrigerator at 4°C for further studies.

Screening for ligninocellulolytic bacteria: The ligninoocellulolytic activity of the bacterial isolates were characterized using lignin related compounds and palm oil mesocarp fibre with congo red agar (POMFCRA) in order to observe their capability to degrade lignin and natural substrate i.e., palm oil mesocarp fibre (POMF). The composition of lignin agar (Ariana *et al.*, 2017) consisted of lignin (gallic acid) - 2.0 g, magnesium sulphate - 0.25 g, potassium dihydrogen phosphate - 0.5 g, gelatin - 2.0 g, agar - 15.0 g, distilled water to make up the volume to 1 litre. Lignin was substituted with gallic acid, a natural substrate.

Palm oil mesocarp fibre (100 g) was boiled in one litre of hot water (9°C) for 15 min. The supernatant of palm oil

mesocarp fibre was used to prepare one litre of Palm oil mesocarp fibre agar (POMF) medium by adding to 15g of agar. This medium was sterilized at 121°C for 15min and dispensed into Petri plates (20 ml/plate). Bacterial isolates were streaked and incubated at room temperature for 48 hours. The cellulolytic index, lignocellulolytic index and ratio between diameters of the clear zone surrounding colonies illustrates the capability of bacterial isolates to degrade lignocellulolytic materials. Further screening was carried out on minimal salt media (MSM) broth containing various lignin - related low molecular weights aromatic compounds (LMWAC 50 mg/l) as sole carbon source. These LMWAC were gallic acid (GA) and tannic acid (TA). The inoculated plates and broths were incubated at 35°C. Growth was observed after 3 days of incubation.

Laccase activity: Laccase activity was tested by performing different experiments based on various substrates like tannic acid and α-naphthol.

Tannic acid: Bacterial isolates were inoculated in 25 ml of nutrient broth and incubated overnight at 35°C for 12 to 24 hrs. These mother cultures were checked by streaking on nutrient agar plates which were then incubated at 35°C for further experiments. The cultures were inoculated in laccase production media and incubate at 35°C for 24 hrs. One ml of the grown culture was transferred to test tube containing 0.3% of tannic acid. The test tubes were incubated at 35°C for 1 hr.

α-naphthol; For the α-naphthol test, 1ml of 0.5% α-Naphthol was added to 1ml of 24 hrs old culture of bacterial isolates from laccase production broth media (Pointing, 1999). The blank test tube contained only medium not inoculated by the isolates served as control. The tubes were kept in incubator shaker at 35°C for 48 hrs at 200 rpm.

Dye decolorization: Preparation of flasks for dye decolorization assay: Bacterial isolates were inoculated in 25 ml of nutrient broth and incubated overnight at 35°C and 200 rpm. These mother cultures were checked by streaking on nutrient agar plates which were then incubated at 35°C for further experiments.

In nutrient broth media, methylene blue was present in the concentration of 25 mg/l. Stock solution of methylene blue dye was prepared. Nutrient broth media and dye were autoclaved at 15 lbs for 20 min. After autoclaving, media and dye were mixed and poured in sterile flasks of 100 ml and overnight grown culture was inoculated in each flask. The flasks were incubated at 35°C for 48 hrs. Flasks were observed at 8 hrs of time intervals by using spectro-photometer. The maximum absorbance wavelength of the dyes was recorded with the help of spectrophotometer. The color of the pellet was also visually inspected to establish whether the dye had adsorbed to the cells rather than being degraded.

The percentage of decolorization efficiency of bacterial isolate was calculated as

$$\text{Decolorization efficiency (\%)} = \frac{\text{(Initial OD - Final OD)}}{\text{Initial OD}} \times 100$$

Statistical analysis: All data were expressed as means ± standard deviation using Microsoft excel. The data recorded for different parameters were analyzed with the help of Fischers LSD Test Calculator on running the Analysis of Variance (ANOVA).

RESULTS AND DISCUSSION

The bacterial isolates obtained from Oil palm mesocarp waste dumped soil through serial dilution and pour plate method were designated as OPMW 1, OPMW 2, OPMW 3, OPMW 4, OPMW 5, OPMW 6, OPMW 7 and OPMW 8.

Lignocellulolytic activity of the isolates: The bacterial isolates were subjected to lignocellulolytic activity using Oil palm fruit waste agar media supplemented with Congo red and with other lignin related compounds *viz.,* methylene blue, gallic acid, tannic acid and α-naphthol supplemented in Minimal salt media (Table 1). The isolates were grown on oil palm mesocarp fruit waste agar media (natural substrate) supplemented with Congo red and the clear zones around the colonies were distinctly observed. All the bacterial isolates showed a significant growth ranging from 1.7 to 3.5 mm colony diameter and 1.5 to 2.5 mm in producing the clear zone (Table 2; Plate 1). The highest lignocellulolytic index was produced by bacterial isolate OPMW 6 (1.5 mm) followed by OPMW 5 (1.2mm), OPMW 1 (0.83 mm), OPMW3(0.71mm) and OPMW 4 (0.71 mm).

Changes in methylene blue color supplemented in nutrient broth from blue to light green or yellow were observed by four bacterial isolates (OPMW1, OPMW 3 and OPMW5 other bacterial isolates showed a less decolourization during their incubation period respectively (Table 2; Fig. 1). Highest percentage of dye decolorization was produced by bacterial isolate i.e. OPMW5 (83.3%) and the lowest rate of decolourization was observed in bacterial isolate OPMW 6 (13.54 %) .

The synthesis of polyphenol oxidase is necessary for lignin depolymerisation. In this study, colour change of the tannic acid medium was used as an indicator of polyphenol oxidase synthesis, which was observed in 6 isolates (OPMW1, OPMW3, OPMW5, OPMW6, OPMW7 and OPMW8). Tannic acid has been used as an indicator for the

ability of soil microorganisms to decompose phenol - like compound (Table 1).

All the 8 tested isolates were able to grow on tannic acid amended media where they produced dark brown zone surrounding their colonies (Table.2). Isolates OPMW5, OPMW6, OPMW8 showed significantly darker brown zone on tannic acid amended media and the culture also turned brown upon the addition of tannic acid in minimal salt media respectively. Utilization of gallic acid as lignin related low molecular weight compound supplemented in minimal salt media showed positive reaction with 4 bacterial isolates (OPMW 2, OPMW 5, OPMW 6 and OPMW 8) by formation of dark colouration and addition of α-naphthol in MSM inoculated broth cultures showed formation of purple blue colour due to production of laccase activity (Table 1).

Table 1 Growth of bacterial isolates on selective media

Bacterial Isolates	OPMFW medium	MSM Broth + Gallic acid	MSM Broth + Tannic acid	Nutrient broth + -Napthol	Nutrient broth + Methylene blue	Gallic acid agar medium
OPMW1	+++	++	++	+	+++	++
OPMW2	++	++	++	+	++	++
OPMW3	+++	++	++	+	+++	++
OPMW4	+++	++	+	++	++	++
OPMW5	+++	+++	+++	+++	+++	+++
OPMW6	+++	+++	+++	+++	+	+++
OPMW7	++	+	+++	++	++	++
OPMW8	++	++	+++	++	++	++

(+++): very fast growth (colonies visible after 12 hours), (++): fast growth (colonies visible after 18 hours) (+): slow growth (colonies visible after 24 hours); OPMFW: Oil Palm Mesocarp Fibre Waste Medium; MSM Broth: Minimal Salt Media Broth

Table 2 *In vitro* degradation of lignin by the bacterial isolates

Bacterial isolates	Lignin (Oil Palm Mesocarp Fruit Waste)			Dye Decolourization (%)
	Diameter of zone (mm)	Diameter of colony (mm)	Ligno cellulolytic Index (mm)	
OPMW1	2.5	3	0.83	72.9
OPMW2	1.5	2.5	0.6	20.8
OPMW3	2	2.8	0.71	52.0
OPMW4	2.3	3.2	0.71	31.2
OPMW5	2.4	2	1.2	83.3
OPMW6	2.5	1.7	1.5	13.54
OPMW7	1.9	3	0.63	21.87
OPMW8	2	3.5	0.57	43.7
SE	0.12	0.21	0.12	9.0
(LSD = 0.38)				

Front view Back view

Fig. 1. Bacterial isolates producing clear zones on oil palm mesocarp fruit extract medium

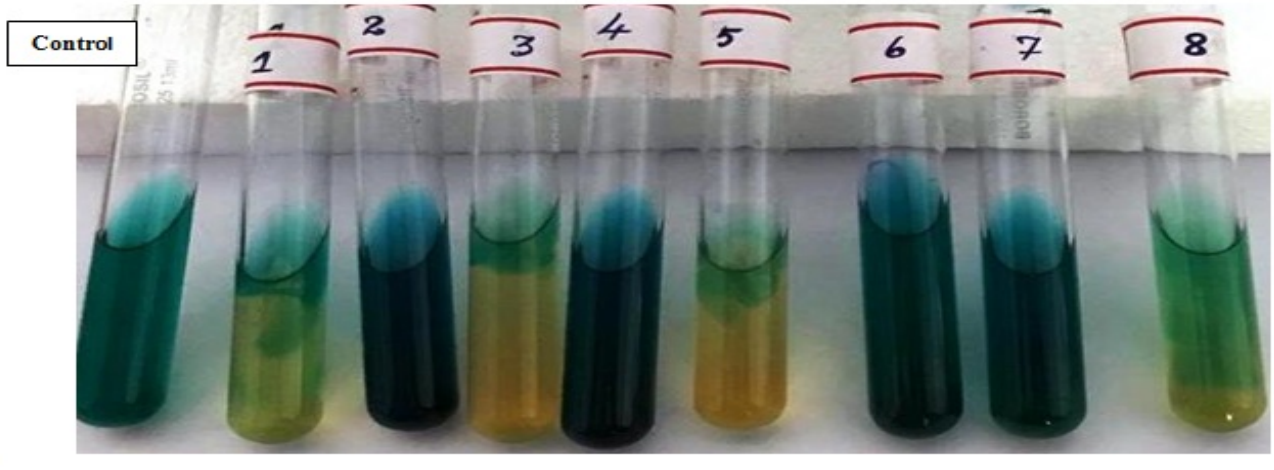

Fig. 2. Dye reduction test carried out with the 8 isolates

The isolates were grown on oil palm mesocarp fruit waste agar media (natural substrate) supplemented with congo red media and the clear zones around the colonies were also distinctly observable (Table 1). Similar results have been reported by Ariana *et al.* (2017) using EFBCRA media in which as much as 430 isolates were successfully collected and 12 of them exhibited promising capability to synthesize lignocellulolytic enzyme, the key for FEB degradation. The present results are in agreement with Zverlov *et al.* (2003) where they indicated that the size of the clear zone is directly proportional to the lignocellulolytic activity. The presence of clear zone is an early suggestion that the isolates have capability as lignocellulosic biomass degrading microbes. These microbes are able to break down the complex of lignocellulosic biomass into monomers which can be used as the main carbon source for their metabolism. Some studies (Chen, 2018; Rawway *et al.,* 2018) demonstrated that adding cellulolytic microorganisms can accelerate the decomposition of organic matter. Therefore, employing indigenous lignocellulolytic microbes will accelerate the oil palm mesocarp waste decomposition process. Gusma wartati (1999) and Wahyuni (2008) reported application of cellulolytic bacteria, fungi or actinomycetes or their consortium with the addition of chicken manure to reduce C/N ratio during the decomposition process of EFB. In the present study, change in methylene blue color supplemented in Nutrient broth from blue to light green or yellow was observed in four bacterial strains. These results are in accordance with the findings of Ferreira-Laeito *et al.* (2007), Bholay (2012) and Rahman *et al.* (2013), who reported that the decolorization of methylene blue has been used as an indicator of lignin peroxidase enzyme activity.

Tannic acid used as an indicator for the ability of soil microorganisms to decompose phenol-like compound and as indicator of polyphenol oxidases used to select ligninolytic microbes (Thormann *et al.,* 2002). A report by Chen *et al.* (2012) also recorded that lignin - degrading bacteria could use lignin as their sole source of carbon without depending on another carbon source for energy.

The results from our study have identified 4 bacterial isolates having the highest lignocellulolytic indices with two isolates (OPMW5 and OPMW6) having the best ligno-cellulolytic activity. Based on enzyme biosynthesis ability, the OPMW5 and OPMW6 were selected as the best isolates as it can produce cellulase and ligninase enzymes. These enzymes are known to accelerate the degradation of oil palm mesocarp fibre substrate. Therefore, if further characterized for their efficacy and validated for their competitiveness to colonize under natural conditions, the identified bacteria could be exploited for faster degradation of the oil palm mesocarp waste and convert it into useful products.

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Co-integration and causality analysis of castor markets in Telangana state

R VIJAYA KUMARI, RAMAKRISHNA GUNDU, VENKATESH PANASA AND SREENIVAS AKULA

College of Agriculture, Professor Jayashankar Telangana State Agril. University, Hyderabad-500 030, Telangana

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ABSTRACT

This study tested long-run spatial market integration between price pairs of castor in five major markets viz., Badepalli, Devarakadra, Gadwal, Nagarkurnool and Narayanpet of Telangana state by adopting econometric tools like Johansen's multivariate co integration approach, Augmented Dickey-Fuller (ADF), Granger causality test, and Vector Error Correction Model (VECM). The study confirmed the presence of co integration, implying the five years price association among the markets. To get the additional evidence as to whether and in which direction price transmission is occurring between the market pairs, Granger causality test was used, which confirmed Devarakadra to be the price-determining market. Devarakadra was found comparatively more efficient as it showed most unidirectional causal relations with other markets. The results showed that Devarakadra market influenced the prices in the other three major markets i.e., Badepalli, Gadwal and Narayanpet.

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

Castor is one of the oldest cultivated crops; however, 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 (Kumar *et al.,* 2021). In 2019-20, the major castor producing countries were India (18.42 lakh tonnes), Mozambique (0.85 lakh tonnes), China (0.36 lakh tonnes), Brazil (0.16 lakh tonnes) and Myanmar (0.13 lakh tonnes). Area under castor reported during 2021-22 was 6.960 lakh ha (17.199 lakh acres) as against 7.336 lakh ha (18.128 lakh acres) during 2020-21. Among states, Gujarat is leading with 5.389 lakh ha (13.316 lakh acres) under castor followed by Rajasthan 1.200 lakh ha (2.965 lakh acres), Andhra Pradesh 0.177 lakh ha (0.437 lakh acres) and Telangana 0.022 lakh ha (0.54 lakh acres). According to Government 1st advance estimates, all India castor production in 2021-22 is at 15.98 lakh tonnes. In Telangana state, Narayanpet 602 ha (1,487 acres), Mahabubnagar 494 ha (1,221 acres), Gadwal 435 ha (998 acres) and Wanaparthy 404 ha (998 acres) are the major castor growing districts (www.agritelangana.gov.in).

An indirect means of analyzing market efficiency is to test for market integration. Three types of market integration are identified: inter-temporal, vertical and spatial. Inter temporalmarket 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 to 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 and 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 bi variate correlation coefficients, co-integration and Granger-Causality. Campiche *et al.* (2007) studied the relation between crude oil prices and variation of agricultural commodities using a vector error correction model. Co integration results showed that corn and soybean prices were co integrated with crude oil price during 2006-2007. Awal and Sabur (2009) examined the pricing efficiency of exportable fresh vegetable markets in Bangladesh and its export markets by using Engle-Granger (EG) test, Co integration Regression

Corresponding author's E-mail: ramalingareddyvijaya@gmail.com

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.* (2021) studied co-integration of major soybean markets in India, observed that Indore market is the price-determining market and it was found comparatively more efficient as it showed most bidirectional causal relations with other markets.

The present investigation was carried out to understand the market integration for castor crop in Telangana state where there are primarily five markets trading castor produce.

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}~ I (1) and P_{jt}~ I (1). There exists a linear combination between these two series, which is stationary i.e. P_{it}

$$(=\tilde{P_{it}} \tilde{\alpha} \hat{\beta} P_{it}) \sim I(0)$$

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 co-integration. The Engle and Granger (1987) procedure is the common way to test co-integration.

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 co-integration, 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 co-integration: 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 co-integration 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 co-integration 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,

$$\Gamma_i = -(I - \Pi_i - \ldots\ldots, T); \ i=1,2,\ldots k-1$$

$$\Pi = -(I - \Pi, - \ldots\ldots\ldots \Pi_k)$$

Both Γi and ΓIi are the n*n matrixes 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 ΔP_t is stationary P_t is integrated at order one I(1) which will make unbalance relation as long as ΓI matrix has a full rank of k. In this respect, the equation can be solved by inversing the matrix $\Gamma\Gamma^{-1}$ for Pt and as a linear combination of stationary variable (Kirchgässner *et al*., 2012). The stationary linear combination of the Pt determines by the rank of IΓ matrix. If the rank r of the matrix ΓI r=0 the matrix is the null and the series underlying is stationary. If the rank of the matrix IΓ is such that 0 < rank of (IΓ) = r < n then there are n × r co-integrating vectors. The central point of the Johansen's procedure is simply to decompose IΓ into two n × r matrices such that IΓ=αβ'. The decomposition of IΓ implies that the β'Pt 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 co-integrating vectors as follows:

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

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

Where, r is the number co-integrated vector, $\bar{\lambda}_1$ is the eigen value and $\bar{\lambda}_{r+1}$ 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 co-integrating vector(s) against the alternative hypothesis of n co-integrating 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, γ_2 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_{jt}. On the other hand, 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 P_{it} 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.

$$\theta_{21} = \ldots = \theta_{2n} = \cdots = \gamma_1 = 0 (\text{No causality from } P_{jt} \text{ to } P_{it})$$
$$\theta_{41} = \ldots = \theta_{4n} = \cdots = \gamma_2 = 0 (\text{No causality from } P_{it} \text{ to } P_{jt})$$

EViews software was used for the analysis

RESULTS AND DISCUSSION

Our price data consisted of monthly modal prices of castor (₹/q) in five major markets *viz.,* Badepalli, Devarakadra, Gadwal, Nagarkurnool and Narayanpet of the Telangana using monthly castor prices over the period from December 2016 to November 2021. The data was taken from the website of government agriculture marketing of Telangana http://tsmarketing.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 ₹ 5671/q prevailed in Nagarkurnool and the minimum price was also found in Nagarkurnool ₹ 3022/q followed by Narayanpet ₹ 3048/q.

Descriptive statistics: Summary statistics result showed that the price of castor remained most volatile in Nagarkurnool followed by Badepalli as measured by coefficient of variation. Gadwal is the biggest of castor markets in Telangana and the prices are dependent upon the demand of the other markets. The highest average prices of castor were found in Badepalli market, while lowest average prices were in Narayanpet (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 Telangana Badepalli, Devarakadra, Gadwal, Nagarkurnool and Narayanpet. All the major markets i.e., Badepalli, Devarakadra, Gadwal, Nagarkurnool and Narayanpet were stationary at first difference I (1).

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 $r > 1$ to $r > 4$ 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, we performed pair-wise Granger causality test for five major castor markets to comprehend causal relation between them. The result of the Granger causality analysis presented in Table 4 explicates that bidirectional causality market pair is Devarakadra-Nagarkurnool. In these cases, the former market in each pair Granger causes the modal price formation in the latter market, which in turn provides the feedback to the former market as well. A unidirectional causality markets pair is Devarakadra-Badepalli, Nagarkurnool- Badepalli, Devarakadra-Gadwal, Devarakadra-Narayanpet, Gadwal-Narayanpet and Nagarkurnool- Narayanpet. It means that a price change in the former market in each pair Granger cause the price formation in the latter market. Badepalli, Gadwal and Narayanpet markets price signals are depending on Devarakadra market because this market geographically present at centre to this markets. 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 estimates of VECM revealed that co-integration equation value of Narayanpet market attained short run equilibrium rapidly. One month lag price of Badepalli market was affecting current prices of itself. One month lag price of Devarakadra market was affecting current prices of

itself and Gadwal market. One month lag price of Nagarkurnool market was affecting current prices of Devarakadra and Narayanpet market. Two month lag price of Nagarkurnool market was affecting current prices of Gadwal market. One month lag price of Narayanpet market was affecting current prices of itself.

This study investigated the spatial market integration and price behavior of castor markets through co integration analysis in Telangana using December 2016 to November 2021 modal monthly price data. All major markets of castor in the Telangana were found to be highly integrated with regard to price movement. The results of ADF unit root test indicated that price series are stationary in first differencing logarithm i.e., Badepalli, Devarakadra, Gadwal, Nagarkurnool and Narayanpet markets were found to be integrated zero order I. Results of Johansen's co integration test showed the price series as co integrated. The result of the Granger causality analysis explicated that bidirectional causality market pair was Devarakadra-Nagarkurnool. Unidirectional causality market pairs were Devarakadra-Badepalli, Nagarkurnool- Badepalli, Devarakadra - Gadwal, Devarakadra - Narayanpet, Gadwal - Narayanpet and Nagarkurnool- Narayanpet. Results of Vector Error Correction Model (VECM) showed that Narayanpet market attained short run equilibrium rapidly. One month lag price of Badepalli market was affecting current prices of itself. One month lag price of Devarakadra market was affecting current prices of itself and Gadwal market. One month lag price of Nagarkurnool market was affecting current prices of Devarakadra and Narayanpet market. Two month lag price of Nagarkurnool market was affecting current prices of Gadwal market. One month lag price of Narayanpet market was affecting current prices of itself.

Table 1 Summary statistics of the monthly modal prices for castor in major markets for Telangana from the period December 2016 to November 2021(in ₹/q)

	Badepalli	Devarakadra	Gadwal	Nagarkurnool	Narayanpet
Mean	4252	4206	4168	4204	3882
Median	4012	3995	4009	4052	3678
Maximum	5530	5611	5604	5671	4828
Minimum	3226	3187	3233	3022	3048
Std. Dev.	570	558	548	602	456
CV (%)	13.40	13.26	13.16	14.32	11.74

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
Badepalli	-1.80	0.37	Non-stationary	-1.77	0.39	Non-stationary
Devarakadra	-0.95	0.76	Non-stationary	- 1.33	0.60	Non-stationary
Gadwal	-0.88	0.78	Non-stationary	-1.04	0.73	Non-stationary
Nagarkurnool	-1.80	0.37	Non-stationary	-1.80	0.37	Non-stationary
Narayanpet	-2.35	0.16	Non-stationary	-2.26	0.18	Non-stationary
	Augmented Dickey-Fuller test results after differencing			Phillips-Perron test results after differencing		
$^{\Delta}$Badepalli	-7.23*	0.00	Stationary	-7.21*	0.00	Stationary
$^{\Delta}$Devarakadra	-6.47*	0.00	Stationary	-6.48*	0.00	Stationary
$^{\Delta}$Gadwal	-7.49*	0.00	Stationary	-7.50*	0.00	Stationary
$^{\Delta}$Nagarkurnool	-9.22*	0.00	Stationary	-8.39*	0.00	Stationary
$^{\Delta}$Narayanpet	-8.96*	0.00	Stationary	-9.78*	0.00	Stationary

Note: *denote significance at 1% levels of significance and Δ denote the first difference of the time series

Table 3 Johansen's co-integration Test results of five major castor market prices in Telangana

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	0.758	228.771	69.819	0.000	80.814	33.877	0.000
At most 1*	r≤1	r>2	0.599	147.957	47.856	0.000	52.148	27.584	0.000
At most 2*	r≤2	r>3	0.549	95.809	29.797	0.000	45.378	21.132	0.000
At most 3*	r≤3	r>4	0.429	50.431	15.495	0.000	31.906	14.265	0.000
At most 4*	r≤4	r=5	0.277	18.525	3.841	0.000	18.525	3.841	0.000

Note: in represent the natural logarithm and * denote the rejection of null hypothesis at 5% level of significance

Tables 4 Market pair wise results of the Granger Casualty test

Markets Pairs	F-Statistic	P-Value	Decision of null hypothesis	Remarks
Devarakadra - Badepalli*	4.51*	0.02*	Do not reject	Unidirectional
Badepalli - Devarakadra	0.13	0.88	Reject	No causality
Gadwal - Badepalli	1.56	0.22	Reject	No causality
Badepalli - Gadwal	0.58	0.56	Reject	No causality
Nagarkurnool - Badepalli*	4.98*	0.01*	Do not reject	Unidirectional
Badepalli - Nagarkurnool*	1.83	0.17	Reject	No causality
Narayanpet - Badepalli	0.84	0.44	Reject	No causality
Badepalli - Narayanpet	0.76	0.47	Reject	No causality
Gadwal - Devarakadra	0.36	0.70	Reject	No causality
Devarakadra - Gadwal*	3.68*	0.03*	Do not reject	Unidirectional
Nagarkurnool - Devarakadra*	4.84*	0.02*	Do not reject	Bi-directional
Devarakadra - Nagarkurnool*	5.34*	0.01*	Do not reject	
Narayanpet - Devarakadra	0.82	0.44	Reject	No causality
Devarakadra - Narayanpet*	4.22*	0.02*	Do not reject	Unidirectional
Nagarkurnool - Gadwal	1.57	0.22	Reject	No causality
Gadwal - Nagarkurnool	2.03	0.14	Reject	No causality
Narayanpet - Gadwal	0.69	0.50	Reject	No causality
Gadwal - Narayanpet*	5.06*	0.01*	Do not reject	Unidirectional
Narayanpet - Nagarkurnool	1.93	0.15	Reject	No causality
Nagarkurnool - Narayanpet*	9.26*	0.00*	Do not reject	Unidirectional

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

Table 5 Vector Error Correction Model for castor prices for major five selected markets in Telangana

	Badepalli	Devarakadra	Gadwal	Nagarkurnool	Narayanpet
C	[0.94773]	[1.27482]	[0.44597]	[0.66615]	[1.97322]
Badepalli (-1)	[2.16827]	[-0.67168]	[-0.71708]	[0.73826]	[-1.48945]
Badepalli (-2)	[0.14948]	[0.45623]	[1.54812]	[-0.43744]	[0.07482]
Devarakadra (-1)	[1.15528]	[2.97854]	[2.17740]	[1.68400]	[0.07015]
Devarakadra (-2)	[0.78113]	[0.61118]	[0.31489]	[0.61901]	[-0.62096]
Gadwal (-1)	[-0.63191]	[0.23018]	[1.72843]	[-0.02019]	[1.06486]
Gadwal (-2)	[-0.67701]	[-0.46047]	[-0.50494]	[0.14380]	[0.46852]
Nagarkurnool (-1)	[1.87122]	[2.25549]	[0.64235]	[1.64894]	[2.77331]
Nagarkurnool (-2)	[-1.37362]	[-1.46396]	[-2.13367]	[-1.12043]	[-0.82567]
Narayanpet (-1)	[1.25662]	[1.56151]	[1.69298]	[0.88742]	[3.10830]
Narayanpet (-2)	[-0.29450]	[-1.66901]	[-0.92905]	[-1.26566]	[0.79924]

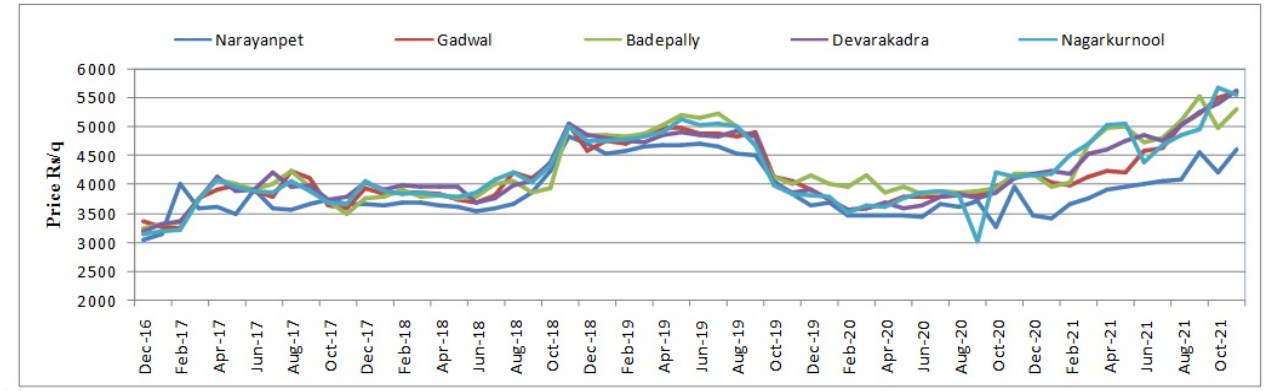

Fig. 1. Price behavior (₹/quintal) of castor crop in major selected markets in Telangana

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Morphological characterization of germplasm accessions of safflower (*Carthamus tinctorius* L.)

HEMLATA OHDAR, RAJEEV SHRIVASTAVA*, BHASKAR CHANDRA SAHOO AND ASMITA PILLAI

College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur-492 012, Chhattisgarh

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ABSTRACT

The present investigation was conducted at Research cum Instructional farm of Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh during *rabi*, 2019-20. The experimental material included 108 germplasm accessions and 4 checks (A-1, PBNS-12, CG Kusum-1 and IGKV Kusum) obtained from Germplasm Maintenance Unit (GMU), Indian Institute of Oilseed Research (IIOR), Hyderabad. In the experiment, the selected germplasm accessions were evaluated for 26 morphological characters in an augmented design to determine distinctiveness among the accessions. Observations were recorded at different growth stages of based on the DUS descriptors for safflower as suggested by IIOR, Hyderabad. First leaf: length of blade, first leaf: width of blade, petal: colour, petal: change in colour (faded stage), leaf: width of blade, leaf: shape, capitulum: width of outer involucral bract of main capitula (cm), capitulum: ratio length/width of outer involucral bract of main capitulum (cm), capitulum: number of spines on outer involucral bract of main capitulum, capitulum: diameter of main capitula (cm), plant: length of longest primary branch (cm), seed: hull content (%) were exhibited highest variation among the germplasm accessions. Genotypes which were unique for a particular character can be used as reference varieties in further DUS characterization experiments.

Keywords: DUS descriptors, Germplasm accessions, Morphological characterization, Safflower

Safflower (*Carthamus tinctorius* L.) is one of the world's oldest crops and is mainly cultivated for the production of vegetable oil. It is primarily an often cross-pollinated crop. Safflower is cultivated in over 60 countries, including India, China, Mexico, the United States, Ethiopia, Argentina, and Australia being the top producers. Safflower is mostly grown in China for its medicinal uses. Safflower has covered an area of 652 m ha worldwide; with a global production of 590 m ha and 9.052 q/ha productivity. The production of safflower is 24.64 m t in an area of 45.89 m ha with productivity of 537 kgha in India (Anonymous, 2019). Safflower is cultivated in an area of 270 hectares with a production of 110 tons and a productivity of 811 kg/ha in Chhattisgarh (Anonymous, 2020).

Safflower is an extensively branched, erect, herbaceous, spiny, thistle-like annual plant that grows to a height of 30 to 150 cm (Singh and Nimbkar, 2006; Neelima *et al.,* 2021). The stem elongates rapidly and branch extensively after the rosette stage. At the end of branches buds are borne and each flower head (capitulum) contains 20-180 individual florets. Each plant can develop 3-50 or more flower heads which has a diameter of 1.25-4 cm. Each flower head commonly contains 15-50 seeds and sometimes it can exceed 100. The oil content of the whole seed varies from 20 to 45% or more (Zehra, 2005). Mature safflower seed contains 27-32% oil, 5-8% moisture, 14-15% protein, 2-7% ash, and 32-40% crude fiber. The oil content of safflower is determined by the thickness of the pericarp; when the pericarp is dense, the oil content of safflower is low; when the pericarp is thin, the oil content of safflower seed increases directly (Weiss, 2000).

Morphological characterization of safflower varieties is required for their protection under Plant Variety Protection (PVP) legislation, although varietal testing for Distinctiveness, Uniformity, and Stability (DUS) is the basis for granting protection to new varieties under the PPV & FR Act 2001. In this study, the DUS descriptors of safflower are as per guidelines of PPV&FRA (Anonymous, 2009).

Characterization of germplasm is essential for providing information on the traits of accessions to ensure maximum utilization of the germplasm collection by the end users. It provides simple grouping of accessions and retrieval of useful germplasm for breeding programmes.

The experiment was carried out at the Research cum Instructional farm of Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh during *rabi,* 2019-20. One hundred eight germplasm accessions were characterized for 26 different morphological characters based on DUS descriptors.

The material was sown on 20th November 2019. The experiment was conducted in augmented block design i.e., a design wherein the test material is not replicated but the checks are replicated in each block. The experimental material comprising of 108 germplasm accessions obtained from Germplasm Management Unit, ICAR-Indian Institute of Oilseeds Research, Hyderabad (Telangana) was raised

*Corresponding author's E-mail: rajeevigkv@gmail.com

along with four checks in 3 blocks. Each block consisted of 36 germplasm entries and 4 checks (A-1, PBNS-12, CG Kusum -1 and IGKV Kusum). All the germplasm accessions were grown in single rows. The length of each row was 4m with row to row spacing of 45 cm and plant to plant distance of 20 cm.

For characterization at different growth stages based on the DUS descriptors for safflower published by PPV&FRA, observations were recorded for 26 morphological characters and different states of expression for each characteristic were recorded (Table 1). Based on the characterisation, some accessions with unique traits were identified among the germplasm accessions (Table 2).

Table 1 Frequency distribution of germplasm accessions (108) and checks (4) of safflower for DUS characteristics

DUS Descriptors	No. of genotypes and frequency (%) under each category
First leaf: length of blade	Very short 3 (2.67), Short 4(3.57), Medium37(33.03), Long 43 (38.39),Very long25(22.32)
First leaf: width of blade	Very narrow 1 (0.89), Narrow 16 (14.28),Medium 46(41.07), Broad 27 (24.10),Very broad 21(18.75)
First leaf: ratio (length/width of blade)	Low 2 (1.78), Medium 9 (18.03), High 65 (58.03),Very high 36 (32.14)
First leaf: dentations	Absent/very weak 6 (5.35), Weak 26 (23.21), Medium 64 (57.14), Strong 16 (14.28)
Days to 50% flowering	Late 36 (32.14), Very late 79 (70.53)
Petal: colour	White 6 (5.35), Pale yellow 5 (4.46), Yellow 85 (75.89), Orange16 (14.28)
Petal: change in colour (faded stage)	Grey white 4 (3.57), Pinkish white 3 (2.67), Golden yellow 18 (16.07),Orange 73 (65.17), Red14 (12.5)
Leaf: length of blade	Short 14 (12.5), Medium 85 (75.89), Long 15 (13.39)
Leaf: width of blade	Very narrow 3 (2.67), Narrow 51 (45.53), Medium 43(38.39), Broad 13 (11.60), Very broad 2 (1.78)
Leaf: ratio (length/width of blade)	Low : 3 (2.67), Medium : 80 (71.42), High : 21 (18.75), Very high 8 (7.14)
Leaf: shape	Fusiform : 57 (50.89), Ovate :6 (5.35), Elliptic : 19 (16.96), Obovate : 30 (26.78)
Leaf: number of spines	Few : 12 (10.71), Medium : 37 (33.03), Many: 36 (32.14), Very many : 27 (24.10)
Leaf: dentations	Weak: 7 (6.25), Medium: 22 (19.64), Strong : 50 (44.64), Very strong: 33 (29.46)
Capitulum: length of outer involucral bract of main capitulum (cm)	Short : 38 (33.92), Medium : 74 (66.07)
Capitulum: width of outer involucral bract of main capitulum (cm)	Narrow : 101 (90.17), Medium: 10 (8.92), Broad : 1 (0.89)
Capitulum: ratio length/width of outer involucral bract of main capitulum (cm)	Low : 19 (16.96), Medium: 65 (58.03), High : 28 (25)
Capitulum: number of spines on outer involucral bract of main capitulum	Absent : 8 (7.14), Sparse: 46 (41.07), Dense: 58 (51.78)
Capitulum: diameter of main capitula (cm)	Small : 13 (11.60), Medium: 87 (77.67), Large : 12 (10.71)
Plant: height of insertion of first branch (from ground level) (cm)	Short : 2 (1.78), Medium : 3 (2.67), Tall : 14 (12.5), Very tall 93 (83.03)
Plant:length of longestprimary branch (cm)	Very short : 66 (58.92), Short: 29 (25.89), Medium: 9 (8.03), Long: 3 (2.67),Very long: 5 (4.46)
Plant: height upto main capitula (cm)	Very short : 2 (1.78), Medium: 6 (5.35), Tall: 22 (19.64), Very tall : 82 (73.21)
Seed: weight of 100 seeds (g)	Very low: 18 (16.07), Low: 51 (45.53), Medium: 24 (21.42), High: 19 (16.96)
Seed: colour	White: 69 (61.60), White yellowish: 32 (28.51), Brown yellowish: 11 (9.82)
Seed: number/main capitula	Medium : 36 (32.14), High: 76 (67.85),
Seed: hull content (%)	Low: 19 (16.96), Medium: 84 (75), High: 9 (8.03)
Seed: oil content (%)	Medium: 47 (41.96), High : 43 (38.39), Very high: 22 (19.64)

The germplasm accessions *viz.,* GMU-5135, GMU-3781, GMU-1654, GMU-1758, GMU-5142, GMU-1437, GMU-2424, GMU-3266, GMU-1397, GMU-6004 and GMU-2380 were identified to be unique and can be used as example genotypes for DUS testing or as a distinguishing trait among the germplasm material. Similar studies were reported Mukta *et al.* (2012), Shinde *et al*. (2014), Yusuf (2018), Janjal *et al*. (2018), Nishat (2016) and Shrivastava *et al*. (2018). The germplasm accessions identified in this study can be used in crossing programme to improve the traits in selected agronomically superior genotypes.

Table 2 Genotypes with unique morphological traits

Unique character	Genotypes
First leaf: length of blade: Very short	GMU-5135 (5.5), GMU-6854 (4.38), GMU-1067(5.74)
First leaf: width of blade: Very narrow	GMU-3781(1.34)
First leaf: ratio (length/width of blade): Low	GMU-1654 (1.97), GMU-7572(2.31),
Petal change in colour (faded stage): Pinkish white	GMU-1758 (pinkish), GMU-2757 (pinkish white), GMU-2648 (pinkish white),
Leaf: width of blade: Very narrow	GMU-3781 (1.93), GMU-7399 (1.9), GMU-7601 (1.77)
Leaf: width of blade: Very broad	GMU-5142 (5.75), GMU-5133(5.17),
Leaf: ratio (length/width of blade): Low	GMU-1437 (2.03), GMU-880 (2.38), GMU-1731(2.24)
Capitulum: width of outer involucral bract of main capitulum (cm): Broad	GMU-2424 (2.6)
Plant: height of insertion of first branch (from ground level) (cm): Short	GMU-3266 (14.2), GMU-4610 (14.3)
Plant: height of insertion of first branch (from ground level) (cm): Medium	GMU-1397 (19.8), GMU-7579 (18.3)
Plant: length of longest primary branch (cm): Long	GMU-6004 (60), GMU-6891 (78), GMU-1397 (57)
Plant: Height upto main capitula (cm): Very short	GMU-2380 (35), GMU-7569(30)

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Effect of boron on growth and productivity of groundnut in coastal sandy soils

K M HANEENA*, P VENKATA SUBBAIAH, CH SUJANI RAO[1] AND K SRINIVASULU

Agriculture College, Acharya N G Ranga Agricultural University, Bapatla - 522 101, Andhra Pradesh

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ABSTRACT

A field experiment was conducted at Agricultural College Farm, Bapatla, to study the effect of boron on growth and productivity of groundnut in coastal sandy soils. The experiment comprised of nine treatments with three replications in randomized block design. The growth and yield parameters of groundnut were significantly improved by the application of boron. Among the treatments, soil application of borax @ 12.5 kg/ha resulted in maximum values of growth and yield parameters of groundnut viz., plant height, number of branches, number of leaves, leaf area index, number of pods/plant, haulm yield, pod yield and kernel yield and most of these parameters were on par with all the other boron applied treatments. Application of boron resulted in higher gross and net returns when compared to control. The highest gross returns was obtained by soil application of Borax @ 12.5 kg/ha whereas, highest net returns and BC ratio is obtained by T6 i.e. RDF + foliar spray of Borax @ 0.1% at 65 DAS. Soil application of Borax @ 7.5 kg/ha along with RDF may be recommended from the study as application of boron along with RDF enhances boron availability in soil, growth parameters, yield parameters, yield, quality parameters, nutrient content and uptake in groundnut in coastal sandy soils.

Keywords: Boron, Groundnut, Growth and yield, Micronutrient

Groundnut (*Arachis hypogaea* L.) is an important leguminous oilseed crop grown in tropics and subtropics. It is used as oil seed as well as food crop. It is known as "king of oilseeds" owing to its high oil content. It contains about 50% oil, 25-30 % protein, 20 % carbohydrate and 5 % fiber and ash which make it a substantial contribution to human. The high-energy value protein content and minerals make groundnut a rich source of nutrition at a comparatively low price. It has the capacity to fix atmospheric nitrogen through symbiotic nitrogen fixing bacteria in root nodules.

India is having the largest area of cultivation of groundnut in the world and it is grown on all soil types mainly as a rainfed crop. But the average productivity is low as compared to United States and China mainly due to unreliable weather conditions and mineral deficiencies. Boron is an essential micronutrient in vegetative and reproductive stages of groundnut. Boron plays a dynamic role in various plant structural, physiological and biochemical functions. Physiological process of plants, such as cell elongation, cell maturation, meristematic tissue development, and protein and nucleic acid synthesis and metabolism of carbohydrates, transport of sugars, synthesis of nucleotide, respiration and pollen viability are affected by boron. Boron empowers pollen viability, absorption of ions and affects the metabolic process of different nutrients, maintains structural integrity of the plant and protects plasma membrane from external damage Due to its underground pod bearing habit, the groundnut, is mainly grown in light-textured soils which are generally deficient in macro- and micro-nutrients (Chaudhari *et al.,* 2021). Among the micronutrients, the deficiency of boron is a common feature of coastal sandy soils (Elayaraja and Singaravel, 2016; Mahapatra *et al.*, 2021). Most of the light textured soils of India where, groundnut is grown are deficient in boron and there is a good response for boron application in these soils (Ansari *et al.*, 2013; Viswakarma *et al.*, 2008). Keeping all these points in view an experiment was conducted to evaluate the effect of boron on growth and productivity of groundnut in coastal sandy soils.

The field experiment was carried out at the Agricultural College Farm, Bapatla (Andhra Pradesh, India) during *rabi* season 2019-20 with nine treatments replicated thrice. TAG-24 variety of groundnut was used as a test crop, with a spacing of 30 cm x 10 cm. The treatments comprised of T1 - RDF (35:40:50 N-P_2O_5-K_2O kg/ha through Urea, SSP and MOP along with 500 kg gypsum), T_2- RDF + soil application of Borax @ 7.5 kg/ha, T_3- RDF + soil application of Borax @ 10 kg/ha, T_4-RDF + soil application of Borax @ 12.5 kg/ha, T_5-RDF + foliar spray of Borax @ 0.1% at 45 DAS, T_6-RDF + foliar spray of Borax @ 0.1% at 65 DAS, T_7-RDF + foliar spray of Borax @ 0.1% @ 45 & 65 DAS, T_8-T_2 + foliar spray of Borax @ 0.1% at 65 DAS and T_9-T_3 + foliar spray of Borax @ 0.1% at 65 DAS. The mean values of various weather parameters pertaining to the crop growth period of previous 20 years and current season were recorded from the India Meteorological Department Observatory, Bapatla, to arrive at a general distribution of different weather parameters over the years

[1]Advanced Post Graduate Centre, Lam, Guntur-522 034, Andhra Pradesh;
*Corresponding author's E-mail: haneenakm201541434@gmail.com

and their deviation in current crop growing season. During experimentation the study area experienced average maximum and minimum temperatures of 26.2°C and 17.9°C, respectively with a total rainfall of 421 mm over 19 rainy days.

The initial composite soil sample was collected from experimental field and analyzed for physical, physico-chemical properties and nutrient status. The results of the analyses indicated that the soil was sandy in texture with neutral reaction (pH 6.77), non-saline (EC 0.28 dS/m) and low in organic matter (1.3 g/kg). The bulk density was 1.69 Mg/m^3. The soil was low in nitrogen (113 kg/ha), phosphorus (21.79 kg P_2O_5/ha) and potassium (112 kg K^2O/ha), sufficient in sulphur (20 ppm), iron (6.01 mg/kg), manganese (4.63 mg/kg) and copper (1.85 mg/kg) and deficient in boron (0.30 mg/kg) and zinc (0.48 mg/kg). A common dose of 35 kg nitrogen/ha was applied as urea, in two equal splits as half at basal and half at 30 DAS by taking the plot size into consideration. A common dose of phosphorus @ 40 kg/ha in the form of single super phosphate and potassium @ 50 kg/ha in the form of muriate of potash was applied as basal dose just before sowing. Boron was applied as soil application of borax @ 7.5 kg/ha, 10 kg/ha and 12.5 kg/ha as basal dose just before sowing and foliar application of borax @ 0.1 % at 45 DAS and 65 DAS as per the treatments (0.1% = 0.1g/100mL).

The crop was planted in the third week of October. The crop was raised with all the standard package of practices and protection measures also timely carried out as required. Five representative plants were selected randomly from each treatment, labelled properly and observations on growth parameters were recorded periodically and yield was recorded at harvest. The cost of cultivation was worked out taking into consideration of various costs *viz.,* non variable costs and variable cost (Treatment costs). The gross returns were worked out by summing the returns from both pod and haulm from the different treatments individually, at the prevailing market prices. The data were analyzed statistically following the analysis of variance (ANOVA) technique as suggested by Panse and Sukhathme (1978) for Randomized block design.

The results of the investigation revealed that application of boron positively influenced plant height at different growth stages of groundnut (Table 1). The maximum plant height (35.28, 48.01 and 53.01 cm at peg penetration, pod development and at harvest stages respectively) was recorded in the treatment T_4 (RDF + soil application of Borax @ 12.5 kg/ha). At peg penetration stage, the treatment T_4 was on par with the treatments T_9 (34.14 cm) followed by T_3 (33.62 cm), T_2 (32.87 cm) and T_8 (32.82 cm). At pod development stage, the treatment which received the highest plant height (T_4) was on par with the treatments, T_3 (45.68 cm) followed by T_9 (45.58 cm), T_2 (43.67 cm), T_8 (43.66 cm), T_5 (44.56 cm) and T_7 (43.91 cm). The highest plant height at harvest stage was obtained by T_4 and this was on par with the treatments T_9 (52.67 cm) followed by T_8 (50.81 cm), T_7 (50.41 cm), T_3 (50.17 cm), T_2 (49.61 cm) and T_5 (49.16 cm).The minimum plant height (28.13, 37.13 and 42.18 cm at peg penetration, pod development and at harvest stages respectively) was recorded in treatment T_1 (RDF). Increased plant height in the treatments that received soil application of boron was more than foliar application treatments. This might be due to application of boron in early stages of plant growth. Higher plant height in the boron treatments might be due to the role of the nutrient in various physiological and biochemical processes contributing to the growth of meristematic region. Yadav and Kumawat (2003) also reported similar results in taramira.

The data indicated that application of boron positively influenced number of primary branches/plant at all the stages of groundnut (Table 1). All the boron treatments resulted in significantly higher number of branches over control at all the growth stages of groundnut. The highest number of branches at peg penetration and pod development stages (5.72 and 7.10 respectively) was reported in the treatment T_4 and it was on par with the treatments T_3, T_9, T_8 and T_2. Whereas, the highest number of branches (7.88) at harvest stage was recorded in the treatment T_9 and this was on par with the treatments T_4, T_3 and T_8. The lowest number of branches was recorded in T_1 at all the stages (3.41, 4.50 and 5.10 at peg penetration, pod development and harvest stages respectively). Increased number of branches/plant in the treatments that received soil application of boron was more than foliar application treatments, but the difference was non-significant. Role of boron in various physiological and biological processes contributing to proper growth of plants to their maximum potential might be the reason for higher number of primary branches (Nadaf and Chidanandappa, 2011). These results were in conformity with the findings of Kabir *et al*. (2013).

The data pertaining to number of compound leaves/plant indicated that, different boron treatments had significant effect on this trait (Table 1). Highest number of leaves at peg penetration and pod development stages (32.97 and 42.53 respectively) was reported in T_4 and this was on par with the treatments T_9 and T_3. On the other hand, T_9 (reported the highest number of leaves (35.52) at harvest stage and this was on par with the treatments T_4 and T_8. The lowest number of branches was recorded in T_1 in all the stages (21.63, 29.12 and 23.13 at peg penetration, pod development and harvest stages respectively). Geethanjali *et al.* (2015) supported the results of significant increase in number of leaves with application of boron by its role in

formation of new plant cells, elevated level of IAA, development of meristematic tissues, cell elongation and tissue differentiation and sugar transportation.

The data revealed positive influences on leaf area index due to boron application in groundnut (Table 1). All the boron treatments resulted in significantly higher leaf area index over control except T_6 at harvest stage. The maximum leaf area index (1.43 and 4.31 at peg penetration and pod development respectively) was recorded in the treatment and it was on par with the treatments T_9 and T_3. The maximum leaf area index at harvest stage (3.60) was recorded in T_9 and this was on par with all the other boron applied treatments except T_5, T_6 and T_7. The minimum leaf area index (0.42, 1.89 and 1.78 at peg penetration, pod development and at harvest stages respectively) was recorded in treatment T_1. Soil application alone or foliar application along with soil application of boron reported significantly higher leaf area index than foliar application alone. Boron plays an important role in physiological processes such as cell division and cell elongation by influencing IAA and nucleic acid metabolisms which in turn improved the plant growth, therefore reflected positive effect in number of leaves and leaf area index (Bameri *et al.,* 2012). Similar results were also reported by Immanuel *et al.* (2019) in groundnut.

The application of boron significantly influenced number of pods/plant of groundnut (Table 1). The highest number of pods/plant (15.52) was recorded in the treatment T_4 and this was on par with T_9 (15.32) followed by T_8 (14.75), T_3 (14.17) and T_7 (13.85). The lowest number of pods per plant (9.28) was recorded in the treatment T_1. Increased number of pods/plant in the treatments that received soil application of boron was more than foliar application treatments. Singh and Vidyachowdary (1996) reported that boron is known to play a role in IAA and carbohydrate metabolism, translocation of sugars, seed development and its application increased the number of pods/plant, yield and its components in groundnut. Higher pods/plant due to boron application might be also due to its role in prevention of flower and pod drop, thereby retaining high number of pods/plant. Naiknaware *et al.* (2015) reported that application of boron increased the number of pegs and pods and finally it helped to get the maximum pod yield of peanut.

The data pertaining to shelling percentage revealed non-significant differences between different treatments (Table 1). The treatments that received boron either through soil application or foliar application increased shelling percentage of groundnut when compared to control (RDF), but the increase was non-significant. Highest shelling percentage (74.58%) was observed in T_4 and the lowest (66.37%) was observed in T1. The increase in shelling percentage might be due to the role of boron in sugar transport and utilization by developing pollen and embryos, fruit and seeds and its unique role in maintaining cell wall structure and function (Perica *et al.,* 2001). The findings were in consonance with the results reported by Ansari *et al.* (2013) in groundnut.

The data pertaining to 100-kernel weight of groundnut revealed non-significant differences between different boron treatments (Table 1). Close observation of data revealed increase in the 100-kernel weight in all the boron treatments when compared to control (RDF). Highest 100-kernel weight (45.81 g) was observed in T_4 and the lowest (37.22) was observed in T_1. This might be due to the effect of boron on flower viability and flower fertility which in turn resulted into decreased number of aborted fruits and increased number of sound mature kernels, ultimately higher weight of 100-kernels. The influence of boron spray on test weight might be due to the increased translocation of assimilates from source to sink. Boron requirement at reproductive stage is more than any other stage, which was met by soil or foliar application of borax ultimately resulted in healthy pods and gained more weight. Kabir *et al.* (2013) also reported similar results in groundnut.

The results of the investigation showed that application of boron significantly influenced haulm yield of groundnut (Table 2). T_4 recorded the highest haulm yield (3398 kg/ha) and this was on par with the treatments T_9 (3382 kg/ha) followed by T_8 (3361 kg/ha), T_3 (3324 kg/ha), T_2 (3286 kg/ha), T_7 (3130 kg/ha), T_6 (3123 kg/ha) and T_5 (3112 kg/ha). The lowest haulm yield (2719 kg/ha) was recorded in T_1. The improvement in haulm yield can be attributed to the role of B in stabilizing certain constituents of cell wall and plasma membrane, enhancement of cell division, tissue differentiation and metabolism of nucleic acids, carbohydrates, proteins, auxins and phenols. These results were in conformation with those of Kabir *et al.* (2013) in groundnut.

The results revealed that there was significant increase in pod yield due to boron application (Table 2). Significantly higher pod yield (2441 kg/ha) was recorded by the treatment T_4 which was 18% over control (RDF), and it was on a par with the treatments T_9 (2434 kg/ha) followed by T_8 (2392 kg/ha), T_3 (2390 kg/ha), T_2 (2357 kg/ha), T_7 (2328 kg/ha), T_6 (2304 kg/ha) and T_5 (2298 kg/ha). The lowest pod yield (2063 kg/ha) was recorded by the treatment T_1. Application of B significantly enhanced chlorophyll content and photosynthetic intensity of the leaves, increased dry matter accumulation of the plants, advanced their flowering and promoted the transport of the photosynthesis from the vegetative organs to the reproductive organs, enhances protein synthesis, thus resulting in significant improvement of the groundnut yield (Qiong *et al.,* 2002). Boron application increased the groundnut yield by increasing the yield contributing parameters and higher number of developed pods. Singh *et al.* (2017) reported

early flowering because of application of boron thus helped in converting more peg to pod and increased number of pods, thus yield.

The data from the experiment indicated that different levels and methods of application of boron significantly influenced kernel yield of groundnut (Table 2). The highest kernel yield (1821 kg/ha) was recorded by the treatment T_4 and it was on a par with all the boron applied treatments. The lowest kernel yield (1367 kg/ha) was recorded by the treatment T_1. Increase in kernel yield with boron application might be also due to its vital role in promotion of photosynthetates transportation from vegetative organs to reproductive organs (Qiong *et al.,* 2002). Increase in shelling percentage and 100-kernel weight also influenced kernel yield. Vishwakarma *et al.* (2008) supported the significant increase in number of pods per plant by application of boron in groundnut.

Table 1 Effect of boron on different characters of groundnut

Treatment	Plant height (cm)			Primary branches/plant			No of leave s/plant			Leaf area index			Number of pods /plant	Shelling Percentage	100-kernel weight
	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3			
T1: RDF	28.13	37.13	42.18	3.41	4.50	5.10	22.28	30.29	27.49	0.42	1.89	1.78	9.28	66.37	37.22
T2: RDF + soil application of Borax @ 7.5 kg /ha	32.87	43.67	49.61	5.47	6.50	7.03	21.88	34.14	31.28	1.18	3.52	3.18	13.08	72.38	44.41
T3: RDF + soil application of Borax @ 10 kg /ha	33.62	45.68	50.17	5.63	6.87	7.51	28.71	37.77	32.59	1.33	3.90	3.21	14.17	72.95	45.21
T4: RDF + soil application of Borax @ 12.5 kg /ha	35.28	48.01	53.01	5.72	7.10	7.82	29.75	38.39	35.52	1.43	4.31	3.54	15.52	74.58	45.81
T5: RDF + foliar spray of Borax @ 0.1% at 45 DAS	29.09	44.56	49.16	3.42	5.21	6.27	1.21	1.58	1.36	0.41	2.79	2.73	13.28	72.27	43.06
T6: RDF + foliar spray of Borax @ 0.1% at 65 DAS	28.99	37.64	44.81	3.41	4.51	5.95	3.62	4.72	4.09	0.44	1.91	1.90	12.10	72.34	43.28
T7: RDF + foliar spray of Borax @ 0.1% at 45 & 65 DAS	29.14	43.91	50.41	3.40	5.23	6.64	7.93	7.58	7.78	0.43	2.80	2.75	13.85	72.42	44.84
T8: T2+ foliar spray of Borax @ 0.1% at 65 DAS	32.82	43.66	50.81	5.48	6.51	7.45	22.28	30.29	27.49	1.24	3.55	3.30	14.75	73.64	44.97
T9: T3+ foliar spray of Borax @ 0.1% at 65 DAS	34.14	45.58	52.67	5.61	6.86	7.88	21.88	34.14	31.28	1.42	3.95	3.60	15.32	74.14	45.77
SEm (±)	1.54	2.02	2.18	0.20	0.26	0.28	28.71	37.77	32.59	0.05	0.16	0.139	0.67	2.92	1.96
CD (0.05%)	4.61	6.06	6.54	0.60	0.77	0.83	29.75	38.39	35.52	0.14	0.47	0.42	2.01	NS	NS
CV (%)	8.48	8.08	7.67	7.47	7.52	7.04	1.21	1.58	1.36	9.01	8.58	8.323	8.61	7.01	7.74

(Note: RDF: 35:40:50 N-P_2O_5-K_2O kg/ha through urea, SSP and MOP along with 500 kg gypsum) stage 1: Peg penetration Stage 2: Pod development Stage 3: Harvest

Table 2 Effect of boron on haulm yield, pod yield and kernel yield of groundnut

Treatment	Haulm yield (kg /ha)	Pod yield (kg /ha)	Kernel yield (kg /ha)
T1: RDF	2719	2063	1367
T2: RDF + soil application of Borax @ 7.5 kg /ha	3286	2357	1702
T3: RDF + soil application of Borax @ 10 kg /ha	3324	2392	1741
T4: RDF + soil application of Borax @ 12.5 kg /ha	3398	2442	1821
T5: RDF + foliar spray of Borax @ 0.1% at 45 DAS	3112	2298	1660
T6: RDF + foliar spray of Borax @ 0.1% at 65 DAS	3123	2304	1668
T7: RDF + foliar spray of Borax @ 0.1% at 45 & 65 DAS	3130	2328	1687
T8: T2+ foliar spray of Borax @ 0.1% at 65 DAS	3361	2390	1751
T9: T3+ foliar spray of Borax @ 0.1% at 65 DAS	3382	2434	1805
SEm (±)	111	71	65
CD@0.05 %	334	213	194
CV (%)	6.02	5.26	6.64

(Note: RDF: 35:40:50 N-P_2O_5-K_2O kg /ha through urea, SSP and MOP along with 500 kg gypsum)

Table 3 Economics of different treatments of groundnut crop as influenced by boron application

Treatment	Cost of cultivation (₹/ha)	Gross returns (₹/ha)	Net returns (₹/ha)	B:C ratio
T1: RDF	41000	94168	53168	2.30
T2: RDF + soil application of Borax @ 7.5 kg /ha	48500	107590	59090	2.22
T3: RDF + soil application of Borax @ 10 kg /ha	51000	109182	58182	2.14
T4: RDF + soil application of Borax @ 12.5 kg /ha	53500	111475	57975	2.08
T5: RDF + foliar spray of Borax @ 0.1% at 45 DAS	42000	104912	62912	2.50
T6: RDF + foliar spray of Borax @ 0.1% at 65 DAS	42000	105175	63175	2.50
T7: RDF + foliar spray of Borax @ 0.1% at 45 & 65 DAS	43000	106278	63278	2.47
T8: T2+ foliar spray of Borax @ 0.1% at 65 DAS	49500	109130	59630	2.20
T9: T3+ foliar spray of Borax @ 0.1% at 65 DAS	52000	111125	59125	2.14
SEm (±)	-	3239	3239	0.07
CD@0.05 %	-	4580	NS	0.21
CV (%)	-	5.26	9.41	5.32

(Note: RDF: 35:40:50 N-P_2O_5-K_2O kg/ha through urea, SSP and MOP along with 500 kg gypsum)

The data revealed that lowest cost of cultivation was recorded in the treatment T_1 and highest cost of cultivation recorded in the treatment T_4 (Table 3). As the rate of fertilizer increased cost of cultivation also increased. The cost of cultivation is higher in soil application compared to foliar application because of higher quantity of boron for soil application.The highest gross returns (₹111475) was obtained in the treatment T_4 followed by T_9 (₹111125) and lowest gross returns (₹94168) was obtained in the treatment T_9 (Table 7). The application of boron resulted in increase in gross returns, this might be due to addition of boron fertilizer resulted in increase in availability of the nutrient to plants resulting in higher yields.

A perusal of the data revealed that application of boron increased the net returns of groundnut crop as compared to control. Highest net returns (₹63278) was fetched by the treatment T_7 followed by T_6 (₹63175) and lowest (₹53168) was recorded in the treatment T_6. Soil application resulted in lower net returns than foliar application because of high cost of borax.

The higher returns per rupee investment (2.50) was obtained by the treatments T_5 and T_6 followed by T_7 (2.47) treatment and lowest (2.08) benefit cost ratio was obtained in the treatment T_4 (Table 3). The higher returns recorded with the foliar applied treatments was due to improvement of growth and yield attributes resulted in higher yield as well as lower cost when compared to soil application. These results are in agreement with the findings of Karthik *et al.* (2021).

ough urea, SSP and MOP along with 500 kg gypsum)

The data pertaining to available boron content of soil are presented in the Table 4. The results of the investigation showed that the application of boron significantly influenced the hot water extractable boron content of soil at different growth stages of groundnut. The perusal of the data clearly indicated that the boron concentration of soil increased from the initial levels due to the application of borax, in all the soil applied treatments and then decreased over the crop period. Among the different levels of boron application treatments significant improvement in boron content was recorded as the boron levels increased from 7.5 kg borax/ha to 12.5 kg borax/ha at all the stages of the crop growth. The maximum available boron content (0.73, 0.65 and 0.59 mg/kg at peg penetration, pod development and harvest stages respectively) was recorded in T_4 and this was superior over all other treatments. The lowest available boron content (0.28, 0.27 and 0.26 mg/kg at peg penetration, pod development and harvest stages respectively) was recorded with T_1. Singh *et al*. (2005) reported significant increase in hot water extractable boron content of soil due to the application of boron. The significant build up of available B status under this boron level might be due to their direct adequate application to soil. Therefore, after meeting the requirement of the crop, the added boron might help to increase the boron status of the soil (Sathya *et al.,* 2009). The available boron content in the soil was sufficient even after the harvest of the crop which was applied as soil application of borax.

Soil application of borax @ 7.5 kg /ha or foliar spray of B @ 0.1% at 45 and 60 DAS along with RDF may be recommended from the study as this improved available boron content in soil and concentration in plants, thus improved growth, yield and quality parameters.

Table 4 Effect of boron on available boron (mg/kg) in soil at different growth stages of groundnut

Treatment		Peg penetration stage	Pod development stage	Harvest stage
T1:	RDF	0.28	0.27	0.26
T2:	RDF + soil application of Borax @ 7.5 kg /ha	0.56	0.50	0.48
T3:	RDF + soil application of Borax @ 10 kg /ha	0.61	0.55	0.52
T4:	RDF + soil application of Borax @ 12.5 kg /ha	0.73	0.65	0.59
T5:	RDF + foliar spray of Borax @ 0.1% at 45 DAS	0.29	0.28	0.27
T6:	RDF + foliar spray of Borax @ 0.1% at 65 DAS	0.30	0.29	0.27
T7:	RDF + foliar spray of Borax @ 0.1% at 45 & 65 DAS	0.31	0.30	0.26
T8:	T2+ foliar spray of Borax @ 0.1% at 65 DAS	0.56	0.52	0.50
T9:	T3+ foliar spray of Borax @ 0.1% at 65 DAS	0.62	0.56	0.53
SEm (±)		0.02	0.02	0.02
CD (P=0.05 %)		0.06	0.06	0.04
CV (%)		7.55	7.80	7.93

Note: RDF: 35:40:50 N-P_2O_5-K_2O kg/ha

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Yield and nutrient uptake of sesame (*Sesamum indicum* L.) as influenced by plant growth regulators

I ANGEL AND R POONGUZHALAN

Pandit Jawaharlal Nehru College of Agriculture and Research Institute, Karaikal-609 603, U.T. of Puducherry

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ABSTRACT

A field experiment was conducted at Pandit Jawaharlal Nehru College of Agriculture and Research Institute, Karaikal to study the effect of plant growth regulators on yield and nutrient uptake of sesame (*Sesamum indicum* L.) during summer 2021. The field experiment with thirteen treatments was conducted on a loamy sand soil in a randomized block design and replicated thrice. The treatments included four plant growth regulators at various concentrations (NAA 50 ppm, NAA 100 ppm, CCC 100 ppm, BA 5 ppm and HA 500 ppm) and water spray at two levels of application (one spray - at flowering stage and two sprays - at flowering and capsule formation stages) and an unsprayed control. The results revealed that spraying NAA 100 ppm twice recorded the highest seed yield (633 kg/ha), haulm yield (1296 kg/ha) as well as the uptake of NPK by sesame when compared to other growth regulators.

Keywords: Nutrient uptake, Plant growth regulators, Sesame, Yield

Sesame (*Sesamum indicum* L.) is one of the most important ancient oilseed crops cultivated extensively in the world. Sesame is a traditional healthy food and its seeds are a good source of fibre which aid in lowering the cholesterol and triglycerides, the main risk factors of heart disease (Langham *et al.*, 2006). Sesame has an excellent nutritive value with long shelf life. India ranks first in the world with 19.47 lakh ha area and 8.66 lakh tonnes production and accounts for one-third of world's sesame production. However, the average yield of sesame in India is low (413 kg/ha) as compared to other countries in the world (535 kg/ha).

The yield of sesame may be increased by using numerous technologies and practices such as use of high yielding varieties, proper nutrient, water, and weed management techniques, application of growth regulators, etc (Rajitha *et al.*, 2021). Plant growth regulators are known to act by controlling or modifying the plant growth processes such as formation of leaves and flowers, elongation of stems, uptake of nutrients, development and maturity (Ewais *et al.*, 2013; Siddik *et al.*, 2015; Singaravel *et al.*, 2016). Bashist (1990) reported that application of CCC (cycocel) increased the nitrogen uptake in sesame while Manal *et al.* (2014) reported that the highest uptake of phosphorus and potassium was achieved by foliar spry of humic acid @ 1.5 g/l. Chlormequat application increased uptake of N, P and K in Brassica juncea (Guroo and Patel, 1993). Habiburrahman Ansari (1996) opined that spraying of GA3 significantly enhanced the uptake of N, P and K by mustard in comparison to water spray. Foliar spray of humic acid @ 6000 ppm resulted in the highest uptake of P and K in peanut (Salwa and Eisa, 2011). The scientific data on the use of plant growth regulators and their effect on sesame yield and nutrient uptake by sesame are scanty. In this context, this study was conducted to find out the effect of different growth regulators *viz*., Naphthalene Acetic Acid (NAA), cycocel (CCC), Benzine Amino Purine (BA) and Humic Acid (HA) at different concentrations on yield and nutrient uptake by sesame.

A field experiment was conducted during summer (March to June) 2021 at Pandit Jawaharlal Nehru College of Agriculture and Research Institute, Karaikal, U.T. of Puducherry. The soil was loamy sand in texture and neutral in reaction (pH=6.63). The soil was medium in organic carbon (0.8%), low in available nitrogen (174 kg/ha), high in available phosphorus (34 kg/ha) and medium in available potassium (115 kg/ha) contents. The treatments consisted four plant growth regulators at various concentrations (NAA @50 ppm, NAA @100 ppm, CCC @100 ppm, BA @5 ppm and HA @500 ppm) and water spray, each at two different levels of application (One spray - at flowering stage and Two sprays - at flowering and capsule formation stages) and an unsprayed control. The field experiment involving 13 treatments was laid out in a randomized block design (RBD) with three replications. Sesame seeds (cv. TMV 7) were sown adopting a seed rate of 5 kg/ha in lines at 30 cm spacing. Thinning was done at 19 DAS and at 28 DAS maintaining one healthy plant at a spacing of 30 cm × 30 cm.

The plant growth regulators were sprayed according to the treatments at two different time points *viz.,* at flowering stage (47 DAS) and at flowering and capsule formation stages (47 and 62 DAS). The data on yield was recorded. The nitrogen, phosphorus and potassium contents of sesame plants at harvest were analyzed following the standard procedures of micro-Kjeldahl, vanado-molybdate and flame

Corresponding author's E-mail: poonguzhalan@yahoo.com

photometric methods (Jackson, 1973), respectively. The uptake was computed by multiplying the nutrient content and the yield. The results were statistically analyzed and the critical difference (CD) values at 5% level of significance were computed to find out the differences between treatment means.

Application of plant growth regulators substantially enhanced the seed and haulm yields of sesame. Significantly higher seed (633 kg/ha) and haulm (1296 kg/ha) yields were recorded when NAA 100 ppm was sprayed twice at flowering and capsule formation stages (Table 1). However, many of the treatments did not differ significantly for seed yield and haulm yield. Application of NAA 100 ppm twice resulted in more number of branches/plant which made the plants to carry more flowers, pods and seeds. Also marked increase in leaf area index (LAI) led to increase in photosynthesis, resulting in greater transfer of assimilates to the seed, causing increase in their weight and finally the seed yield. Similar observations were reported by Siddik *et al.* (2015). The next best growth regulator in terms of seed and haulm yields was spraying of humic acid 500 ppm once at flowering. The higher seed yield and haulm yield might also be attributed to its more vegetative growth and bearing capacity leading to more yield potentiality. Increase in seed yield (Kandil and Esmail, 2015) and haulm yield (Singaravel *et al.,* 2016) by the application humic acid has been reported earlier.

The nutrient uptake by sesame was significantly higher when growth regulators were applied as compared to control (Table 1). Among the different growth regulators, application of NAA 100 ppm twice at flowering and capsule formation stages recorded significantly higher N uptake (38.7 kg/ha) than other growth regulators. The lowest N uptake was recorded under control (20.9 kg/ha). Application of cycocel 100 ppm at flowering and capsule formation stages resulted in the highest P uptake (7.6 kg/ha) and was on par with single spray of NAA 100 ppm or cycocel 100 ppm at flowering. Unsprayed control registered the lowest P uptake (4.8 kg/ha). Lone (2001) also found that spraying 400 ppm cycocel resulted in the highest uptake of N, P and K by mustard. Gangadhar and Brar (2022) also recorded maximum phosphorus uptake in seed and straw of Brassica napus with the application of NAA 40 ppm while the minimum phosphorus uptake was observed in treatment without growth regulator. The K uptake by sesame was also higher under two sprays of NAA 100 ppm at flowering and capsule formation (23.3 kg/ha) which however was on par with single spray of humic acid 500 ppm, cycocel 100 ppm and NAA 50 ppm at flowering. The lowest K uptake was registered under control (15.6 kg/ha). Adam *et al.* (2012) also reported an increase in NPK uptake due to application of NAA 100 ppm. The highest nutrient uptake in growth regulator applied sesame might be attributed to enhanced vegetative growth as indicated by higher dry matter production and higher seed and haulm yields.

From the results of this study, application of NAA 100 ppm at flowering and capsule formation stages is found to be a suitable agronomic practice for increasing the yield and nutrient uptake by sesame.

Table 1 Effect of plant growth regulators on yield and nutrient uptake of sesame

Treatment	Seed yield (kg/ha)	Haulm yield (kg/ha)	Nutrient uptake (kg/ha)		
			Nitrogen	Phosphorus	Potassium
T1 : NAA 50 ppm at F	620a	1207a	30.1	7.1	20.7
T2 : NAA 50 ppm at F & CF	582a	1038bc	26.3	6.5	18.6
T3 : NAA 100 ppm at F	595a	1173ab	26.2	6.7	19.7
T4 : NAA 100 ppm at F & CF	633a	1296a	38.7	7.5	23.3
T5 : CCC 100 ppm at F	615a	1204a	31.5	7.3	22.3
T6 : CCC 100 ppm at F & CF	589a	1085b	30.9	7.6	22.7
T7 : BA 5 ppm at F	507b	961c	25.8	5.8	18.1
T8 : BA 5 ppm at F & CF	490b	951c	22.5	6.5	18.0
T9 : HA 500 ppm at F	623a	1225a	26.6	7.2	22.7
T10 : HA 500 ppm at F & CF	603a	1201a	30.5	6.8	21.7
T11 : Water Spray at F	473b	862c	21.6	5.4	16.8
T12 : Water Spray at F & CF	480b	943c	21.2	5.9	18.4
T13 : Control (No spray)	462b	858c	20.9	4.8	15.6
SEd(±)	27	90	2.60	0.76	1.41
CD (P=0.05)	55	185	5.36	1.57	2.91

F: Flowering CF: Capsule formation Mean values with same alphabets did not differ significantly

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Pathogenicity of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium* (*Nomuraea*) *rileyi* against castor hairy caterpillar, *Euproctis fraterna* (Lepidoptera: Lymantriidae)

E BHARATHI AND P DURAIMURUGAN*

ICAR-Indian Institute of Oilseeds Research, Rajendranagar, Hyderabad-500 030, Telangana

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ABSTRACT

The castor hairy caterpillar, *Euproctis fraterna* is the most destructive insect pest of castor, damaging the crop during both *kharif* and *rabi* seasons. Biocontrol agents, particularly entomopathogens are potential alternatives to chemical pesticides for safe and eco-friendly insect pest management. Pathogenicity of the entomopathogenic fungi *viz*., *Beauveria bassiana* and *Metarhizium* (*Nomuraea*) *rileyi* were evaluated against larvae of the *E. fraterna* under laboratory conditions. Among the entomopathogenic fungi, *B. bassiana* recorded high pathogenicity against *E. fraterna* (80 ± 5.8% larval mortality), while moderate pathogenicity was observed with *M. rileyi* (60 ± 5.8% larval mortality) treated at the concentration of 10^8 conidia/ml. *B. bassiana* recorded the least median lethal concentration (LC_{50}) for *E. fraterna* (4.4×10^5 conidia/ml) as compared to *M. rileyi* (LC_{50} at 5.8 x 10^6 conidia/ml). *B. bassiana* also recorded the shortest median lethal time (LT_{50} value of 3.9 days) to cause the larval mortality in *E. fraterna*, while *M. rileyi* recorded LT_{50} value of 4.5 days. The entomopathogenic fungi, *B. bassiana* can be used in IPM programme as a potential bio-control agent against *E. fraterna* in castor

Keywords: *Beauveria bassiana*, Entomopathogenic fungi, *Euproctis fraterna*, *Metarhizium rileyi*, Pathogenicity

Castor (*Ricinus communis* L.) is one of the most important non-edible oilseed crop that supports quite a large number of agro-based industries including lubricating oils, cosmetics, paints, medicine, biopolymers and so on. The current castor production in the country is 16.5 lakh tonnes from 8.9 lakh hectares with a productivity of 1851 kg/ha (DAC&FW, 2021). One of the major constraints in exploiting higher productivity in castor is the damage due to lepidopteran insect pests *viz.,* semilooper, tobacco caterpillar, hairy caterpillars and capsule borer (Duraimurugan and Alivelu, 2017). It is assessed that castor yields are abridged by 17.2 to 63.3% due to the insect pests during *kharif* season (Lakshminarayanana and Duraimurugan, 2014). Castor hairy caterpillar, *Euproctis fraterna* Moore (Lepidoptera: Lymantriidae) is one of the major threats to castor production during both *kharif* and *rabi* seasons. The caterpillars feed gregariously on leaves, leading to severe defoliation. Under severe infestation, they also feed on capsules and reduce the crop yield. Chemical insecticides have been used widely to control the pest. However, the broad spectrum chemical insecticides are unsafe to potential parasitoids of lepidopteran pests in castor ecosystem (Duraimurugan *et al.,* 2017). Biocontrol agents, particularly entomopathogens, offer considerable promise for eco-friendly insect pest management (Duraimurugan and Vimala Devi, 2021). Entomopathogenic fungi are potentially the most versatile biocontrol agents, because they have wide host ranges, infect at different ages and stages of their hosts and often cause natural epizootics. These fungi have an advantage over other entomopathogens such as bacteria and viruses as they can infect their host not only through diet, but also directly from the spiracles and insect cuticle (Vimala Devi and Duraimurugan, 2013). The present investigation was undertaken to evaluate the pathogenicity of entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium* (*Nomuraea*) *rileyi* isolates against castor hairy caterpillar, *Euproctis fraterna*.

Laboratory culture of castor hairy caterpillar, *Euproctis fraterna* was established from larvae collected in castor fields at the Research Farm, ICAR-Indian Institute of Oilseeds Research (IIOR), Hyderabad during October and November 2020. The culture was maintained on castor leaves at ambient conditions (27±2ºC, 60-70% RH) and used for the experiment. Plants of castor cultivar, VP-1 raised in field without exposure to insecticides were used for the laboratory bioassays.

Indigenous isolates of *Beauveria bassiana* (ITCC 4513) and *Metarhizium* (*Nomuraea*) *rileyi* (a local isolate) from ICAR-IIOR collection was used for the experiment. The isolates of *B. bassiana* and *M. rileyi* were cultured on sterilized PDA and SMAY medium slants (Hi-Media, India), respectively. The slants were incubated at 28°C for 10 days. After sporulation, aerial conidia were harvested by flooding the plate with sterile distilled water containing 0.02% Tween-80. Spore suspensions were prepared and conidial count was determined using improved Neubauer haemocytometer. The conidial suspension of 108 conidia per ml was prepared by counting the spores in the improved Neubauer counting chamber and serial dilutions of the suspension used for bioassay studies.

Corresponding author's E-mail: p.duraimurugan@icar.gov.in

The fungal strains of *B. bassiana* and *M. rileyi* were tested against larvae of castor hairy caterpillar, *E. fraterna* using leaf dip method. Initial virulence test for both the entomopathogenic fungi was carried out with a single concentration (10^8 conidia/ml) of conidial suspension. To assess the Lethal Concentration (LC_{50}) for 50%, inoculum concentration ranging from 10^2 to 10^8 conidia/ml were tested for both the entomopathogenic fungi against 6-day-old larvae of *E. fraterna* along with an untreated control. Bioassays with different fungal isolates were carried out by dipping the castor leaves in conidial suspensions plus 0.02% Tween at each concentration, while distilled water plus 0.02% Tween 20 was used for the control. The leaves were dipped in solutions of respective treatments for about 30 seconds and the excess fluid was drained off. The leaves with the petiole immersed in water in a vial and allowed for shade drying. Each vial having the treated castor leaves was kept separately in a glass jar (30 cm x 15 cm) and the larvae of hairy caterpillar was released. Ten larvae of 6-day-old *E. fraterna* were released per replication. Likewise three replications were maintained for each treatment. The glass jars were covered with muslin cloth and kept at ambient conditions (27±2ºC, 60-70% RH). Observations on larval mortality were recorded every day for 5 days. The cadavers were washed in sterile water and placed on moist tissue paper in Petri-plates and observed for mycelial growth and sporulation to confirm mortality due to infection by the entomopathogenic fungi. Per cent larval mortality was assessed and the Lethal Concentration (LC_{50}) for 50% was estimated using Probit Regression analysis using Statistical Packages for Social Sciences (SPSS).

Among indigenous entomopathogenic fungi evaluated, both the isolate of *Beauveria bassiana* (ITCC 4513) and *Metarhizium* (*Nomuraea*) *rileyi* (a local isolate) was found pathogenic to larvae of castor hairy caterpillar, *E. fraterna*. *Beauveria bassiana* was considered as more potent against E. fraterna with higher mean per cent mortality (80% @ 10^8 conidia/ml at 5 days after treatment) as compared to *Metarhizium* (Nomuraea) *rileyi* (60% @ 10^8 conidia/ml at 5 days after treatment) (Table 1). The values of least median lethal concentration (LC_{50}), fiducially limits corresponding to different LC_{50} and LC_{90} values and chi-square of the entomopathogenic fungi were presented in Table 1. Among the entomopathogenic fungi, *B. bassiana* recorded the least median lethal concentration (LC_{50}) for castor hairy caterpillar, *E. fraterna* (4.4×10^5 conidia/ml), while *M. rileyi* had shown their LC_{50} at 5.8×10^6 conidia/ml. LC_{90} also followed the trend of LC_{50}, *B. bassiana* recorded the lower LC_{90} of 2.8×10^{10} conidia/ml as compared to 7.6×10^{14} conidia/ml observed in *M. rileyi* against larvae of *E. fraterna* at 5 days after treatment.

Table 1. Percent mortality and concentration-mortality response of castor hairy caterpillar, *Euproctis fraterna* exposed to *Beauveria bassiana* and *Metarhizium rileyi*

Entomo pathogenic Fungi	% Mortality ± SD (@ $1x10^8$)	LC_{50} (Conidia/ml)	Fiducial limits		LC_{90} (Conidia/ml)	Fiducial limits		Chi-Square
			Lower limit	Upper limit		Lower limit	Upper limit	
Beauveria bassiana	80 ± 5.8	4.4×10^5	2.2×10^4	8.8×10^6	2.8×10^{10}	1.4×10^9	5.6×10^{11}	0.700
Metarhizium rileyi	60 ± 5.8	5.8×10^6	4.4×10^4	7.7×10^8	7.6×10^{14}	5.7×10^{12}	1.0×10^{14}	0.760

Table 2. Time-mortality response of castor hairy caterpillar, *Euproctis fraterna* exposed to *Beauveria bassiana* and *Metarhizium rileyi*

Entomo pathogenic Fungi	LT_{50} (Days)	Fiducial limits		LT_{90} (Days)	Fiducial limits		Chi-Square
		Lower limit	Upper limit		Lower limit	Upper limit	
Beauveria bassiana	3.9	3.2	4.7	7.1	5.8	8.5	0.013
Metarhizium rileyi	4.5	3.5	5.7	9.6	7.6	12.1	0.283

Larval mortality due to the entomopathogenic fungi occurred 3-5 days after treatment. There were no symptoms in the early stages of the infection, but later on, the larvae became sluggish and slowed down their development. The diseased larvae were also found less responding to physical stimuli. In comparison to the untreated control, the infected larvae remained small and shrunken. The values of median lethal time (LT_{50}), fiducially limits corresponding to different LT_{50} and LT_{90} values and chi-square for the entomopathogenic fungi against E. fraterna were calculated and presented in Table 2. *B. bassiana* recorded the shortest median lethal time (LT_{50} value of 3.9 days) as compared to

M. rileyi (LT_{50} value of 4.5 days) to cause the larval mortality in *E. fraterna* treated at 10^8 conidia/ml.

The approach to pest management has seen a significant change over the years from chemical control to integrated pest management with more emphasis currently being on bio-intensive pest management. This paradigm shift is due to the outcome of the research on eco-friendly pest management that was driven by the ill-effects of injudicious use of chemical pesticides on human health, food and environment. In the bio-intensive pest management, microbial agents play a significant role. Entomopathogenic fungi are potentially the most versatile microbial agents, because they have wide host ranges, infect at different ages and stages of their hosts and often cause natural epizootics. Among the entomopathogenic fungi tried in the present investigation revealed high pathogenicity of *B. bassiana* against castor hairy caterpillar (*E. fraterna*) with 80% larval mortality, while moderate pathogenicity (60% larval mortality) was observed with *M. rileyi* as categorized by Krutmuang (2017). Differences in pathogenicity between fungal species have been observed for other insect species (Moorehouse *et al.,* 1993; Asi *et al.,* 2013). Our results can be compared with findings of Prasad and Syed (2010), who reported 86.7% larval mortality to *Helicoverpa armigera* when treated with 0.25 x 10^8 spore/ml of *B. bassiana.* Similarly, *B. bassiana* showed 100% mortality against groundnut caterpillar, *Spodoptera litura* at 10^9 spores/ml with LC_{50} value of 0.5 x 10^6 spores/ml (Joseph *et al.,* 2010). In support of the present investigation on *M. rileyi,* Sapna Bai *et al.* (2010) observed LC_{50} values from 2.1 x 10^5 to 38.9 x 10^5 conidia/ml of *Metarhizium* isolates against Bihar hairy caterpillar, *Spilarctia obliqua.* The obtained results confirmed the possibility of using *B. bassiana* in controlling *E. fraterna* during high humid conditions (60% or higher). The entomopathogenic fungi can be used in rotation with chemical insecticides in preventing the development of resistance or incorporated in IPM programme as a potential bio-control agent against *Euproctis fraterna* in castor.

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Comparative evaluation of selected neonicotinoids and conventional insecticide against aphids (*Aphis gossypii*) and whiteflies (*Bemisia tabaci*)

R SRINIVASAN* AND M R RAJAN

Department of Biology, Gandhigram Rural Institute, Gandhigram-624 302, TamilNadu

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ABSTRACT

Polyphagous sucking insect pests like aphids (*Aphis gossypii*) and whiteflies (*Bemisia tabaci*) causes major problem in fibre crops, oilseed crops, pulses and vegetables. These sucking insect pests cause serious economic losses directly and indirectly by acting as vectors in transmitting several viral diseases in cultivated crops. Neonicotinoids *viz*., imidacloprid 17.6 SL @ 0.28 ml, acetamiprid 20SP @ 0.1 g, thiacloprid 21.7SC @ 0.28 mL, thiamethoxam 25 WG @ 0.2 g and clothianidin 50 WDG @0.08 g along with monocrotophos 36 SL @ 1 ml per litre water and control (water alone) were tested against nymphs and adults of *A. gossypii* and *B. tabaci* following methods recommended by Insecticide Resistance Action Committee (IRAC). Among different treatment tested on A. *gossypii*, imidacloprid was found to be superior with 100% mortality on both nymphs and adults at 72h after treatment. When these treatments were tested against *B. tabaci*, acetamiprid was found to be superior to other neonicotinoids and monocrotophos, resulting in 100% mortality of nymphs and adults at 72h after treatment. Outcome of the studies suggest that neonicotinoids are significantly superior to monocrotophos for management of sucking insect pests. Imidacloprid and acetamiprid, that are systemic, selective with less mammalian toxicity are comparatively safer than conventional insecticide like monocrotophos and hence may be included in IPM practices to manage polyphagous sucking insect pests like aphids and whiteflies in different crop ecosystems.

Keywords: *Aphis gossypii, Bemesia tabaci,* Efficacy, Insecticides, Neonicotinoids

The sucking insect pests *viz.,* aphids, *Aphis gossypii* Glover (Hemiptera: Aphididae) and whiteflies, *Bemisia tabaci* Gennadius (Hemiptera : Aleyrodidae) are highly polyphagous and are deleterious and serious threat to several crops like fibre crops, oilseeds, pulses and vegetables. These sucking insect pests that occur at all the stages of crop growth and responsible for direct and indirect yield losses. They have the ability to build up to serious proportions as a result of rapid and prolific breeding in cultivated crops.

A. gossypii is an agriculturally important insect pests and a remarkable species in terms of geographical and host plant range. It is extremely polyphagous infesting over 900 plant species in the world (Blackman and Eastop, 2000). *A. gossypii* vector many plant diseases which cause substantially greater losses than damage caused by direct feeding injury. This is often the most damaging feature of an aphid infestation. This aphid transmits more than 80 kinds of agriculturally important virus diseases (Miyazaki, 2001). Damage is indirect through contamination with aphid honeydew. Honeydew causes economic loss through physical contamination and through providing a nutrient source for fungi that contaminate produce and reduce photosynthesis rates by blocking sunlight. *B. tabaci* has been found globally distributed except in Antarctica (De Barro *et al.,* 2000). *B. tabaci* remains a serious pest of many agricultural and ornamental crops worldwide, being recorded on a large range of host plant species (Nomikou *et al.,* 2001; Cuthbertson *et al.,* 2010). As a vector, *B. tabaci* is also know to transmit several viral diseases like mosaic, vein clearing, leaf curl, etc., in cotton, pulses, vegetables and oilseed crops. *B. tabaci* also secrete honey dew that attracts sooty mould fungus that affects photosynthesis by covering the leaf surface.

Due to continuous use of insecticides *B. tabaci* and *A. gossypii* has developed varying levels of resistance to almost all the conventional insecticides like organophosphates, carbamates, synthetic pyrethroids (Ahmad *et al.,* 2000, 2001and 2003). This led to several problems like toxic residues, elimination of natural enemies, environmental disharmony and development of resistance. To overcome those problems, identification of new molecules with selective insecticidal properties, low toxicity to non-target, well suited in the IPM practices, an effective chemical management strategy against sucking pests utilizing selected neonicotinoids with low rate and novel mode of action attracted the attention. Its broad-spectrum activity against sucking pests and systemic property appear to be useful for management of *A. gossypii* and *B. tabaci.* Neonicotinoids, are the newer major class of insecticides, have outstanding potency and systemic action for crop protection against piercing-sucking pests. Some of the popular neonicotinoids are acetamiprid, clothianidin, imidacloprid, thiacloprid, and thiamethoxam. They possess lower mammalian toxicity, less resurgence problems, environmental protection, pest management selectivity and

*Correspondence author's E-mail: agrisrini@gmail.com

less toxicity to natural enemies (Kunkel *et al.,* 1999). Keeping in view, the importance of polyphagous sucking insect pests and their management through relatively safer means and considering the endless range of uses of neonicotinoids due to their unique physiochemical properties and translocation rates, combined with residual activity, making them highly effective against sucking insect pests, including aphids, whiteflies, and plant hoppers (Jeschke *et al.,* 2011), the present studies were undertaken to compare effect of application of selected neonicotinoids and a conventional insecticide on sucking insect pests *viz., A. gossypii* and *B. tabaci.*

To assess the bio efficacy of neonicotinoids, test solutions were prepared at the doses *viz.,* imidacloprid 17.6 SL @ 0.28 ml, acetamiprid 20SP @ 0.1 g, thiacloprid 21.7SC @ 0.28 mL, thiamethoxam 25 WG @ 0.2 g and clothianidin 50 WDG @0.08 g. Monocrotophos 36% SL (standard check) and control (untreated check) was also maintained. Freshly test solutions of desired concentration was prepared every time for each Insecticides at the time of experimentation just before the start of treatments against aphids and whiteflies. The dilutions required were prepared from the formulated products only with distilled water. 250 mL of every required insecticides was prepared. For untreated control, only distilled water was used. The aphids were collected from the cotton plants maintained in mylar cages and were exposed to test insecticides of each treatments following leaf dip method (method no.1) recommended by Insecticide Resistance Action Committee (IRAC) (Rani, 2005). The test liquid solutions were agitated to obtain uniform homogenous solution and then the fresh cotton leaves were dipped into the test liquids for five seconds. A small piece of damp cotton wool was placed around the petiole of each leaf to maintain the turgidity of leaf. About 50 apterous aphids were released per cotton leaf in petri dishes. A control was run which was sprayed with distilled water. The entire set up was replicated three times and repeated three times using new solutions and different groups of aphids. Mortality was recorded after 24, 48 and 72 hours of release and moribund insects were also counted as dead.

The whiteflies (nymphs/adults) collected from cotton plants grown in mylar cages were exposed to each treatments following leaf dip method (method No. 8) recommended by Insecticide Resistance Action Committee (IRAC). Two cups were used, i.e. one as inner test chamber and the other as outer water reservoir. The cup which serves as the inner test chamber was taken and a hole was made in the centre of the bottom side of the cup. Then unsprayed cotton leaves were selected and the petiole was cut to a length of approximately 4 cm. The leaves were dipped for 5 seconds in prepared solutions. Then the leaves left for drying in the open air for approximately 5 minutes. The petiole of the test leaf was passed through the inner cup until it protrudes by approximately 1.0 cm. 50 Whiteflies (nymphs/adults) were released in each such inner cup for each replication. Then perforated lid of the cup was placed, so as to avoid escape of test insects. A small amount of water was placed in a second cup and the test cup placed inside, so that it was supporting the protruding petiole with adequate moisture, thereby preventing wilting of cotton leaf.

The treated leaf was carefully taken out from the cups, 24 hours after the treatment and the mortality of whiteflies was recorded. Moribund insects were also considered as dead. A control was also maintained at each time of experimentation where in the leaves were dipped in distilled water. The entire set up was replicated three times and repeated three times using new solutions and different groups of Whiteflies. Mortality was recorded at an interval of 24, 48 and 72 hours of release and moribund insects were also counted as dead.

The corrected percent mortality was calculated using Abbott's formula:

$$\text{Corrected percent mortality} = \frac{T - C}{100 - C} \times 100$$

T- Percent mortality in treatment; C- Percent mortality in control

Perusal of data on the effect of neonicotinoids against *Aphis gossypii* and *Bemisia tabaci* revealed that the all the neonicotinoids were found to be superior than standard check, monocrotophos against both nymphs and adults. Among two stages tested, nymphs were found to be slightly more susceptible than adults. Lab studies on aphids revealed that imidacloprid recorded maximum mortality of nymphs and adults at 24, 48 and 72 hours after treatment (HAT). Against aphid nymphs, imidacloprid with 100% mortality was followed by thiamethoxam, acetamiprid, clothianidin and thiacloprid with 98.4%, 93.3%, 88.3% and 82.7% mortality respectively. monocrotophos treatment recorded the lowest percent mortality at 71.1%after 72 hours of treatment (Fig. 1). Similar trends continued for adult aphids also, with imidacloprid recording maximum mortality (Fig. 2). The present investigation on neonicotinoid insecticides against aphids is in line with the finding of Misra (2002); Kumar *et al.* (1999); Sreelatha and Divakar (1997). Goshal *et al.* (2013) has also reported similar findings and revealed that imidacloprid 17.8 SL was superior to other neonicotinoid insecticides against aphids.

In the bioassay with different neonicotinoids and standard chemical against whitefly nymph and adult, the acetamiprid recorded maximum of 84.2% mortality of nymphs and 80% mortality of adults at 72 hours after treatment. acetamiprid was followed by thiamethoxam, imidacloprid, clothianidin and thiacloprid with 79.6%, 76.4%, 71.8% and 64.3% mortality respectively. Monocrotophos treatment recorded the lowest percent mortality at 58.1% after 72 hours of treatment (Fig. 3). Similar trends continued for adult whiteflies also, with acetamiprid recording maximum mortality (Fig. 4).

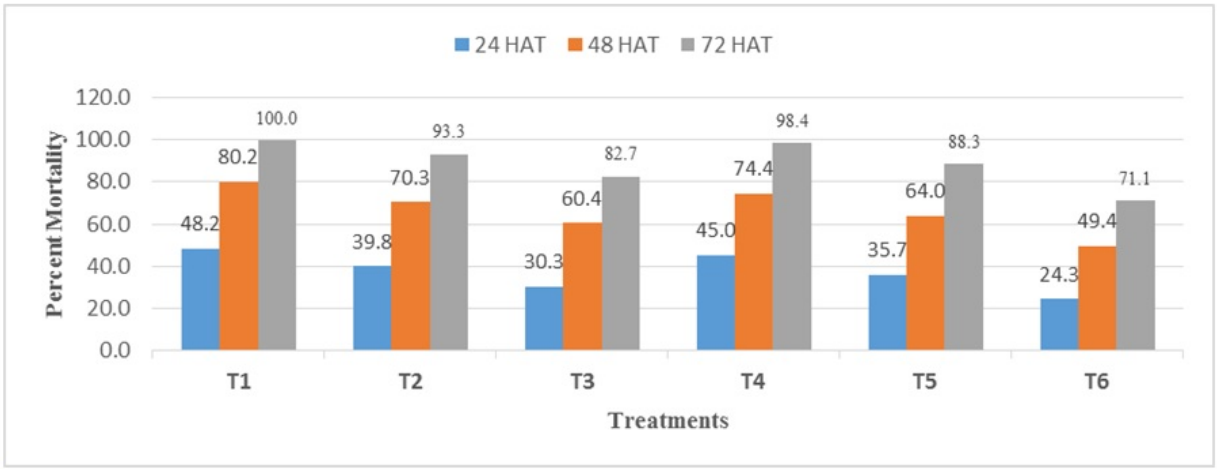

Fig. 1. Effect of neonicotinoids on the nymphal mortality percentage of aphid, *Aphis gossypii*

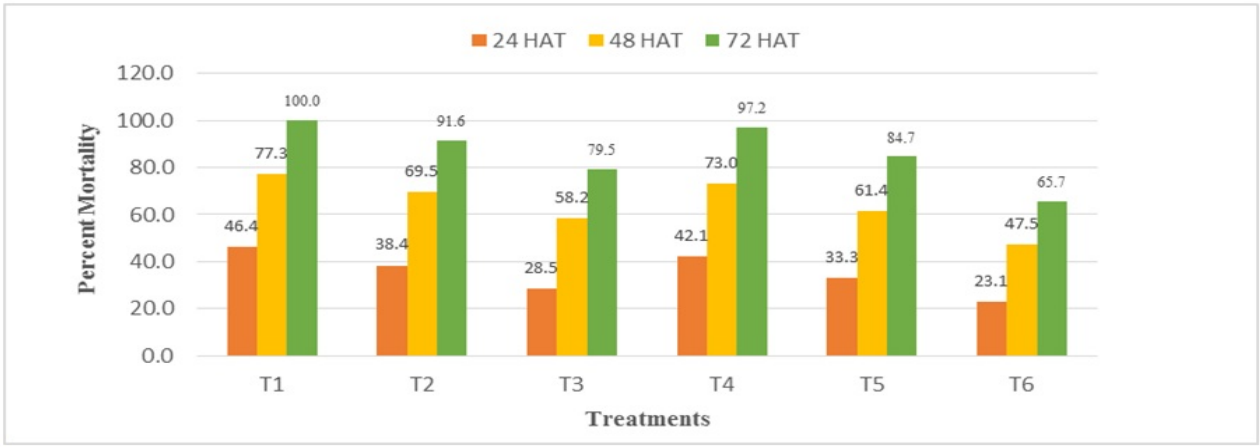

Fig. 2. Effect of neonicotinoids on the adult mortality percentage of aphid, *Aphis gossypii*

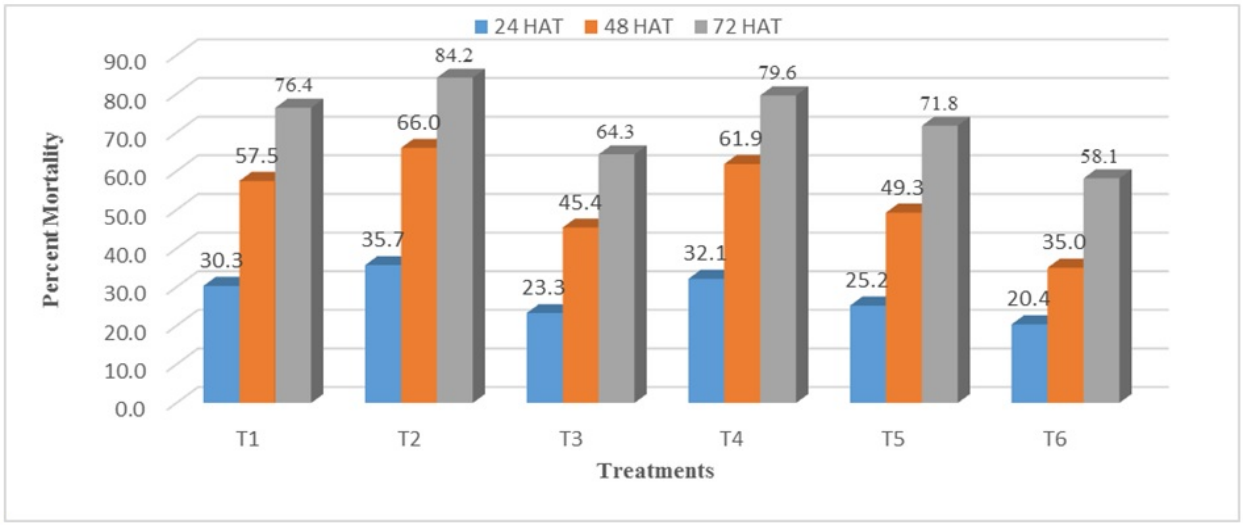

Fig. 3. Effect of neonicotinoids on the nymphal mortality percentage of whitefly, *Bemisia tabaci*

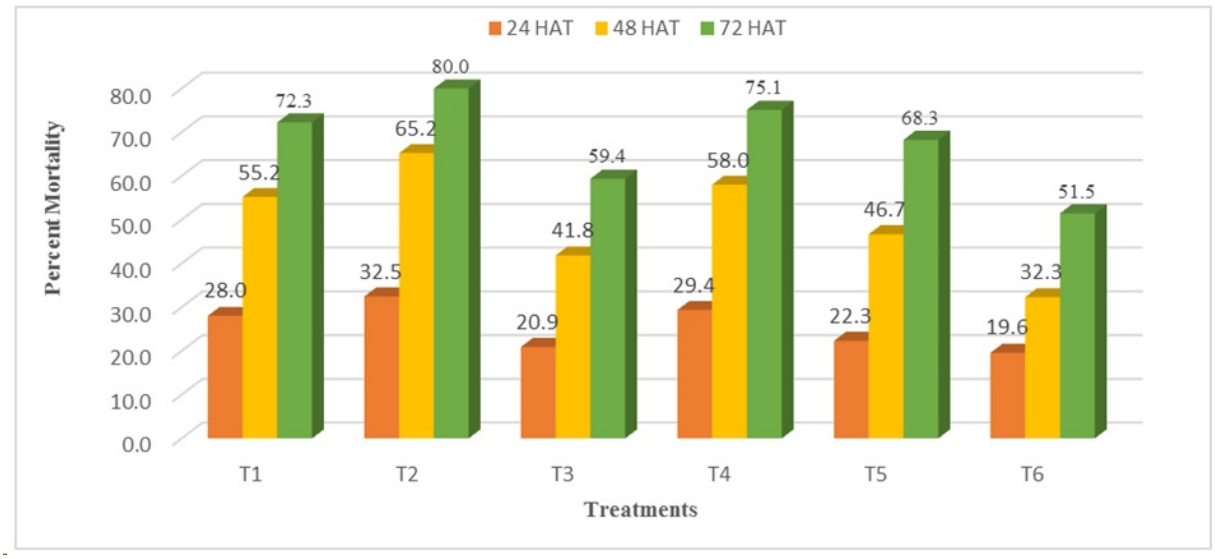

Fig. 4. Effect of neonicotinoids on the adult mortality percentage of whitefly, *Bemisia tabaci*

The results of the present investigation on neonicotinoid insecticides against whiteflies was supported by the findings of Muhammad *et al.* (2005) who reported that acetamiprid was superior to other neonicotinoids in controlling whiteflies. Results are also in line with the finding of Nath and Sinha (2011). The research findings suggest that the neonicotinoids are very effective in management of polyphagous sucking insect pests, *A. gossypii* and *B. tabaci* compared to conventional insecticide like monocrotophos. Hence, considering the systemic, selective, broad spectrum activity against sucking insect pests and low mammalian toxicity, neonicotinoids *viz.,* imidacloprid and acetamiprid may be recommended as a part of integrated pest management (IPM) package for the management of polyphagous sucking insect pests *viz., A. gossypii* and *B. tabaci.*

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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

Language of the Journal is English. Generally, the length of an article should not exceed 3,000 words in the case of 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 manuscript in the form of soft copy either as an e-mail attachment to editorisor@gmail.com (preferred because of ease in handling during review process) or in a **compact disk (CD) (in MS Word document; double line space; Times New Roman; font size 12).** In exceptional cases, where the typed manuscript is being submitted as hard copy, typing must be done only on one side of the paper, leaving sufficient margin, at least 4 cm on the left hand side and 3 cm on the other three sides. Faded typewriter ribbon should not be used. Double space typing is essential throughout the manuscript, right from the **Title** through **References** (except tables), foot note etc. Typed manuscript complete in all respects, is to be submitted to the Editor, Journal of Oilseeds Research, Directorate of Oilseeds Research, Rajendranagar, Hyderabad-500 030. 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, and phrases and in other ways. These marks should be used in proper manner if the reader of a paper is to understand exactly the intended meaning. Receipt of the manuscript (in the form of either soft or hard copy) 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:

Rao C R 1968. *Advances in Statistical Methods in Biometrical Research*, pp.40-45, John Wiley & Sons, New York.

Kanwar J S and Raychaudhuri S P 1971. *Review of Soil Research in India*, pp 30-36. Indian Society of Soil Science, New Delhi.

Mukherjee J N 1953. The need for delineating the basic soil and climatic regions of importance to the plant industry. *Journal of the Indian Society of Soil Science*, **1** : 1-6.

Khan S K, Mohanty S K and Chalam A B, 1986. Integrated management of organic manure and fertilizer nitrogen for rice. *Journal of the Indian Society of Soil Science*, **34** : 505-509.

Bijay-Singh and Yadvinder-Singh 1997. Green manuring and biological N fixation: North Indian perspective. In: Kanwar J S and Katyal J C (Ed.) *Plant Nutrient Needs, Supply, Efficiency and Policy Issues 2000-2025*. National Academy of Agricultural Sciences, New Delhi, India, pp.29-44.

Singh S, Pahuja S S and Malik R K 1992. Herbicidal control of water hyacinth and its effect on chemical composition of water *(in) Proceedings of Annual Weed Science Conference*, held during 3-4 March 1992 by the Indian Society of Weed Science, at Chaurdhary Charan Singh Haryana Agricultural University, Hisar, 127p.

AICRP on Soybean 1992. *Proceedings of 23rd Annual Workshop of All-India Co-ordinated Research Project on Soybean*, held during 7-9 May 1992 at University of Agricultural Sciences, Bangalore, Karnataka, National Research Centre for Soybean, Indore, pp.48.

Devakumar C. 1986. Identification of nitrification retarding principles in neem (*Azadirachta indic*a A.Juss.) seeds. Ph D Thesis, Indian Agricultural Research Institute, New Delhi.

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 Symbol for SI Unit Symbol Remarks quantity physical quantity for SI Unit

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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