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CONTENTS

Review Article

Versatile roles of ubiquitous calcium-dependent protein kinases (CDPKs) in plants

SUJATHA THANKESWARAN PARVATHY

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

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ABSTRACT

Calcium-dependent protein kinases (CDPKs) are a class of multifunctional serine/threonine (Ser/Thr) protein kinases involved in Ca^{2+} signalling in plants and mediate the signalling cascade triggered by changes in concentration of intracellular free calcium (Ca^{2+}), to result in intracellular signalling and various physiological responses of plants to biotic as well as abiotic stress conditions, during plant growth and development, but primarily involved in plant innate immunity. CDPKs are group of calcium-binding proteins with kinase activity that are only found in plants and some protozoans. CDPKs form large gene family having multigenes with diversified roles, the structure, and functions of which are elucidated in many plants. The short review delineates major classes of calcium sensor proteins and discusses the structure and multitude functions of CDPKs in plants.

Keywords: Abiotic and biotic stress, Growth, Signaling, Calcium binding proteins, Protein Kinase, Functions

Perception of stimuli and activation of a signalling cascade during the multiple facets of growth and development and to environment is an intrinsic characteristic feature of all living organisms. Exposure to unfavourable stimuli or stress condition activates different signalling cascades in both plants and animals. Plants are constantly exposed to environmental changes and have to integrate a variety of biotic and abiotic stress stimuli. In plants, calcium acts as a second messenger in both abiotic and biotic stress signalling (Das and Pandey, 2010). Specific responses to different stimuli could be achieved through variations in the amplitude, duration, location and frequency of these Ca^{2+} spikes (McAinsh and Hetherington, 1998). As calcium (Ca^{2+}) is ubiquitous in stress signalling, it may be an important node at which cross-talk between pathways can occur. Protein kinase C and calmodulin-dependent kinases are major mammalian calcium-dependent signalling molecules, and in plants CDPKs play similar function (Roberts and Harmon, 1992).

CDPKs have been identified throughout the plant kingdom, in some ciliates and apicomplexan parasites including the malaria parasite, *Plasmodium falciparum* but are notably absent from the sequenced eukaryotic genomes of yeast, worms, flies, mice and humans (Cheng *et al.,* 2002; Harper and Harmon, 2005; Hrabak *et al.,* 2003; Wurzinger *et al.,* 2011). CDPKs are a large multigene family of calcium binding protein kinases, involved in diverse functions in the plants, ranging from stress signalling to hormone-regulated growth and developmental processes. Individual isoforms of CDPKs have different functions and participate in multiple distinct signalling pathways. CDPKs are unique since they have both Ca^{2+}-binding and signalling capabilities within a single gene product (Hamel *et al.,* 2014). This combination probably arose following the early fusion of an upstream protein kinase (PK) gene and a downstream CaM gene to enable immediate and efficient translation of input Ca^{2+} signals into appropriate output phosphorylation events (Zhang and Choi, 2001).

In this short review, the major classes of calcium binding proteins in plants are outlined with emphasis on the CDPKs, their structure functions and interaction or cross talk with other signalling pathways or kinases.

Calcium binding proteins in plants

In plants, diverse Ca^{2+}-binding proteins serve as sensors to monitor cellular Ca^{2+} changes. Four major families of calcium-binding proteins have been identified in plants: calmodulins (CaM), calmodulin-like proteins (CML), calcineurin B-like proteins (CBL) and calcium-dependent protein kinases (CDPKs), where CaM, CBL and CDPKs play a crucial role in abiotic stress signalling in plants (Luan *et al.,* 2002; Sanders *et al.,* 2002; Valmonte *et al.,* 2014). CaMs and CBLs unlike CDPKs, are calcium-binding proteins, which do not have any protein kinase enzyme activity and interact with a target protein kinase termed as CaM kinases and CBL-interacting protein kinase (CIPK) respectively. Decoding of Ca^{2+} signals is thus mediated by different gene families in plants *viz.,* by calmodulins (CAMs) and calmodulin-dependent protein kinases (CaMKs), by

E-mail: sujatha.parvathy@icar.gov.in

calcineurin B like proteins (CBLs) and CBL interacting protein kinases (CIPKs)/SnRK3s), by Ca^{2+} dependent protein kinases (CDPKs) and CDPK-related kinases (CRKs and by Ca^{2+} and calmodulin dependent protein kinases (CCaMKs) (Hashimoto and Kudla, 2011; Batistic and Kudla, 2012).

Calmodulins, the primary class of calcium sensors in eukaryotes have 2 separate globular domains, each having a pair of EF-hands (helix-loop-helix structural domain), connected by a flexible helical region, so that each CaM binds to 4 Ca^{2+} ions. Generally, CaM has no catalytic activities of its own, but when Ca^{2+} binds to the EF-hand motif, the configuration of CaM changes, exposing the hydrophobic regions that in turn bind to downstream target proteins known as CaM-binding proteins (CaMBPs). Thus, CaM also regulates the activities of CaMBPs (Zhang *et al.,* 2014b). Besides CaMs, there is another class of Ca^{2+} sensor proteins in plants termed CaM-like proteins (CMLs) which share at least 16 per cent amino acid identity with CaM, contain EF-hand motifs but have no other known functional domains. The expression of CMLs is spatially (tissue-specific) or temporally-regulated (developmental stage-specific) and inducible or varying with various environmental stimuli, indicating distinct roles of different CMLs (McCormack *et al.,* 2005). On the contrary, expression pattern of CaM genes is uniform and high. The third category of Ca^{2+} sensor proteins are Calcineurin B-like proteins (CBLs) which are similar to the regulatory β subunit of calcineurin as well as neuronal calcium sensors. CBLs also have EF-hand motifs for Ca^{2+}-binding and interact with CBL-interacting protein kinases (CIPKs) to relay Ca^{2+} signals (Batistic and Kudla, 2009).

Another class of canonical Ca^{2+} binding protein is Ca^{2+} dependent protein kinase. CDPKs have 4 EF-hand motifs for Ca^{2+} binding which is fused to the C-terminus of a Ser/Thr kinase domain with a junction of an auto inhibitory domain (Harmon *et al.,* 2000). CRKs from *Arabidopsis* and maize do not bind calcium and are not regulated by calcium (Furumoto *et al*., 1996). Calcium and calmodulin (CaM)-dependent protein kinase (CCaMK), though plant-specific are distinct from plant CDPKs, but highly similar to animal CaM-dependent protein kinase II (CaMKII), with a serine/threonine kinase domain, an overlapping autoinhibitory/CaM-binding (CaMB) domain and three visinin-like EF-hand calcium binding motifs (Patil *et al*., 1995; Mitra *et al*., 2004). CCaMK activity requires both free Ca^{2+} and Ca^{2+} bound to CaM (Ca^{2+}/CaM)., while CDPKs do not require calmodulin for their activation and Ca^{2+} binds directly the calmodulin domain of CDPKs. The EF-hand domain and CaMB domain act as Ca^{2+}-triggered switch and autophosphorylation-triggered molecular switch, respectively (Takezawa *et al*., 1996; Sathyanarayanan *et al*., 2000). The calcium sensor proteins play a crucial role in stress signaling in plants.

CDPKs in plants

CDPKs constitute a large multigene family of multi-functional proteins. The calcium-sensor domain and a protein kinase effector domain are combined in one molecule. CDPKs perceive rapid intracellular changes of Ca^{2+} ion concentration, relay them into specific phosphorylation events to induce further downstream stress responses (Romeis and Herd, 2014). The binding of Ca^{2+} to the EF-hand motif induces a conformational change removing this auto-inhibition of kinase activity, leading to phosphorylation of specific substrates CDPKs. Variations in Ca^{2+} concentrations are reflected as physiological response mediated by the phosphorylation events. Thus, CDPKs are sensors of Ca^{2+} flux in plants in response to the stresses and act as signalling mediators that regulate downstream components in calcium signalling pathways.

Calcium-dependent protein kinase (CDPK) activities were first reported in pea shoot membranes by Hetherington and Trewavas (1982). The first biochemical evidence that CDPKs are calmodulin independent was provided in soybean (Harmon *et al*,. 1987). The first CDPK cDNA clones however, were isolated from *Arabidopsis* (Harper *et al*., 1991) and carrot (Suen and Choi, 1991). Genome-wide searches and analyses of CDPKs have identified 34 and 29 CDPK genes in *Arabidopsis* and rice respectively (Cheng *et al*., 2002; Hrabak *et al*., 2003; Asano *et al*., 2005).

The numerous paralogues of CDPKs may be dispersed in the genome in different chromosomes and/or clustered together. For example, in *Arabidopsis* 34 CDPK genes are identified, chromosome IV has the most CDPKs (11), whereas chromosome III has the least (4). The only region that contains no CDPKs is the short arm of chromosome II. Interestingly, one gene cluster on the short arm of chromosome IV contains five genes (AtCPK 21, 22, 23, 27 and 31) organized in tandem in the same transcriptional orientation and amino acid sequences are highly homologous (61%- 82% identity and 74%-89% similarity) suggesting that they arose relatively recently by gene duplication and may have similar or overlapping functions (Cheng *et al*., 2002).

Genomic sequencing projects and extensive expressed sequence tag (EST) projects also indicate the presence of multigene families of CDPKs in other plants, including soybean, tomato, rice, and maize (Harmon *et al*., 2000). Genome wide identification and functional analysis of CDPKs have been carried out in many crops. The number of CDPKs in various plant species are enlisted in Table 1.

CDPKs in oilseed crops: There are 22 CDPKs predicted in castor, 70 in sunflower, 33 in sesame 58-61 soybean, 150 loci for *Brassica rapus* (NCBI database www.ncbi.nlm.nih.gov, accessed 14-11-2017). The large families of protein kinases enable the organisms to diversify the response to various environmental signals without a need for a different signalling mechanism.

Table 1 CDPK isoforms in plants

Plant species	Number of CDPKs	Reference
Arabidopsis thaliana	34	Cheng *et al.,* 2002
Rice (*Oryza sativa*)	29	Asano *et al.,* 2005
Wheat (*Tricitcum aestivum*)	20	Li *et al.,* 2008
Maize (*Zea mays*)	35	Ma *et al.,* 2013
Barley (*Hordeum vulgare*)	28	Yang *et al.,* 2017
Soybean (*Glycine max*)	50	Hettenhausen *et al.,* 2016
Canola (*Brassica napus*)	25	Zhang *et al.,* 2014a
Cucumber (*Cucumis sativus*)	19	Xu *et al.,* 2015
Melon (*Cucumis melo*)	18	Zhang *et al.,* 2017
Tomato (*Solanum lycopersicum*)	29	Wang *et al.* 2016
Pepper (*Capsicum annuum*)	31	Cai *et al.,* 2015
Grapes (*Vitis vinifera*)	19	Zhang *et al.* 2015
Cotton (*Gossypium raimondii*)	41	Liu *et al.,* 2014
Cassava (*Manihot esculenta*)	27	Hu *et al.,* 2016
Populus (*Populus trichocarpa*)	20	Zuo *et al.,* 2013

Domain structure of plant CDPKs: CDPKs are Ser/Thr protein kinases. The CDPK family members harbor four distinct domains in their protein structure *viz.,* an N-terminal variable domain (N), a Ser/Thr protein kinase domain (K), an autoinhibitory domain (A), and a regulatory calmodulin-like calcium binding domain (Fig. 1). The auto-inhibitory domain or inhibitory junction domain also called as CAD (CDPK activation domain) is formed from a pseudo substrate region. (Harper *et al.,* 2004; Harper and Harmon, 2005; Schulz *et al.,* 2013).

CDPKs have a highly conserved structure. Isoform-specific differences are mainly restricted to the N-terminal variable domain, which in many CDPKs also includes a fatty acylation site (Hrabak, 2000). myristoylation and palmitoylation at these sites have been found in CDPKs and necessary for targeting to the membrane (Ellard-Ivey *et al*., 1999; Martin and Busconi, 2000).

Fig. 1. Structure of plant CDPKs, N terminal variable domain (N), kinase domain (K), Auto-inhibitory Junction domain (A) and CaM-LD is Calmodulin like domain (CaM-LD)

The 3 domains except the N-terminal domain are conserved across the paralogous genes, especially the kinase domain. For example, alignments of the predicted amino acid sequences of all 34 *Arabidopsis* CDPKs reveal a high conservation of the kinase (44%-95% identity and 60%-98% similarity), auto-inhibitory (23%-100% identity and 42%-100% similarity) and calmodulin-like (27%-97% identity and 50%-98% similarity) domains, whereas the N-terminal variable domain shows little sequence similarity. The N terminal variable domain determines substrate specificity so that substrate specificity of CDPK can be engineered by manipulating the variable N-terminal domain (Ito *et al.,* 2010). The C-terminal domains of CDPKs and (CCaMKs) are calcium-binding, regulatory domains. The calcium-binding domain of the archetypal CDPK is similar to calmodulin in sequence (~40% identity) and contains four elongation factor (EF)-hand calcium-binding motifs (Harmon *et al.,* 2000). The four EF-hand Ca^{2+}-binding motifs of the CaM-LD are organized into two lobes with distinct Ca^{2+} affinities resulting in different roles in CDPK regulation. CRKs do not have any functional EF hands, but with degenerate calcium binding sites, CCaMKs have visinin like domains and CaMKs have C terminal domain for protein-protein interactions (Harmon *et al.,* 2001). EF-hands are found in pairs in calcium sensor proteins; mainly to stabilize the protein and facilitate high-affinity binding to Ca^{2+} (Gao *et al*., 2014).

Based on the alignment of amino acid sequences and sequence homology, the CDPKs of *Arabidopsis* were found to cluster into four subgroups (I-IV). Subgroup II is the most complex, with 13 members and subgroup IV is the least complex, with three members. This pattern of grouping was observed, when the phylogenetic tree was constructed based on the sequences of kinase domain only (Harmon *et al.,* 2001). Subgroups I through III are closer in sequence identity to each other than to subgroup IV. Whether such clustering pattern has any role in functional differences between the subgroups is not known. Upon the inclusion of rice and wheat CPKs, Group II and III were separated into subgroups (IIa, IIb, IIIa and IIIb) (Asano *et al.,* 2005; Li *et al.,* 2008). Based on phylogenetic analysis, CDPK gene family is believed to result from fusion of a CaMK and a calmodulin and this facilitates the direct activation of CDPKs by Ca^{2+} (Harper *et al*., 1991; Suen and Choi, 1991; Harmon *et al.,* 2000; Zhang and Choi, 2001). CDPKs function as monomers unlike the analogous mammalian CaMKII which is a multi-subunit protein (Roberts and Harmon, 1992).

Mode of action of plant CDPKs: Developmental cues and environmental stimuli generate second messenger signals such as transient and dynamic alterations in Ca^{2+}. Calcium has limited diffusion potential in the cytosol, and hence rapid and efficient calcium detection occurs when calcium sensor proteins are located at cell membranes. Transient cytosolic calcium signals generated in response to environmental signals or internal stimuli are decoded by CDPKs (DeFalco *et al.,* 2010).The calmodulin-like domain of CDPKs directly binds calcium, inducing intramolecular conformational changes that lead to calcium-specific activation of the catalytic domain. A change of the CaM globular structure, allows the interaction of CaMs with their target proteins (Gao *et al.,* 2014).

In the basal or resting state, the intramolecular interaction between the auto-inhibitory junction region and the catalytic centre maintains the kinase in an inactive state by a pseudo-substrate mechanism (Harper *et al.,* 2004). The C-terminal lobe of the CaM-LD (Calmodulin Like Domain) has high Ca^{2+} affinity, while N terminal lobe of CaM-LD has low Ca^{2+} affinity. The autoinhibitory junction domain blocks the action of reactive kinase center and prevents substrate phosphorylation. At low Ca^{2+} level, C terminal lobe interacts with the auto-inhibitory region to stabilize the structure (Zhang *et al.,* 2014b).

N-lobe of the CaM-LD plays an important role in triggering CDPK activation (Wernimont *et al.,* 2010). CDPKs are activated by a calcium signal that is perceived by Ca^{2+} binding to the C-terminal EF-hands. Binding of Ca^{2+} to the low-affinity N-terminal lobe of the CaM-LD induces a conformational change in CDPKs, that leads to binding of the calmodulin-like domain to the junction domain, and retraction of the junction domain from the kinase domain, releasing the auto-inhibition (Hamel *et al.,* 2014). Thus, the reactive kinase centre is exposed for substrate phosphorylation (Freymark *et al.,* 2007; Boudsocq and Sheen, 2013). Ca^{2+} sensitivity of CDPK activities were found to vary with different substrates *in vivo* (Lee *et al.,* 1998). Deleting both the autoinhibitory domain and the CaM-LD generates a constitutively active form of CDPK (Romeos *et al.,* 2001). On the other hand, the highly divergent amino-terminal variable domain is critical for correct subcellular localization of the enzyme and in substrate recognition (Lu and Hrabak, 2013).

Cellular localisation and spatial regulation of CDPKs : The CDPKs show subcellular localizations in cytosol, nucleus, plasma membrane, endoplasmic reticulum, peroxisome, mitochondrial outer membrane, and oil bodies indicating their diverse functions (Harper *et al.,* 2004). Although none of the CDPKs has transmembrane domains, the majority of CDPKs have potential myristoylation and palmitoylation motifs at the beginning of their N-terminal V domain that may be responsible for membrane association (Asai *et al.,* 2013). Several CDPKs have been shown to be myristoylated *in vitro* (Lu and Hrabak, 2002) indicating specific cellular localisation. CDPKs are localized to different cellular membranes, including plasma membrane, endoplasmic reticulum membrane, and peroxisome membrane (Lu and Hrabak 2002; Dammann *et al.,* 2003). Calcium-stimulated kinase activity has been detected in both soluble and microsomal fractions of plant cells, although the specific membrane was not determined (Battey, 1990; Abo-El-Saad and Wu, 1995; MacIntosh *et al.,* 1996; Martin and Busconi, 2000). In many cases, CDPK-like activity was associated with the plasma membrane in oats, red beet and tobacco (Schaller *et al.,* 1992; Baizabal-Aguirre and de la Vara, 1997; Iwata *et al.,* 1998). An *Arabidopsis* CDPK was associated with the endoplasmic reticulum (Sheen *et al.,* 2002). The variable domain of CDPK5 of potato was found to confer subcellular localization to plasma membrane and substrate recognition, activation and phosphorylation of plasma membrane NADPH Oxidase (Asai *et al.,* 2013).

The N-terminal variable domain is also subjected to *in vivo* phosphorylation. In domain-swap experiments, where N-terminal variable domains were exchanged between NtCDPK2 and NtCDPK3 in *Nicotiana benthamiana*, it was observed that *in vivo* phosphorylation pattern was solely determined by the N-terminal variable domain, irrespective of the protein kinase domain (Witte *et al.,* 2010).

Tissue localisation is mostly associated with the functions of the CDPKs. Relatively few signal transduction genes are expressed in a strictly cell-specific manner in roots, guard cells and mesophyll cells. The *CPK3* (At4g23650) and *CPK6* (At2g17290), genes in *Arabidopsis* are guard cell-specific, involved in guard cell ion channel regulation and transduce stomatal ABA signaling (Mori *et al.,* 2006). Cultivar-and tissue-specific expression was exhibited by 12 out of 29 CDPK genes in rice (Wan *et al.,* 2007). In rice, the CDPK gene OsCPK18 was detected in cortical cells, but not epidermal cells, of the *Arbuscular mycorrhizal* (AM) fungus *G. intraradices*-inoculated rice roots and localised in plasma membrane (Campos-Soriano *et al*., 2011).

Functions of CDPKs in plants

The second messenger Ca^{2+} is a ubiquitous intracellular signaling molecule that regulates many growth and developmental processes (Hepler and Wayne, 1985; Braun and Schulman, 1995). CDPKs are involved in plant signaling during various physiological pathways and stress conditions (Xu *et al.,* 2010). Redundancies in CDPK genes is a major challenge in molecular or genetic analysis of CDPK functions. (Mori *et al.,* 2006). Loss-of-function and gain-of-function studies revealed that signalling pathways leading to cold, salt, drought or pathogen resistance are mediated by specific CDPK isoforms. Multiple CDPK isoforms exhibit distinct Ca^{2+} sensitivities, consistent with their roles in decoding different Ca^{2+} signals (Lee *et al.,* 1998; Boudsocq *et al.,* 2012). Each CDPK isoform may be functionally specialized. Expression levels of CDPKs are spatially and temporally controlled throughout development, and also vary in response to external stimuli. Expression of CDPKs are up-regulated by abiotic stresses, pathogens and hormones (Saijo *et al.,* 2000; Romeis *et al.,* 2001; Asano *et al.,* 2005; Wan *et al.,* 2007; Li *et al.,* 2008). Although the regulation of CDPK expression has been reported in a variety of plant species, little is known about the biological functions of specific CDPKs.

CDPKs of land plants have significantly expanded through gene duplications and novel CDPK homologs have evolved to achieve highly specialized functions. The CDPK

isoforms may have specific or overlapping functions. Many CDPKs may be involved in same biological function or a single isoform of CDPK may have multiple roles. For example in *Arabidopsis,* six CPDKs from Group I have been shown to play a role in innate immunity such as CPK1/2, 4/5/6/11 and seven *Arabidopsis* CDPKs from Group I (CPK4/6/11), Group II (CPK3/21/23), and Group III (CPK10) have been shown to be involved in regulatory network controlling stomatal movements in response to drought and abscisic acid (Hamel *et al.,* 2014). In *Arabidopsis,* 10 CDPKs genes (Group I, CPK20/25; Group II, CPK17/19/22/34; Group III, CPK14/24; and Group IV, CPK16/18) show weak or no expression in mesophyll cells while most show high and specific expression in pollen, for example, CPK17/34 regulate polarized tip growth in pollen tubes. The functions of *Arabidopsis* CDPKs belonging to different groups are summarised in Table 2.

Table 2 Functions of members of four different clades of CDPKs (CPKs) of *Arabidopsis* grouped based on amino acid sequence homology

	Locus ID	Chromosome	Function	Reference
Group-I				
AtCPK1	At5G04870	V	Salicylic acid-mediated defence response (to fungi)	Coca and San Segundo, 2010
AtCPK2	At3G10660	III	Pollen tube growth and regulation	Gutermuth *et al*., 2013
AtCPK4	At4G09570	IV	ABA, Salt/ drought tolerance	Zhu *et al*., 2007
AtCPK5	At4G35310	IV	MAMP	
AtCPK6	At2G17290	II	Stomatal closure, Salt/drought tolerance	Mori *et al*., 2006 Xu *et al*., 2010
AtCPK11	At1G35670	I	ABA, cold, drought, stomata, Salinity, MAMP	Urao *et al*., 1994
AtCPK12	At5G23580	V	ABA	Zhao *et al*., 2011
AtCPK20	At2G38910	II	Pollen tube growth	Gutermuth *et al*., 2013
AtCPK25	At2G35890	II	Constitutive activity	Boudsocq *et al*., 2012
AtCPK26	At4G38230	IV	ABA signaling	TAIR
Group-II				
AtCPK3	At4G23650	IV	ABA-induced stomatal closure, salinity, defence against herbivore attack	Xu *et al*., 2010 Mori *et al*., 2006
AtCPK9	At3G20410	III	-	
AtCPK15	At4G21940	IV	-	
AtCPK17	At5G12180	V	Pollen tube growth, Florigen	Myers *et al*., 2009 Kawamoto *et al*., 2015
AtCPK19	At1G61950	I		
AtCPK21	At4G04720	IV	Stomata osmotic stress	Franz *et al*., 2011
AtCPK22	At4G04710	IV	Stomata, drought, salinity	
AtCPK23	At4G04740	IV	Salinity	Ma and Wu, 2007
AtCPK27	At4G04700	IV	-	
AtCPK29	At1G76040	I	-	
AtCPK31	At4G04695	IV	-	
AtCPK33	At1G50700	I	Flowering	Kawamoto *et al*., 2015
AtCPK34	At5G19360	V	Pollen tube growth	Myers *et al*., 2009
Group-III				
AtCPK7	At5G12480	V	Stomata	
AtCPK8	At5G19450	V	Stomata	
AtCPK10	At1G18890	I	ABA, Cold, Drought, Salinity, Stomata	Urao *et al*., 1994; Zou *et al*., 2010
AtCPK13	At3G51850	III	Defence against herbivore attack	Kanchiswamy *et al*., 2010
AtCPK14	At2G41860	II	-	
AtCPK24	At2G31500	II	-	
AtCPK30	At1G74740	I	ABA , abiotic stress	Sheen, 1996
AtCPK32	At3G57530	III	ABA, stomata	Choi *et al*., 2005
Group-IV				
AtCPK16	At2G17890	II	-	
AtCPK18	At4G36070	IV	-	
AtCPK28	At5G66210	V	Stem elongation, Vascular development Defense signaling (JA signaling)	Matschi *et al*., 2013; Monaghan *et al*., 2014; Matschi *et al*., 2015

(Modified from Boudsocq and Sheen, 2013)

Biotic stress

Defence response: An early event during plant defense response is the elevation of calcium concentration in plant cells. CDPKs decode the encoded Ca^{2+} signals into specific physiological responses to survive pathogen attack. Calcium (Ca^{2+}) signaling is essential in both the primary innate immune response PAMP-triggered immunity (PTI) through the detection of evolutionarily conserved pathogen-associated molecular pattern (PAMP)s and effector-triggered immunity (ETI) triggered by the recognition of specific pathogen effector proteins. Plant perception of pathogen-associated molecular patterns (PAMPs) triggers a phosphorylation relay leading to PAMP-triggered immunity (PTI). Surface-localized immune receptors are required for the PAMP-induced Ca^{2+} burst and directly regulates ROS production. CDPKs become activated in response to pathogen-associated molecular pattern (PAMP) stimulation (Gao *et al.,* 2014). A *Phytophthora*-associated PAMPS from *P. infestans* (PiPE) was found to bind to CDPK2 in potato for the induction of hypersensitive reaction (Furuichi *et al.,* 2014). The effector-triggered immunity (ETI) mediated by recognition of pathogen-derived virulence effector factors, recognised by plant resistance (R) proteins, is accompanied by increase in cytosolic Ca^{2+} levels and the CDPKs phosphorylate a specific subgroup of WRKY transcription factors for restriction of pathogen growth (Gao and He, 2013). OsCPK4 of rice regulates immunity and provides resistance to rice blast fungus, by preventing fungal penetration, enhancing production of reactive oxygen species (ROS), callose deposition and defence gene expression (Bundo and Coca, 2016). A CDPK, CPK5 had dual function in PAMP immune signaling or pathogen resistance and CPK5/ NADPH oxidase-mediated, Reactive Oxygen Species (ROS)-based, signal propagation, required for the constitution of distal immune reactions. (Dubiella *et al.,* 2013).

Ca^{2+} signaling not only plays positive, but also negative roles in regulating defence responses (Zhang *et al.,* 2014b). In *Arabidopsis* Ca^{2+} dependent protein kinase CPK28 acts as a negative regulator of immune signalling and attenuates PAMP-triggered immune responses as well as antibacterial immunity. CPK28 interacts with and phosphorylates the plasma-membrane-associated cytoplasmic kinase BIK1, and regulates the optimal level BIK1, through a process of continuous degradation. Plants over-expressing CPK28 accumulate less BIK1 protein and display impaired immune signalling. (Monaghan *et al.,* 2015), CPK28 confers developmental processes by the tissue-specific balance of JA and GA without affecting JA-mediated defence responses (Matschi *et al.,* 2015). An extensive review ion functions of CDPKs in plant innate immunity is given by Gao *et al. (*2014).

Systemic defence signalling: CDPKs are involved not only in rapid local immune response but long-term distal immune responses as well. Thus, CDPKs have dual function in plasma-membrane/calcium-mediated signal propagation and in phytohormone signaling-dependent systemic resistance mediated by salicylic acid or jasmonic acid, for long term resistance to bacterial pathogens and herbivores (Freymark *et al.,* 2007; Romeis and Herd, 2014). Herbivore or insect pest- induced signals rapidly spread over the leaf and leads to a strong Ca^{2+}-dependent transmembrane potential (Vm) depolarization in the damage zone. Upon insect attack, the cytosolic Ca^{2+} increases, which in turn activates the calcium-sensing proteins such as CDPKs. In *Arabidopsis,* two CPKs (CPK3 and CPK13) were involved in herbivory-induced signaling network, while in tobacco, NtCDPK2 regulated the activation of stress-induced MAP kinases (Ludwig *et al.,* 2005; Kanchiswamy *et al.,* 2010; War *et al.,* 2012). Tobacco CDPKs NaCDPK4 and NaCDPK5 were involved in plant-herbivore interactions largely through jasmonic acid (JA) signalling. Jasmonic acid is a hormone regulating plant defence against herbivores. Herbivore attack induces increased levels of JA which in turn activates production of metabolites for resistance to insect/ herbivore attack or wounding. Silencing of CDPK genes increased JA levels and increased Salicylic acid -induced protein kinase activity (Yang *et al.,* 2012).

Abiotic stress

Cytosolic free calcium is a common second messenger in abiotic stress signalling. Salinity, water availability and hypoxia are signalled through calcium. Nutrient deficiency such as potassium, nitrate and boron deficiency and heavy metals are stress factors, signalled through cytosolic free calcium in roots (Wilkins *et al.,* 2016). AtCPK6 is functionally redundant and positive regulator involved in salt/drought stress tolerance in *Arabidopsis* (Xu *et al.,* 2010). CPK21 from *Arabidopsis thaliana* is biochemically activated *in vivo* in response to hyperosmotic stress (Franz *et al.,* 2011). In rice, CDPK7 (OsCPK13-1) was involved in enhanced tolerance to cold, salt and drought stresses (Saijo *et al.,* 2000). OsCPK21 was associated with positive regulation of the signalling pathways that are involved in the response to ABA and salt stress (Asano *et al.,* 2011).

Multiple abiotic stress factors: CDPK genes in grapes (*Vitis amurensis*) were involved in tolerance different abiotic stress such as low and high temperature stress, freezing as well as to drought stress (Dubrovina *et al.,* 2015). The 19 CDPK genes of cucumber were similarly involved in abscisic acid, salt, cold, drought, heat, and water logging responses, possibly by different mechanisms (Xu *et al.,* 2015). On the other hand in rice, all 29 CDPK genes (OsCPK1-29) had

multiple stress-responsive cis-elements in the 1 kb promoter region upstream of genes, but only 11 were regulated by chilling temperature, dehydration, salt, rice blast infection and chitin treatment (Wan *et al.,* 2007).

Phytohormone signalling: CDPKs are involved in phytohormone signalling such as abscisic acid, ethylene, auxins, gibberellins, and brassinosteroids. Abscisic acid (ABA), a drought-inducible plant hormone, regulates Ca^{2+} elevations in guard cells and other cells (Mori *et al.,* 2006). Both AtCPK3 and AtCPK6 were functioning in ion channel regulation and in ABA-regulated signaling of guard cells in *Arabidopsis* (Mori *et al.,* 2006). In *Arabidopsis,* AtCPK32 was reported to regulate ABA-responsive gene expression through a leucine zipper transcription factor ABF4 (Choi *et al.,* 2005). Concentration of cytosolic free Ca^{2+} regulates guard cell ion channels and proton pumps such that Ca^{2+} elevation activates stomatal closing mechanisms. Thus ABA-induced stomatal closing is dependent on Ca^{2+} levels. Impairment of ABA signal transduction was observed in stomata of calcium-dependent protein kinase (CDPK) quadruple mutant plants (Brandt *et al.,* 2015). S-type anion channels are activated during stomatal closure and guard cell CDPKs (CPK21 and 23) were found to stimulate the SLAC1 (Slow Anion Channel Associated 1), where Ca^{2+}-sensitive activation of SLAC1, was assigned to the CPK21 pathway only because CPK23 was rather Ca^{2+}-insensitive. Stress hormone ABA hence activates guard cell anion channels in a calcium-dependent, as well as calcium-independent manner. Open stomata 1 protein kinase (OST1) and ABI1 protein phosphatase (ABA insensitive 1) represent key components of calcium-independent ABA signalling (Geiger *et al.,* 2010; Maierhofer *et al.,* 2014). A closely related pair of CDPKs (AtCPK4 and AtCPK11) were positive regulators in the ABA signalling processes involved in seed germination, seedling growth, guard cell regulation and tolerance to salt stress (Zhu *et al.,* 2007). CPK10 functions in abscisic acid- and Ca^{2+}-mediated stomatal regulation in response to drought stress (Zou *et al.,* 2010), while CPK8 functions in ABA-mediated stomatal regulation in responses to drought stress through regulation of Catalase 3 (CAT3) activity (Zou *et al.,* 2015).

A Ca^{2+}-dependent protein kinase, (CDPK1) decodes the Ca^{2+} signal produced by GAs and regulates the intracellular localization of RSG in plant cells (Nakata *et al.,* 2009).

Symbiotic relations: Expression of two distinct cpk genes, the OsCPK18 and OsCPK4 genes, is rapidly induced during the pre-symbiotic phase of the rice/*Arbuscular mycorrhizal* (AM) fungus, *Glomus intraradices* interaction (Campos-Soriano *et al.,* 2011). Silencing CDPK1, resulted in significantly reduced root hair and root cell lengths and both rhizobial and mycorrhizal symbiotic colonization in Medicago trunculata (Ivashuta *et al*., 2005)

Growth and Development

Stem elongation: CPK28 functions as a developmentally controlled regulator for co-ordinated stem elongation and secondary growth. In *Arabidopsis* cpk28 mutants showed altered expression of NAC transcriptional regulators NST1 and NST3 as well as of GA3ox1, a key regulator of gibberellic acid homeostasis (Matschi *et al.,* 2013). A calcium-dependent protein kinase, (CDPK1) was identified as an RSG (Repression of Shoot Growth) kinase that promotes 14-3-3 binding of RSG by phosphorylation of the Ser-114 of RSG. CDPK1 decodes the Ca^{2+} signal produced by GAs and regulates the intracellular localization of RSG in plant cells (Ishida *et al.,* 2008; Nakata *et al.,* 2009).

StCDPK3, from a stolon cDNA library of potato had expressed in early stolons, while StCDPK1 expressed upon stolon swelling, indicating sequential activation of StCDPK3 and StCDPK1 and the subcellular localisation of StCDPK1 might be critical regulatory steps of calcium signalling during potato tuber development (Raices *et al.,* 2003).

Flowering: CDPKs were believed to play a predominant role in stress signalling in plants especially in biotic and abiotic stress responses. The multitude of functions of the CDPKs are not confined to stress responses but also in growth and development of plants especially in flowering. CDPKs are involved in regulation of flowering time. A ternary complex of FT (FLOWERING LOCUS T), the central component of florigen, with 14-3-3 and FD (FLOWERING LOCUS D) is formed at the shoot apex which promotes flowering. CPK33 phosphorylates the threonine residue of FD which is essential for the florigen function. A kinase-dead form of CPK33 caused a clear delayed-flowering phenotype (Kawamoto *et al.,* 2015).

Pollen tube growth: In *Arabidopsis thaliana,* pollen tube tip growth involves the redundant activity of two Ca^{2+}-dependent protein kinase (CPKs) isoforms, CPK17 and CPK34 targeting the plasma membrane CPK17 and CPK34, transduce Ca^{2+} signals to increase the rate of pollen tube tip growth and facilitate a response to tropism cues (Myers *et al.,* 2009).

CDPK signaling: Not necessarily Calcium dependent?

In *Arabidopsis,* CDPKs show highly variable calcium-dependencies for their kinase activities: seven CPKs from subgroups 1 and 2 were sensitive to calcium with different intensities, while six CPKs from subgroup 3 had low or no calcium sensitivity to two generic substrates while CPK25 was calcium-independent. Although all CDPKs bound calcium, the calcium-independence could be correlated with significant alterations in the predicted

EF-hands of these kinases, while functional EF-hands were absent from CPK25 (Boudsocq *et al.,* 2012)

In the presence of Ca^{2+} and Mg^{2+}, alternaric acid, a host-specific toxin produced by *Alternaria solani,* stimulated *in vitro* phosphorylation of CDPK2 from potato and Solanapyrone-A (SpA), a non-host-specific toxin produced by *A. solani,* inhibited the phosphorylation of CDPK2. However, SpA stimulated CDPK2 phosphorylation in the absence of these cations, suggesting that CDPK2 may mediate SpA-induced signalling independent of Ca^{2+} and Mg^{2+} (Hassan *et al.,* 2013). CDPK signalling cascade is activated independent of calcium. Calcium binding is always not necessary in CDPK signalling.

Convergence of Calcium signalling with other pathways

The CDPK and MAPK (Mitogen-activated Protein Kinase) pathways are the two major pathways that are widely used to adapt the cellular metabolism to a changing environment and involved in signalling of abiotic and biotic stress in animal, yeast and plant cells. MAPKs and CDPKs are both involved in cross-tolerance between biotic and abiotic stress responses, for example, wounding or over-expression of pathogen-induced MYB transcription factors increased salt tolerance in tomato (Abuqamar *et al.,* 2009) while in *Arabidopsis,* AtMKK9 induced ethylene and camalexin biosynthesis and increased salt sensitivity (Xu *et al.,* 2008). CDPKs were involved in cross tolerance where wounding improved salt tolerance in tomato (Capiati *et al.,* 2006). Early studies of CDPKs involved in the biotic stress response in *Arabidopsis* and tobacco indicated a cross-talk of CDPK and MAPK activities (Ludwig *et al.,* 2005; Mehlmer *et al.,* 2010). Elevated CDPK signaling compromised stress-induced MAPK activation, and this inhibition required ethylene synthesis and perception, indicating that CDPK and MAPK pathways do not function independently but a concerted activation of both pathways controls response specificity to biotic and abiotic stress (Ludwig *et al.,* 2005). A recent study in *Arabidopsis* however revealed that CDPKs and MAPKs act differentially in innate immune signalling to control early genes involved in the synthesis of defense peptides and metabolites, cell wall modifications and redox signaling and showed no direct cross-talk between CDPK and MAPK activities (Boudsocq *et al.,* 2010). Similar results were also reported for CDPK and MAPK activities in the salt stress response in *Arabidopsis* (Wurzinger *et al.,* 2011). A novel regulatory mechanism of MAPK phosphorylation and activation besides the canonical MAPK cascade was reported in rice (*Oryza sativa*) where CPK18, was an upstream kinase of MAPK (MPK5) *in vitro* and *in vivo* (Xie *et al.,* 2014).

Details on plant protein kinases is available at the PlantP database. The NSF 2010 Project in *Arabidopsis* was initiated to define phosphorylation networks that were related to the function of CDPKs and four closely related families; CDPK-related kinases (CRKs), phosphoenolpyruvate carboxylase kinases (PPCKs), PPCK-related kinases (PEPRKs), and SNF-1 related kinases (SnRKs). The 84 protein kinases with gene identification numbers are enlisted at http://www.arabidopsis.org and gene information available at http://plantsp.genomics.purdue.edu. The presence of closely related kinases in genomic regions known to have arisen by genome duplication indicates that they probably diverged from common ancestors (Hrabak *et al.,* 2003).

WRKYs belong to a large family of plant specific transcription factors with a conserved sequence motif WRK, adjacent to a zinc-finger motif which binds to the W-box (TTGACC/T) in the promoter region of target genes. WRKY transcription factors play key roles in diverse physiological processes, including plant innate immunity. Plant disease resistance (R) proteins, often carrying cytosolic nucleotide-binding leucine-rich repeats (NB-LRR) domains sense the effectors or perturbations of host targets and elicit effector-triggered immunity (ETI). Effector triggered immunity is often triggered by increase in cytosolic Ca^{2+} levels. In addition to MAP kinases, CPKs could directly phosphorylate and activate WRKYs. WRKYs are bona fide substrates of CPKs and phosphorylated by CPKs at the conserved threonine residues in their DNA binding domain. Activation of CPK4, 5, 6 and 11 phosphorylates a specific subgroup of WRKY transcription factors to regulate transcriptional reprogramming to restrict pathogen growth (Gao and He, 2013).

Using yeast two-hybrid assays the interacting partners/proteins of CDPKs were elucidated. AtCPK4 was found to interact with 14 redundant proteins while AtCPK11 interacted with 24 proteins out of which 13 were redundant (Uno *et al.,* 2009).

Conclusion

The redundancy and close homology of various isoforms within CDPK gene families, poses a major challenge not only in allocating defined biological functions to specific CDPK isoforms, but also to integrate CDPK signalling with other signal networks. Due to functional redundancy among the CDPKs, loss-of-function approach would not be advantageous to characterise and define the functions of individual CDPKs. The function of CDPKs of the multigene families can be identified through determination of their sub-cellular location, downstream targets or target sequences in substrate proteins that are phosphorylated by each kinase, and other proteins with which the kinases associate, thereby understanding the overlap in kinase function and cross-talk between signalling pathways. How CDPKs recognise specific substrates; still needs to be elucidated. Substrate specificity of CDPKs is an emerging area of research, which is

significant in understanding the downstream signalling machinery. CDPKs may have distinct stimulus-specific and pathway-specific roles and may as well as undergo post translational modifications. The versatile roles of CDPKs make them ideal candidates for genetic manipulation for incorporating multiple traits in crop plants. Future challenges will be to elucidate their specific or redundant physiological functions in plants.

REFERENCES

Abo-El-Saad M and Wu R 1995. A rice membrane calcium-dependent protein kinase is induced by gibberellin. *Plant Physiology*, **108**: 787-793.

Abuqamar S, Luo H, Laluk K, Mickelbart M V and Mengiste T 2009. Crosstalk between biotic and abiotic stress responses in tomato is mediated by the AIM1 transcription factor. *Plant Journal*, **58**(2): 347-360

Asai S, Ichikawa T, Nomura H, Kobayashi M, Kamiyoshihara Y, Mori H, Kadota Y, Zipfel C, Jones J D G and Yoshioka H 2013. The variable domain of a plant calcium-dependent protein kinase (CDPK) confers sub-cellular localization and substrate recognition for NADPH Oxidase. *Journal of Biological Chemistry*, **288**: 14332-14340.

Asano T, Hakata M, Nakamura H, Aoki N, Komatsu S, Ichikawa H, Hirochika H and Ohsugi R 2011. Functional characterisation of OsCPK21, a calcium-dependent protein kinase that confers salt tolerance in rice. *Plant Molecular Biology*, **75**:179-191.

Asano T, Tanaka N, Yang G, Hayashi N and Komatsu S 2005. Genome-wide identification of the rice calcium-dependent protein kinase and its closely related kinase gene families: comprehensive analysis of the CDPKs gene family in rice. *Plant and Cell Physiology*, **46**(2): 356-366.

Baizabal-Aguirre V M and de la Vara L E G 1997. Purification and characterisation of a calcium-regulated protein kinase from beet root (*Beta vulgaris*) plasma membranes. *Plant Physiology*, **99**: 135-143.

Batisti O and Kudla J 2009. Plant calcineurin B-like proteins and their interacting protein kinases. *Biochimica et Biophysica Acta*, **1793**: 985-992.

Batisti O and Kudla J 2012. Analysis of calcium signaling pathways in plants. *Biochimica et Biophysica Acta*, **1820**: 1283-1293.

Battey N H 1990. Calcium-activated protein kinase from soluble and membrane fractions of maize coleoptiles. *Biochemical and Biophysical Research Communications*, **170**: 17-22.

Brandt B, Munemasa S, Wang C, Nguyen D, Yong T, Yang P G, Poretsky E, Belknap T F, Waadt R, Aleman F and Schroeder J I 2015. Calcium specificity signaling mechanisms in abscisic acid signal transduction in *Arabidopsis* guard cells. *Elife*, **20**: 4.

Boudsocq M, Droillard M J, Regad L and Laurière C 2012. Characterization of *Arabidopsis* calcium-dependent protein kinases: activated or not by calcium? *Biochemical Journal*, **447**(2): 291-299.

Boudsocq M, Willmann M R, McCormack M, Lee H, Shan L, He P, Bush J, Cheng S H and Sheen J 2010. Differential innate immune signalling via Ca($^{2+}$) sensor protein kinases. *Nature*, **464** (7287): 418-422.

Boudsocq M and Sheen J 2013. CDPKs in immune and stress signaling. *Trends in Plant Science*, **18**: 30-40.

Braun A P and Schulman H 1995. The multifunctional calcium/calmodulin-dependent protein kinase: from form to function. *Annual Review of Physiology*, **57**: 417-445.

Bundo M and Coca M 2016. Enhancing blast disease resistance by over-expression of the calcium-dependent protein kinase OsCPK4 in rice. *Plant Biotechnology Journal*, **14**(6): 1357-1367.

Cai H, Cheng J, Yan Y, Xiao Z, Li J, Mou S, Qiu A, Lai Y, Guan D and He S 2015. Genome-wide identification and expression analysis of calcium-dependent protein kinase and its closely related kinase genes in *Capsicum annuum. Frontiers in Plant Science*, **6**: 737.

Campos-Soriano L, Gomez-Ariza J, Bonfante P and San Segundo B 2011. A rice calcium-dependent protein kinase is expressed in cortical root cells during the presymbiotic phase of the arbuscular mycorrhizal symbiosis. *BMC Plant Biology*, **11**: 90.

Capiati D A, País S M and Téllez-Iñón M T 2006. Wounding increases salt tolerance in tomato plants: evidence on the participation of calmodulin-like activities in cross-tolerance signalling. *Journal of Experimental Botany*, **57**(10): 2391-2400.

Choi H I, Park H J, Park J H, Kim S, Im M Y, Seo H H, Kim Y W, Hwang I and Kim S Y 2005. *Arabidopsis* calcium-dependent protein kinase AtCPK32 interacts with ABF4, a transcriptional regulator of abscisic acid-responsive gene expression, and modulates its activity. *Plant Physiology*, **139**: 1750-1761.

Coca M and San Segundo B 2010. AtCPK1 calcium-dependent protein kinase mediates pathogen resistance in *Arabidopsis. Plant Journal*, **63**: 526-540.

Cheng S H, Willmann M R, Chen H C and Sheen J 2002. Calcium signaling through protein kinases. The *Arabidopsis* calcium-dependent protein kinase gene family. *Plant Physiology*, **129**: 469-485.

Dammann C, Ichida A, Hong B, Romanowsky S M, Hrabak E M, Harmon A C, Pickard B G and Harper J F 2003. Subcellular targeting of nine calcium-dependent protein kinase isoforms from *Arabidopsis. Plant Physiology*, **132**(4): 1840-1648.

Das R and Pandey G K 2010. Expressional analysis and role of calcium regulated kinases in abiotic stress signaling. *Current Genomics*, **11**: 2-13.

DeFalco T A, Bender K W and Snedden W A 2010. Breaking the code: Ca^{2+} sensors in plant signalling. *Biochemical Journal*, **425**: 27-40.

Dubiella U, Seybold H, Durian G, Komander E, Lassig R, Witte C P, Schulze W X and Romeis T 2013. Calcium-dependent protein kinase/NADPH oxidase activation circuit is required for rapid defense signal propagation. *Proceedings of the National Academy of Sciences of the United States of America*, **110**(21): 8744-8749.

Dubrovina A S, Kiselev K V, Khristenko V S and Aleynova O A 2015. VaCPK20, a calcium-dependent protein kinase gene of wild grapevine *Vitis amurensis* Rupr., mediates cold and drought stress tolerance. *Journal of Plant Physiology*, **185**: 1-12.

Ellard-Ivey M, Hopkins R B, White T and Lomax T L 1999. Cloning, expression and N-terminal myristoylation of CpCPK1, a calcium-dependent protein kinase from zucchini (*Cucurbita pepo* L.). *Plant Molecular Biology*, **39**: 199-208.

Franz S, Ehlert B, Liese A, Kurth J, Cazalé A C and Romeis T 2011. Calcium-dependent protein kinase CPK21 functions in abiotic stress response in *Arabidopsis thaliana*. *Molecular Plant,* **4**: 83-96.

Freymark G, Diehl T, Miklis M, Romeis T and Panstruga R 2007. Antagonistic control of powdery mildew host cell entry by barley calcium dependent protein kinases (CDPKs). *Molecular Plant-Microbe Interactions*, **20**(10): 1213-1221.

Furuichi N, Yokokawa K, Okamura H and Ohta M 2014. PiPE, a Phytophthora-associated PAMPS from *P. infestans*, binds to a Ca^{2+}-Dependent Protein Kinase (CDPK) in potato for the induction of hypersensitive reaction. *Journal of Clinical and Experimental Pathology*, **4**: 156.

Furumoto T, Ogawa N, Hata S and Izui K 1996. Plant calcium-dependent protein kinase-related kinases (CRKs) do not require calcium for their activities. *FEBS Letters*, **396**: 147-151.

Gao X and He P 2013. Nuclear dynamics of *Arabidopsis* calcium-dependent protein kinases in effector-triggered immunity. *Plant Signal Behavior*, 8(4): e23868. doi: 10.4161/psb.23868.

Gao X, Cox Jr K L and He P 2014. Functions of calcium-dependent protein kinases in plant innate immunity. *Plants*, **3**: 160-176.

Geiger D, Scherzer S, Mumm P, Marten I, Ache P, Matschi S, Liese A, Wellmann C, Al-Rasheid K A S, Grill E, Romeis T and Hedrich R 2010. Guard cell anion channel SLAC1 is regulated by CDPK protein kinases with distinct Ca^{2+} affinities. *Proceedings of the National Academy of Sciences of the United States of America*, **107**(17): 8023-8028.

Gutermuth T, Lassig R, Portes M, Maierhofer T, Romeis T, Borst J W, Hedrich R, Feijo J A and Konrad K R 2013. Pollen tube growth regulation by free anions depends on the interaction between the anion channel SLAH3 and calcium-dependent protein kinases CPK2 and CPK20. *Plant Cell*, **25**(11): 4525-4543.

Hamel L P, Sheen J and Sequin A 2014. Ancient signals: comparative genomics of green plant CDPKs. *Trends in Plant Sciences*, **19**: 79-89.

Harmon A C, Putnam-Evans C and Cormier M J 1987. A calcium-dependent but calmodulin-independent protein kinase from soybean. *Plant Physiology*, **83**: 830-837.

Harmon A C, Gribskov M, Gubrium E and Harper J F 2001. The CDPK superfamily of protein kinases. *New Phytologist*, **151**: 175-183.

Harmon A C, Gribskov M and Harper J F 2000. CDPKs-a kinase for every Ca^{2+} signal. *Trends in Plant Science*, **5**: 154-159.

Harper J F, Breton G and Harmon A 2004. Decoding Ca^{2+} signals through plant protein kinases. *Annual Review of Plant Biology*, **55**: 263-288.

Harper J F and Harmon A 2005. Plants, symbiosis and parasites: a calcium signalling connection. *Nature Reviews Molecular Cell Biology,* **6**: 555-566.

Harper J F, Sussman M R, Schaller G E, Putnam-Evans C, Charbonneau H and Harmon A C 1991. A calcium-dependent protein kinase with a regulatory domain similar to calmodulin. *Science*, **252**: 951-954.

Hashimoto K and Kudla J 2011. Calcium decoding mechanisms in plants. *Biochimie*, **93**: 2054-2059.

Hassan A, Hatsugai N and Shah M M 2013. A phytotoxin Solanapyrone-A downregulates calcium-dependent protein kinase activity in potato. *Genetics and Molecular Research Journal*, **12**(2): 1540-1545.

Hepler P K and Wayne R O 1985. Calcium and plant development. *Annual Review of Plant Biology*, **36**: 397-439.

Hetherington A M and Trewavas A J 1982. Calcium dependent protein kinase in pea shoot membranes. *FEBS Letters*, **145**: 67-71.

Hettenhausen C, Sun G, He Y, Zhuang H, Sun T, Qi J and Wu J 2016. Genome-wide identification of calcium-dependent protein kinases in soybean and analyses of their transcriptional responses to insect herbivory and drought stress. *Scientific Reports*, 6.18973. doi:10.1038/srep18973.

Hrabak E M 2000. Calcium-dependent protein kinases and their relatives. *Advances in Botanical Research*, **2**: 185-223.

Hrabak E M, Chan C W, Gribskov M, Harper J F, Choi J H, Halford N, Kudla J, Luan S, Nimmo H G, Sussman M, Thomas M, Waalker-Simmoons K, Zhu J K and Harmon A C 2003. The *Arabidopsis* CDPK-SnRK superfamily of protein kinases. *Plant Physiology*, **132**: 666-680.

Hu W, Hou X, Xia Z, Yan Y, Wei Y, Wang L, Zou M, Lu C, Wang W and Peng M 2016. Genome-wide survey and expression analysis of the calcium-dependent protein kinase gene family in cassava. *Molecular Genetics and Genomics*, **291**: 241-253.

Ito T, Nakata M, Fukazawa J, Ishida S and Takahashi Y 2010. Alteration of substrate specificity: The variable N-terminal domain of tobacco Ca^{2+}-dependent protein kinase is important for substrate recognition. *Plant Cell*, **22**: 1592-1604.

Ishida S, Yuasa T, Nakata M and Takahashi Y 2008. A tobacco calcium-dependent protein kinase CDPK1, regulates the transcription factor repression of shoot growth in response to Gibberellins. *Plant Cell*, **20** (12): 3273-3288.

Ivashuta S, Liu J, Liu J, Lohar D P, Haridas S, Bruna Bucciarelli B, VandenBosch K A, Vance C P, Harrison M J and Gantt S J 2005. RNA interference identifies a calcium-dependent protein kinase involved in *Medicago truncatula* root development. *Plant Cell,* **17**(11): 2911-2921.

Iwata Y, Kuriyama M, Nakakita M, Kojima H, Ohto M and Nakamura K 1998. Characterisation of a calcium dependent protein kinase of tobacco leaves that is associated with the plasma membrane and is inducible by sucrose. *Plant Cell Physiology*, **39**: 1176-1183.

Kanchiswamy C N, Takahashi H, Quadro S, Maffei M E, Bossi S, Bertea C, Zebelo S A, Muroi A, Ishihama N, Yoshioka H, Boland W, Takabayashi J, Endo Y, Sawasaki T and Arimura G 2010. Regulation of *Arabidopsis* defense responses against *Spodoptera littoralis* by CPK-mediated calcium signaling. *BMC Plant Biology*, **10**: 97.

Kawamoto N, Endo M and Araki T 2015. Expression of a kinase-dead form of CPK33 involved in florigen complex formation causes delayed flowering. *Plant Signaling and Behavior,* **10** (12): e1086856. doi: 10.180/15592324.205. 186856.

Lee S S, Cho H S, Yoon G M, Ahn J W, Kim H H and Pai H S 2003. Interaction of NtCDPK1 calcium-dependent protein kinase with NtRpn3 regulatory subunit of the 26S proteasome in Nicotiana tabacum. *Plant Journal*, **33**: 825-840.

Li A, Wang X, Leseberg C H, Jia J and Mao L 2008. Biotic and abiotic stress responses through calcium-dependent protein kinase (CDPK) signaling in wheat (*Triticum aestivum* L). *Plant Signaling and Behavior*, **3**: 654-656.

Liu W, Li W, He Q, Daud M K, Chen J and Zhu S 2014. Genome-wide survey and expression analysis of calcium-dependent protein kinase in *Gossypium raimondii.* PLoS One, 9: e98189. doi: 10.1371/journal.pone.0098189.

Lu S X and Hrabak E M 2002. An *Arabidopsis* calcium-dependent protein kinase is associated with the endoplasmic reticulum. *Plant Physiology*, **128**: 1008-1021.

Lu S X and Hrabak E M 2013. The myristoylated amino-terminus of an *Arabidopsis* calcium-dependent protein kinase mediates plasma membrane localization. *Plant Molecular Biology*, **82**(3): 267-278.

Luan S, Kudla J, Rodriguez-Concepcion M, Yalovsky S and Gruissem W 2002. Calmodulins and calcineurin B-like proteins: calcium sensors for specific signal response coupling in plants. *Plant Cell*, **14** (Suppl): S389-S400.

Ludwig A A, Saitoh H, Felix G, Freymark G, Miersch O, Wasternack C, Boller T, Jones J D G and Romeis T 2005. Ethylene- cross-talk between calcium-dependent protein kinase and MAPK signaling controls stress responses in plants. *Proceedings of the National Academy of Sciences of the United States of America*, **102**: 10736-10741.

Ma P, Liu J, Yang X and Ma R 2013. Genome-wide identification of the maize calcium-dependent protein kinase gene family. *Biotechnology and Applied Biochemistry*, **169**: 2111-2125.

Ma S Y and Wu W H 2007. At CPK23 functions in *Arabidopsis* responses to drought and salt stresses. *Plant Molecular Biology*, **65**: 511-518.

MacIntosh G C, Ulloa R M, Raices M and Tellez-Inon M T 1996. Changes in calcium-dependent protein kinase activity during *in vitro* tuberization in potato. *Plant Physiology*, **112**: 1541-1550.

Maierhofer T, Diekmann M, Offenborn J N, Lind C, Bauer H, Hashimoto K S, Al-Rasheid K A, Luan S, Kudla J, Geiger D and Hedrich R 2014. Site- and kinase-specific phosphorylation-mediated activation of SLAC1, a guard cell anion channel stimulated by abscisic acid. *Science Signaling*, **7**(342): ra86. doi: 10.1126/scisignal.2005703.

Martin M L and Busconi L 2000. Membrane localization of a rice calcium-dependent protein kinase (CDPK) is mediated by myristoylation and palmitoylation. *Plant Journal*, **24**: 429-435.

Matschi S, Werner S, Schulze W X, Legen J, Hilger H H and Romeis T 2013. Function of calcium-dependent protein kinase CPK28 of *Arabidopsis thaliana* in plant stem elongation and vascular development. *Plant Journal*, **73**: 883-896.

Matschi S, Hake K, Herde M, Hause B and Romeis T 2015. The calcium-dependent protein kinase CPK28 regulates development by inducing growth phase-specific, spatially restricted alterations in jasmonic acid levels independent of defense responses in *Arabidopsis. Plant Cell*, **27**(2): 591-606.

McAinsh M R and Hetherington A M 1998. Encoding specificity in Ca^{2+} signaling systems. *Trends Plant Science*, **3**: 32-36.

McCormack E, Tsai Y C and Braam J 2005. Handling calcium signaling: *Arabidopsis* CaMs and CMLs. *Trends in Plant Science*, **10**: 383-389.

Mehlmer N, Wurzinger B, Stael S, Hofmann-Rodriques D, Csaszar E, Pfister B, Bayer R and Teige M 2010. The Ca $^{(2+)}$-dependent protein kinase CPK3 is required for MAPK-independent salt-stress acclimation in *Arabidopsis. Plant Journal*, **63** (3): 484-498.

Monaghan J, Matschi S, Romeis T and Zpfel C 2015. The calcium-dependent protein kinase CPK28 negatively regulates the BIK1-mediated PAMP-induced calcium burst. *Plant Signaling and Behavior*, **10**(5): e1018497. doi: 10.1080/ 15592324.2015.1018497.

Mori I C, Murata Y, Yang Y, Munemasa S, Wang Y F, Andreoli S, Tiriac H, Alonso J M, Harper J F, Ecker J R, Kwak J M and Schroeder J I 2006. CDPKs CPK6 and CPK3 function in ABA regulation of guard cell S-type anion- and $Ca^{(2+)}$-permeable channels and stomatal closure. *PLOS Biology*, **4**: e327.

Mitra R M, Gleason C A, Edwards A, Hadfield J, Downie J A, Oldroyd G E D and Long S R 2004. A Ca^{2+}/calmodulin-dependent protein kinase required for symbiotic nodule development: Gene identification by transcript-based cloning. *Proceedings of the National Academy of Sciences of the United States of America*., **101**: 4701-4705.

Myers C, Romanowsky S M, Barron Y D, Garg S, Azuse C L, Curran A, Davis R M, Hatton J, Harmon A C and Harper J F 2009. Calcium-dependent protein kinases regulate polarized tip growth in pollen tubes. *Plant Journal*, **59** (4): 528-539.

Nakata M, Yuasa T, Takahashi Y and Ishida S 2009. CDPK1, a calcium-dependent protein kinase, regulates transcriptional activator RSG in response to gibberellins. *Plant Signaling and Behavior*, **4**: 372-374.

Patil S, Takezawa D and Poovaiah B W 1995. Chimeric plant calcium/calmodulin-dependent protein-kinase gene with a neural visinin-like calcium-binding domain. Proceedings of the *National Academy of Sciences of the United States of America*, **92**: 4897-4901.

Raíces M, Gargantini P R, Chinchilla D, Crespi M, Téllez-Iñón M T and Ulloa R M 2003. Regulation of CDPK isoforms during tuber development. *Plant Molecular Biology*, **52**(5): 1011-1024.

Roberts D M and Harmon A C 1992. Calcium-modulated proteins-targets of intracellular calcium signals in higher-plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, **43**: 375-414.

Romeis T, Ludwig A A, Martin R and Jones J D G 2001. Calcium-dependent protein kinases play an essential role in a plant defence response. *EMBO Journal*, **20**: 5556-5567.

Romeis T and Herde M 2014. From local to global: CDPKs in systemic defense signaling upon microbial and herbivore attack. *Current Opinion in Plant Biology*, **20**: 1-10.

Saijo Y, Hata S, Kyozuka J, Shimamoto K and Izui K 2000. Over-expression of a single Ca^{2+} dependent protein kinase confers both cold and salt/drought tolerance on rice plants. *Plant Journal*, **23**: 319-327.

Sanders D, Pelloux J, Brownlee C and Harper J F 2002. Calcium at the crossroads of signaling. *Plant Cell*, **14** (Suppl): S401-S417.

Sathyanarayanan P V, Cremo C R and Poovaiah B W 2000. Plant chimeric Ca^{2+}/calmodulin-dependent protein kinase: role of neural visinin-like domain in regulating autophosphorylation and calmodulin affinity. *Journal of Biological Chemistry*, **275**: 30417-30422.

Schaller G E, Harmon A C and Sussman M R 1992. Characterisation of a calcium- and lipid-dependent protein kinase associated with the plasma membrane of oat. *Biochemistry*, **31**: 1721-1727.

Schulz P, Herde M and Romeis T 2013. Calcium-dependent protein kinases: Hubs in plant stress signaling and development. *Plant Physiology*, **163** (2): 523-530.

Sheen J 1996. Ca^{2+}-dependent protein kinases and stress signal transduction in plants. *Science*, **274**: 1900-1902.

Sheen J, Lu X and Hrabak E M 2002. An *Arabidopsis* calcium-dependent protein kinase is associated with the endoplasmic reticulum. *Plant Physiology*, **128**(3): 1008-1021.

Suen K L and Choi J H 1991. Isolation and sequence analysis of a cDNA clone for a carrot calcium-dependent protein kinase: homology to calcium/calmodulin-dependent protein kinases and to calmodulin. *Plant Molecular Biology*, **17**: 581-590.

Takezawa D, Ramachandran S, Paranjape V and Poovaiah B W 1996. Dual regulation of a chimeric plant serine/threonine kinase by calcium and calcium/calmodulin. *Journal of Biological Chemistry*, **271**: 8126-8132.

Uno Y, Rodriguez Milla M A, Maher E and Cushman J C 2009. Identification of proteins that interact with catalytically active calcium-dependent protein kinases from *Arabidopsis. Molecular Genetics and Genomics*, **281**: 375-390.

Urao T, Katagiri T, Mizoguchi T, Yamaguchi-Shinozaki K, Hayashida N and Shinozaki K 1994. Two genes that encode Ca^{2+}- dependent protein kinases are induced by drought and high salt stresses in *Arabidopsis thaliana. Molecular Genetics and Genomics*, **244**: 331-340.

Wan B, Lin Y and Mou T 2007. Expression of rice Ca^{2+}-dependent protein kinases (CDPKs) genes under different environmental stresses. *FEBS Letters*, **581**: 1179-1189.

Wang J P, Xu Y P, Munyampundu J P, Liu T Y and Cai X Z 2016. Calcium-dependent protein kinase (CDPK) and CDPK-related kinase (CRK) gene families in tomato: genome-wide identification and functional analyses in disease resistance. *Molecular Genetics and Genomics*, **291**(2): 661-676.

War A R, Paulraj M G, Ahmad T, Buhroo A A, Hussain B, Ignacimuthu S and Sharma H C 2012. Mechanisms of plant defense against insect herbivores. *Plant Signaling and Behavior*, **7**: 1306-1320.

Wilkins K A, Matthus E, Swarbreck S M and Davies J M 2016. Calcium-mediated abiotic stress signaling in roots. *Frontiers in Plant Science*, **7**: 1296.

Witte C P, Keinath N, Dubiella U, Demoulière R, Seal A and Romeis T 2010. Tobacco calcium-dependent protein kinases are differentially phosphorylated in vivo as part of a kinase cascade that regulates stress response. *Journal of Biological Chemistry*, **285**: 9740-9748.

Wernimont A K, Artz J D, Finerty P Jr, Lin Y H, Amani M, Allali-Hassani A, Senisterra G, Vedadi M, Tempel W, Mackenzie F, Chau I, Lourido S, Sibley L D and Hui R 2010. Structures of apicomplexan calcium-dependent protein kinases reveal mechanism of activation by calcium. *Nature Structural and Molecular Biology*, **17**: 596-601.

Wurzinger B, Mair A, Pfister B and Teige M 2011. Cross-talk of calcium-dependent protein kinase and MAP kinase signalling. *Plant Signaling and Behavior*, **6**: 8-12.

Xie K, Chen J, Wang W and Yang Y 2014. Direct phosphorylation and activation of a mitogen-activated protein kinase by a calcium-dependent protein kinase in rice. *Plant Cell*, **26**(7): 3077-3089.

Xu J, Li Y, Wang Y, Liu H, Lei L, Yang H, Liu G and Ren D 2008. Activation of MAPK kinase 9 induces ethylene and camalexin biosynthesis and enhances sensitivity to salt stress in *Arabidopsis. Journal of Biological Chemistry*, **283**(40): 26996-27006.

Xu J, Tian Y S, Peng R H, Xiong Ai-Sheng, Zhu B, Jin X F, Gao F, Fu X Y, Hou X L and Yao Q H 2010. AtCPK6, a functionally redundant and positive regulator involved in salt/drought stress tolerance in *Arabidopsis. Planta,* **231**: 1251-1260.

Xu X, Liu M, Lu L, He M, Qu W, Xu Q, Qi X and Chen X 2015. Genome-wide analysis and expression of the calcium-dependent protein kinase gene family in cucumber. *Molecular Genetics and Genomics*, **290**:1403-1414.

Yang D H, Hettenhausen C, Baldwin I T and Wu J 2012. Silencing *Nicotiana attenuata* calcium-dependent protein kinases, CDPK4 and CDPK5, strongly up-regulates wound- and herbivory-induced jasmonic acid accumulations. *Plant Physiology*, **159**: 1591-1607.

Yang Y, Wang Q, Chen Q,Yin X, Qian M, Sun X and Yang Y 2017. Genome-wide survey indicates diverse physiological roles of the barley (*Hordeum vulgare* L.) calcium-dependent protein kinase genes. *Scientific Reports*, **7**: 5306.

Zhang H, Liu W Z, Zhang Y, Deng M, Niu F, Yang B, Wang X, Wang B, Liang W, Deyholos M K and Jiang Y Q 2014a. Identification, expression and interaction analyses of calcium-dependent protein kinase (CPK) genes in canola (*Brassica napus* L.). *BMC Genomics*, **15**: 211.

Zhang H, Wei C, Yang X, Chen H, Yang, Y, Mo Y, Li H, Zhang Y, Ma J, Yang J and Zhang X 2017. Genome-wide identification and expression analysis of calcium-dependent protein kinase and its related kinase gene families in melon (*Cucumis melo* L.). *PLoS One*, **12**(4): e0176352. doi: 10.1371/journal.pone.0176352.

Zhang K, Han Y T, Zhao F L, Hu Y, Gao Y R, Ma Y F, Zheng Y, Wang Y J and Wen Y Q 2015. Genome-wide identification and expression analysis of the CDPK gene family in grape, *Vitis* spp. *BMC Plant Biology*, **15**: 164. doi: 10.1186/s12870-015-0552-z.

Zhang L, Du L and Poovaiah B W 2014. Calcium signaling and biotic defense responses in plants. *Plant Signaling and Behavior*, **9**: e973818. doi: 10.4161/15592324.2014.973818.

Zhang X S and Choi J H 2001. Molecular evolution of calmodulin-like domain protein kinases (CDPKs) in plants and protists. *Journal of Molecular Evolution*, **53**: 214-224.

Zhao R, Sun H L, Mei C, Wang X J, Yan L, Liu R, Zhang X F, Wang X F and Zhang D P 2011. The *Arabidopsis* Ca^{2+}-dependent protein kinase CPK12 negatively regulates absisic acid signaling in seed germination and post-germination growth. *New Phytologist,* **192**: 61-73.

Zhu S Y, Yu X C, Wang X J, Zhao R, Li Y, Fan R C, Shang Y, Du S Y, Wang X F, Wu F Q, Xu Y H, Zhang X Y and Zhang D P 2007. Two calcium-dependent protein kinases, CPK4 and CPK11, regulate abscisic acid signal transduction in *Arabidopsis. Plant Cell*, **19**: 3019-3036.

Zou J J, Li X D, Ratnasekera D, Wang C, Liu W X, Song L F, Zhang, W-Z and Wu W H 2015. *Arabidopsis* calcium-dependent protein kinase and catalase 3 function in Abscisic acid-mediated signaling and H_2O_2 homeostasis in stomatal guard cells under drought stress. *Plant Cell*, **27**(5): 1445-1460.

Zou J J, Wei F J, Wang C, Wu J J, Ratnasekera D, Liu W X and Wu W H 2010. *Arabidopsis* calcium-dependent protein kinase CPK10 functions in abscisic acid- and Ca^{2+}-mediated stomatal regulation in response to drought stress. *Plant Physiology*, **154**: 1232-1243.

Zuo R, Hu R, Chai G, Xu M, Qi G, Kong Y and Zhou G 2013. Genome-wide identification, classification, and expression analysis of CDPK and its closely related gene families in poplar (*Populus trichocarpa*). *Molecular Biology Reports*, **40**(3): 2645-2662.

Generation mean analysis of yield and mineral nutrient concentrations in peanut (*Arachis hypogaea* L.)

B C AJAY*, H N MEENA, A L SINGH, M C DAGLA, NARENDRA KUMAR, S K BERA, K GANGADHAR AND A D MAKWANA

ICAR-Directorate of Groundnut Research, Ivnagar road, Junagadh-362 001, Gujarat

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ABSTRACT

Present study was undertaken to study the inheritance pattern of yield and mineral nutrients (Iron, Phosphorus, Potassium and Zinc) using five parameter generation mean analysis (P_1, P_2, F_1, F_2 and F_3) in two peanut crosses (Girnar-3 × FDRS-10 and TG-37A × FDRS-10). Scaling and joint scaling tests were significant for most characters studied indicating that additive-dominance model alone is not enough to explain the inheritance of characters studied. Both additive and dominance variance played important role for most of the traits. Traits PY, HY, HKW, SHP and RDW are governed by additive gene whereas K_{shoot}, K_{root}, Fe_{shoot}, Fe_{root}, P_{shoot} and P_{root} were governed by both additive and non-additive gene effects. Positive estimates of 'i' for Zn, K and P in cross-1 (Girnar-3 × FDRS-10) indicates that parents employed were phenotypically diverse. Therefore cross-1 holds better chance for identifying genotypes with high mineral concentrations without compromising yield levels. Hence, pedigree method of breeding could be followed for improving yield and selection could be followed in later generation when population is stable to select genotypes with high mineral concentrations.

Keywords: Gene action, Generation mean analysis, Mineral elements, Peanut, Yield

In India, peanut shares 2.66 per cent of gross cropped area and producing 25 per cent of world peanut production. There are fluctuating trends in area and production of peanut in India; however, on an average it is grown in an area of 4.56 million hectare producing 6.77 million tons of pods (DAC, 2015). Peanut being a drought tolerant in nature suffers from nutrient deficiencies resulting in low yield. On an average peanut crop with 2.0 to 2.5 t/ha of yield requires 20-25 kg P, 80-100 kg K, 3-4 kg Fe and 150-200g zinc (Singh, 1999). Higher peanut yield was attributable to enhanced uptake of mineral elements such as N, P, K, etc. (Chang and Sung 2004; Dinh *et al.,* 2014). On the contrary peanut farmers in most part of semi-arid region use very less fertilizer resulting in severe nutrient deficiencies and yield loss. The iron and zinc deficiencies cause 14-40 per cent (Singh *et al.,* 2004) and 15-20 per cent (Singh, 2001) yield loss, respectively. Increasing phosphorus application increased leaves and stem weight/plant, pods and seeds per plant, as well as N, P and K contents (El-Habbasha *et al.,* 2005).

Large amount of variability has been reported in peanut genetic stocks for yield (Upadhyaya, 2003) and accumulation of mineral elements (Singh and Chaudhari, 2006; Singh *et al.,* 2011). Till date studies related to inheritance pattern of P, K, Zn and Fe in peanut are very scarce. Knowledge of gene action and heritability involved in several quantitatively inherited traits helps in deciding appropriate breeding schemes for crop improvement. To know genetic mechanism for accumulation of these mineral elements knowledge of gene action and genetic variance are important (Akhshi *et al.,* 2014). Generation mean analysis is one such useful tool for estimation of gene effects for polygenic traits which can estimate epistatic gene effects such as additive × additive, dominance × dominance and additive × dominance effects (Kearsey and Pooni, 1996). Hence, present study was designed to study genetic variability and inheritance pattern of mineral nutrients such as P, K, Zn and Fe concentrations in shoot and root tissues of peanut in addition to yield and yield contributing characters.

MATERIALS AND METHODS

Materials used for this study consisted of 5 generations, i.e. parents (P_1, P_2), F_1, F_2 and F_3, from two crosses of peanut namely Girnar-3 × FDRS-10 (Cross-1) and TG-37A × FDRS-10 (Cross-2). Girnar-3 is a high yielding variety released for west Bengal, Orissa and Manipur regions whereas TG-37A is a high yielding variety released for Rajasthan, Uttar Pradesh, Punjab, Gujarat, Orissa, West Bengal, Bihar and north-eastern regions (Rathnakumar *et al.,* 2013). FDRS-10 has the ability to absorb and translocate higher amount of P into seeds but yields are low (Krishna, 1997). Hence, genotypes were selected to combine high yielding and efficient in absorption and translocation of P.

Hybridization was carried out during July, 2011 at ICAR-Directorate of Groundnut Research, Junagadh. Flowers were emasculated during evening hours and pollination was done next day early morning. Morphological

*E-mail: ajaygpb@yahoo.co.in

traits such as plant type, flower colour and pod characters were used as markers to check the trueness of F_1 plants. Fifty per cent F_1 seeds generated by hybridization during *kharif* 2011 were raised during summer 2012 and rest were retained for sowing during summer 2013. F_2 population generated during summer 2012 were divided into two parts and 50 per cent of F_2 population were raised during *kharif* 2012 and rest were retained for summer 2013. F_3 population generated were raised during summer 2013. Families from two crosses were grown in randomised complete block design with two replications. A replication consisted of one row of P_1 and P_2 (Parental lines), one row of F_1 generation, two rows of F_2 (i.e. 8 plants of P_1, P_2 and F_1 families and 32 plants for F_2 family). F_3 generation consisted of 152 plants in cross Girnar-3 × FDRS-10 and 80 plants in TG-37A × FDRS-10. Crop was harvested at maturity, shoot and root samples were dried to a constant weight in hot air oven at 65°C. One gram sample of shoot and root were digested separately with 3:1 nitric and perchloric acid mixture. Concentration of P in digests was measured spectroscopically using Fiske and Subbarao (1925) blue colour method. Fe, K and Zn estimation was done using atomic absorption spectrophotometer (Perkin Elmer AA400).

Statistical analysis: The two crosses were analysed separately for components of means, variance, heritability and genetic advance as per cent mean (%). The presence of epistasis was detected using C and D scaling tests proposed by Mather (Mather, 1949) and Hayman and Mather (Hayman and Mather, 1955). Formulas used for calculating these scales are given in Table 1.

There are reports indicating the inadequacy of Mather's scaling test in explaining additive-dominance model (Deb and Khaleque, 2009). Hence joint scaling test was also employed to test the adequacy of additive-dominance model. Joint scaling test is based on 3-parameter model - *m* (mean of F_2 generation), *d* (pooled additive effects) and *h* (pooled dominance effects) - estimated from 5 generations using weighted minimum square method as proposed by Cavalli (Cavalli, 1952). The Chi-square (χ^2) test was employed to test the goodness of fit of observed generation means with expected means. If the 2 test was significant five generation mean analysis was performed to estimate other gene effects like 'i' (additive × additive) and 'l' (dominance × dominance) epistatic effects in addition to '*m*', '*d*', and '*h*'. The formulas used for five parameter model was given by Hayman (Hayman 1958) and are presented in Table 1 (Sharma, 1998). Dominance effect ('*h*') and dominance epistatic effect ('*l*') with the same sign the have complementary where as different signs indicated duplicate epistasis (Kearsey and Pooni, 1996).

Variance analysis: Heritable [additive (D)] and non-heritable [dominance (H) and Environment (E)] components of variance were derived as per the formula suggested by Mather and Jinks (Mather and Jinks, 1971). After solving the equation for total variance in F_2 and F_3 D and H components were obtained and are given below:

$$\text{Total variance in } F_2 = V_{F2} = \frac{1}{2}D + \frac{1}{4}H + E$$

$$\text{Total variance in } F_3 = V_{F3} = \frac{3}{4}D + \frac{3}{16}H + E$$

$$\text{Variance among } F_3 \text{ families} = V_{F3a} = \frac{1}{2}D + \frac{1}{16}H + E$$

$$\text{Variance within } F_3 \text{ families} = V_{F3W} = \frac{1}{4}D + \frac{1}{8}H + E$$

$$E = \bar{V}P_1 + \bar{V}P_2 + \bar{V}F_1$$

$$\text{Additive Variance (D)} = 4(V_{F3} - V_{F2} + \frac{1}{16}H)$$

$$\text{Dominance Variance (H)} = 16(3V_{F2} - 2V_{F3} - E)/6$$

$$\text{Average degree of dominance} = (H/D)^{1/2}$$

Heritability: Heritability in narrow sense (h^{2n}_s) was expressed as the ratio of additive variance to the phenotypic variance (h^2_n = D/(D+H+E)) in F_2 and F_3 generation. Heritability was classified into low (0-30%), moderate (30%-60%) and high (>60%) according to Robinson *et al*. (1949).

RESULTS AND DISCUSSION

Results of two crosses analysed for genetic components of variance, gene action and heritability involved in inheritance of Zinc (Zn), Potassium (K), Iron (Fe), Phosphorus (P), shoot weight per plant (HY), root weight per plant (RDW), pod yield per plant (PY), shelling per cent (SHP) and 100 kernel weight (HKW) are furnished below:

Mean of Girnar-3 was higher for Zn_{shoot}, K_{shoot}, Fe_{shoot} Fe_{root}, RDW, PY and SHP over TG-37A and FDRS-10 whereas FDRS-10 was superior over other parents for K_{root}, P_{shoot} , P_{root}, HY (Table 2). The mean F_1 of the cross-1 was greater than both parents for Fe_{root}, PY and HKW and F_1 means of cross-2 was higher than parents for K_{root}, Fe_{shoot}, P_{shoot}, HY, RDW, PY, SHP and HKW. F_2 means for Zn_{root}, K_{root} was higher than both the parents in both crosses; lower than both the parents in cross-I for Zn_{shoot} and K_{shoot} and in cross-2 it was high and between two parents for K_{shoot} and Zn_{shoot} respectively. In cross-1 means values of Fe_{shoot}, Fe_{root}, P_{shoot} and P_{root} of F_2 generation were in between the parents, but in cross-2 F_2 mean values of Fe_{shoot} and P_{root} was higher than both the parents. F_3 means for Zn_{root}, K_{root} was higher than both the parents in both crosses; lower than both the parents in cross-I for Zn_{shoot} and K_{shoot} and was in between

two parents in cross-2. F_3 mean values of Fe_{shoot}, Fe_{root}, P_{shoot} and P_{root} were in between two parents in both the crosses except for P_{shoot} in cross-1.

Scaling and joint scaling test: Results of scaling test revealed that C and D scales were significant for Zn_{shoot}, K_{shoot}, K_{root}, Fe_{root}, P_{shoot}, P_{root}, PY and SHP in both the crosses. In cross-1 only C test was significant for Zn_{root}, Fe_{root} and HY and both C and D were significant for Fe_{root} and HY in cross-2. For RDW and HKW both C and D scale test were significant in cross-2 and non-significant in cross-1. Significance of one and/or both the C and D scale test indicates the presence of epistasis for all the characters studied. Non-significant C and D indicate that additive-dominance model was adequate for the respective characters and crosses. The Cavalli's (1952) joint scaling (χ^2) test was done to test significance of observed generation means over expected means based on 3-parameter model (Table 3). 2 values were significant for all the characters indicating that epistasis is present and additive-dominance model alone is not sufficient.

Table 1 Formulae used for scaling test and five parameter model of generation mean analysis

Components	Estimate	SE	df
Scaling test			
C	$\bar{P}_1+\bar{P}_2+2\bar{F}_1-4\bar{F}_2$	$(\bar{V}_{P1}+\bar{V}_{P2}+4\bar{F}_1+16\bar{F}_2)^{0.5}$	$df(P_1)+df(P_2)+df(F_1)+df(F_2)$
D	$4\bar{F}_3- 2\bar{F}_2 - \bar{P}_1- \bar{P}_2$	$(16\bar{V}_{F3}+4\bar{V}_{F2}+\bar{V}_{P1}+\bar{V}_{P2})^{0.5}$	df(F3)+ df(F2)+ df(P1)+df(P2)
Genetic Variance			
m	$\bar{F}_2$	$(\bar{V}_{F2})^{0.5}$	$df(F_2)$
d	$(\bar{P}_1-\bar{P}_2)/2$	$[(\bar{V}_{P1} + \bar{V}_{P2})/4]^{0.5}$	$df(P_1)+df(P_2)$
h	$1/6(4\bar{F}_1 + 12\bar{F}_2 -16\bar{F}_3)$	$[1/36(16\bar{V}_{F1} + 144\bar{V}_{F2} + 256\bar{V}_{F3}]^{0.5}$	$df(F_1)+df(F_2)+ df(F_3)$
i	$\bar{P}1-\bar{F}2-1/2(\bar{P}1-\bar{P}2)+$ $½(4\bar{F}_1+12\bar{F}_2-16\bar{F}_3)/6$ $¼(16\bar{F}_3-24\bar{F}_2+8\bar{F}_1)/3$	$[\bar{V}P1+\bar{V}F2+1/4(\bar{V}P1+\bar{V}P2)$ $+1/4(16\bar{V}_{F1}+144\bar{V}_{F2}+256\bar{V})/36$ $-1/16(256\bar{V}_{F3}+576\bar{V}_{F2}+64\bar{V}_{F1})/3]^{0.5}$	$df(P_1)+ df(F_2)+ df(P_1)+df(P_2)+ df(F_1)+df(F_2)+ df(F_3)+ df(F_1)+ df(F_2)+ df(F_3)$
l	$1/3(16\bar{F}_3–24\bar{F}_2+8\bar{F}_1)$	$[1/9(256\bar{V}_{F3}+576\bar{V}_{F2}+ 64\bar{V}_{F1})]^{0.5}$	$df(F_1)+df(F_2)+ df(F_3)$

Where VP_1, VP_2, VF_1, VF_2, VF_3 are the variances of P_1, P_2, F_1, F_2, F_3 populations respectively

Genetic components of variance and gene action: In cross-1 additive (D) component of variance was higher than dominance (H) component for most of the traits except K_{shoot}, SHP and HKW (Table 4). In cross-2 dominance (H) component of variance was higher than additive (D) component for most of the traits except K_{shoot}, Fe_{root}, and RDW. The H component was negative for most of the traits in cross-1. Average degree of dominance was more than unity and narrow sense heritability was low for most of the traits in both the crosses.

The gene actions such as mean (m), additive (d), dominance (h), additive × additive (i) and dominance × dominance (l) for different traits among cross-1 and 2 are presented in Table 5. In the inheritance of Zn_{shoot}, 'd' had significant influence in both the crosses whereas in epistatic interactions 'i' type of gene action was more predominant. Inheritance of Zn_{root} was mainly governed by additive type of gene action and epistatic interactions were not significant. In the inheritance of K_{shoot}, K_{root}, Fe_{shoot}, Fe_{root}, P_{shoot} and P_{root}, gene effects such as 'm', 'd', 'h' and 'l' had significant influence in both crosses whereas component 'i' had significant influence in Fe_{shoot}, Fe_{root}, P_{shoot} and P_{root} in cross-1 and in cross-2 'i' component was significant only in Fe_{root} and P_{root}. In the inheritance of Zn_{shoot} gene effects such as 'm', 'd' and 'i' were prominent in both crosses whereas inheritance of Zn_{root} was governed by 'm' and 'd' in cross-1 and by 'm', 'd' and 'l' in cross-2.

In the inheritance of HY, 'd' was significant in cross-1 whereas in cross-2 both 'd' and 'h' type of gene actions were significant. Among epistatic interactions, 'l' type gene effect was significant. For RDW only 'd' was significant and none of the epistatic interactions were significant in both crosses. For PY, 'd' and 'l' were significant in cross-1 whereas in cross-2 components 'm', 'd', 'i' and 'l' were found to be significant. For SHP, 'm', 'd', 'i' and 'l' components were significant in both the crosses. For HKW, 'd' component was significant in both the crosses and 'h' component was significant in cross-2. Among epistatic interactions both 'i'

and 'l' were significant in cross-2 and in cross-1 only 'I' type of gene effects was significant. Duplicate type of gene action was more predominant for most of the traits in both the crosses except for Zn_{root}, K_{shoot} and Fe_{shoot} in cross-1 and Zn_{shoot}, K_{root} and RDW in cross-2.

Scaling and joint scaling tests were significant for most of the traits in both crosses (Table 1). This indicates that higher value interactions (inter-allelic interactions) play important role in the expression of characters and additive-dominance alone is not sufficient (Shahid, 1996; Ajay *et al.,* 2012). In such cases, populations have to be forwarded to next generations in order to arrive at the best fit model (Mather and Jinks, 1982).

Table 2 Average Zinc (Zn), Potassium (K), Iron (Fe), Phosphorus (P) and yield related traits among five generations in two peanut crosses

Sample	Zn Conc (ppm)		K Conc (ppm)		Fe Conc (ppm)		P conc (ppm)		HY (g/plt)	RDW (g/plant)	PY (g/plant)	SHP (%)	HKW
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root					
Cross-1: Girnar-3 × FDRS-10													
Girnar-3 (P1)	67.0	14.3	22420.0	10400.0	1431.0	918.3	1180.0	500.0	19.3	3.3	14.5	71.5	33.8
FDRS-10 (P2)	61.4	10.1	16350.0	13850.0	249.2	344.2	1820.0	1000.0	30.4	2.1	9.0	63.0	35.3
F_1	16.1	22.4	10390.0	9325.5	1087.8	1022.5	928.0	827.5	24.8	2.9	16.6	71.2	36.8
F_2	21.0	19.4	14554.5	21740.0	714.1	665.0	1400.0	1040.0	44.5	3.4	6.0	64.4	34.8
F_3	19.4	18.0	13705.4	14282.1	798.1	827.4	964.6	695.3	37.6	3.1	16.0	68.1	36.7
Cross-2: TG-37A × FDRS-10													
TG-37A (P1)	18.6	18.7	11690.0	9057.0	162.8	723.8	1140.0	560.0	16.1	2.7	6.9	66.5	35.9
FDRS-10 (P2)	61.4	10.1	16350.0	13850.0	249.2	344.2	1820.0	1000.0	30.4	2.1	9.0	63.0	35.3
F_1	31.2	15.7	9104.0	14030.0	283.3	374.9	830.0	1130.0	34.9	7.3	13.8	68.4	38.7
F_2	23.8	31.6	17295.0	16830.0	785.8	283.0	1810.0	1385.0	39.4	3.8	9.4	63.8	29.1
F_3	25.2	22.1	14708.5	18060.7	543.5	446.1	1292.0	1040.0	27.1	4.3	17.4	66.4	36.3

Table 3 Scaling and joint scaling test (χ^2) for Zinc (Zn), Potassium (K), Iron (Fe), Phosphorus (P) and yield related traits in two peanut crosses

Sample	Zn Conc (ppm)		K Conc (ppm)		Fe Conc (ppm)		P conc (ppm)		HY (g/plt)	RDW (g/plant)	PY (g/plant)	SHP (%)	HKW
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root					
Cross-1: Girnar-3 × FDRS-10													
C	76.6*	-8.2*	-1332.0*	-44059*	999.5*	647.5*	-744.0*	-1005.0*	-78.8*	-2.2	32.6*	19.2*	3.3
D	-92.8*	9.0	-13057*	-10601*	84.0	717.1*	-1941.7*	-798.8*	11.9	0.4	28.3*	9.0*	8.2
χ^2	22686**	83103**	10452**	1278.1**	178418**	2472.3**	2459**	1325.2**	85961**	853289**	1308.3**	4335.2**	2946.3**
Cross-2: TG-37A × FDRS-10													
C	47.2*	-66.1*	-22932.0*	-16353.0*	-2164.8*	685.7*	-3220.0*	-1400.0*	-41.3*	2.1*	5.9	10.9*	32.0*
D	-26.8*	-3.6	-3795.9*	15675.9*	190.4*	150.3*	-812.0*	-490.0*	-17.1*	3.8	35.2*	8.5*	15.8*
χ^2	21375**	514**	10903**	339856**	231144**	155182**	49938**	12954**	1116071**	4016.5**	553**	33689**	2639**

C and D Mather's Scaling test *,** Significant at $P \leq 0.05$ and $P \leq 0.01$, respectively

Table 4 Genetic variance components and allied parameters for Zinc, Potassium (K), Iron (Fe), Phosphorus (P) and yield related traits in two peanut crosses

Sample	Zn Conc (ppm)		K Conc (ppm)		Fe Conc (ppm)		P conc (ppm)		HY (g/plt)	RDW (g/plant)	PY (g/plant)	SHP (%)	HKW
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root					
Cross-1: Girnar-3 × FDRS-10													
D	21.0	14.3	-8464.3	6085.6	877.9	1713.8	210.0	129.3	12.4	0.5	1.0	-1.7	-11.5
H	-40.9	-28.4	41942.7	-12435.9	-1320.7	-3720.9	-403.2	-256.6	-24.2	-0.1	24.7	10.4	54.2
E	0.0	0.04	337.9	450.7	3.0	73.2	4.8	6.3	0.5	0.0	0.3	0.0	0.0
$(H/D)^{0.5}$	1.4	1.44	2.3	1.4	1.6	1.5	1.6	1.9	1.4	1.4	5.0	2.5	2.2
h^2_{ns} (%)	34.0	32.5	16.2	35.0	25.1	31.0	27.0	19.5	33.2	33.9	2.6	13.8	17.6
Cross-2: TG-37A × FDRS-10													
D	-20.0	-12.4	6251.0	-2544.9	-361.3	467.2	-1646.9	-37.1	-4.5	2.1	-20.6	-1.8	-4.1
H	67.6	69.2	-6047.2	12256.2	1443.3	-847.9	6717.5	645.1	31.0	-4.6	105.0	8.5	25.1
E	6.7	0.2	51.3	734.1	70.0	0.6	61.4	23.8	0.3	0.1	0.4	0.0	0.1
$(H/D)^{0.5}$	1.8	2.4	1.0	2.2	2.0	1.3	2.0	4.2	2.6	1.5	2.3	2.2	2.5
h^2_{ns} (%)	21.2	15.2	50.6	16.4	19.3	35.5	19.5	5.3	12.7	30.6	16.3	17.4	13.9

D - additive variance; H - dominance variance; E - environmental variance; h^2ns - narrow sense heritability; $(H/D)^{0.5}$ - degree of dominance

Table 5 Gene effects and epistasis for Zinc, Potassium (K), Iron (Fe), Phosphorus (P) and yield related traits in two peanut crosses

Sample	Zn Conc (ppm)		K Conc (ppm)		Fe Conc (ppm)		P conc (ppm)		HY (g/plt)	RDW (g/plant)	PY (g/plant)	SHP (%)	HKW
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root					
Cross-1: Girnar-3 × FDRS-10													
m	21.0*	19.4*	14554.5	21740.0*	714.1*	665.0*	1340.0*	1040.0*	44.5*	3.4*	6.0*	64.4*	34.8*
d	2.8*	2.1*	3035.0*	-1725.0*	590.9*	287.1*	-320.0*	-250.0*	-5.6*	0.6*	2.8*	4.3*	-0.8*
h	0.9	5.55	-513.2*	11634.6*	25.2*	-195.1*	848.2*	779.1*	5.2	0.3	-19.5	-5.2	-3.7
i	49.1*	-4.67	8468.2*	14314.8*	-221.2*	-583.8*	1413.2*	696.1*	5.1	0.1	-24.2	-9.1*	-5.9*
l	-21.4	1.2	-15477.3*	-72151.9*	1430.3*	1801.3*	-2545.1*	-2381.1*	-88.3*	-2.4	80.5*	37.2*	15.1
Epistasis	D	C	C	D	C	D	D	D	D	D	D	D	D
Cross-2: TG-37A × FDRS-10													
m	23.8*	31.6*	17295.0*	16830.0*	785.8*	283.0*	1810.0*	1385.0*	39.4*	4.3*	9.4*	63.8*	29.1*
d	40.0*	14.4*	14020.0*	11453.5*	206.0*	534.0*	1180.0*	940.0*	23.3*	2.4*	7.9*	64.8*	35.6*
h	1.2	14.7	1439.5*	-5158.9*	311.8*	-374.4*	729.5*	751.5*	30.0*	2.0	-18.6*	-3.8	-12.8*
i	-51.4*	3.2	-10035.9*	-21565.7*	-18.6	-556.8*	-148.8	-764.0*	-12.2	-5.0	-33.3*	-70.4*	-51.1*
l	27.0	-92.0*	-35280.8*	-893.7*	-2606.2*	1103.6*	-5322.2*	-2494.8*	-77.1*	7.8	54.2	25.6*	63.2*
Epistasis	C	D	D	C	D	D	D	D	D	C	D	D	D

M - mean of the F_2 generation; d - additive gene effect; h - dominance gene effect; I - additive × additive gene effect; l - dominance × dominance gene effect; C - complementary gene action and D - duplicate gene action.
*, ** Significant at P≤0.05 and P≤0.01, respectively

Additive variance indicates average effects of individual alleles at segregating loci whereas dominance variance represents summation of variance due to interaction effects between two alleles at different loci. If the trait has high additive variance it may not follow strictly additive model. There is a possibility that traits may follow dominance model even when additive variance is high. Though additive variance is a major factor it is not always the best measure in the inheritance of a trait (Abney *et al.,* 2001; Ajay *et al.,* 2012). Hence it is possible, for instance, to have a trait that is heavily influenced by genetics but has a relatively low additive variance. In the present study, additive variance (D) was more prominent than dominance variance (H) for most of the traits (Table 3) in cross-1 whereas in cross-2 'H' was predominant than 'D'. Predominance of additive variance indicates that there is difference between homozygotes at a locus with positive and negative alleles being distributed between parents. Dominance genetic variance for these mineral nutrients have been reported in peanut kernels (Ajay *et al.,* 2016). For some of the traits negative H component have been observed. Previously negative H component have been reported in many crops like chickpea (Deb and Khaleque, 2009), bread wheat (Aglan and Farhat, 2014), soybean (Ribeiro *et al.,* 2009) and pigeonpea (Ajay *et al.,* 2012). Mather (1949) has inferred that this negative value of H arises due to sampling error and/or genotype and environment interactions (Robinson *et al.,* 1955).

Estimates of 'D' and 'H' components for the characters studied were not free from bias due to the presence of epistatic gene effects as indicated by scaling and joint scaling test (Table 2). Under such circumstances 'D' is affected by the presence of 'i' which often inflates the variance of F_2 and its subsequent generations (Mather and Jinks, 1982). H is also affected by 'j' and 'l' when genes interact and 'l' increases the variance of F_2 when having the same sign with 'h' and decreases it when it is in the opposite sign. For yield related traits such as PY, HY, RDW, SHP and HKW and for Znshoot and Znroot residual effect 'm' and additive effect 'd' was significant and dominance effect 'h' was non-significant. Significance of additive effect suggests that effective selection for PY could be practiced even in the early generations (Venuprasad *et al.,* 2011). To exploit additive effect simple selection techniques or hybridization followed by pedigree method is suggested for improvement of yield. For K_{shoot}, K_{root}, Fe_{shoot}, Fe_{root}, P_{shoot} and P_{root} all the gene effects were significant though some of the gene effects were negative indicating that both additive and non-additive gene effects are present for these traits. Improvement of such traits requires recombination breeding followed by postponing selection to later generations.

Positive 'i' estimates suggest that the sum of the interactions from dispersed pairs of genes is less than half the sum of all interactions. Conversely, when the contribution from dispersed pairs is more than half, 'i' will have negative sign (Mather and Jinks, 1982). For PY estimates of 'i' was negative in both crosses indicating that parents were in dispersed form and phenotypically parents are not contrasting. For Zn_{shoot}, K_{shoot}, K_{root}, P_{shoot}, P_{root} and HY estimates of 'i' was positive in cross-1 than in cross-2. This indicates that parents employed in cross-1 were phenotypically contrasting and genes were in associated form for zinc, phosphorus and potassium concentrations. Hence cross-1 holds better chance for identifying genotypes with high mineral concentrations without compromising yield levels.

Gene interaction is considered to be complementary when the 'h' and 'l' estimates have the same signs and duplicate when the signs differ (Mather and Jinks, 1982). Both patterns were found among these results, with duplicate gene action being more prominent. Peanut being an allo-tetraploid with two genomes and four sets of chromosomes (Seijo *et al.,* 2004; 2007) most of the characters were controlled by duplicate gene factors. Previous studies have also indicated duplicate gene action among tetraploid crops such as Eragrostis tef (Tefera and Peat, 1997) and cotton (Nidagundi *et al.,* 2012). This is further supported by the fact that most of the traits were governed by over-dominance type of gene action with low to moderate narrow sense heritability. As selection based on progeny performance exploits only additive component of genetic variances, bi-parental mating followed by recurrent selection or diallel selective mating, which allows inter-mating among the selected segregates in the different cycles, would be useful to recover superior homozygote in later generations (Eshighi and Akhundovoa, 2010). Selection intensity and progress in improving population performance may be greater under complementary interaction than under duplicate interaction (Ajay *et al.,* 2012).

Present study concludes the presence of additive variance in the inheritance of mineral concentrations and yield related traits. Additive gene effect governed yield traits and Fe, K and P contents were governed by both additive and non-additive gene effects. Hence, pedigree method of breeding could be followed for improving yield and selection could be followed in later generation when population is stable to select genotypes with high mineral concentrations.

REFERENCES

Abney M, McPeek M S and Ober C 2001. Broad and narrow heritabilities of quantitative traits in a founder population. *American Journal of Human Genetics*, **68**:1302-1307.

Aglan M A and Farhat W Z E 2014. Genetic studies on some earliness and agronomic characters in advanced generations in bread wheat (*Triticum aestivum* L.). *International Journal of Plant and Soil Science*, **3**: 790-798.

Ajay B C, Gnanesh B N, Ganapathy K N, Byre Gowda M, Prasad P S, Veerakumar G N, Venkatesha S C, Abdul Fiyaz R and Ramya K T 2012. Genetic analysis of yield and quantitative traits in pigeonpea (*Cajanus cajan* L. Millsp.). *Euphytica*, **186**: 705-714.

Ajay B C, Meena H N, Singh A L, Dagla M C, Narendra Kumar, Bera S K, Makwana A D and Kalariya K A 2016. Genetic variability and inheritance of iron, phosphorus, potassium and zinc contents in segregating generations of peanut (*Arachis hypogaea* L.). *Journal of Oilseeds Research*, **33**(2): 102-107.

Akhshi N, Cheghamirza K, Ahmadi H and Firouzabadi F N 2014. Generation mean analysis to estimate genetic parameters for morphological traits in common bean (*Phaseolus vulgaris* L.). *Journal of Biodiversity and Environmental Science*, **4**: 254-261.

Cavalli L L 1952. An analysis of linkage in quantitative inheritance. In: Reive E C R and Waddington C H (eds), *HMSO*, London, pp. 135-144.

Chang C S and Sung J M 2004. Nutrient uptake and yield responses of peanuts and rice to lime and fused magnesium phosphate in an acid soil. *Field Crops Research*, **89**: 319-325.

Deb A C and Khaleque M A 2009. Nature of gene action of some quantitative traits in chickpea (*Cicer arietinum*). *World Journal of Agricultural Science*, **5**(3): 361-368.

DAC 2015. Agriculture Statistics at Glance, Department of Agriculture Cooperation and Farmers Welfare, http://eands.dacnet.nic.in/PDF/Agricultural-Statistics-At-Glance 2014.

Dinh H T, Kaewpradit W, Jogloy S, Vorasoot N and Patanothai A 2014. Nutrient uptake of peanut genotypes with different levels of drought tolerance under midseason drought. *Turkish Journal of Agriculture and Forestry*, **38**: 495-505.

El-Habbasha S F, Kandil A A, Abu-hagaza N S, Abd-El-Haleem A K, Khalfallah M A and Behairy T G 2005. Effect of phosphorus levels and some biofertilizers on dry matter, yield and yield attributes of groundnut. *Bulletin of Faculty of Agriculture Cairo University*, **56**: 237-252.

Eshghi R and Akhundova E 2010. Inheritance of some important agronomic traits in hulless barley. *International Journal of Agricultural Biology*, **12**: 73-76.

Fiske C H and Subbarao Y 1925. The colorimetric determination of phosphorus. *Journal of Biological Chemistry*, **66**: 375-400.

Hayman B I 1958. The separation of epistatic from additive and dominance variation in generation means. *Heredity*, **12**: 371-390

Hayman B I and Mather K 1955. The description of genetic interaction in continuous variation. *Biometrics*, **11**: 69-82.

Kearsey M J and Pooni H S 1996. *The genetical analysis of quantitative traits* (1st edition). Chapman & Hall, London, pp. 381.

Krishna K R 1997. Phosphorus uptake and utilization efficiency in peanut. *Peanut Science*, **24**: 1-6.

Mather K 1949. *Biometrical Genetics,* Dover publications Inc, New York.

Mather K and Jinks J L 1971. *Biometrical genetics.* Chapman and Hall ltd, London.

Mather K and Jinks J L 1982. *Biometrical genetics: the study of continuous variation* (3rd edn), Chapman and Hall, London.

Nidagundi J M, Patil S S, Salimath P M, Kajjidoni S T, Patil B C and Hegde M G 2012. Genetic analysis of seed cotton yield and its component traits in *Gossypium hirsutum* L. *Karnataka Journal of Agricultural Science*, **25**: 260-261.

Rathnakumar A L, Singh R, Parmar D L and Misra J B 2013. *Groundnut: a crop profile and compendium of notified varieties in India*. Directorate of Groundnut Research, Junagadh, Gujarat, India, pp.118.

Ribeiro A S, de Toledo J F F and Ramalho M A P 2009. Selection strategies of segregant soybean populations for resistance to Asian rust. *Pesquisa Agropecuária Brasileira,* **44**: 1452-1459.

Robinson H F, Comstock R E and Harney P H 1949. Estimates of heritability and degree in corn. *Agronomy Journal*, **41**: 353-359.

Robinson H F, Comstock R E and Harney P H 1955 Genetic variance in open pollinated varieties of corn. *Genetics*, **40**: 45-60.

Seijo J G, Lavia G I and Fernandez A 2007. Genomic relationships between the cultivated peanut (*Arachis hypogaea,* Leguminosae) and its close relatives revealed by double GISH. *American Journal of Botany*, **94**: 1963-1971.

Seijo J G, Lavia G I, Fernandez A, Krapovickas A, Ducasse D and Moscone E A 2004. Physical mapping of the 5S and 18S-25S rRNA genes by FISH as evidence that *Arachis duranensis* and A. ipaensis are the wild diploid progenitors of *A. hypogaea* (Leguminosae). *American Journal of Botany*, **91**: 1294-1303.

Shahid M A 1996. Genomic composition, gene action and genotype-interaction in hexaploid wheat (*Triticum aestivum* L.) dissertation, Rajshahi University, Bangladesh.

Sharma J R 1998 *Statistical and Biometrical Techniques in Plant Breeding*. New Age International, India, pp. 440.

Singh A L 2001. Yield losses in groundnut due to micronutrient deficiencies in calcareous soils of india. In: *Plant-nutrition: food security and sustainability of agro-ecosystems through basic and applied research*. Horst W J, Schenk M K, Burkert A, Claassen N, Flessa H, Frommer W B, Goldbach H, Olfs H W, Romheld V (Eds.), Proceedings of 14th International Plant Nutrition Colloquium, 27 July to 3rd August 2001, Hannover, Germany, Kluwer Academic publisher, Dordrecht, Netherlands, pp. 838-839.

Singh A L, Basu M S and Singh N B 2004. *Mineral Disorders of Groundnut. National Research Centre for Groundnut*, Junagadh, Gujarat, India, pp. 85.

Singh A L and Chaudhari V 2006. Macronutrient requirement of groundnut: Effects on the growth and yield components. *Indian Journal of Plant Physiology*, **11**: 401-409.

Singh A L, Chaudhari V and Patel C B 2011. Identification of High Zinc Density Groundnut Cultivars to Combat Zinc Malnutrition in India. In: *Proceeding of Zinc Crops 2011* (3rd International Zinc Symposium 2011) "Improving Crop Production and Human Health" Hyderabad, India, 10-14 October, 2011. http://www.zinccrops2011.com

Singh A L 1999. Mineral nutrition of groundnut. *Advances in Plant Physiology*, **2**: 161-200.

Tefera H and Peat W E 1997. Genetics of grain yield and other agronomic characters in t'ef (Eragrostis tef Zucc. Trotter). I. Generation means and variances analysis. *Euphytica*, **96**: 185-191.

Upadhyaya H D 2003. Phenotypic diversity in groundnut (*Arachis hypogaea* L.) core collection assessed by morphological and agronomical evaluations. *Genetic Resource and Crop Evolution*, **50**: 539-550.

Venuprasad R, Aruna R and Nigam S N 2011. Inheritance of traits associated with seed size in groundnut. *Euphytica*, **181**: 169-177.

Combining ability of new pistillate lines for plant growth attributes, seed yield and its components in castor (*Ricinus communis* L.)

C LAVANYA, T MANJUNATH AND K T RAMYA

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

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ABSTRACT

Combining ability of four newly developed pistillate lines were tested using Line x Tester analysis. Sixteen hybrids generated using four pistillate lines and four testers were studied along with parents and three checks to estimate combining ability for nine characters in castor. Non-additive gene action was preponderant in all the characters studied except for seed yield, which indicated a negative estimate. Among the female parents, IPC-15 (DPC-15) was a good general combiner for earliness and short plant height while IPC-19 (IPC-19) for late duration and tall plant height. Three pistillate lines, DPC-15 (IPC-15), DPC-19 (IPC-19) and DPC-21 (IPC-21) were identified as good general combiners for total and effective primary spike length. Among the eight parents studied, DCS-107 was a good general combiner for seed yield and other traits like total primary spike length, effective primary spike length and 100-seed weight. None of the hybrids were good specific combiners for seed yield and several other yield contributing characters while three hybrids *viz.,* DPC-16 x DCS-107 (1800 kg/ha), DPC-15 x DCS-107 (1646 kg/ha) and DPC-21 x DCS-107 (1595 kg/ha) were identified as the top three performing hybrids. Among them, DPC-21 x DCS-107 (DCH-1720), has been tested in the All India Coordinated multi location trials for three years while DPC-15 x DCS-107 (PHT-12-2) was tested in three locations (2014-15) and found promising.

Keywords: Castor, Combining ability, GCA, Pistillate lines, SCA

India is the leading country in castor area (10.61 lakh ha), production (17.51 lakh tonnes) with a productivity of 1652 kg/ha followed by China and Brazil. Castor, a member of Euphorbiaceae or spurge family (2n=20), is sexually polymorphic; mostly monoecious while pistillate, pistillate with interspersed staminate flowers (ISF) and sex revertants are also most common. Monoecious is the most common occurrence in nature while identification of complete female plants in a back cross population triggered the study of sex variants in castor (Shifriss, 1960; Zimmerman and Smith, 1966; Kulkarni and Ankineedu, 1966; Lavanya 2002; Lavanya and Gopinath, 2008). TSP-10-R became background genotype to develop a dwarf line VP-1, with increased expression of pistillateness under favorable environment but revert to monoecious under different environment. This sex reversal mechanism was advantageous to maintain the female line. With this mechanism, heterosis breeding and hybrid seed production strengthened in India. Heterosis breeding, taking female parents as VP-1 or a genotype with VP-1 as background lead to development of most commonly used commercial hybrids like GCH-4, GCH-7, DCH-177, DCH-32, PCH-111, YRCH-1, RHC-1 etc. However, VP-1 is highly susceptible to wilt and therefore researches are continuously thriving to broaden the genetic base of pistillate lines. At ICAR-Indian Institute of Oilseeds Research, Hyderabad diverse pistillate lines are developed using pistillate sources from wild germplasm and inter varietal hybridization followed by pedigree method of selection for 12-15 years. Selection is done during *rabi* season under shorter day and average temperature between 25-30°C and higher nitrogen levels. As a result four genotypes showing stable pistillateness were identified with diverse background. A good combining parent will lead to the identification of best performing and combining hybrid for the target trait. Therefore present study was conducted to test the combining ability of four pistillate lines using four male parents.

MATERIALS AND METHODS

Four stable diverse pistillate lines *viz.,* IPC-15 (DPC-15), DPC-16, IPC-19 (DPC-19), IPC-21 (DPC-21) were crossed with four monoecious/male parents *viz.,* DCS-81, DCS-107, DCS-108, RG-1526 using line x tester mating design during *rabi* season of 2011-12. The experimental material including 16 hybrids, eight parents along with three checks *viz.,* DCH-177, DCH-519 and GCH-7 was evaluated in a randomized block design with two replications at ICAR-Indian Institute of Oilseeds Research farm in *kharif* 2012 under rainfed conditions. Each genotype was raised in a four row plot of 3.6 x 6 m^2 with a spacing of 90 x 60 cm. The crop was managed with all recommended agronomic practices followed by timely plant protection measures to raise a healthy crop. Observations were recorded on five randomly selected competitive plants from each entry per replication on plant height up to primary spike (cm), number of nodes to the primary, secondary 1 and secondary 2 spike, total primary spike length (cm), effective primary spike

length (cm), number of effective spikes per plant and hundred seed weight (g). Seed yield was calculated on net plot (1.8 x 4.8 m^2) and expressed in kg/ha. Statistical analysis for testing the variation among parents and crosses and combining ability analysis was performed as per the procedures suggested (Cochran and Cox, 1957; Kempthorne, 1957).

RESULTS AND DISCUSSION

The analysis of variance of combining ability revealed significant differences among hybrids and parents for all the characters studied except for seed yield (Table 1). Several studies on combining ability inferred that mean sum of squares due to males and females indicated the magnitude of general combining ability while the interaction between males and females indicated specific combining ability (Chandra Mohan *et al.,* 2006; Kavani *et al.,* 2016).

The present study indicated that the mean sum of squares of lines and testers were significant for all the characters studied except for 100-seed weight and seed yield for lines (females) and total spike length and effective spike length for males (testers) when tested against line x tester mean squares. This indicated the involvement of non-additive type of gene action for majority of the characters. Similar findings were reported by Chandra Mohan *et al.* (2006), Madariya *et al.* (2008), Barad *et al.* (2009), Patel *et al.* (2010), while majority of the researchers documented the importance of both additive and dominance type of gene action (Madariya *et al.,* 2008; Kavania *et al.,* 2010; Venkata Ramana Rao *et al.,* 2010; Padhar *et al.,* 2010). The variance component due to specific combining ability (σ^2 SCA) was greater than general combining ability (σ^2 GCA) for all the characters except for seed yield which has shown negative estimate. The ratio of variance due to GCA to that of SCA (σ^2 GCA/σ^2 SCA) was also less than unity indicating the preponderance of non-additive gene action for the characters except for seed yield. Similar findings were reported by Kavania *et al.* (2016), Ramesh *et al.* (2000), Kavani *et al.* (2001), Solanki *et al.* (2004) for effective spike length and Tank *et al.* (2003) and Thakker *et al.* (2005) for 100-seed weight.

The GCA effects of eight parents (4 lines and 4 testers) for nine characters revealed that none of the parents were good general combiners for all the characters studied (Table 2). Among the lines, IPC-15 (DPC-15) was a good general combiner for early flowering to primary and secondaries and short plant height as indicated by significant *gca* effects in negative direction for number of nodes to the primary, secondary 1, secondary 2 and plant height up to primary spike. It was also a good general combiner for number of effective spikes per plant. DPC-16 was a good general combiner for 100-seed weight and also short plant height as indicated by *gca* effects in negative direction.

Several studies on plant growth attributes in castor indicated that on an average every node takes four days to develop while the number of nodes to primary spike was an indicator of the duration of flower initiation of the primary spike (Shifriss, 1961; Moshkin, 1986; Lavanya and Gopinath, 2008). Sequential order of raceme development in castor was also utilized further to measure flower initiation of the secondary spikes, 1 and 2 by counting the number of nodes to secondary 1 and secondary 2 (Ramana *et al.,* 2005).

Two pistillate lines, IPC-19 and IPC-21 were good general combiners for tall plant height, total spike length and effective spike length of primary. In addition, IPC-19 was also identified as a good general combiner for late flowering as indicated by significant *gca* effects in positive direction for number of nodes to primary and secondaries. None of the female lines were good combiners for seed yield.

Among the male lines, DCS-107 was identified as a good general combiner for seed yield, total primary spike length, effective primary spike length and 100-seed weight. It was also a good general combiner for tall plant height as indicated by *gca* effects in positive direction. Another male line, RG-1526, was a good combiner for late flowering to primary but early flowering to secondary 1 and 2 as indicated by *gca* effects in positive and negative direction simultaneously. Among the testers, DCS-108 was the only male line with significant *gca* effects for number of effective spikes per plant while it was also a good combiner for early flowering and short plant height to primary as indicted by significant *gca* effects in negative direction.

A perusal of per se performance of 27 genotypes for nine characters (Table 3), indicated that, DCS-107, the only parent with good combining ability for seed yield, was also successful in generating hybrids with high *per se* performance ranging from 1346 kg/ha (DPC-19 x DCS-107) to 1800 kg/ha (DPC-16 x DCS-107). None of the crosses showed significant *sca* effects for seed yield and yield attributes (Table 4). The top five crosses for high seed yield along with the status of *gca* effects for female and male parents are presented in Table 5. Among them, four crosses involved medium x high combiners indicating the presence of non-additive gene effects. The present study confirmed the earlier reports on the role of at least one good combiner (Lavanya and Chandra Mohan, 2003; Lavanya *et al.,* 2006). The cross IPC-19 x DCS-81, based on medium x medium combiners indicated epistatic gene action for seed yield.

The top three heterotic hybrids *viz.,* DPC-16 x DCS-107 (39.72%, 1800.31 kg/ha), DPC-15 x DCS-107 (27.7%, 1646 kg/ha) and DPC-21 x DCS-107 (23.8%, 1595 kg/ha) with high mean seed yield per plot as the significant outcome of the study on combining ability. Among them, two hybrids, DCH-1720 (DPC-21 x DCS-107) and PHT-12-3 (DPC-15 x DCS-107) were tested in multi-location trials and proven for their stable heterosis in genotype x environment (G x E) interaction (Anonymous, 2014; 2016).

Table 1 Analysis of variance for combining ability of nine characters in castor

Source of variance	d.f.	Mean sum of squares								
		Number of nodes to			Plant height to primary	Total spike length (cm)	Effective spike length (cm)	Number of effective spikes per plant	100 seed weight (g)	Seed yield (kg/ha)
		Primary	Secondary1	Secondary 2						
Replications	1	0.72	0.196	0.7945	393.66*	73.54	85.3	36.51**	0.76	70970
Treatments	26	9.05**	1.3**	1.56**	1189.2**	328.0**	352.82**	14.05**	27.75**	177530
Parents	7	18.6**	2.25**	2.13**	1075**	531.75	592.7**	22.76**	49.71**	140690
Hybrids	15	3.75*	0.53*	0.83**	735**	206.5**	267.5**	8.42*	20.25**	102954
checks	2	19.33**	1.31*	1.95**	3454**	112.96*	78.6	8.06	8.72	360470
Lines (L)	3	9.01*	1.24**	2.32**	3007**	851.4**	1009.4*	31.47**	13.7	22586
Testers (T)	3	4.7*	0.888**	1.555**	397.4*	52.4	154.6	7.79*	67.3**	383994*
L x T	9	1.79	0.168	0.098	90.9	42.9	57.8	0.950	6.76	36063
Error	26	1.08	0.161	0.208	78.5	23.22	29.9	2.62	2.91	217686
σ^2 GCA		0.102	0.02	0.038	33.6	8.521	10.92	0.39	0.703	3484
σ^2 SCA		1.92	0.29	0.533	520.9	140.5	181.4	5.13	12.702	-37392*
σ^2 GCA & SCA		0.05	0.07	0.07	0.06	0.06	0.06	0.08	0.06	-0.09

* Significant at 5 per cent level; ** Significant at 1 per cent level

Table 2 Estimates of general combining ability (*gca*) effects of eight parents for nine characters in castor

GCA effects	Number of nodes to			Plant height to primary	Total spike length	Effective spike length	Number of effective spikes per plant	100 seed weight	Seed yield
	Primary	Secondary1	Secondary 2						
Lines									
DPC-15	-1.42**	-0.52**	-0.62**	-21.8**	-11.62**	-13.13**	2.38**	0.097	-26.7
DPC-16	-0.09	-0.08	-0.29	-9.37**	-5.77**	-5.57**	0.73	1.48*	70.8
DPC-19	1.08**	0.33*	0.39*	21.8**	8.73**	9.11**	-0.95	-1.7**	-51.8
DPC-21	0.43	0.28	0.51**	9.42**	8.76**	9.59**	-2.17**	0.122	7.7
S.E (*gca* for line)	0.37	0.142	0.16	3.13	1.71	1.94	0.573	0.61	165
S.E. (gi-gj) line	0.52	0.20	0.23	4.43	2.41	2.74	0.81	0.85	233.3
Testers									
DCS-81	-0.49	0.08	0.14	-2.22	-1.76	-2.54	-0.04	2.2**	-55.84
DCS-107	0.58	0.43**	0.52	7.15*	3.64*	5.66**	-1.27*	1.56**	320.97*
DCS-108	-0.77*	-0.155	-0.11	-8.82**	-0.06	1.15	1.13*	-4.22**	-95.78
RG-1526	0.68*	-0.35*	-0.54**	3.89	-1.82	-4.27*	0.18	0.46	-169.35
S.E. (*gca* for tester)	0.37	0.142	0.16	3.13	1.71	1.94	0.573	0.61	165
S.E. (gj-gj) tester	0.52	0.2	0.23	4.43	2.41	2.74	0.81	0.85	233.3

* Significant at 5 per cent level; * * Significant at 1 per cent level

A perusal of *per se* performance and *gca* effects of the female parents indicated that *per se* performance was also a good indicator of *gca* effects (Table 6). IPC-15 (DPC-15) with its low number of nodes to primary and secondaries (7.7, 6.1, 6.3) was a good general combiner for short plant height and early flowering to primary and secondaries. IPC-15, with its very short total and effective primary spike length (9 cm) was a poor combiner for the spike length while a good general combiner for number of effective spikes per plant with high *per se* performance (6.8 spikes per plant).

Among the late maturing pistillate lines, IPC-19 (DPC-19), with its high number of nodes to primary (16.5), secondary 1 (6.8), secondary 2 (7.3), tall plant height (73.5 cm), long total and effective primary spike length (62.5 cm) was also a good combiner for all the above six traits. IPC-21 (DPC-21) with its tall plant height (79.1 cm), long total and effective primary spike length (53.2 cm) was also a good combiner for all the three traits. The present study also identified the significant contribution of testers to the two major characters like seed yield and 100-seed weight while lines contributed a major share to all the remaining characters (Table 7).

The present study identified IPC-15 and DPC-16 as suitable pistillate lines for generation of early maturing hybrids while IPC-19 and IPC-21 for medium to late maturing hybrids with medium to tall plant height.

Table 3 Mean performance of 27 genotypes for nine characters in castor

Treatment	Number of nodes to			Plant height upto primary spike (cm)	Total spike length (cm)	Effective spike length (cm)	Number of effective spikes per plant	100-seed weight (g)	Seed yield (kg/ha)
	Primary	Secondary 1	Secondary 2						
DPC-15 x DCS-81	10.5	5.7	6.6	40.5	28.6	23.35	12.7	25.55	1205.43
DPC-15 x DCS-107	12.5	6.2	6.7	45.1	41.2	41.2	10.9	24.85	1645.56
DPC-15 x DCS-108	12.3	5.6	6.3	41.5	37.8	37.8	12.8	22.2	1117.5
DPC-15 x RG-1526	11.4	4.9	5.4	43.55	31.48	21.73	13.5	24.4	1027.38
DPC-16 x DCS-81	13.5	6.2	6.7	61.4	44.1	42	10.7	30.05	1126.36
DPC-16 x DCS-107	12.7	6.3	6.9	54.2	45.88	45	9.3	24.75	1800.31
DPC-16 x DCS-108	11.1	5.48	6.3	47.3	39.3	35.4	12.8	21.6	1196.48
DPC-16 x RG-1526	14.7	6.2	6.4	57.6	33.2	31.9	10.5	26.15	1262.56
DPC-19 x DCS-81	13.4	6.6	7.2	77.9	53.9	51.3	9.5	22.85	1368.3
DPC-19 x DCS-107	15.4	6.9	7.9	100.4	52.5	52.5	7.9	25.95	1345.8
DPC-19 x DCS-108	13.2	6.4	7.2	72.8	57.25	57.05	9.7	17.55	1108.33
DPC-19 x RG-1526	14.7	5.9	6.7	94	56.8	52.2	9.5	23.45	1072.72
DPC-21 x DCS-81	13	6.3	7.5	69.3	51.9	49.8	7.3	26.95	1179.04
DPC-21 x DCS-107	14.1	6.8	8	86.9	60.53	60.53	7.2	27.3	1594.72
DPC-21 x DCS-108	12.7	6.4	7.2	61.1	50.93	50.93	9.6	18.4	1297.04
DPC-21 x RG-1526	14.3	6.1	6.8	78.38	56.8	53.7	7.6	24.45	1062.41
DPC-15	7.7	6.1	6.3	16.4	9	9	6.8	21.65	1394.6
DPC-16	14.5	6.4	7.3	58.1	35.25	35.25	7.7	23.4	947.01
DPC-19	16.5	6.8	7.25	73.5	62.45	62.45	4.8	27	825.4
DPC-21	16	8.7	9.05	79.1	53.17	53.17	4.3	19.65	787.19
DCS-81	12.5	5.8	6.8	66.73	28.7	21.3	12.5	29.4	1499.14
DCS-107	16.2	8.3	9.2	85.2	44	43.1	5.7	26.4	1122.28
DCS-108	11.8	6.2	7.18	53.9	30.1	29.2	10.4	13.9	1030.62
RG-1526	15.8	6.6	7.3	88.4	36.3	29.6	12.8	26.35	858.33
DCH-177	11.83	6.8	7.5	65.65	45.55	39.45	6.5	29.3	604.94
DCH-519	13	6.2	7.3	80.5	57.2	41	9.7	25.5	510.56
GCH-7	17.7	7.8	9.1	143.9	59.6	51	6	25.9	1288.52
General Mean	13.45	6.43	7.19	68.27	44.57	41.51	9.21	24.26	1158.46
CV(%)	7.72	6.23	6.35	12.97	10.81	13.18	17.58	7.03	40.27
SE (d)	1.038	0.401	0.456	8.858	4.818	5.473	1.619	1.705	466.583
LSD at 1%	2.8835	1.1136	1.2683	24.614	13.389	15.207	4.4995	4.7374	NS

Table 4 Estimates of specific combining ability (*sca*) effects of 16 hybrids for nine characters in castor

SCA effects	Number of nodes to			Plant height upto primary spike	Spike length		Number of effective spikes per plant	100-seed weight	Seed Yield
	Primary	Secondary1	Secondary2		Total	Effective			
DPC-15 x DCS-81	-0.68	0.02	0.21	0.06			0.27	-0.89	12.31
DPC-15 x DCS-107	0.24	0.17	-0.06	-4.72	2.79	4.52	-0.31	-0.96	75.61
DPC-15 x DCS-108	1.394*	0.155	0.163	7.66	3.095	5.63	-0.81	2.17	-35.68
DPC-15 x RG-1526	-0.96	-0.352	-0.313	-2.998	-1.48	-5.03	0.84	-0.31	-52.24
DPC-16 x DCS-81	0.99	0.08	-0.013	8.56	5.24	5.96	-0.08	2.22	-164.23
DPC-16 x DCS-107	-0.88	-0.17	-0.19	-8.1	1.62	0.77	-0.26	-2.45*	132.91
DPC-16 x DCS-108	-1.13	-0.41	-0.16	0.995	-1.26	-4.322	0.84	0.18	-54.17
DPC-16 x RG-1526	1.02	0.51*	0.363	-1.411	-5.61	-2.41	-0.51	0.05	85.49
DPC-19 x DCS-81	-0.28	0.07	-0.19	-6.16	0.55	0.57	0.39	-1.78	200.35
DPC-19 x DCS-107	0.64	0.02	0.14	6.97	-6.25	-6.42	0.02	1.94	-198.96
DPC-19 x DCS-108	-0.21	0.11	0.06	-4.65	2.2	2.64	-0.58	-0.68	-19.67
DPC-19 x RG-1526	-0.16	-0.2	-0.013	3.84	3.5	3.21	0.17	0.54	18.28
DPC-21 x DCS-81	-0.03	-0.18	-0.013	-2.398	-1.38	-1.4	-0.58	0.48	-48.42
DPC-21 x DCS-107	-0.01	-0.03	0.113	5.83	1.85	1.13	0.54	1.47	-9.56
DPC-21 x DCS-108	-0.16	0.16	-0.063	-3.998	-4.04	-3.95	0.54	-1.66	109.52
DPC-21 x RG-1526	0.14	0.05	-0.04	0.57	3.58	4.23	-0.51	-0.29	-51.54
S.E. (*sca* effect)	0.73	0.28	0.323	6.26	3.41	3.87	1.145	1.21	329.9
S.E. (sij-skl) tester	1.04	0.401	0.46	8.86	4.82	5.47	1.62	1.71	466.6

* Significant at 5 per cent level

Table 5 Top five ranking crosses for seed yield per plot and the *gca* status of parents in castor

Cross	*Per se* performance (kg/ha)	gca status	
		Female	Male
DPC-16 x DCS-107	1800.31	M	H
DPC-15 x DCS-107	1645.56	M	H
DPC-21 x DCS-107	1594.72	M	H
DPC-19 x DCS-81	1368.3	M	M
DPC-19 x DCS-107	1345.8	M	H

M-Medium, H-High *gca* effects

Table 6 *Per se* performance and estimates of *gca* effects of eight parents in castor

	Number of nodes to						Plant height up to primary (cm)		Primary spike length (cm)				Number of effective spikes per plant		100-seed weight (g)		Seed yield (kg/ha)	
	Primary		Secondary1		Secondary2				Total		Effective							
	Mean	*gca*	Mean	*gca*	Mean	*gca*	Mean	*gca*	Mean	*gca*	Mean	*gca*	Mean	*gca*	Mean	*gca*	Mean	*gca*
DPC-15	7.7	L	6.1	L	6.3	L	16.4	L	9	H	9	H	6.8	H	21.65	M	1394.6	M
DPC-16	14.5	M	6.4	M	7.3	M	58.1	L	35.25	L	35.25	L	7.7	M	23.4	H	947.01	M
DPC-19	16.5	H	6.8	H	7.25	H	73.5	H	62.45	H	62.45	H	4.8	M	27	L	825.4	M
DPC-21	16	M	8.7	M	9.05	H	79.1	H	53.17	H	53.17	H	4.3	L	19.65	M	787.19	M
DCS-81	12.5	M	5.8	H	6.8	M	66.73	M	28.7	M	21.3	M	12.5	M	29.4	H	1499.14	M
DCS-107	16.2	M	8.3	M	9.2	M	85.2	H	44	H	43.1	H	5.7	L	26.4	H	1122.28	H
DCS-108	11.8	L	6.2	LM	7.18	M	53.9	L	30.1	M	29.2	M	10.4	H	13.9	L	1030.62	M
RG-1526	15.8	H	6.6	L	7.3	L	88.4	M	36.3	M	29.6	L	12.8	M	26.35	M	858.33	M

L-Low, M-medium, H-High *gca* effects

Table 7 Proportional Contribution of lines and testers in a 4 x 4 line x tester analysis of castor

	Number of nodes to			Plant height up to primary	Primary spike length (cm)		Number of effective spikes per plant	100-seed weight	Seed yield
	Primary	Secondary 1	Secondary 2		Total	Effective			
Lines	48.1	47.03	55.65	81.77	82.5	75.5	74.72	13.51	4.39
Testers	23.3	33.81	37.3	10.81	5.08	11.6	18.52	66.5	74.59
Line x Tester	28.6	19.2	7.07	7.42	12.46	12.96	6.77	20.02	21.02

REFERENCES

Barad Y M, Pathak A R and Patel B N 2009. Heterosis for yield attributing traits in castor (*Ricinus communis* L.). *Journal of Oilseeds Research*, **26**(2): 102-103.

Chandramohan Y, Vishnuvardhan Reddy A and Nageshwar Rao T 2006. Combining ability in castor (*Ricinus communis* L.). *Journal of Oilseeds Research*, **23**(2): 178-183.

Cochran W G and Cox G M 1957. *Experimental Designs.* John Willey & Sons, New York, pp. 107-108.

Kavani R H, Golakia P R and Dhadu K H L 2001. Combining ability analysis in castor (*Ricinus communis* L.). *Journal of Oilseeds Research*, **18**(1): 24-27.

Kavani R H, Vacchani J H, Madariya R B, Jadeja S R and Dobariya K L 2016. Combining ability for seed yield and its components in castor (*Ricinus communis* L.,). *Journal of Oilseeds Research*, **33**(1): 22-25.

Kavani R H, Padhar P R, Chovatia V P, Patel M B and Dobaria K L 2010. Heterosis and combining ability analysis for seed yield and its components in castor (*Ricinus communis* L.). *Journal of Oilseeds Research*, **27**(Special issue) : 105-107.

Kempthorne O 1957. *An Introduction to Genetic Statistics.* John Wiley and Sons Inc., New York.

Kulkarni L G and Ramanamurthy G V 1977. *Castor.* Indian Council of Agricultural Research, New Delhi,105 p.

Lavanya C 2002. Sensitivity and variations of sex expression in response to environmental changes in castor. *Indian Journal of Plant Breeding*, **62**(3): 232-237.

Lavanya C and Chandramohan Y 2003. Combining ability and heterosis for seed yield and yield components in castor (*Ricinus communis* L.). *Journal of Oilseeds Research*, **20**(2): 220-224.

Lavanya C and Gopinath V 2008. Inheritance studies for morphological characters and sex expression in pistillate lines of castor (*Ricinus communis* L.). *Indian Journal of Genetics and Plant Breeding*, **68**(3): 275-282.

Lavanya C, Ramanarao P V and Venkatagopinath V 2006. Studies on combining ability and heterosis for seed yield and yield components in castor, *Ricinus communis* L. hybrids. *Journal of Oilseeds Research*, **23**(2): 174-177.

Madariya R B, Vadderia M A, Mehta D R and Kavani R H 2008. Combining ability analysis over environments for seed yield

and its components in castor (*Ricinus communis* L.). *Crop Improvement*, **35**(2): 163-166.

Moshkin V A 1986. *Castor*. Oxonian Press Pvt. Ltd., New Delhi.

Padhar P R, Chovatia V P, Barad V G, Patel M B and Dobariya K L 2010. Combining ability by line x tester analysis in castor (*Ricinus communis* L.). *Journal of Oilseeds Research*, **27**(Special issue): 44-46.

Patel D K, Patel C J and Patel P J 2010. Study of combining ability for yield and its components in castor (*Ricinus communis* L.) through line x tester analysis. *Journal of Oilseeds Research*, **27**(Special issue): 51-53.

Ramesh T, Venkateswarlu O, Durgaprasad M M K and Sankaraiah M 2000. Estimation of genetic parameters in castor. *Journal of Oilseeds Research*, **17**(2): 234-238.

Shiffriss O 1960. Conventional and unconventional systems controlling sex variations in *Ricinus*. *Journal of Genetics*, **57**: 573-578.

Solanki S S, Deora V S and Singh D P 2004. Combining ability of new castor (*Ricinus communis* L.). *Journal of Oilseeds Research*, **21**(2): 274-276.

Tank C J, Jaimini S N and Ravindra Babu Y 2003. Combining ability analysis over environments in castor (*Ricinus communis* L.). *Crop Research*, **26**(1): 119-125.

Thakkar D A, Jadon B S, Patel K M and Patel C J 2005. Heterosis over environments for seed yield and other attributes in castor, *Ricinus communis* L. *Journal of Oilseeds Research*, **22**(2): 324-326.

Venkata Ramana Rao P, Gouri Shankar V and Vishnuvardhan Reddy A 2010. Combining ability analysis in castor (*Ricinus communis* L.). *Journal of Oilseeds Research*, **27**(Special issue): 102-104.

Venkataramana P, Lavanya C and Ratnasree P 2005. Combining ability and heterosis studies under rainfed conditions in castor (*Ricinus communis* L.). *Indian Journal of Genetics and Plant Breeding*, **65**(4): 325-326.

Zimmerman L M and Smith J D 1966. Production of F_1 seed in castor beans by use of sex genes sensitive to environment. *Crop Sciences*, **6**: 406-409.

Correlations among seed traits: implications for breeding high oil yield in safflower (*Carthamus tinctorius* L.)

K SAISANTHOSH[1,2], T JOSEPH RAJU[1,2], P KADIRVEL[2*], K KESHAVALU[1], RAZIA SULTANA[1], PRADUMAN YADAV[2] AND N MUKTA[2]

[1]College of Agriculture, Prof. Jayashankar Telangana State Agril. University, Hyderabad-500 030, Telangana

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ABSTRACT

Seed traits are critical determinants of oil yield and quality in safflower. In this study, correlations among a set of seed traits (physical, physiological, biochemical) were studied and compared in a subset of germplasm and a pedigree based population (F_3). Some previously published correlations among physical (seed size, weight, hull content, hull type) and biochemical (oil content, fatty acid composition) traits in germplasm could be redetected in F_3 population and some disappeared. In germplasm, physiological traits (germination, vigour) did not show correlation with oil content while vigour was positively correlated with seed size and test weight. Contrary to observations in germplasm, seed weight was not correlated with hull content and oil content in F_3 population. This is an interesting observation because it raises the possibility of improving seed weight and oil content simultaneously and without affecting the hull proportion, which is critical for breeding high oil yield in safflower.

Keywords: *Carthamus tinctorious,* Genetic improvement, Oilseed crop, Trait relationships

Safflower (*Carthamus tinctorius* L.) crop is a source of healthy edible oil with more than 90% unsaturated fatty acids (Li and Mündel, 1996). India, Kazakhstan, Mexico, Russian Federation and USA are major safflower growing countries with total area of about 0.94 million ha and production of about 0.73 million tonnes (FAOSTAT, 2014). It has the potential to contribute for edible oil requirement in the developing countries. For instance, India has a large deficit of edible oils (~50%) to meet the domestic demand. This can be addressed by promoting crops like safflower, which is drought tolerant and well adapted to resource poor dry land environments. Development of cultivars with high oil yield coupled with high quality is the top priority in safflower breeding research. Genetic enhancement of safflower for oil yield and quality requires simultaneous improvement in seed yield, oil content and unsaturated fatty acids (oleic and linoleic acid). Though the fatty acid content can be easily manipulated in breeding programmes, it is a great challenge to combine seed yield and oil content to achieve desirable oil yield due to genetic complexities (Rao *et al.*, 1977; Golkar *et al.*, 2011). Therefore, knowledge on relationships among various seed yield related traits and seed traits per se are crucial to identify a suitable combination of traits and strategies for breeding cultivars with high oil yield potential in safflower.

Only a few studies have explored the relationships among seed yield components and oil content in safflower. Capsule number, capsule weight and hull content were observed to be some of the most critical determinants of seed yield and oil content in safflower (Rao *et al.*, 1977). The hull content (proportion of hull expressed as per cent of the whole seed) had negative relationship with oil content. Historically, hull mutants (striped, thin and partial) were known to be positively correlated with oil content and have been exploited for breeding commercial safflower cultivars (Mündel and Bergman, 2009). To date, no study has been conducted to relate physiological traits such as germination, vigour etc. with oil content in safflower. Snider *et al.* (2014) observed that seed size and oil content determine seedling vigour in cotton. Understanding such trait relationships would help breeders in selecting plants with high oil content without compromising other desirable agronomic attributes, which is critical for achieving high oil yield. Keeping this view, main objective of this study was to analyze correlations among a set of seed traits related to oil yield and quality in safflower in a sub set of germplasm accessions and F_3 families produced from a cross between two diverse genotypes.

[2]ICAR-Indian Institute of Oilseeds Research, Hyderabad-500030, India
*Correspondence: E-mail: kadirvel.palchamy@icar.gov.in

MATERIALS AND METHODS

Plant material: A set of 61 safflower germplasm accessions and a set of 90 F_3 families produced from the cross between a high yielding Indian variety A-1 (with 26% oil content) and a germplasm accession EC-755673-1 (with 40% oil content) from Mexico were used in this study. The germplasm set included five Indian safflower varieties (A-1, Bhima, NARI-57, HUS-305, CO-1), the variety Centennial from USA, 36 exotic accessions from Mexico and USA and 19 Indian accessions. A-1, Bhima, NARI-57 and Centennial were used as checks. The germplasm set was selected on the basis of fair representation of variability for seed traits. The

genotypes were maintained at ICAR-Indian Institute of Oilseeds Research (ICAR-IIOR), Hyderabad, India.

Trait measurements: Field trial for the germplasm set was laid out in Augmented Randomized Block Design (Augmented RCB) in three blocks. Each block consisted of 19 genotypes with checks. Spacing of 45 cm x 20 cm and row length of 5 m were adopted. Bulked seeds from 25 plants per genotype were used for data collection. In case of F_3 family evaluation, a set of 90 F_2 plants was grown and seeds (F_3) were harvested from each plant for data collection. The trial was conducted during October 2014 to February 2015 at the research farm of IIOR, Hyderabad (17.37°N, 78.48°E). A range of seed traits (physical, physiological and biochemical) were measured (at moisture content of about 7%) using the procedures described below.

Physical traits - Seed size was determined in terms of length x breadth ratio (LBR) and length x breadth product (LBP) based on seed length, width and thickness data from 10 randomly selected seeds (Alexander *et al.,* 2001). Test weight (TW) (g) was determined by weighing 100 randomly selected seeds. Bulk density (BD) (gm^{-3}) was determined by filling a 1000 ml container with seeds from a height of about 15 cm, striking the top level and then weighing the content (Deshpande, 1993). Hull content (HC) (%) was calculated as the ratio of the seed hull to the total seed using a random sample of 100 seeds as per the seed soaking procedure (Rao *et al.,* 1977). Hull type (HT)-normal (0) or striped (1) was recorded as per safflower descriptors (IBPGR 1983). All measurements were repeated three times.

Physiological traits - Standard germination (%) was determined by 'between paper' method (bp) as described by International Seed Testing Association (ISTA) rules (ISTA, 1999). The first and final germination counts were recorded on 4^{th} and 14^{th} day of germination test, respectively. Subsequently, root length (cm), shoot length (cm) and seedling dry weight (mg) were determined using 10 normal seedlings on 14^{th} day of germination test. Based on the above parameters, the seedling vigour indices [Seedling vigour index I (SVI-I) = Germination (%) x Seedling length (cm) and Seedling vigour index II (SVI-II) = Germination (%) x Seedling dry weight] (Abdul-Baki and Anderson, 1973) were determined and expressed as whole number. Speed of germination (SG) was determined based on daily counts of germination until no further germination was observed (up to 14 days in this case). Index of the speed of germination was calculated by adding the quotients of the daily counts divided by the number of days of germination (Maguire, 1962). Field emergence (%) was determined by sowing 100 seeds on a well prepared seed bed (with 4 cm depth and spacing of a spacing of 45 cm between rows and 15 cm between the plants) at adequate moisture conditions. Number of seedlings of at least 4 cm high, which emerged above the soil surface on 14^{th} day after sowing were counted and expressed in percentage. Four replicates (100 seeds each) were maintained for each genotype for all the physiological trait measurements.

Biochemical traits - Oil content (%) was measured on whole seeds (~ 20 g of sample) using Nuclear Magnetic Resonance Spectroscopy (NMR-MQC-5 Analyzer, Oxford, London) as described by Yadav and Murthy (2016). Fatty acid composition (%) was determined using an Agilent 7860A gas chromatograph equipped with a flame ionization detector (FID) (Kadirvel *et al.,* 2017).

Data analysis: Data were analyzed as per Augmented RCB Design as implemented in the software, Plant Breeding Tools (PBTools v 1.3) (IRRI, 2013). Least square means and range were obtained. Significance of genotypic effect on trait variation was tested by analysis of variance (ANOVA). Frequency distribution graphs of traits were drawn by the software, MYSTAT V.12 (https://systatsoftware.com). Simple correlation analysis based on Pearson correlation coefficients (*r*) was performed using 'Data Analysis' option implemented in MS Excel.

RESULTS AND DISCUSSION

Range of variability for physical and biochemical traits in germplasm (data not shown) were comparable with the previous reports in safflower (Johnson *et al.,* 1999; Erica *et al.,* 2004; Usha Kiran *et al.,* 2017). Frequency distribution of traits namely seed size, hull content, test weight, bulk density, physiological traits, oil content, palmitic acid, stearic acid content showed quantitative nature of variation. Hull type (normal/striped), oleic acid content and linoleic acid content were qualitative with distinct variation (Fig.1A), which has been well documented in safflower germplasm (Johnson *et al.,* 1999; Mündel and Bergman, 2009). To our knowledge, this is one of the very few reports in safflower germplasm on the variability for seed physiological traits. The F_3 population was studied only for the seed physical and biochemical traits, which varied significantly in the germplasm set (data not shown). The F_3 population showed typical quantitative variation for seed size, hull content, test weight and oil content whereas the oleic acid and linoleic acid contents were qualitative (Fig. 1B). Similar findings have been reported in safflower using segregating populations (Yermanos *et al.,* 1967; Hamdan *et al.,* 2012). Over all, both germplasm set and F_3 population fairly represented the variability for the concerned seed traits, which is critical for the study.

Simple correlation coefficients among traits estimated in the germplasm set and F_3 population are presented in Table 1 and 2, respectively. Overall, the study revealed some important correlations among traits contributing for oil yield potential and quality in safflower, which were consistent in both germplasm set and a pedigree based population (F_3

families). Those correlations included: positive correlation between seed size and test weight, negative correlation between hull content and oil content, positive correlation between striped hull and oil content and negative correlation between oleic acid content and linoleic acid content. However, some correlations namely seed size with striped hull, hull content with striped hull, test weight with oil content and oil content with fatty acid composition remained inconsistent i.e. found in germplasm set and missing in F_3 population, which require more investigation. Nevertheless, an interesting observation was that seed weight did not correlate with hull content and oil content in F_3 population, which was hitherto found correlated in germplasm in earlier studies. In germplasm, physiological traits did not correlate with oil content. However, vigour was found to be correlated with seed size and weight.

Plant breeders use correlation coefficients to understand genetic relationships among quantitative traits and to guide them in progeny selections with desirable combination of traits. However, correlations among traits are subjective and affected by various statistical and biological factors. For instance, the strength of Pearson correlation is likely to be affected by amount of variability in the data, presence of outliers, characteristics of the sample and measurement error etc. The value of correlation will be greater if there is more variability among the observations than if there is less variability when other things being equal (Goodwin and Leech, 2006). Toebe *et al.* (2015) demonstrated that larger sample size was needed to estimate the correlation coefficient between weakly correlated traits while smaller sample size was enough to estimate the correlation coefficient between highly correlated traits. Based on this perspective, our hypotheses were that (1) a sample population with skewed representation of trait variability in germpalsm materials could lead to misleading correlations, which would affect breeding plans and (2) this could probably be circumvented by verifying the correlations (found in germplasm) further in a pedigree based population produced by crossing two diverse unrelated genotypes.

In this study, some of the previously published correlations in germplasm could be redetected in the F_3 population; hence, they were consistently found in both the cases. Hull percent was negatively correlated with oil content in safflower (Rao *et al.,* 1977; Rudolphi *et al.,* 2012; Rahim *et al.,* 2014). Striped hull type was reported to be associated with high oil content and has been exploited for development of high oil (>40%) safflower cultivars (Mündel and Bergman, 2009). Negative association between oleic acid and linoleic acid content has been well documented in safflower.

It was observed that some trait correlations in the present study were inconsistent with the previous reports involving germplasm. Rao *et al.* (1977) observed that hull percent and seed size (g) (seed weight) were strongly correlated but in this study it was found that both traits were weakly correlated in germplasm and were not correlated in F_3 population. As observed by Rao *et al.* (1977) seed size (g) (seed weight) was negatively correlated with oil content in germplasm but did not show correlation in F_3 population. Stearic acid content showed negative correlation with oleic acid but was not correlated in F_3 population and vice versa with linoleic acid content. These results supported our view that correlations in germplasm could be 'weaker or incidental' if variability is poorly represented while the pedigree based population could reveal stronger correlations. However, it is important to note that the correlations in F_3 populations need to be considered cautiously because the population would be segregating which could affect the results. In a similar study, Meru and McGregor (2013) reported negative correlations between seed size and seed/kernel oil percentage in watermelon using F_3 population. A correlation study using advanced generation populations, for example, recombinant inbred lines would be more desirable.

To our knowledge, no relationship of physiological traits (germination, speed of germination, vigour) with the oil content of the freshly harvested seeds found in this study was the maiden observation in safflower germplasm. However, vigour was found to be positively correlated with seed size and weight. Similarly, it has been reported that larger seeds had better germination (Mirshekarnezhad *et al.,* 2013) and rate of germination and field emergence were correlated with higher seed yield (Soleymani, 2017) in safflower.

No correlation of seed weight with hull content and oil content would be interesting for breeding purposes because that raises the possibility of improving seed weight and oil content simultaneously and without affecting the hull proportion. In soybean, Masetri *et al.* (1998) reported that seed size (g/100 seeds) was not correlated with oil content but showed association with individual fatty acids: stearic, oleic and linoleic acid content using a set of 18 genotypes. In this study, we observed that oil content was negatively correlated with linoleic acid content while it was positively correlated with oleic acid content. In contrast, Rudophi *et al.* (2012) reported that oil content was positively correlated with linoleic acid content and negatively correlated with oleic acid content. The inconsistency of correlations between oil content and fatty acid composition, particularly in safflower, could be attributed to the following issues: (1) use of a small number of genotypes for correlation study and (2) use of only low oil and high linoleic type genotypes. When genotypes with high oil and high oleic acid content were included in this study, positive correlation between oleic acid content and oil content appeared. Therefore, inclusion of genotypes, which are incidentally low oil/high linoleic acid content and high oil/high oleic acid content could perhaps have contributed for the differences in the correlations.

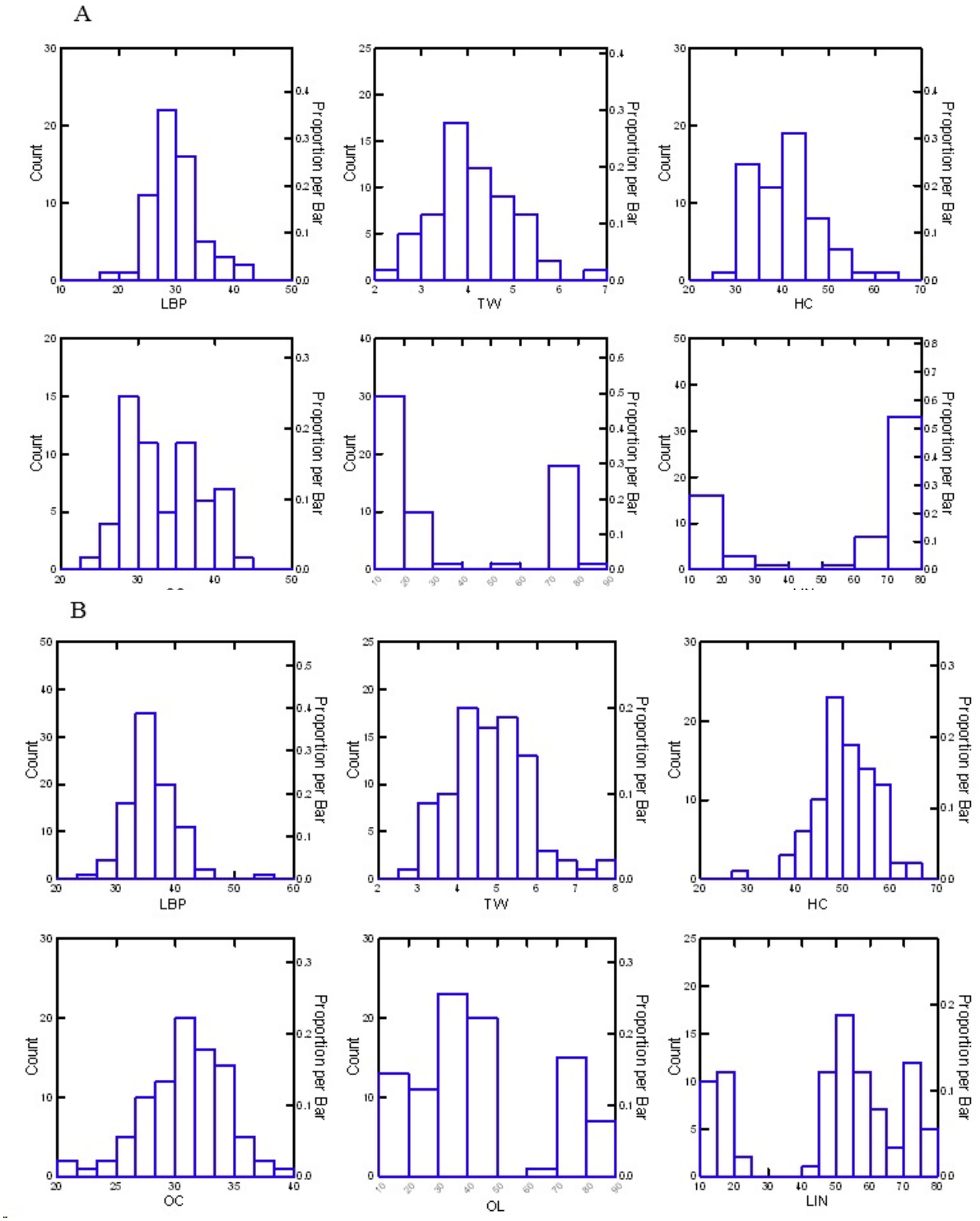

Fig. 1. Frequency distribution of seed traits in a germplasm set (A) and a F_3 population (B) of safflower. LBP-Length x breadth Product, TW-Test weight (g), HC-Hull content (%), OC-Oil content (%), OL-Oleic acid content (%), LIN-Linoleic acid content (%)

Table 1 Simple correlation among seed morphological, physiological and biochemical traits in safflower germplasm

Traits	LBR	LBP	HC	TW	BD	HT	SG	SVI-2	PM	ST	OL	Lin
LBP	-0.187											
HC	-0.091	0.259										
TW	-0.288	0.656**	0.390*									
BD	-0.318	0.159	0.117	0.605**								
HT	0.389*	-0.073	-0.465**	-0.317	-0.321							
SG	0.472**	-0.253	-0.163	-0.380*	-0.262	0.059						
SVI-2	-0.052	0.472**	-0.037	0.504**	0.149	-0.175	-0.294					
PM	-0.181	-0.051	-0.014	0.022	0.064	-0.005	-0.159	-0.047				
ST	-0.029	0.010	0.012	0.105	0.163	0.014	-0.112	0.076	0.632**			
OL	0.364*	-0.182	-0.176	-0.364*	-0.256	0.182	0.378*	-0.099	-0.785**	-0.609**		
Lin	-0.369*	0.189	0.181	0.372*	0.259	0.186	-0.383*	0.103	0.772**	0.594**	-0.999**	
OC	0.239	-0.300	-0.754**	-0.509**	-0.221	0.458**	0.341*	-0.003	-0.132	-0.186	0.449**	-0.455**

Correlation coefficients at 0.1 % and 1 % level of significance are 0.414 (**) and 0.330 (*), respectively for n=61. LBR-Length breadth ratio, LBP-length breadth product, HC-Hull content (%), TW-Test weight (g), BD-Bulk density (tm^{-3}), HT-Hull type (striped/normal), SG-Speed of germination, SVI-2-Seedling vigour index-2, PM-Palmitic acid (%), ST-Stearic acid (%), OL-Oleic acid (%), LIN-Linoleic acid (%)

Table 2 Simple correlation among seed morphological and biochemical traits in F_3 families of the cross: A-1 x EC-755673-1 in safflower

Traits	HT	LBP	LBR	TW	HC	OC	PM	ST	OL
LBP	-0.272*								
LBR	0.311*	-0.330*							
TW	-0.148	0.604**	-0.257*						
HC	-0.092	0.155	0.048	0.008					
OC	0.373**	-0.204	-0.026	0.195	-0.364**				
PM	-0.049	-0.009	-0.057	-0.187	0.090	-0.249*			
ST	-0.063	0.015	-0.113	-0.042	0.114	-0.191	0.276**		
OL	-0.032	-0.119	0.082	0.085	-0.156	0.235*	-0.803**	-0.212*	
LIN	0.036	0.123	-0.079	-0.080	0.155	-0.229*	0.787**	0.179	-0.999**

Critical values of correlation coefficients at 0.1% and 1% level of significance are 0.347 (**) and 0.275 (*), respectively for n=90. HT - Hull type, LBP - Length breadth product, LBR - Length breadth ratio, TW - Test weight (g), HC - Hull content (%), OC - Oil content (%), PM - Palmitic acid (%), ST - Stearic acid (%), OL - Oleic acid (%), LIN -Linoleic acid (%)

Interestingly, correlation analysis in F_3 population did not strongly support the relationship of fatty acid composition with the oil content suggesting that they are not related; therefore, improvement of either linoleic or oleic acid content may not affect oil content in safflower. Similar observations on the relationships between oil content and fatty acid composition have been reported in safflower by other researchers (Liu *et al.,* 2016).

Correlations are important for plant breeders to make selection decisions. Determining linear correlations among traits may not be sufficient and the direction of their effects would be critical as well to indicate selection criteria in breeding programmes. The correlations among traits may occur due to linkage or pleiotrophy. Therefore, it is essential that biological basis of such correlations need to be understood. For instance, the genetic/molecular basis of negative correlation between oleic acid and linoleic acid content in safflower has been explained in detail. During fatty acid biosynthesis, oleic acid is converted to linoleic acid by fatty acid desaturase enzyme (*FAD2-1*) coded by *fad2-1* gene. High oleic mutants carry a single base mutation in *fad2-1* gene that affects *FAD2-1* activity, which eventually leads to high oleic level (Liu *et al.,* 2013). Therefore, breeding of high oleic safflower cultivars will inevitably be having low linoleic acid content. With advancements in genetics and genomics research, it has become possible to explain such trait relations at molecular level using either germplasm or pedigree based populations (Chen and Lübberstedt, 2010).

In conclusion, it was observed that trait correlations varied between germplasm set and a pedigree based F_3 population. Some trait correlations remained consistent in both germplasm set and F_3 population but some disappeared. Importantly, seed weight was not correlated with hull content and oil content in a pedigree based population (F_3), which was hitherto known to be correlated in germplasm in earlier studies. This is an interesting observation because it raises the possibility of improving seed weight and oil content simultaneously and without affecting the hull proportion, which is critical for breeding high oil yield in safflower.

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REFERENCES

Abdul-Baki A A and Anderson J D 1973. Vigour determination in soybean by multiple criteria. *Crop Science,* **13**: 630-633.

Alexander H M, Cummings C L, Kahn L and Snow A A 2001. Seed size variation and predation of seeds produced by wild and crop-wild sunflowers. *American Journal of Botany*, **88**(4): 623-627.

Chen Y and Lübberstedt T 2010. Molecular basis of trait correlations. *Trends in Plant Science*, **15**: 454-461.

Deshpande S D, Bal S and Ojha T P 1993. Physical properties of soybean. *Journal of Agricultural Engineering Research*, **56**: 89-98.

Erica B, Cuniberti A and Susana M 2004. Moisture dependent physical and compression properties of safflower seed. *Journal of Food Engineering*, **2**: 134-140.

FAOSTAT 2014. http://faostat.fao.org

Fernández-Martinez J, del Rio M and de Haro A 1993. Survey of safflower (*Carthamus tinctorius* L.) germplasm for variants in fatty acid composition and other seed characters. *Euphytica*, **69**: 115-122.

Golkar P, Arzani A and Rezaei A M 2011. Genetic analysis of oil content and fatty acid composition in safflower (*Carthamus tinctorious* L.). *Journal of the American Oil Chemists Society*, **88**: 975-982.

Goodwin L D and Leech N 2006. Understanding correlation: factors that affect the size of r. *The Journal of Experimental Education*, **74**(3): 251-266.

Hamdan Y A S, Garciamoreno M J, Fernandez-Martinez J M, Velasco L and Perezvich B P 2012. Mapping of major and modifying genes for high oleic acid content in safflower. *Molecular Breeding*, **30**: 1279-1293.

IBPGR 1983. *Safflower Descriptors.* Rome: International Board for Plant Genetic Resources (IBPGR), p. 22.

IRRI 2013. *Plant Breeding Tools (PBTools) Version 1.3.* Manila: International Rice Research Institute.

ISTA 1999. *International Rules for Seed Testing.* Seed Science and Technology, 27: Supplement, 333 pp.

Johnson R C, Bergman J W and Flynn C R 1999. Oil and meal characteristics of core and non-core safflower accessions from the USDA collection. *Genetic Resources and Crop Evolution*, **46**: 611-618.

Kadirvel P, Ravi D, Mukta N, Montoya-Coronado M C L, Ghuge S B, Singh J, Singh V, Shinde S K, Deshmukh S N, Yadav P and Varaprasad K S 2017. Genetic distinctiveness of safflower cultivars of India and Mexico as revealed by SSR markers. *Plant Genetic Resources*, **15**: 474-487.

Li D and Mündel H H 1996. Safflower. *Carthamus tinctorius* L. Promoting the conservation and use of underutilized and neglected crops. 7. Rome: Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute.

Liu L, Guan L L, Wu W and Wang L 2016. A review of fatty acids and genetic characterization of safflower (*Carthamus tinctorius* L.) seed oil. *Organic Chemistry Current Research*, **5**: 1.

Liu Q, Cao S, Zhou X R, Wood C, Green A and Singh S 2013. Nonsense-mediated mRNA degradation of CtFAD2-1 and development of a perfect molecular marker for olol mutation in high oleic safflower (*Carthamus tinctorius* L.). *Theoretical and Applied Genetics*, **126**: 2219-2231.

Maestri D M, Labuckas D O, Guzman G A and Giorda L M 1998. Correlation between seed size, protein and oil contents, and fatty acid composition in soybean genotypes. *Grasas y Aceites*, **49**: 450-453.

Maguire J D 1962. Speed of germination aid in selection and evaluation for seedling emergence and vigour. *Crop Science*, **2**: 176-177.

Meru G and McGregor C 2013. Genetic mapping of seed traits correlated with seed oil percentage in watermelon. *Hortscience*, **48**(8): 955-959.

Mirshekarnezhad B, Abbas Akbari G, Sadeghi H 2013. Affiliation of seed size with germination aspects and morphological traits in safflower (*Carthamus tinctorius* L.). *Journal of Cereals and Oilseeds*, **4**(5): 58-64.

Mündel H H and Bergman J W 2009. *Safflower*. In: Vollmann J and Rajcan I (eds.). Oil Crops, Handbook of Plant Breeding 4, DOI 10.1007/978-0-387-77594-4_14, Springer Science+Business Media, LLC

MYSTAT V.12 available at https://systatsoftware.com

Rahim A D A 2014. Dimension, geometric, agricultural and quality characteristics of safflower seeds. *Turkish Journal of Field Crops*, **19**: 7-12.

Rao V R, Ramachandram M and Arunachalam V 1977. An analysis of association of components of yield and oil in safflower (*Carthamus tinctorius* L.). *Theoretical and Applied Genetics*, **50**: 185-191.

Rudolphi S, Becker H C, Schierholt A and Ehbrecht S V W 2012. Improved estimation of oil, linoleic and oleic acid and seed hull fractions in safflower by NIRS. *Journal of American Oil Chemists Society,* **89**: 363-369.

Snider J L, Collins G D, Whitaker J, Chapman K D, Horn P and Grey T L 2014. Seed size and oil content are key determinants of seedling vigor in *Gossypium hirsutum*. *The Journal of Cotton Science,* **18**: 1-9.

Soleymani A 2017. Safflower (*Carthamus tinctorius* L.) seed vigor tests for the prediction of field emergence. *Industrial Crops and Products*, http://dx.doi.org/10.1016/j.indcrop.2017.03.022.

Toebe M, Filho A C, Lopes S J, Burin C, da Silveira T R and Casarotto G 2015. Sample size in the estimation of correlation coefficients for corn hybrids in crops and accuracy levels. *Bragantia, Campinas* **74**(1): 16-24.

Usha Kiran B, Mukta N, Kadirvel P, Alivelu K, Senthilvel S, Kishore P and Varaprasad K S 2017. Genetic diversity of safflower (*Carthamus tinctorius* L.) germplasm as revealed by SSR markers. *Plant Genetic Resources*, **15**: 1-11.

Yadav P and Murthy I Y L N 2016. Calibration of NMR Spectroscopy for accurate estimation of oil content in sunflower, safflower and castor seeds. *Current Science*, **110**: 73-76.

Yermanos D M, Hemstreet S and Garber M J 1967. Inheritance of quality and quantity of seed-oil in safflower (*Carthamus tinctorius* L.). *Crop Science*, **7**: 417-422.

Effect of different depths of irrigation water on yield and water use pattern of summer groundnut (*Arachis hypogaea* L.)

AMAR NATH MALLIC, SUNIL KUMAR GUNRI, SRIJITA PAUL, MANASHI BARMAN, ASISH MUKHERJEE AND AMRITA SENGUPTA

Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia-741 252, West Bengal

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ABSTRACT

Field experiment was conducted during summer season of 2013 and 2014 to evaluate the effect of different depths of irrigation water on yield and water use pattern of summer groundnut. Plant height increased with increasing the quantity and uniform depth (50 mm) of irrigation water during all through growth period. Towards maturity presence of moisture had no greater influence to plant growth but had significant role to developmental processes. Depth of irrigation had greatly influenced on dry matter production rather than total quantity of irrigation water applied. Increasing irrigation depth to higher (40 mm) from lower (20 mm) depth did not influence the crop growth rate significantly. Irrigation water applied either uniform or different depth during various growth stages significantly influenced the yield attributes *viz.,* number of pods per plant, shelling percentage etc. and skipping of irrigation water at any stage did not influence the yield attributes. Yield of groundnut also increased with lesser quantity of irrigation water when irrigation water was applied in judicious manner with different depths during different growth stages. Skipping of any depth of irrigation water from 30 DAE to 65 DAE the pod yield was reduced significantly about 20-26 per cent and moisture stress at early growth stage to early pod development stage was detrimental. Total water use or water expense was higher under T_7 (379.0 mm) where 160 mm irrigation water was applied under various depths during different growth stages followed by skipping of 20 mm irrigation water at 30 DAE but water expense efficiency was higher under T_5 where, 180 mm of irrigation water was applied in different depth of combinations during different growth stages of the crop followed by higher pod yield. So it may be concluded that moisture stress at early growth stage to early pod development stage was detrimental and to meet the physiological demand of the crop judicious management of irrigation water *viz.,* shallow depth of irrigation water at early part of the growth along with medium depth of irrigation water towards later growth stages (T_5) is recommended for good harvest of the crop.

Keywords: Depth of irrigation, Economics, Groundnut, Growth, Yield

Groundnut is a good source of oil, protein and food for people; and fodder for cattle. Amongst all oilseeds crops, groundnut accounts for more than 40 per cent area and 60 per cent production in the country. In recent years, the area under summer groundnut has increased owing to its higher assured productivity and profit. The crop is subjected to soil moisture deficits of varying degree and duration, which occasionally result in substantial loss in yield. So, summer groundnut in India is grown mainly in pure stands with irrigation (Damodaram and Hegde, 2000). Since the irrigated crop is not dependent on the vagaries of rainfall, so it is the single most effective measure for rapid and sustained increase in production of groundnut. Groundnut during rabi and summer has opened up new areas in west Bengal as more than 70 per cent area in West Bengal is under *rabi* and summer seasons and there is ample scope for increasing productivity through refinement of crop management/production technology like timely sowing, irrigation management, need based optimum fertilization and better plant protection measures. In view of the above fact it was considered worthwhile to undertake the investigations in order to evaluate the productivity enhancement of groundnut through need based irrigation management practices.

MATERIALS AND METHODS

A field experiment was conducted at District seed farm, Kalyani under Bidhan Chandra Krishi Viswavidyalaya, West Bengal. The soil of the experimental field was sandy loam in texture with pH 7.1. The total rainfall received during the cropping period was 150.2 mm and 82.4 mm, respectively. The experiment was laid out in a randomized block design with three replications contained 11 treatments *viz.,* T_1 = 20 mm depth of irrigation applied at 15, 30, 40, 50, 65 and 80 DAE; T_2 = 30 mm depth of irrigation applied at 15, 30, 40, 50, 65 and 80 DAE; T_3 = 40 mm depth of irrigation applied at 15, 30, 40, 50, 65 and 80 DAE; T_4 = 50 mm depth of irrigation applied at 15, 30, 40, 50, 65 and 80 DAE; T_5 = 20 mm at 15 and 30 DAE, 30 mm at 40 and 50 DAE, 40 mm at 65 and 80 DAE; T_6 = Same as T_5 only skipped 20 mm irrigation at 15 DAE; T_7 = Same as T_5 only skipped 20 mm irrigation at 30 DAE; T_8 = Same as T_5 only skipped 30 mm irrigation at 40 DAE; T_9 = Same as T_5 only skipped 30 mm irrigation at 50 DAE; T_{10} = Same as T_5 only skipped 40 mm irrigation at 65 DAE; T_{11} = Same as T_5 only skipped 40 mm irrigation at 80 DAE. Groundnut variety TG51 was sown on 10th and 8th February during 2013 and 2014, respectively

with a spacing of 30 cm x 10 cm. Immediately after sowing a common irrigation was given uniformly in all the treated plots for uniform germination followed by scheduling of irrigation as per the treatments. Recommended dose of inorganic fertilizer (20:60:40 kg/ha of N:P_2O_5 and K_2O) along with farm yard manure (FYM) @ of 7.5 tonnes/ha was applied.

Measurement of water expenses efficiency

$$\text{Economic yield produced in kg per unit area} = \frac{\text{Water expense efficiency}}{\text{Total water expense in mm per unit area}}$$

RESULTS AND DISCUSSION

Crop growth: Different depth of irrigation water applied during different growth stages had the profound effect on plant height and significant differences were observed at early stage. Plant height at 45 DAE had the significant variations among the different depths of irrigation water applied to groundnut. From the table1 it was found that plant height increased with increasing the quantity of irrigation water and significantly higher plant height was recorded in T_4 (25.4 cm) where maximum quantity of irrigation water along with uniform depth of irrigation water (50 mm) was applied during all through growth period. The treatment T_4 was statistically at par with T_2 and T_3 where 30 mm and 40 mm respectively, uniform depth of irrigation water was applied. Skipping of irrigation water at early to maximum vegetative stage (15-50 DAE) of any depth of irrigation significantly decrease the plant height but at later stage towards maturity (65 to 80 DAE) skipping of irrigation water did not reduce the plant height significantly. Induced moisture stress at early stage was detrimental for stem elongation, leaf production and leaf area duration, flower induction, root proliferation etc. which ultimately affect the vegetative growth of groundnut. Firake and Shinde (2000) opined alike. Plant height at harvest did not vary significantly due to application of different depth of irrigation water with or without skipping of irrigation at any stage. This was due to at later stage either presence of more or less quantity of moisture had no greater influence to plant growth but had significant role to developmental processes (Sridhara *et al.,* 1996).

Dry matter production at early stage (45 DAE) of the crop growth, irrigation management either uniform or different depths with or without skipping of irrigation of any stage did not influence the dry matter production significantly. But dry matter production at later stage (75 DAE) varied significantly and highest dry matter was found in the treatment T_5 (applied 180 mm irrigation water with different depth of irrigation) which was statistically on par with T_4 (applied 300 mm irrigation water with uniform depth of irrigation) but these two treatments were significantly superior to rest of the treatments. Irrespective of quantity of irrigation water applied to groundnut, skipping of irrigation water of any depth at any stage of the crop growth significantly reduced the plant dry matter. Similar observation was also reported by Kumar and Narda (2001). Management of irrigation water during different growth stages under various depth of irrigation had greatly influenced the dry matter production rather than total quantity of irrigation water applied.

The magnitude of the crop growth rate varied due to several factors among them moisture supply through irrigation water is the most critical factor. Crop growth rate did not vary significantly either uniform or different depths of irrigation applied during 46-60 DAE but at later part towards maturity 61-75 DAE crop growth rate was faster and significantly higher crop growth rate was found in T_5 (7.0 g/m^2/day) which was statistically at par with T_4 (6.5 g/m^2 /day) and T_3 (5.2 g/m^2 /day), Skipping of irrigation water from lower (20 mm) to higher (40 mm) depth at any stage of application did not influence the crop growth rate significantly. Nodule numbers per plant did not vary significantly due to application of uniform or different depth of irrigation water with or without skipping of irrigation of any stage but the nodule dry weight varied significantly.

The number of pods per plant under T_3 and T_5 were very close and although they were statistically significant. The treatment T_3 received maximum amount of irrigation water (240mm) with uniform depth of irrigation where T_5 received 180 mm of irrigation water but different combinations of depth of irrigation water was judicious as per growth stage of the crop leads to higher number of pods and this was happened due to maximum dry matter partioning as compared to T_3 where maximum depth of irrigation water (40) were applied uniformly in all the stages continue to longer maximum vegetative growth leads to less dry matter partioning. Number of pods per plant is one of the most important yield attributing character but germination per cent or number of plants per unit area is also important factor for deciding yield of the crop. Here, T_3 treatment received uniform moisture supply due to uniform depth of irrigations leads to higher germination per cent or higher plant stand but T_5 due its different depth of irrigation compensate the plant populations by increasing number of pods per plant.

Highest number of pods/plant was recorded with the treatment T_5 (24.9) received different depth of irrigation water at different physiological growth stages without escaping the irrigation water of any crop stage. In all the cases the treatment T_5 was significantly superior to rest of the treatments. Irrigation water applied either uniform or different depth during various growth stages significantly influenced the shelling percentage only skipping of irrigation water at any stage did not influence the shelling percentage

significantly. Hundred kernel weight remain unaffected due to treatments.

Pod yield increased with increasing the amount of irrigation water applied through maintaining uniform depth of irrigation at all the physiological growth stages (T_1-T_4). Yield of groundnut also increased with lesser quantity of irrigation water when irrigation water was applied in judicious manner with different depths during different growth stages (T_5). Butskipping of any depth of irrigation water from 30DAE to 65DAE (T_7-T_{10}) the pod yield was reduced significantly about 20-26 per cent. Skipping of irrigation at very initial stage i.e., 15 DAE (T_6) as well as towards harvesting stage i.e. 80 DAE (T_{11}) did not affect the pod yield significantly. This might be due to presence of sufficient moisture during sowing time in the soil and also rains (9mm) occurred at initial stage (15 DAE). In addition prevailing of low temperature at initial stage the evapo-transpiration loss was less and also due to lower crop growth rate the moisture demand was minimum. During later stage towards maturity the moisture demand was more but due to occurrence of rainfall during this time, the crop received the sufficient moisture to fulfill their growth and development. The crop was more susceptible to stress from 40 DAE to 65 DAE (T_8-T_{10}). So from the table it may be concluded that the different depths of irrigation water applied to summer groundnut during different growth stages had the profound effect to increase the pod yield and moisture stress at early growth stage to early pod development stage was detrimental. It not only hampers the yield but also hindrance or delay the flower induction which ultimately determines the yield. Similar finding was also reported by Babalad and Kulkarni (1990) who found that lowest pod yield (2.91 t/ha) was obtained with irrigation only at flowering, pegging and pod formation (60 mm depth of water applied at each growth stage). Depending upon the climatological factor and to meet the physiological demand of the crop judicious management of irrigation water *viz.,* shallow depth of irrigation water at early part of the growth along with medium depth of irrigation water at later growth stages is essentially required to establish good harvest of the crop.

Table 1 Effect of depth of irrigation and water use pattern on growth of groundnut (Pooled over 2013 and 2014)

Treatment	Irrigation days after emergence (mm)						Plant height (cm)		Drymatter production (g/m^2)		Crop growth rate (g/m^2/day)		No. of nodules/ plant	Nodule dry weight (mg/plant)
	15 DAE	30 DAE	40 DAE	50 DAE	65 DAE	80 DAE	45 DAE	At harvest	45 DAE	75 DAE	46-60 DAE	61-75 DAE	45 DAE	45 DAE
T_1	20	20	20	20	20	20	22.9	54.6	299.2	396.9	4.1	2.5	92.5	0.44
T_2	30	30	30	30	30	30	24.5	57.8	319.3	420.5	3.8	3.0	118.0	0.32
T_3	40	40	40	40	40	40	25.0	58.4	320.8	450.8	3.5	5.2	106.7	0.40
T_4	50	50	50	50	50	50	25.4	59.7	322.7	468.0	3.3	6.5	115.2	0.39
T_5	20	20	30	30	40	40	24.8	59.6	323.5	491.5	4.6	7.0	117.2	0.40
T_6	20	20	30	30	40	40	22.0	59.2	287.1	427.0	5.0	4.1	107.7	0.33
T_7	20	20	30	30	40	40	20.7	58.9	287.6	415.2	4.7	3.2	93.5	0.26
T_8	20	20	30	30	40	40	22.2	59.4	291.7	392.5	5.0	1.7	115.8	0.26
T_9	20	20	30	30	40	40	22.1	59.9	298.6	393.1	5.0	2.1	116.8	0.27
T_{10}	20	20	30	30	40	40	23.8	59.3	302.5	394.2	4.7	1.4	114.4	0.33
T_{11}	20	20	30	30	40	40	23.9	59.3	306.2	433.8	5.0	3.6	127.4	0.25
SEm±							0.828	1.672	14.04	13.78	0.95	1.07	7.86	0.03
CD (P=0.05)							1.990	NS	NS	33.13	NS	2.57	NS	0.07

*Bold marked depth of irrigation (mm) was skipped during different growth stages

Water use pattern: Total water use or water expense was higher under T_7 (379.0 mm) where 160 mm irrigation water was applied under various depths during different growth stages followed by skipping of 20 mm irrigation water at 30 DAE. The treatment T_7 was higher to all other treatments under higher or lower quantity of irrigation water was applied. Different depth of irrigation water during different growth stages was more important rather than maintaining uniform depth of irrigation under higher or lower quantity of irrigation water used. Water expense efficiency was found higher under the treatment T_5 where 180 mm irrigation water was applied throughout the growth period without skipping any depth of irrigation water. Similar observation was also reported by Taha and Gulati (2001), who stated that optimum scheduling of irrigation led to increase in pod yield and water use efficiency. Decreasing trend of water expense efficiency was found under the treatments (T_7-T_{10}) where irrigation was skipped at any growth stage except T_6 and T_{11} where irrigation was skipped at initial stage (15 DAE) and later growth stage towards maturity (80 DAE), respectively.

Economics: Higher gross return, net return and net return cost ratio was recorder under the treatments T_4 (2.55) which was statistically at par with T_2,T_3 T_5, T_6 and T_{11} but significantly superior to T_1, T_7, T_8,T_9 and T_{10}. So skipping of irrigation during different growth stages was not desirable to achieve maximum profit except skipping at very initial stage (15 DAE) and at later stage towards maturity (80 DAE) (Table 3).

The table 4 clearly indicated that pod yield have a significant strong positive relationship with various yield attributing characters taken for the observation. Among these, shelling percentage features the maximum correlation (0.914 and 0.772, respectively) with yield in both the years. However, when observed in a pooled analysis over two year, hundred kernel weight occupies the maximum correlation with pod yield (0.927) followed by shelling percentage, number of pod per plant and pod dry weight per plant. Shelling percentage would be the highest contributor over pod yield in both the year. Although others factors have significant positive correlation with yield still are excluded from the regression equation (Table 5). Pooled data to get the combining effect of the agronomic factors revealed that the kernel weight would be the sole factor contributor responsible for pooled yield over two years.

Table 2 Effect of depth of irrigation and water use pattern on yield attributes and yields of groundnut (Pooled over 2013 and 2014)

Treatment	Irrigation days after emergence (mm)						Yield attributes			Yield (kg/ha)	
	15 DAE	30 DAE	40 DAE	50 DAE	65 DAE	80 DAE	No. of pods /plant	Shelling (%)	100 kernel weight (g)	Dry pod	Haulm
T_1	20	20	20	20	20	20	22.2	68.9	44.8	3557	4312
T_2	30	30	30	30	30	30	22.9	69.2	45.6	3892	4774
T_3	40	40	40	40	40	40	24.3	70.0	45.9	4165	4998
T_4	50	50	50	50	50	50	23.1	69.6	45.1	4187	5068
T_5	20	20	30	30	40	40	24.9	71.0	46.3	4164	4966
T_6	**20**	20	30	30	40	40	22.8	69.1	45.1	3816	4292
T_7	20	**20**	30	30	40	40	22.0	68.2	44.5	3690	4346
T_8	20	20	**30**	30	40	40	20.6	68.3	43.7	3232	3928
T_9	20	20	30	**30**	40	40	20.3	67.4	43.4	3159	3823
T_{10}	20	20	30	30	**40**	40	19.0	68.0	43.8	3269	3931
T_{11}	20	20	30	30	40	**40**	20.5	69.3	45.0	3849	4649
SEm±							0.13	0.76	1.85	148.53	170.96
CD (P=0.05)							0.31	1.83	NS	357.11	411.01

* Bold marked depth of irrigation (mm) was skipped during different growth stages

Table 3 Effect of depth of irrigation and water use pattern on yield attributes and yield of groundnut (Pooled over 2013 and 2014)

Treatment	Irrigation days after emergence (mm)						Economics			Water use pattern	
	15 DAE	30 DAE	40 DAE	50 DAE	65 DAE	80 DAE	Gross return (Rs/ha)	Net return (Rs/ha)	Net return: Cost ratio	Water expense (mm)	Water expense efficiency (kg/ha/mm)
T_1	20	20	20	20	20	20	124478	83154	2.02	305.2	11.7
T_2	30	30	30	30	30	30	136220	94884	2.30	323.1	12.5
T_3	40	40	40	40	40	40	145775	104451	2.53	349.6	12.5
T_4	50	50	50	50	50	50	146528	105192	2.55	377.9	11.7
T_5	20	20	30	30	40	40	145740	104410	2.53	334.7	12.9
T_6	20	20	30	30	40	40	133560	92756	2.31	312.8	12.6
T_7	20	20	30	30	40	40	129150	88346	2.17	379.0	9.8
T_8	20	20	30	30	40	40	113103	72299	1.77	360.1	9.1
T_9	20	20	30	30	40	40	110565	69755	1.71	334.3	9.5
T_{10}	20	20	30	30	40	40	114415	73611	1.81	361.8	9.2
T_{11}	20	20	30	30	40	40	134715	83153	2.30	321.3	12.1
SEm±							5198.2	3763.8	0.09		
CD (P=0.05)							12497	9048.8	0.23		

*Bold marked depth of irrigation was skipped during different growth stages

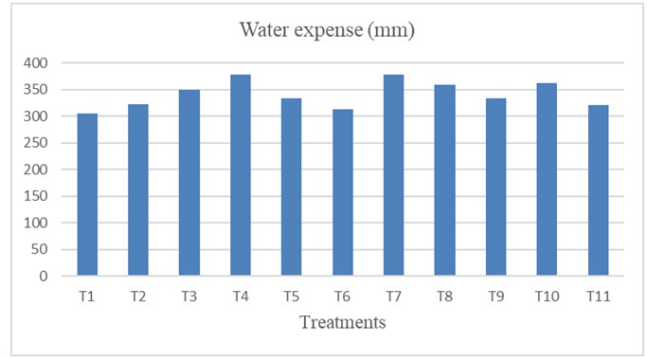

Fig. 1. Effect of depth of irrigation and water use pattern on water expense (mm)

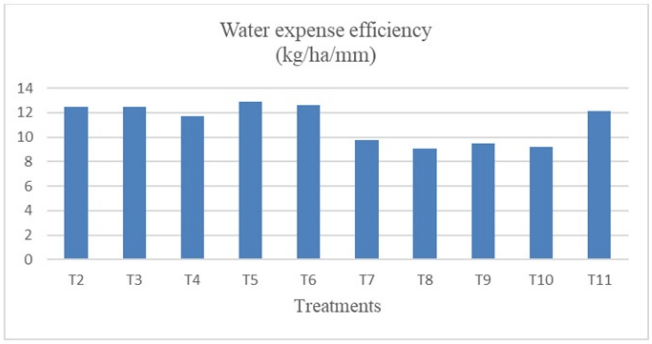

Fig. 2. Effect of depth of irrigation and water use pattern on water expense efficiency (kg/ha/mm)

Table 4 Pearson correlation matrices between dry pod yield of groundnut with different yield attributing characters

Year 2013	Y	X_1	X_2	X_3	X_4
Y	1.000	0.897**	0.812**	0.914**	0.891**
X_1		1.000	0.947**	0.891**	0.927**
X_2			1.000	0.880**	0.851**
X_3				1.000	0.934**
X_4					1.000
Year 2014					
Y	1.000	0.720**	0.654*	0.772**	0.748**
X_1		1.000	0.946**	0.670*	0.662*
X_2			1.000	0.717**	0.744**
X_3				1.000	0.792**
X_4					1.000
Pooled Year					
Y	1.000	0.847**	0.755**	0.890**	0.927**
X_1		1.000	0.947**	0.838**	0.886**
X_2			1.000	0.843**	0.862**
X_3				1.000	0.939**
X_4					1.000

Y = Dry pod weight, X_1 = No. of pod/plant, X_2 = Pod dry weight/plant, X_3 = Shelling percentage, X_4 = Hundred kernel weight,
*means significant at 5% level, **means significant at 1% level

Table 5 Step wise multiple linear regression analysis between dry pod yields of groundnut with different agronomic characters

Year	Regression equation	Variables to be included	Variables to be excluded
2013	$Y = -16249.0+286.81\ X_3$ $R^2 = 0.835$	X_3:Shelling percentage	X_1: No. of pod/plant X_2: Pod dry weight/plant X_4:Hundred kernel weight
2014	$Y = -18567.7+326.47\ X_3$ $R^2 = 0.596$	X_3:Shelling percentage	X_1: No. of pod/plant X_2: Pod dry weight/plant X_4: Hundred kernel weight
Pooled	$Y=-13380.1+381.511\ X_4$ $R^2 = 0.859$	X_4:Kernel weight	X_1: No. of pod/plant X_2: Pod dry weight/plant X_3: Shelling percentage

REFERENCES

Babalad H B and Kulkarni G N 1990. Scheduling of irrigation to summer groundnut (*Arachis hypogaea* L.). *Karnataka Journal of Agricultural Sciences*, **3**(1-2): 150-152.

Damodaram T and Hegde D M 2000. *Oilseeds Situation: A Statistical Compendium.* Directorate of Oilseed Research, Rajendranagar, Hyderabad.

Firake N N and Shinde S H 2000. Performance of planting geometry and micro-sprinkler layouts in summer groundnut. *Journal of Maharashtra Agricultural Universities*, **25**(2): 206-208.

Kumar S and Narda N K 2001. Performance of summer planted bunch type groundnut under trickle irrigation regimes. *Journal of Research*, **38**(1/2): 82-90.

Sridhara C J, Thimmegowda S and Krishnamurthy N 1996. Effect of moisture stress and irrigation levels on growth and pod yield of groundnut. *International Journal of Tropical Agriculture*, **14**(1/4): 219-222.

Taha M and Gulati J M L 2001. Influence of irrigation on yield and moisture utilization of groundnut (*Arachis hypogaea*). *Indian Journal of Agronomy*, **46**(3): 523-527.

Phenophasic heat units requirement and growth variations of safflower (*Carthamus tinctorius* L.) varieties under different sowing dates

S V KHADTARE, S K SHINDE AND V M TORADMAL

AICRP on Safflower, Krishak Bhawan, ZARS, Solapur-413 002, Maharashtra

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ABSTRACT

The field experiment was conducted at the dry farming research station, Solapur having three different sowing dates and seven varieties of safflower. Early sown safflower requires 7-9 days more for attaining physiological maturity than latest sown safflower. Among the varieties, SSF-708 was found significantly more efficient over other genotypes in duration and yield. The deviation in heat unit requirement among early and late sown safflower due to sowing dates was <100°C. Early sown safflower completed the life cycle by obtaining 1684°C heat units. Variety SSF-708 attained physiological maturity at 1492°C and NARI-57 the late maturing variety attended physiological maturity at 1628°C heat units. Similar trend was noticed in helio and photothermal units requirement of safflower. An increasing trend in leaf area upto 50 per cent flowering and gravitating trend towards the physiological maturity was noticed over sowing dates. Higher dry matter (DMP) was noticed under crop sown during first and second fortnight of September. At the end of physiological maturity, SSF-748 found better (94.32 g/plant) and SSF-708 (91.44 g/plant) was second in order for dry matter production.

Keywords: Dry matter partitioning, GDD, HTU, PTU, LAI, Safflower

Safflower (*Carthamus tinctorius* L.) is an annual, broadleaf oilseed crop of the family compositae adapted chiefly to dryland or irrigated cropping systems (Rohini and Sankara, 2000). Safflower was originally grown for the flowers that were used in making red and yellow dyes for clothing and food preparation (Cho and Tae, 2000). With earlier maturity, crop development of winter safflower would occur when temperatures are lower and moisture more plentiful than spring sown safflower (Yazdi-Samadi and Zali, 1979). India ranks first in the world with an area of 1.78 m hectares accounting for 47 per cent area and with a production of 1.14 m tones accounting for 27 per cent of world's production in 2014. Maharashtra is the largest producer of Safflower having 63 per cent (61 m tonnes) production from the largest growing area of 67 per cent (107 m ha). The productivity of the crop showed impressive advance increasing from just 203 kg/ha during 1962-63 to 641 kg/ha during 2013-14 (Anonymous, 2015).

Safflower can perform well under various climatic and soil conditions. Temperature variations in the field can be treated by sowing crops at different dates in the season. Temperature is a major environmental factor that determines the rate of plant growth and development. In addition, it's resistant to some diseases and susceptible to humidity (Odivi *et al.,* 2013). The rate of plant development is mainly temperature and photoperiod driven (Ritche and NeSmith, 1991). Regarding growth conditions, safflower is not selective and is more tolerant to drought and low temperatures (e.g. -12°C) than other oilseed crops. It was reported that the sowing date of safflower vary depending on ecological conditions (Ozel *et al.,* 2004).

Winter crops are vulnerable to high temperature during reproductive stages and differential response of temperature change (rise) to various crops has been noticed under different production environments (Kalra, 2008). Documented increases in global temperatures have stimulated interest on the direct effects of temperature and other climatic variables on plant growth, yield and quality of oil crops. Genotypes behave differently under different environmental conditions (Allard and Bradshaw, 1964). Growing degree days (GDD) are a heuristic tool in phenology. The concept was originally suggested by Reaumur over 200 years ago. GDD and helio-thermal units (HTU) are a measure of heat accumulation and used by agricultural scientists to predict crop development rates (Holmes and Robertson, 1959; Yasiri *et al.,* 2014). Though accumulation of growing degree days and photothermal units (PTU) for each developmental stage is relatively constant and independent of sowing date, crop variety may modify it considerably (Phadnawis and Saini, 1992). Having wider adaptability, different safflower genotypes require different total number of cumulative degree days or heat units for growth, development and maturity. The heat unit system or growing degree days (GDD) assumes a direct and linear relationship between growth of plant and temperature (Miller *et al.,* 2001) . The crop heat unit (CHU) system suggests that the temperature response of development differs between the day and the night. Despite this flaw, the CHU system works well and is recognized around the world as one of the best heat unit systems to quantify the effect of temperature on crop development (Parthasarathi, 2013). Sowing date is among the predictable and major non

monetary agronomic factor influencing leaf area and dry matter production in safflower (Tomar, 1995). Hence, a field investigation was carried at AICRP, Safflower, Solapur for two consecutive years to know the phase-wise heat unit requirement and to find to determine its effect on leaf area and dry matter production of safflower.

MATERIALS AND METHODS

The field experiment was conducted in post rainy season for two years at dry farming research station, Solapur during 2012-13 and 2013-14. The centre comes under scarcity zone and geographically situated at 17º 41´ North latitude and 75º 56´ East longitude at 483.6 meter above mean sea level (MSL). The rainfall of this region is characterized by inadequate, ill distributed and erratic nature. The annual normal rainfall is 723.4 mm in 40 to 45 rainy days while, the rabi normal is 237.8 mm (Anonymous, 2015). Normal maximum temperature and minimum temperature were in the range 29.8 to 34.5 and 13.3 to 21.8°C, respectively and number of bright sunshine hours and hours of photoperiod were in the range of 0.8-10.1 hr and 11.3-12.2 hrs respectively during the crop growth period for both the years. The experiment composed of three sowing windows i.e. first fortnight of September, second fortnight of September and First fortnight of October. Seven different varieties of safflower were used for the study. The experiment was laid out in split plot design and replicated four times. The crop was fertilized with the recommended dose of fertilizers (50:25:0 kg NPK/ha) through urea and single super phosphate at the time of sowing by band placement. Safflower seeds were hand dibbled at 45 x 20 cm spacing. All the agronomic practices were followed to maintain the crop condition satisfactory. The observations were recorded at the end of each physiological growth stages as suggested by Mundel *et al.* (2004).

Meteorological data was obtained from the agrometeorological observatory of DFRS, Solapur. Daily maximum, minimum, mean temperature, number of bright sunshine hours and photoperiod during the study of 2012-13 and 2014-15 were taken into consideration for phase wise calculation of GDD, HTU and PTU. The base temperature used for growing degree days (GDD) computations was 10°C. Thermal response of sowing dates can be quantified by using the heat unit or thermal time concept and calculated according to the equation

$$GDD = \sum_{i=1}^{n} = \frac{Timax + Timin}{2} - Tb$$

Where, Timax = Daily maximum temperature of day i (°C), Timin = Daily minimum temperature of day i (°C), Tb = Base temperature of safflower and, heliothermal units were calculated by the formula given by Ritchie and Nesmith, (1991).

HTU = Bright sunshine hours x GDD

The photo-thermal units were calculated by following formula as given by Wilsie (1962). PTU= Day length X GDD

Leaf area is an important variable for most ecophysiological studies in safflower concerning light interception, evapotranspiration, photosynthetic efficiency and plant growth. The leaf area in present investigation was determined by direct method using leaf area meter of LAI -305°C canopy analyser (Li-COR, Inc., Lincoln, NE). The leaves were detached from the uprooted plant and then the leaf area was measured in cm^2. The leaf area index was calculated by using formula

LAI= Leaf area (cm^2)/Ground area (cm^2)

For determining stage wise dry matter per plant, uprooted plant from each plot was washed and was sun dried and then allowed drying in thermostatically controlled hot air oven at 60±2°C till constant weight was recorded. The dry matter study was carried out at the end of each growth stage.

RESULTS AND DISCUSSION

Temperature is the key factor that influences plant growth and development. Differences among the genotypes for heat units depicted by each genotype have varying maturity periods. The shifting of sowing dates corresponds to fluctuations in temperature causing either increasing or shortening of the growth periods. Data perusal to growing degree days, heat units and photothermal units required to complete the phenological growth stages are furnished in Table 1 to 3. Data exhibited that, numerically higher cumulative degree days required to attain the physiological maturity were reported under early sown safflower (1684°C). Whereas, midlate sown safflower attended the physiological maturity on cumulative accumulation of degree days of 1596°C (88°C>D_1). Higher cumulative GDD requirement under early and midlate sown safflower was attributed to increased degree days (496°C) between rosette termination and flower initiation stages under D_1 and between germination and rosette termination (452°C) stages under midlate sown safflower. This variation in GDD among the different stages might be due to the variation in temperature prevailed during these phenological growth stages. Arslan *et al.* (1997) reported that high temperatures at germination and subsequent stages resulted in early completion of physiological maturity. The accumulation of HTU is a bright sunshine hour (BSS) driven phenomena in the life cycle of a

crop (Amrawat *et al.,* 2013; Reda *et al.,* 2013). The data in Table 2 showed the differences in stage wise HTU requirement of safflower. The midlate sown safflower (D_2: SF Sept) accumulated maximum HTU (14667°C) and early sown safflower (D_1) was second in order (14427). The safflower sown during first fortnight of October was matured on accumulation of 14069°C HTU. Variation in accumulation of HTU could be attributed to the bright sunshine hours (BSS) during different growth phases. In case of early sowing (D_1), less number of BSS was observed during germination and upto the end of rosette termination than midlate sown safflower (D_2).

Data on photothermal units (PTU) requirement to complete the phenophases of safflower genotypes under different sowing windows are given in Table 3. The PTU for a day represents the product of GDD and the possible sunshine hours as the day length (Thavaprakash *et al.,* 2007). It may inferred from the data that the PTU requirement differ from genotype to genotype. Further, it was also found that in general a progressive delay in sowing causes a decrease in PTU requirement of constituent phenophases as well as for the crop duration. So, the safflower sown during first fortnight of September showed higher PTU requirement over midlate (D_2) and late (D_3) sown safflower. The similar results are also noticed by Kumar (2008) and Pal *et al.* (2013).

The GDD, HTU and PTU are good estimators of safflower growth stages. Though accumulation of photo thermal units for each developmental stage is relatively constant and independent of sowing date, crop variety may modify it considerably (Phadnawis and Saini, 1992). The data also revealed visible differences among the genotypes for the degree days and heat unit requirements. Among different varieties under investigation, A-1, Phule Kusuma, SSF-748, PBNS-12 and NARI-6 had completed the phenological growth stage on receiving the cumulative GDD between the range of 1545 and 1588°C. Variety NARI-57 required numerically higher degree days (1628°C). Significantly lower leaf area of var. NARI -57 lead to lesser water loss through evapo-transpiration. The increased soil moisture availability period might be the reason of duration stretched during germination to rosette termination (472°C). While, variety SSF 708 found most efficient in case of calories requirement (1492°C). The results keep pace with the earlier findings of Reda *et al.* (2013). Variety SSF-708 has completed each growth stage on receiving decreased growing degree days. This implies an earliness of a genotype SSF-708. Highest heliothermal and photothermal units required under NARI-6 (14932 and 19325, respectively) and lowest were reported under SSF-708 (13689 and 17699, respectively).

Data on leaf area and leaf area index (LAI) recorded at the end of each growth stages are presented in Table 4. An increasing trend in leaf area upto 50 per cent flowering and gravitating trend towards physiological maturity was noticed over the sowing dates. Data further revealed that, crop planted during first and second fortnight exhibited at par leaf area upto flower initiation and D_1 at 50 per cent flowering resulted in significantly superior over rest of the sowing. A drastic reduction in LA at physiological maturity was attributed to leaf drop due to senescence. Among the different varieties under consideration, SSF-748 found significantly superior in leaf area and LAI at the end of all the growth stages. Second in order was PBNS -12 at the end of rosette termination while flower initiation onwards SSF -748 was closely followed by SSF -708. Significantly lowest leaf area and Index was observed under NARI -57. Varieties SSF 748 and SSF 708 might be tolerant to prevailing high temperature and well developed root system of these two varieties allowed the better and expanded photosynthetic apparatus over the crop growth period. These findings are in accordance with the earlier findings of Ashkani *et al.* (2007).

Table 1 Stage-wise heat unit requirement of safflower-growing degree days (°C)

Treatments	Phenological growth stage					
	Sowing-Germination	Germination - Rosette	Rosette - Bud Initiation	Bud Initiation-50% flowering	50% flowering-Physiological maturity	Total
Sowing dates						
D_1:FF Sept	88	431	496	171	498	1684
D_2:SF Sept	81	452	441	168	454	1596
D_3:FF Oct	83	418	386	137	399	1423
Varieties						
Annigeri-1	82	432	443	163	451	1571
SSF-708	82	410	424	144	432	1492
P. Kusuma	87	437	452	151	459	1586
SSF- 748	82	427	437	159	440	1545
PBNS-12	87	416	439	161	459	1562
NARI-57	87	472	442	170	457	1628
NARI-6	82	442	449	166	449	1588

FF - First fortnight; SF - Second fortnight

Table 2 Stagewise heliothermal units (°C) requirement of safflower

Treatments	Phenological growth stage					Total
	Sowing - Germination	Germination - Rosette	Rosette - Bud Initiation	Bud Initiation – 50% flowering	50% flowering Physiological maturity	
Sowing dates						
D_1:FF Sept	625	3491	4122	1558	4631	14427
D_2:SF Sept	640	3887	3925	1630	4585	14667
D_3:FF Oct	689	3929	3744	1397	4309	14069
Varieties						
Annigeri-1	634	3754	3950	1565	4523	14426
SSF-708	634	3563	3779	1381	4332	13689
P. Kusuma	673	3798	4030	1449	4604	14554
SSF- 748	634	3711	3896	1527	4412	14180
PBNS-12	673	3615	3914	1546	4604	14351
NARI-57	673	4102	3941	1633	4583	14932
NARI-6	634	3841	4004	1594	4503	14576

FF - First fortnight; SF - Second fortnight

Data perusal to stage wise dry matter production (g/plant) of different safflower genotypes sown at three different sowing windows are given in Table 6. Data showed the significant changes in dry matter production (DMP) per plant from rosette termination stage onwards. Higher DMP was noticed under crop sown during first fortnight of September (D_1) and it was closely followed by D_2 sown crop upto flower initiation. The respective DMP values under D_1 and D_2 were 3.06 and 2.83, respectively at the end of rosette termination and 69.92 and 62.83, respectively at the flower initiation. At 50 per cent flowering and physiological maturity, highest values of DMP were noticed under crop sown at D_1 alone. Crop physiological process dependent on integrated atmospheric parameters (Ko *et al.,* 2010). Under desired conditions of D_1 sowing, a desirable vegetative period and dry matter was increased significantly during formation of reproductive organs.

Late planting date (D_3) in addition to reducing vegetative growth, dry matter production also decreased due to undesired environmental conditions and lack of suitable transforming presented matter other plant parts, as a results of increasing temperature at the end of each growth stage (Emami *et al.,* 2011).

Dry matter yield was significantly influenced ($p < 0.05$) with different genotypes under investigation. Data showed the differential response of varieties with prevailed weather condition during the growth stages. At rosette termination, PBNS-12 (3.46 g/plant) was significantly higher and was at par with NARI- 57 (3.22 g/plant) and SSF -708 (3.15 g/plant). From flower initiation onwards, cv. SSF-748 produced significantly higher dry matter. At the end of physiological maturity stage, SSF-748 found better (94.32 g/plant) and SSF-708 (91.44 g/plant) was remaining second in order.

Table 3 Stagewise photothermal units (°C) requirement of safflower

Treatments	Phenological growth stage					Total
	Sowing - Germination	Germination - Rosette	Rosette - Bud Initiation	Bud Initiation – 50% flowering	50% flowering- Physiological maturity	
Sowing dates						
D_1:FF Sept	977	4870	5704	2069	6125	19746
D_2:SF Sept	923	5153	5204	2066	5630	18976
D_3:FF Oct	988	4877	4619	1690	4921	17094
Varieties						
Annigeri-1	938	4947	5201	1989	5576	18652
SSF-708	938	4690	4974	1755	5342	17699
P. Kusuma	996	5005	5309	1841	5675	18826
SSF- 748	938	4889	5129	1940	5441	18337
PBNS-12	996	4760	5153	1965	5675	18549
NARI-57	996	5414	5189	2076	5650	19325
NARI-6	938	5064	5273	2026	5552	18853

FF - First fortnight; SF - Second fortnight

Table 4 Leaf area index of safflower under different sowing dates and varieties at different physiological growth stages (Data of two years)

Treatments	Leaf area (cm^2) and LAI									
	Germination		Rosette		Bud Initiation		50% flowering		Physiological maturity	
Sowing dates	LA	LAI	LA	LAI	LA	LAI	LA	LAI	LA	LAI
D_1:FF Sept	4.26	0.005	279	0.31	1557	1.73	1573	1.75	733	0.81
D_2:SF Sept	5.09	0.006	240	0.27	1402	1.56	1443	1.60	641	0.71
D_3:FF Oct	4.87	0.005	180	0.20	1110	1.23	1136	1.26	432	0.48
SEm±	0.36		19		55		37		17	
CD (P=0.05)	NS		55		166		110		51	
Varieties										
Annigeri-1	4.57	0.005	198	0.22	1432	1.59	1486	1.65	641	0.71
SSF-708	5.96	0.007	287	0.32	1642	1.82	1663	1.85	593	0.66
P. Kusuma	4.61	0.005	233	0.26	1246	1.38	1257	1.40	478	0.53
SSF- 748	5.16	0.006	312	0.35	1781	1.98	1806	2.01	795	0.88
PBNS-12	4.42	0.005	299	0.33	1391	1.55	1414	1.57	542	0.60
NARI-57	4.38	0.005	139	0.15	911	1.01	936	1.04	473	0.53
NARI-6	4.09	0.005	161	0.18	1091	1.21	1124	1.25	692	0.77
SEm±	0.68		20		59		51		54	
CD (P=0.05)	NS		58		178		153		162	
CV %	6.34		10.4		12.4		16.3		18.4	

FF - First fortnight; SF - Second fortnight

Table 5 Dry matter production of safflower under different sowing dates and varieties at different physiological growth stages (Mean data of two years)

Treatments	Dry matter production (g/plant)				
	Germination	Rosette	Bud Initiation	50 % flowering	Physiological maturity
Sowing dates					
D_1:FF Sept	0.011	3.06	69.92	88.94	99.32
D_2:SF Sept	0.014	2.83	62.83	77.36	86.17
D_3:FF Oct	0.015	2.75	57.33	59.73	70.59
SEm±	NS	0.09	3.19	5.19	5.88
CD (P=0.05)	0.006	0.3	9.5	15.6	17.6
Varieties					
Annigeri-1	0.009	3.03	61.29	73.21	82.35
SSF-708	0.014	3.15	72.88	82.63	91.44
P. Kusuma	0.016	2.63	56.42	69.74	78.88
SSF- 748	0.011	2.21	76.32	85.18	94.32
PBNS-12	0.013	3.46	53.39	59.34	68.48
NARI-57	0.014	3.22	66.97	79.87	95.5
NARI-6	0.016	2.47	56.24	77.39	86.53
SEm±	0.003	0.10	2.32	2.05	1.72
CD (P=0.05)	NS	0.32	6.97	6.16	5.15
CV %	6.18	9.31	14.67	17.26	19.73

FF - First fortnight; SF - Second fortnight

REFERENCES

Allard R W and Bradshaw A D 1964. Implications of genotype-environment interaction in applied plant breeding. *Crop Science*, **4**: 503-507.

Amrawat T, Solanki N S, Sharma S K, Jajoria D K and Dotaniya M L 2013. Phenology growth and yield of wheat in relation to agrometeorological indices under different sowing dates. *African Journal of Agricultural Research*, **8**(49): 6366 -6374.

Anonymous 2015. Director's Report of AICRP on Safflower. In: *Group Meeting on Safflower* held at RVSKVV, Indore. pp. 27-29.

Anonymous 2015a. Economic Survey 2014-15, Statistical Appendix. www.indiabudget.nic.in /es2014-15/estat.1.pdf.

Anonymous 2015b. Decadal and pentacle analysis of rainfall at Solapur. In: Annual Report of AICRP on Agrometeorology presented in annual group meeting on Agromrteorology held at BCKV, Kalyani. pp. 27-29.

Arslan B, Yildirim B, Ilbas A I, Dede O, and Okut N 1997. The effect of sowing date on yield and yield characters of varieties of safflower (*Carthamus tinctorius* L.). 4th International Safflower Conference, Bari, Italy, pp. 125-131.

Ashkani J, Pakniyat H and Ghotbi V 2007. Genetic evaluation of several physiological traits for screening of suitable spring safflower (*Carthamus tinctorius* L.) genotypes under stressed and non stressed irrigation regimes. *Pakistan Journal of Biological Sciences*, **10**(4): 2320-2326.

Cho M H and Tae R H 2000. Purification and characterization of Precarthamin Decarboxylase from the yellow petals of *Carthamus tinctorius* L. *Archives of Biochemistry and Biophysics*, **382**: 238-244.

Emami T, Naseri R. Falahi H. and Kazami E 2011. Response of yield, yield components and oil content of safflower (cv. Sina) to planting date, plant spacing on row in rainfed conditions of western Iran. *American-Eurasian Journal of Agricultural and Environmental Science*, **10**(6): 947-953.

Holmes R M and Robertson G W 1959. Heat Units and Crop Growth. *Canada Department of Agriculture.* Publication No. 1042. pp. 35.

Kalra N 2008. Effect of increasing temperature on yield of some winter crops in north- west India, *Current Science*, **94**(1): 82-88.

Kumar A, Pandey V, Shekh A M and Kumar M 2008. Growth and yield response of soybean (*Glycine max* L.) in relation to temperature, photoperiod and sunshine duration at Anand, Gujarat, India. *American-Eurasian Journal of of Agronomy*, **1**(2): 45-50.

Miller P, Lanier W and Brandt S 2001. Using growing degree days to predict plant stages E-5, In: Montguide M T 200/03AG/ 7 2001. http://store.msuextension.org/publications/AgandNaturalResources/MT200103 AG .pdf.

Ozel A T, Demurbulek M, Atilla G R and Opur O 2004. Effects of different sowing date and intrarow spacing on yield and some agronomic traits of safflower (*Carthamus tinctorius* L.) under Harran plains arid conditions. *Turkey Journal of Agriculture*, **28**: 413-419.

Odivi A G, Hadi H, Bahrampour B and Saeidi M 2013. Effect of sowing date on yield and its components, oil and protein concentration and some agronomical traits of safflower (*Carthamus tinctorius.* L.). *Technical Journal of Engineering and Applied Sciences*, **3**(14): 1405-1410.

Pal R K, Rao M N N and Murty N S 2013. Agro-meteorological indices to predict plant stages and yield of wheat for foot hills of western Himalayas. *International Journal of Agriculture and Food Science Technology*, **4**(9): 909-914.

Parthasarathi T, Velu G and Jeyakumar P 2013. Impact of crop heat units on growth and developmental physiology of future crop production: *A Review. Journal of Crop Science and Technology*, **2**(1): 1-8.

Phadnawis N B and Saini A D 1992. Yield models based on sowing time and phonological development. *Annals of Plant Physiology*, **6**: 52-55.

Reda S, Ashraf A A, Mohsen E, Hasnaa A, Gouda H and Hafez S 2013. Impact of temperature fluctuation on yield and quality traits of different safflower genotypes. *Scientific Research and Review Journal*, **1**(3): 74-87.

Ritchie J T and Nesmith D S 1991. Temperatures and Crop Development. Modeling Plant and Soil Systems. In: *Agronomy Monograph No. 31,* Madison, WI 5711, USA.

Rohini V K and Sankara K R 2000. Embryo transformation, a practical approach for realizing transgenic plants of safflower (*Carthamus tinctorius* L.). *Annals of Botany*, **86**: 1043-1049.

Thavaprakaash N, Jagannathan R, Velayudham K and Gurusamy L 2007. Seasonal influence on phenology and accumulated heat units in relation to yield of baby corn. *International Journal of Agricultural Research,* **2**: 826-831.

Tomar S S 1995. Effect of soil hydrothermal regimes on the performance of safflower planted on different dates. *Journal of Agronomy and Crop Science*, **175**: 141-152.

Wilsie C P 1962. *Crop Adoptation and Distribution.* Freeman W. H. and Co., London, pp. 52-59

Yasari T, Shahsavari M and Rezae M 2014. Safflower growing degree days in different temperature regimes. *Scientific Journal of Crop Science*, **3**(12): 413-419.

Yazdi-Samadi B and Zali, A A 1979. Comparison of winter and spring-type safflower. *Crop Science*, **19**: 783-785.

Selection of *Trichoderma* strains for salinity stress and evaluation for imparting salinity tolerance in sunflower

A AZIZ QURESHI, R D PRASAD, A KULKARNI, T NAVANEETA, P LAKSHMAMMA, K S VARAPRASAD AND A VISHNUVARDHAN REDDY

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

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ABSTRACT

The role of *Trichoderma* fungi in imparting tolerance to various fungal diseases is well known. The idea of this study was to assess the potentiality of *Trichoderma* in imparting tolerance to abiotic stress, in particular to salinity. In a laboratory study at Indian Institute of Oilseeds Research, Hyderabad, 15 best strains were selected out of 30. Out of the short listed, five potential isolates *viz., T. harzianum* Th-4d, *T. asperellum* Ta-v5, *T. asperellum* Ta-N13, *T. asperellum* Ta-A5 and *T. asperellum* Ta-A7 were found prominent towards salt stress tolerance. These were able to grow and sporulate, survived and revived during 7 days of continuous stress up to 1.5 *M* NaCl concentration in growth medium. *Trichoderma* isolates Ta-A5 and Ta-A7 recorded highest mean spore count (4.77 logcfu/ml) followed by Ta-N13 > Th-4d >Ta-v5. The significant colony diameter (25.0 mm) at 7th day was observed in isolate Ta-A5. In nutrient broth, the highest sodium concentration in mycelium was recorded in Ta-N13 (860 µg/g) and followed by Th-4d (852 µg/g). In the green house trial, seed priming of sunflower with salinity tolerant isolate Ta-N13 produced highest vigour index (1848) followed by isolates Ta-A5 (1664) and Th-4d (1632). During the field evaluation of saline tolerant *Trichoderma* isolates, it was noticed that isolate Th-4d was found prominent over others when it was seed treated, while Ta-N13 was found effective when it was applied as soil based application in sunflower at 45 days.

Keywords: Abiotic stress, Salinity tolerance, Sunflower, *Trichoderma* isolates

Worldwide, salinity is one of the most severe abiotic stresses that limit crop growth and productivity. Around 20 per cent of worlds irrigated land is salt affected, with 2,500-5,000 km^2 of production area lost every year as a result of salinity (UNEP, 2009). About 60 per cent of salt affected soils are of sodic and saline sodic in nature which has increased steadily over decades in the northwest plains of the Indo-Gangetic basin and in China's Yellow River basin (Gupta and Abrol, 2000). Salt affected soil is progressively being exacerbated by agronomic practices such as excessive irrigation and fertilizer application, especially in arid regions (Villa-Castorena *et al.,* 2003). At higher salt levels, the crop yields are reduced so drastically that crop cultivation is not economical without soil amendments. The addition of salts to water lowers its osmotic potential, resulting in decreased availability of water to roots and thus exposes plants to secondary osmotic stress. This implies that all the physiological responses associated with the drought stress can also be invoked by salt stress. Adverse effects of salinity on plant growth may be due to ion cytotoxicity (mainly due to Na^+, Cl^-, SO_{4-2}) and osmotic stress, which results in nutritional deficiencies and metabolic imbalance (Zhu, 2002). Salt stress leads to inhibition of growth and development, reduction in photosynthesis, respiration and protein synthesis and disturbs nucleic acid metabolism (Levine *et al.,* 1990; Bray *et al.,* 2000). Decrease in growth at higher sodium concentration due to decrease in the uptake of K^+ and Ca^{2+} has also been reported (Sairam and Tyagi, 2004). Salt stress is an important abiotic factor that highly impacts the microbial ecosystem of soil and limiting crop productivity (Poosapati *et al.,* 2014). High alkalinity (pH > 8.5) and high exchangeable sodium percentage (ESP > 15) of the soil render it inhospitable rhizophere environment for normal crop production and there is minimal productivity in such soil (Chhabra, 1995). The utilization of salt-affected soil for agriculture has become necessary to meet the rise in food demand. One of the possible strategy to counteract the adverse effect of salinity is to exploit the avenues of bio-agents or bio-inoculants (Egamberdieva, 2012). Incorporation of *Trichoderma* through seed bio-priming treatments in many cereal and vegetable crops has resulted in increased levels of plant growth hormones and improved seed performance even under adverse soil conditions (Singh *et al.,* 2003). Bio-control agent, *Trichoderma,* releases a variety of compounds that induce resistance responses to biotic and abiotic stresses (Harman *et al.,* 2004; Cardona and Rodriguez, 2006). Seed coating of wheat seeds with salinity tolerant *Trichoderma* strains reduced the detrimental effects of salinity stress in wheat (Laxmi Rawat *et al.,* 2011). Farm lands in arid ecology largely suitable of oilseed crops is being subjected to salinity and alkalinity due to excess irrigation in command areas and poor quality tube well irrigation. To face the future threat of salinity and keep the pace of oilseeds production it is essential to develop

technologies that can impart tolerance capacity in the oilseed crops against salinity. Envisaging the above background, laboratory studies were conducted with various *Trichoderma* collection, for preliminary screening against salinity stress in growth medium. Further, potential salinity tolerant *Trichoderma* isolates were tested for germination and seedling emergence of sunflower in natural saline soil in green house conditions. Finally, field trials were conducted to evaluate *Trichoderma* isolates for imparting salinity tolerance in sunflower.

MATERIALS AND METHODS

Selection of *Trichoderma* isolates for salt stress tolerance: Initially, 30 *Trichoderma* isolates collected from different agro-ecosystem were subjected to salinity stress. Based on initial screening in Nutrient agar medium for salinity up to 2.0 *M* NaCl concentration, 15 isolates were further short listed. Among the 15, five potential isolates *viz., T. harzianum* Th-4d, *T. asperellum* Ta-v5, *T. asperellum* Ta-N13, *T. asperellum* Ta-A5 and *T. asperellum* Ta-A7 were found prominent towards salinity tolerance. These were able to grow and sporulate, survived and revived during 7 days of continuous stress up to 1.5 *M* NaCl concentration in growth medium. The colony forming units of potential *Trichoderma* isolates were expressed as log values. Colony diameter was also recorded for denoting the growth of isolates.

Characterization of identified isolates: Morphological characteristics *viz.,* growth pattern, sporulation pattern and spore colour of the colonies were recorded by growing isolates in the nutrient agar growth medium by adopting standard techniques at different salinity stress levels.

In another assay, a 2 factorial CRD (Factor 1 = salinity; Factor 2 = *Trichoderma* isolates) was adopted to study the salt tolerance by *Trichoderma* mycelium with two level of salinity in nutrient broth. The isolates were allowed to grow up to 21 days to study the growth of mycelium in terms of fresh and dry weight and the ability to tolerate the level of salinity due to NaCl by measuring sodium concentration in the mycelium. The mycelium mat was thoroughly washed to remove excess sodium from the surface and powdered after proper drying. Required quantity of mycelium was subjected to wet digestion by using Di-acid mixture (9 : 6 :: HNO_3 : $HClO_4$). In the digest, sodium concentration was estimated by adopting flame photometry technique (Jackson, 1976).

Evaluation of potential isolates for imparting tolerance in sunflower against salinity: Greenhouse experiment: The five potential salt tolerant *Trichoderma* isolates were evaluated for imparting tolerance in sunflower in natural saline soil. The soil was black and clayey textured having electrical conductivity (EC) of 6.0 dS/m. Green house experiment was conducted by adopting completely randomised block design to study the germination of sunflower. Seed priming of sunflower seeds with five *Trichoderma* isolates was done with kaolinite based inoculum with the initial load of 1×10^8 cfu/ml. After air drying, the seeds were sown in disposable plastic trays, the single seedling per hole was allowed to grow up to 15 days and growth parameters were recorded. After 15th day, plant height, shoot fresh weight, dry matter yield and vigour index were recorded. The equation VI = [(Root length + Shoot length) × Germination%] was adopted to work out vigour index (VI).

Field experiment: Field experiments were conducted in natural saline soil with salinity value of 6.0 dS/m at Agricultural Research Station, Gangavathi, UAS, Raichur during late *kharif* season (i.e. Mid rainy season) in 2015-16 to evaluate the salinity tolerant *Trichoderma* strains on the growth parameters of sunflower. Experiments were set up to evaluate the effect of *Trichoderma* isolates in imparting salinity tolerance in sunflower through i) soil application and ii) seed priming with randomized block design with four replications and consisting of 6 *Trichoderma* treatments (T_1 = control i.e. no *Trichoderma;* T_2 = Th-4d; T_3 = Ta-N13; T_4 = Ta-v5; T_5 = Ta-A5 and T_6 = Ta-A7, respectively). The data obtained from laboratory, green house and field trials was analyzed using MSTATC statistical software.

RESULTS AND DISCUSSION

Assessment of *Trichoderma* isolates against salinity stress: In the initial screening, 15 *Trichoderma* isolates were subjected to salinity stress up to 2.0 *M* NaCl. The results for viable colony forming units (cfu) were expressed as Log transformed values per millilitre (i.e. Logcfu/ml) for the different isolates against salinity levels and are presented in Table 1. The highest values were noticed for *T. asperellum* isolates: Ta-A5 and Ta-A7 (4.77) followed by Ta-N13 (4.6) and *T. harzianum* isolate: Th-4d (4.56).The results indicated variation in growth response, ability to produce viable colonies only up to 1.5 *M* NaCl salt stress. Among the 15 isolates, five were found to be prominent and survive under salt stress, the mean value of Logcfu/ml was in the following order Ta-A5 = Ta- A7 (4.77) >Ta-N13 (4.6) > Th-4d >(4.56) >Ta-v5 (4.55). This might be due to the ability of their mycelium to extrude excessive sodium ions beyond certain concentration. The ability to develop extrusion systems to keep levels of intracellular sodium below toxic concentration in the cell of *Trichoderma* species was reported by Gunde-Cimerman *et al.* (2009).

Effect of salt stress on the growth of potential *Trichoderma* colonies: The data presented in Table 2 indicate the colony diameter of potential *Trichoderma* isolates recorded at 7th day to salinity stress. Significantly

highest colony diameter with 25.0 mm was noticed in isolate Ta-A5 at salinity stress of 1.5 *M* NaCl. Other potential isolates which had produced high colony diameter were in the following order: Ta-N13 (19.0 mm) > Ta-v5 (18.0 mm) > Th-4d (17.0 mm) > Ta-A7(14.3 mm). In a study, Sowmya *et al.* (2014) indicated in their findings that thermo-tolerant *Trichoderma* isolate TaDORS3 had produced 3.0 cm mean colony radii when subjected to salinity of 0.75 *M* NaCl. Further increase in salinity decreased the colony radii. Similarly, Zehra *et al.* (2017) reported that at all the tested concentration of salt, *T. harzianum* was able to grow up to 400 µ*M* NaCl concentrations but sporulation was inhibited at high concentration of salt (1000 µM).

Morphological characters of the potential *Trichoderma* isolates under salt stress: The data presented in Table 3 variation in the morphological characters of salinity tolerant colonies recorded when subjected to different levels of salinity stress from zero to 1.5 *M* NaCl concentration. In control (i.e. zero salinity), the growth pattern was flat and compact colonies with dark green colour. With the increase in the salinity the growth pattern changed to cottony, with loose mycelia and moderately dispersed colonies in the growth medium. The colour of the colonies also changed from dark green to green, to yellowish green and finally to white at highest salinity level of NaCl. The modifications of morphological aspects and antagonistic capacity of *T. harzianum* due to presence of sodium chloride in the medium was also reported by Regragui (2005).

Table 1 Effect of different salinity levels on conidial germination determined by colony forming units (cfu) on saline tolerant *Trichoderma* isolates after 7 days incubation

Trichoderma isolate	Log cfu/ml at different salinity (NaCl)		
	0.5 *M*	1.0 *M*	1.5 *M*
T. harzianum - Th4d	4.95	4.03	3.92
T. asperellum-Tv5	4.80	4.61	3.43
T. asperellum –TN13	4.70	4.82	3.83
T. asperellum- TaDOR7316	4.19	4.37	3.82
T. asperellum -TaDOR222	4.74	4.59	3.73
T. asperellum - Tv2	3.13	1.23	0.00
T. asperellum-T12	3.52	3.67	0.00
T. asperellum -TS12	3.67	2.00	0.00
T. asperellum - GS1	3.87	0.00	0.00
T. asperellum -TS1	3.85	2.91	0.00
T. asperellum-TS2	3.72	2.56	0.00
T. asperellum - A6	4.70	4.58	3.70
T. asperellum -TaDOR673	4.70	4.58	3.99
T. asperellum - A5	4.99	4.82	4.05
T. asperellum - A7	4.99	4.84	4.05

Table 2 Colony growth of *Trichoderma* isolates under different salinity stress after 7 day incubation

Trichoderma isolate	Colony diameter (mm)					
	Control	0.1 *M*	0.2 *M*	0.5 *M*	1.0 *M*	1.5 *M*
T. harzianum-Th4d	90.0	88.7	72.7	60.5	39.7	17.0
T. asperellum-Tv5	90.0	88.3	75.0	61.0	45.7	19.0
T. asperellum-TN13	90.0	90.0	88.0	60.5	41.0	11.3
T. asperellum-TaDOR7316	90.0	90.0	89.0	63.0	43.3	18.0
T. asperellum-TaDOR222	90.0	90.0	89.7	70.0	46.3	12.0
T. asperellum-Tv2	90.0	88.3	76.0	75.0	42.3	11.7
T. asperellum-T12	90.0	89.0	73.3	75.0	46.0	11.7
T. asperellum-TS12	90.0	88.7	75.3	75.5	51.7	10.7
T. asperellum-GS1	90.0	90.0	88.0	78.0	51.0	11.0
T. asperellum-TS1	90.0	88.7	77.7	59.5	51.3	12.3
T. asperellum-TS2	90.0	88.0	80.0	63.0	50.7	10.0
T. asperellum -A6	90.0	89.3	89.0	65.0	47.0	11.7
T. asperellum -TaDOR673	90.0	89.7	79.7	77.0	49.7	11.3
T. asperellum -A5	90.0	90.0	84.3	87.5	55.3	25.0
T. asperellum-A7	90.0	90.0	89.7	85.0	44.0	14.3
CD (0.05)	0	1.0	2.7	3.1	3.9	1.3
CV (%)	0	0.68	2	2.6	5	6.7

Table 3 Morphological characteristics of saline tolerant *Trichoderma* isolates at different salinity levels in nutrient medium

Trichoderma isolate	Growth pattern				Sporulation pattern				Spore colour			
	0.0 *M*	0.5 *M*	1.0 *M*	1.5 *M*	0.0 *M*	0.5 *M*	1.0 *M*	1.5 *M*	0.0 *M*	0.5 *M*	1.0 *M*	1.5 *M*
Th-4d	F	F	F	F	++++	++++	++++	+++	DG	G	YG	W
Ta-v5	F	C	C	C	++++	++++	+++	++	DG	G	YG	W
Ta-N13	F	F	F	F	++++	++++	++++	+++	DG	YG	W	W
Ta-A5	F	F	F	F	++++	++++	++++	+++	DG	G	YG	W
Ta-A7	F	C	C	C	++++	++++	+++	++	DG	G	YG	W

Note: C- cottony, F- Flat, +- little disperse, ++- moderately disperse, +++- good disperse, ++++- compact, DG- dark green, G- green, YG- yellowish green, W- white

Table 4 Effect of salt stress on fungal mat parameters of saline tolerant *Trichoderma* isolates

Trichoderma isolate (TI)	Salinity level (SL)								
	Mycelium fresh weight (g)			Mycelium dry weight (g)			Sodium concentration in mycelium (µg/g dw)		
	0.0 *M*	1.5 *M*	Mean	0.0 *M*	1.5 *M*	Mean	0.0 *M*	1.5 *M*	Mean
Th-4d	13.04	9.28	11.16	3.02	1.26	2.14	450	852	651
Ta-v5	6.88	6.07	6.47	2.14	1.07	1.60	102	120	111
Ta-N13	12.08	7.37	9.72	1.93	0.76	1.34	420	860	640
Ta-A5	16.21	8.26	12.23	3.12	1.13	2.12	500	833	666
Ta-A7	9.96	6.23	8.09	1.48	0.40	0.94	230	840	535
Mean	11.63	7.44	--	2.33	0.92	--	340	701	--
CD (0.05)									
TI		0.24			0.06			3.61	
SL		0.15			0.42			2.26	
TI x SL		0.34			0.09			5.11	

Table 5 Effect of seed priming with saline tolerant *Trichoderma* isolates on growth of sunflower seedlings at15 days

Treatment	SL (cm)	RL (cm)	FW (g)	DW (g)	G (%)	VI
Control	9.6	4.3	3.2	0.4	60	834
Th-4d	13.4	7.0	5.9	1.0	80	1632
Ta-v5	12.4	6.7	5.1	0.7	70	1337
Ta-N13	14.1	9.0	6.1	1.2	80	1848
Ta-A5	13.9	6.9	5.5	1.0	80	1664
Ta-A7	12.9	5.6	4.9	0.9	80	1480
CD (0.05)	0.9	0.8	0.5	0.3	--	--
CV (%)	4.9	8.0	6.5	23		

SL = Shoot length; RL = Root length; FW = Fresh weight; DW = Dry matter weight; G = Germination%; VI = Vigour index

Table 6 Growth of sunflower in saline soil (EC=6.0 dS/m) due to two mode of application of saline tolerant *Trichoderma* isolates at 45 days

Saline tolerant *Trichoderma* strain	Mode of application of *Trichoderma*							
	Soil application				Seed priming			
	SL (cm)	RL (cm)	FW (g)	DW (g)	SL (cm)	RL (cm)	FW (g)	DW (g)
Control	63.1	12.2	131.1	32.9	57.0	12.0	170.1	37.6
T. harzianum-Th-4d	81.6	16.5	193.8	48.4	71.2	14.3	295.8	51.2
T. asperellum-Ta-N13	81.4	14.9	210.0	47.3	74.6	14.0	244.8	48.5
T. asperellum-Ta-v5	75.8	16.4	160.6	35.7	89.4	16.1	180.0	41.0
T. asperellum-Ta-A5	97.2	17.4	254.0	52.4	86.4	17.0	224.6	46.8
T. asperellum-Ta-A7	83.0	16.2	215.2	41.2	71.4	16.7	212.6	40.5
CD (0.05)	11.9	2.7	13.8	6.1	12.8	2.4	15.6	8.9
CV (%)	8.2	9.4	10.7	7.9	9.7	8.9	11.2	11.1

SL = Shoot length; RL = Root length; FW = Fresh weight; DW = Dry matter weight

Mycelium growth and sodium concentration: Potential *Trichoderma* isolates were grown in broth at two levels of salinity to study the sodium concentration in mycelium mat and also recorded their fresh and dry weight. The data pertaining to above mention parameters has been presented in Table 4. The results indicated that Isolate Th-4d had produced highest fresh weight (9.28 g) and dry weight (1.26 g) of mycelium mat when subjected to highest salinity stress of 1.5 *M* NaCl up to 21 days. But, significantly highest sodium level in the mycelium mat was observed in isolate Ta-N13 (860 µg/g dw) and it was followed by Th-4d (852 µg/g dw).

This might be due to their capacity to grow in saline environment by producing high biomass of fungal/mycelia mat while tolerating the sodium ions up to certain level and extrude the excess. Similarly, Gunde-Cimerman *et al.* (2009) had reported that some of the Trichoderma species have ability to develop extrusion systems to keep levels of intracellular sodium below toxic concentration to cell.

Evaluation of *Trichoderma* isolates for imparting salinity tolerance in natural saline soils under green house and field conditions

Evaluation in green house experiment: The five potential Trichoderma isolates were evaluated in green house conditions for studying their ability to impart salinity tolerance to sunflower seedlings in saline soils with EC value of 6.0 dS/m. Seed priming with *Trichoderma* isolate Ta-N13 had produced significantly highest shoot length (14.0 cm), root length (9.0 cm) and dry matter weight (1.2 g) of sunflower seedlings at 15 days. It had also produced 46 per cent higher shoot and 33 per cent root growths over NaCl alone treated sunflower (i.e. without salt tolerant *Trichoderma* isolate) (Table 5 and Fig. 1), which might be due to highest vigour index value (1848) produced with Ta-N13 and could also be due to the overall ability of this isolate to grow in saline environment. Parvaiz Ahmed *et al.* (2015) corroborated that supplementation of *Trichoderma harzianum* to NaCl treated mustard seedlings showed elevation by 13.8, 11.8, and 16.7 per cent in shoot, root length and plant dry matter weight respectively, as compared to plants treated with NaCl (200 mM) alone.

Evaluation in field experiment: The results pertaining to growth response of sunflower for the two methods of *Trichoderma* application in natural saline soils has been depicted in Table 6. Variation in the performance of Trichoderma isolates for imparting salinity tolerance in sunflower at 45 days was quite visible due to application modes. In soil based application, the isolate Ta-A5 was found superior in improving the growth parameters of sunflower *viz*., shoot length = 97cm; root length =17cm; fresh weight =254 g/plant and dry matter weight = 52 g/plant in natural saline soils with EC = 6.0dS/m. On the other hand, the isolate Th-4d had produced superior fresh weight (296 g/plant) and dry weight (51 g/plant) of sunflower in the same soils over other isolates when it was applied through seed priming (Table 6). It was quite interesting to notice that there was seldom difference in the effect of isolates Th-4d due to seed priming and Ta-A5 due to soil based application on dry matter weight production of sunflower at 45 day stage.

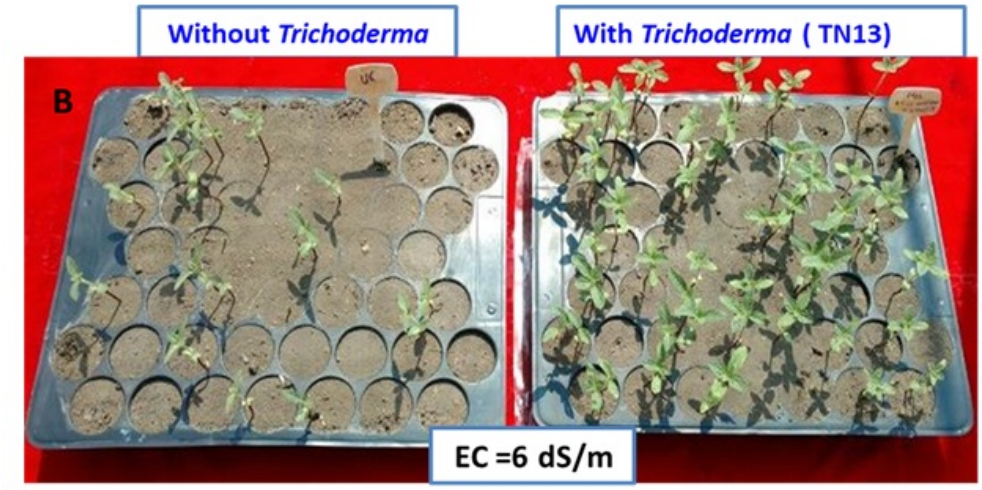

Fig. 1 Effect of *Trichoderma* isolate, TN 13 on the growth of sunflower seedling under salinity stress (EC=6 dS/m)

In this study, we could identify five potential *Trichoderma* isolates with the ability to impart tolerance to salinity. In green house experiment, isolate Ta-N13 was found to be superior in terms of better germination, seedling vigour, shoot and root growth and highest dry matter of sunflower seedlings when grown in natural saline soils with salinity of 6.0 dS/m. This could be supported with the findings that above isolate showed better tolerance to salinity in nutrient medium and produced spores even at 1.5 M NaCl concentration. Further, field trials could prove that isolate Ta-N13 was effective for soil based application, while, isolate Th-4d effective as seed priming for imparting salinity tolerance in sunflower when grown in field conditions.

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REFERENCES

Bray E A, Bailey-Serres J and Weretilnyk E 2000. Responses to abiotic stresses. In: Buchanan BB, Gruissem W and Jones RL (eds) *Biochemistry and Molecular Biology of Plants.* ASPP, Rockville, pp. 1158-1203.

Cardona R and Rodriguez H 2006. Effects of *Trichoderma harzianum* fungus on the incidence of the charcoal rot disease on sesame. *Revista de la Faculated de Agronomia* (LUZ), **23**: 42-47.

Chhabra R 1995. Nutrient requirement for sodic soil. *Fertilizer News,* **40**:13-21

Egamberdieva D 2012. *Pseudomonas chlororaphis:* a salt-tolerant bacterial inoculant for plant growth stimulation under saline soil conditions. *Acta Physiologiae Plantarum*, **34**: 751-756.

Gunde-Cimerman N, Ramos J and Plemenitaš A 2009. Halotolerant and halophilic fungi. *Mycology Research*, **113**: 1231-1241

Gupta R K and Abrol I P 2000. Salinity build-up and changes in the rice-wheat system of the Indo-Gangetic Plains. *Experimental Agronomy*, **36**: 273-284.

Harman G E, Howell C R, Vitarbo A, Chet I and Lorito M 2004. *Trichoderma* species -opportunistic, a virulent plant symbionts. *Nature Reviews Microbiology*, **2**:43-56.

Jackson M L 1976. *Soil Chemical Analysis.* Prenice Hall of India Pvt. Ltd., New Delhi. pp. 498.

Laxmi Rawat, Singh Y, Shukla N and Kumar J 2011. Alleviation of the adverse effects of salinity stress in wheat (*Triticum aestivum* L.) by seed bio-priming with salinity tolerant isolates of *Trichoderma harzianum. Plant and Soil,* **347**: 387-400.

Levine R L, Garland D, Oliver C, Amici A, Clement I, Lenz, A G, Ahn B W, Shalteil S and Stadtman E R 1990. Determination of carboxyl content in oxidatively modified proteins. *Methods Enzymology*, **186**: 464-478.

Parvaiz Ahmad, Abeer Hashem, Elsayed Fathi Abd-Allah, A A Alqarawi, Riffat John, Dilfuza Egamberdieva and Salih Gucel 2015. Role of *Trichoderma harzianum* in mitigating NaCl stress in Indian mustard (*Brassica juncea* L) through antioxidative defense system. *Front. Plant Science*, **14**: 868.

Regragui and Lahlou H 2005. Effect of salinity on in vitro *Trichoderma harzianum* against *Verticillium dahliae. Pakistan Journal of Biological Sciences*, **8**: 872-876.

Sairam R K and Tyagi A 2004. Physiology and molecular biology of salinity stress tolerance in plants. *Current Science*, **86**:407-421.

Singh U S, Zaidi N W, Joshi D, Varshney S and Khan T 2003. Current status of *Trichoderma* as a biocontrol agent. In: Ramanujam B, Rabindra RJ (eds) *Current Status of Biological Control of Plant Diseases using Antagonistic Organisms in India.* Project Directorate of Biological Control, Bangalore, pp 13-48.

Sowmya Poosapati, Prasad Durga Ravulapalli, Navaneetha Tippirishetty, Dinesh Kumar Vishwanathaswamy and Sarada Chunduri 2014. Selection of high temperature and salinity tolerant *Trichoderma* isolates with antagonistic activity against *Sclerotium rolfsii. Springer Plus*, **3**: 641.

UNEP 2009. The environmental food crisis: The environments' role in averting future food crisis.

Villa-Castorena M, Ulery A L, Valencia E A C and Remmenga M D 2003. Division S-4-soil fertility and plant nutrition. *Soil Science Society of America Journal*, **67**: 1781-1789.

Zehra M K, Dubey M, Meena and Upadhya R S 2017. Effect of different environmental conditions on growth and sporulation of some *Trichoderma* species. *Journal of Environmental Biology*, **38**: 197-203.

Zhu J K 2002. Salt and drought signal transduction in plants. *Annual Review of Plant Biology*, **53**: 247-273.

Effect of Rice Bran Oil Spread (RBOS) as a fat substitute on the sensory properties of baked products

RIZWANA SHAIK, APARNA KUNA[1], MOHIBBE AZAM[2] AND MANORAMA KANURI[1]

Post-Graduate and Research Centre, Professor Jayashankar Telangana State Agricultural University, Rajendranagar-500 030, Hyderabad, Telangana

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ABSTRACT

Studies were carried out to replace Hydrogenated Fat (HF) with Rice Bran Oil (RBO) and two varieties of Rice Bran Oil Spread - RBOS1 and RBOS2 in the preparation of cake, cookies, piecrusts, muffins and doughnuts. Sensory evaluation of all the baked products prepared with HF, RBO, RBOS1 and RBOS2 was carried out using a 9 point hedonic score card and results indicated that cakes were most liked followed by piecrust and muffins prepared with RBOS2. There was no significant difference in overall acceptability of baked products made with HF and RBOS2 indicating that RBOS2 can be used for development of baked products without any compromise on the sensory attributes. All baked products made with RBOS2 were well accepted by the respondents compared to HF indicating that RBOS2 can be popularized as a baking fat.

Keywords: Hydrogenated fat, Rice bran oil, Rice bran oil spread, Sensory evaluation

Rice bran oil is an excellent cooking medium because it is nutritionally superior, contains more micronutrients, longer shelf life, more stable at higher temperature, gives better taste and flavour to food items; frying takes less time, so saves energy and economical due to 15 per cent less absorption of oil during frying (Sharma, 2002). Rice bran oil has garnered attention from consumers in recent years, owing to its high concentrations of health-promoting compounds, ranging from tocopherols and tocotrienols to phytosterols and γ-oryzanol (Sen *et al.,* 2006). Tocotrienols also act as antioxidants and display anticancer, cholesterol lowering and neuroprotective properties that are distinct from tocopherols (Sen *et al.,* 2007). γ-Oryzanol has been shown previously to act as an antioxidant (Miller and Engel, 2006; Juliano *et al.,* 2005) and is also effective at lowering cholesterol (Yokoyama, 2004). Rice bran oil specifically has been shown to protect against lipid peroxidation *in vitro* (Lee *et al.,* 2005). Many of the same molecules in rice bran oil that impart antioxidant activity in biological tissues also inhibit lipid oxidation on the shelf life of rice bran oil leading to relative resistance to oxidative degradation at both ambient and elevated temperatures, thereby giving longer product life to foods made with rice bran oil. While commodity oils have traditionally been used in the manufacture of spreads, rice bran oil itself is now being explored, for use in spreads, as cooking oil and as bakery fat (Eady *et al.,* 2011).

In the baking industry, a number of functions are induced by lipids (Ghotra *et al.,* 2002; Rogers, 2004). It includes tenderization, mouthfeel, structural integrity, lubrication, air incorporation, heat transfer and shelf life extension. Because of their functional properties (e.g. creaming ability), plastic shortenings, which are often made by partial hydrogenation, are commonly used in the baking industry (Zhou *et al.,* 2011). However, trans fat generated during the hydrogenation process increases the risk of coronary heart disease (Dhaka *et al.,* 2011). The Food Safety and Standards Act, 2006 recommend consuming less than 10 per cent of calories from saturated fatty acids by replacing them with monounsaturated and polyunsaturated fatty acids, and to reduce trans fat intake not more than 5 per cent by weight (FSSAI, 2006). In recent years, the food industry has been making efforts to reduce trans fat by blending oils (high oleic and low linolenic) with fully hardened oils (palm), or by randomizing through interesterification (Wassell and Young 2007; Jeyarani *et al.,* 2009; Sahri and Dian, 2011; Musavi *et al.,* 2011). However, because of its supreme characters such as plasticity, which leads to desired physical qualities of baked products, lipids containing trans fat are still widely used in food industry (Jeyarani *et al.,* 2009). Recently consumers have become more concerned about the health implications of trans fats which are produced in the baked products due to usage of hydrogenated fat at high baking temperatures. Studies have shown that rice bran oil semisolid fraction (RBOF) can also be incorporated into baked food formulations with improvement in oxidative stability in baked foods.

Baking is a developing industry in India, which is growing in size. Foods that are convenient, with good taste, reasonably priced and carry a favourable nutritional image are in great demand. Among bakery products, fat is one of the major ingredients. The functional and nutritional properties of RBOS has appeared well suited to its usage as shortening in various baked goods. The present work was

[1]MFPI - Quality Control Laboratory, PJTSAU, Rajendranagar, Hyderabad
[2]ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad

carried out to evaluate the suitability of rice bran oil and two varieties of rice bran oil spread (RBOS1 and RBOS2) in baked products like cakes, cookies, piecrust, muffins and doughnuts and study the sensory acceptability.

MATERIALS AND METHODS

Raw materials such as hydrogenated fat, refined wheat flour, sugar, baking powder were procured from the local market. Two types of rice bran oil spread *viz.,* RBOS1 and RBOS2 were developed and standardized using edible gelators (mixture of β- sitosterol and gamma oryzanol) at the ICAR-Indian Institute of Rice Research, Hyderabad for the product development of cake. RBOS1 was a hard variant of RBO spread with 5 per cent edible gelator whereas; RBOS2 was a soft variant of the RBO spread with 2 per cent edible gelator. Cakes, cookies, piecrusts, muffins and doughnuts made with HF served as control, whereas those made with RBO, RBOS1 and RBOS2 served as experimental samples. Cakes, cookies, doughnuts and muffins were made following the standard creaming method (Gisslen, 2008) and piecrusts were prepared using standard flaky dough procedure (Gisslen, 2008) using HF, RBO, RBOS1 and RBOS2.

Sensory evaluation: The sensory assessments were conducted in a purpose-built, six-booth sensory evaluation laboratory. A panel of 30 members consisted of staff and graduate students of the Department of Foods and Nutrition, Post Graduate and Research Centre, Professor Jayashankar Telangana State Agricultural University and ICAR-Indian Institute of Rice Research, Hyderabad. The panelists had no knowledge of the project objectives. Sensory evaluation of cake, cookies, piecrusts, muffins and doughnut was conducted on five different days. Cake, cookies, piecrusts, muffins and doughnut samples (prepared with HF, RBO, RBOS1 and RBOS2) were coded using random three-digit numbers and served with the order of presentation counter-balanced. Panelists were provided with a glass of water and instructed to rinse and swallow water between samples. They were given written instructions and asked to evaluate the products for acceptability based on its colour, texture, taste, sponginess, flavour and overall acceptability using nine point hedonic scale (0 = Dislike extremely to 9 = Like extremely; Meilgard *et al.,* 1999).

RESULTS AND DISCUSSION

Cakes: The results of sensory evaluation of cake developed with HF, RBO, RBOS1 and RBOS2 are given in Table 1. The results of colour of cake made with HF (7.80 ± 0.78), RBO (7.87 ± 0.74), RBOS1 (7.67 ± 0.82) and RBOS2 (8.00 ± 0.66) had no significant difference. Similarly, there were no significant differences in flavour of the cakes made with HF (7.40 ± 0.74), RBO (7.53 ± 1.13), RBOS1 (7.60 ± 0.91) and RBOS2 (7.67 ± 0.62). Cakes made with HF (7.60 ± 1.12), RBO (7.93 ± 0.97), RBOS1 (7.53 ± 0.74) and RBOS2 (8.00 ± 0.66) did not vary significantly in taste. There was a significant ($p<0.05$) difference observed in the texture of cake made with HF (7.00 ± 0.78) and RBOS2 (7.73 ± 0.59). The results indicated that there was no significant difference in sponginess of cake made with HF (7.47 ± 1.06), RBO (7.67 ± 0.90) and RBOS2 (7.80 ± 0.78). The cake made with RBOS2 was rated high on 9 point hedonic scale for sponginess, as compared to RBOS1 cake which was rated as lowest. Overall acceptability of the cake made with RBOS2 was rated high (8.133±0.516) as compared to other cakes indicating that, RBOS2 can be utilized for cake preparation over other fat sources used in the study.

Texture properties of bakery products have been always of great importance since firmness of the cake defines its quality. Important quality parameters of cakes like texture, colour, taste, have an important effect on structure and the taste of cakes (Wilderjans *et al.,* 2013) and, lipids in baking contribute to products' quality characteristics such as tenderness, moist mouthfeel, lubricity, flavour, structure and shelf life (Matsakidou *et al.,* 2010; Dogan *et al.,* 2007). The cake quality is related to its aerated structure, which is formed by the incorporation of air during whipping as well as the development of bubbles during cooking (Psimouli and Oreopoulou, 2011). Cakes prepared with RBOS2 had good sensory characters due to the spongy texture, which was the effect of good aeration developed during baking. Results of our study indicate that RBOS2 can be incorporated as a lipid source to impart desired sensory attributes.

Cookies: The results of sensory evaluation of cookies developed with HF, RBO, RBOS1 and RBOS2 are given in Table 2. As per the results obtained, it was seen that there was a significant difference ($P<0.05$) between the colour of the cookies made with HF, RBO, RBOS1 and RBOS2. The colour of cookies made with RBOS2 was rated high with a score of 7.60 ± 0.74, on a 9 hedonic scale compared to other cookies. RBOS2 cookies were given highest score (7.07 ± 0.46) for texture compared to RBOS1 (6.73 ± 1.10), RBO (6.60 ± 0.91) and HF (6.87 ± 0.64) cookies on 9 point hedonic scale. The taste and flavour attributes received significantly ($p<0.01$) higher score for cookies made with RBOS2 and least score for cookies made with RBO as compared to other cookies. Jacob and Leelavathi (2007) studied the effect of fat types on cookie dough and cookie quality and reported that cookies containing liquid oil had relatively harder texture compared to bakery and hydrogenated fat. Our results showed no significant difference in the overall acceptability of cookies made with RBOS2 (7.40 ± 0.51) and HF (7.13 ± 0.64) as compared to cookies made with RBO (6.40 ± 0.63) and RBOS1 (6.93 ± 0.59).

Piecrust: The results of sensory evaluation of piecrust developed with HF, RBO, RBOS1 and RBOS2 are given in Table 3. The results of piecrust made from RBOS2 (8.00 ± 0.66) had significantly highest rating in colour, texture, tenderness, taste, flavour and overall acceptability on a 9 point hedonic scale. It was also found that there was a significant difference ($p<0.05$) between the colour, texture, tenderness and flavour made with HF, RBO, RBOS1 and RBOS2. Pie crusts made with RBO (7.13 ± 0.52) received the lowest rating in overall acceptability, flavour, colour, taste and texture.

In piecrust production, shortening and flour are first pinched together. Then water is added to form the crust dough. During this process, the role of lipid is to prevent excessive gluten formation and create pockets in the dough (Stauffer, 1998). Gluten in dough is formed by the interaction of flour and water (Patient, 1994). The majority of gluten is formed when water is added in the second stage of mixing, after the shortening and flour have been combined. RBOS2 with 2 per cent edible gelator was a semi fluid like fat spread which limited the gluten development in the flour which leads to highly rated sensory texture compared to other fats used in the study. Lipids with a more fluid character tend to limit gluten development, since flour particles are covered by lipid and have less chance to interact with water (Pyler, 1988). However, solid lipids like HF used in the study are more likely to be coated by flour particles than vice versa, and form pockets of lipid within the dough. Therefore, flour proteins are able to interact with water, leading to increased gluten development and tougher pie crusts when made with HF. The lowest ratings of pie crust made with RBO might be due to its crumbly texture, which comes from its underdeveloped gluten as RBO is a lipid with more fluid character. The high acceptability of pie crust made with RBOS2 might be due to its tenderness. Ghotra *et al.* (2002) reported that in pie crusts, lipids with a proper solid character and melting point help contribute to desirable flakiness and tenderness.

Muffin: The results of sensory evaluation of muffins developed with HF, RBO, RBOS1 and RBOS2 are given in Table 4. Muffins prepared with HF were awarded higher scores for colour and texture in comparison to RBO, RBOS1 and RBOS2 muffins. It was found that there was a significant ($p<0.01$) difference between colour and texture made with HF, RBO, RBOS1 and RBOS2 fats. Maximum scores for sponginess (7.67 ± 6.82) and taste were awarded to RBOS2 (7.80 ± 0.86) muffin, whereas minimum score was awarded to muffins prepared with RBOS1 on a 9 point hedonic scale. It was also observed that there was no significant difference in sponginess of muffins made with HF, RBO, RBOS1 and RBOS2. Overall acceptability of RBOS2 muffins (7.87 ± 0.64) was scored best on 9 point hedonic scale in comparison to other muffins. Many researches are in favour of replacements of hydrogenated vegetable fats in muffins. These substitutions may vary in their ingredients, and this replacers come from lipids (Sowmya *et al.,* 2009), fibers (Lee *et al.,* 2011) and hydrocolloids (Zambrano *et al.,* 2005; Gómez *et al.,* 2007). RBOS2 being a combination of rice bran oil and hydrocolloids can be best suitable in preparation of muffins.

Doughnut: The results of sensory evaluation of doughnuts developed with HF, RBO, RBOS1 and RBOS2 are given in Table 5. As per the results obtained, it was observed that, there was no significant difference between colour and texture of doughnuts made with HF, RBO, RBOS1 and RBOS2. The colour score of doughnuts made with HF (7.47 ± 1.13) and RBOS2 doughnut (7.47 ± 0.83) were similar and higher as compared to other fats used in the study. It was also observed that there was no significant difference in taste of doughnut made with RBOS1 (7.07 ± 0.96), HF (6.73 ± 1.16) and RBOS2 (7.20 ± 0.78); HF (6.73 ± 1.16) and RBO (6.40 ± 0.91). The flavour of doughnut made with RBOS2 (7.61 ± 0.96) was rated higher than other fats used in this study, while RBO (6.07 ± 1.71) doughnut was rated least.

The overall acceptability of doughnuts made with RBOS2 (7.53 ± 0.74) was scored high and was considered as best among all the fats. The results show that the taste, flavour and overall acceptability of RBOS2 doughnuts was significantly higher ($P<0.05$) as compared to other doughnuts indicating that, RBOS 2 can be used as a potential fat source for preparation of doughnuts. Tan and Mittal (2006) and Bouchon *et al.* (2003) reported that doughnuts fried for shorter period of time had lower oil uptake and higher moisture, which is similar to doughnuts prepared with RBOS2 in our study.

Overall acceptability of the baked products: The results of overall acceptability of cakes, cookies, piecrust, muffin and doughnut is given in Fig. 1. Among all the products, RBOS2 cake was rated significantly higher on a 9 point hedonic score card indicating that though the colour, flavour, taste did not differ much, texture, sponginess and over all acceptability was definitely superior when made with RBOS2. Overall acceptability of the cookies, piecrust, muffins and doughnuts made with RBOS2 were rated high as compared to other products indicating that the RBOS2 can be utilized for preparation of baked products over other fat sources. Consumption of the HF products might prove to be harmful if consumed in large amounts and at higher frequencies due to the presence or formation of transfatty acids. Hence, RBOS2 can be promoted as a healthy alternative to hydrogenated fat for preparation of baked products.

Table 1 Sensory evaluation of cake

Sensory attributes	HF	RBO	RBOS1	RBOS2	F Value	SE Value	CD value
Colour	7.80 ± 0.78	7.87 ± 0.74	7.67 ± 0.82	8.00 ± 0.66	0.96NS	0.141	0.404
Texture	7.00 ± 0.76b	7.80 ± 1.01a	7.20 ± 0.78b	7.73 ± 0.59a	6.53**	0.154	0.440
Sponginess	7.47 ± 1.06ab	7.67 ± 0.90a	7.20 ± 0.94b	7.80 ± 0.78a	2.15*	0.177	0.507
Taste	7.60 ± 1.12	7.93 ± 0.96	7.53 ± 0.74	8.00 ± 0.66	1.64NS	0.182	0.521
Flavour	7.40 ± 0.74	7.53 ± 1.13	7.60 ± 0.91	7.67 ± 0.62	0.51NS	0.160	0.457
Overall acceptability	7.73 ± 0.80ab	7.93 ± 0.96a	7.47 ± 0.64b	8.13 ± 0.52a	3.90*	0.144	0.411

Note: Values are expressed as Mean ± SD, *significant at 5% level, **significant at 1% level, NS- non significant
HF -Hydrogenated Fat; RBO - Rice Bran Oil; RBOS1 - Rice Bran Oil Spread 1; RBOS2 - Rice Bran Oil Spread

Table 2 Sensory evaluation of cookies

Sensory attributes	HF	RBO	RBOS1	RBOS2	F value	SE Value	CD value
Colour	7.20 ± 0.94a	6.73 ± 1.03b	7.00 ± 0.76ab	7.60 ± 0.74a	2.95*	0.212	0.607
Texture	6.87 ± 0.64	6.60 ± 0.91	6.73 ± 1.10	7.07 ± 0.46	0.88Ns	0.212	0.606
Taste	7.13 ± 0.74ab	6.27 ± 0.80c	6.73 ± 0.80bc	7.33 ± 0.62a	7.00**	0.178	0.508
Flavour	6.73 ± 0.88ab	6.33 ± 0.82b	6.60 ± 0.63b	7.13 ± 0.64a	3.68*	0.173	0.495
Overall acceptability	7.13 ± 0.64ab	6.40 ± 0.63c	6.93 ± 0.59b	7.40 ± 0.51a	8.41**	0.146	0.416

Note: Values are expressed as Mean ± SD, *significant at 5% level, **significant at 1% level, NS- non significant HF -Hydrogenated Fat; RBO - Rice Bran Oil; RBOS1 - Rice Bran Oil Spread 1; RBOS2 - Rice Bran Oil Spread 2.

Table 3 Sensory evaluation of piecrust

Sensory attributes	HF	RBO	RBOS1	RBOS2	F Value	SE Value	CD Value
Colour	7.80 ± 0.56a	7.20 ± 0.94b	7.53 ± 0.52ab	8.00 ± 0.54a	4.18*	0.169	0.483
Texture	7.73 ± 0.46a	6.93 ± 0.59b	7.40 ± 0.99ab	7.67 ± 0.72a	3.85*	0.185	0.528
Tenderness	7.33 ± 0.62ab	7.20 ± 0.78b	7.13 ± 0.52b	7.80 ± 0.68a	2.72*	0.182	0.520
Taste	7.00 ± 0.54bc	6.73 ± 0.88c	7.27 ± 0.46ab	7.73 ± 0.96a	6.34**	0.169	0.483
Flavour	7.40 ± 0.51ab	7.13 ± 0.64b	7.47 ± 0.64a	7.67 ± 0.62a	1.90*	0.159	0.455
Overall acceptability	7.53 ± 0.52bc	7.13 ± 0.52c	7.67 ± 0.49ab	8.00 ± 0.66a	6.24**	0.143	0.409

Note: Values are expressed as Mean ± SD, *significant at 5% level, **significant at 1% level, NS- non significant
HF -Hydrogenated Fat; RBO - Rice Bran Oil; RBOS1 - Rice Bran Oil Spread 1; RBOS2 - Rice Bran Oil Spread 2.

Table 4 Sensory evaluation muffin

Sensory attributes	HF	RBO	RBOS1	RBOS2	F value	SE value	CD value
Colour	8.00 ± 0.76a	7.67 ± 0.62a	7.20 ± 0.68b	7.87 ± 0.74a	5.84**	0.144	0.413
Texture	7.93 ± 0.89a	7.60 ± 0.74ab	7.27 ± 0.59b	7.13 ± 0.74b	4.77**	0.164	0.468
Sponginess	7.53 ± 0.52	7.60 ± 0.83	7.40 ± 0.63	7.67 ± 6.82	0.40Ns	0.181	0.516
Taste	7.33 ± 0.49ab	7.73 ± 0.88a	7.07 ± 0.80b	7.80 ± 0.86a	3.89*	0.175	0.500
Flavour	7.53 ± 0.52a	7.40 ± 0.91a	6.73 ± 0.59b	7.53 ± 0.74a	4.13*	0.188	0.538
Overall acceptability	7.80 ± 0.41a	7.73 ± 0.46a	7.27 ± 0.59b	7.87 ± 0.64a	3.84*	0.138	0.396

Note: Values are expressed as Mean ± SD, *significant at 5% level, **significant at 1% level, NS- non significant
HF -Hydrogenated Fat; RBO - Rice Bran Oil; RBOS1 - Rice Bran Oil Spread 1; RBOS2 - Rice Bran Oil Spread 2.

Table 5 Sensory evaluation of doughnut

Sensory attributes	HF	RBO	RBOS1	RBOS2	F value	SE value	CD value
Colour	7.47 ± 1.13	7.13 ± 0.92	7.00 ± 0.76	7.47 ± 0.83	1.37NS	0.203	0.579
Texture	7.60 ± 0.83	7.07 ± 1.10	7.40 ± 0.89	7.47 ± 0.92	1.20NS	0.206	0.590
Taste	6.73 ± 1.16ab	6.40 ± 0.91b	7.07 ± 0.96a	7.20 ± 0.78a	2.42*	0.230	0.657
Flavour	6.67 ± 1.40ab	6.07 ± 1.71b	6.93 ± 1.00a	7.61 ± 0.80a	2.53*	0.278	0.795
Overall acceptability	7.20 ± 0.94a	6.80 ± 0.86b	7.00 ± 0.93ab	7.53 ± 0.74a	2.36*	0.203	0.580

Note: Values are expressed as Mean ± SD, *significant at 5% level, **significant at 1% level, NS- non significant
HF -Hydrogenated Fat; RBO - Rice Bran Oil; RBOS1 - Rice Bran Oil Spread 1; RBOS2 - Rice Bran Oil Spread

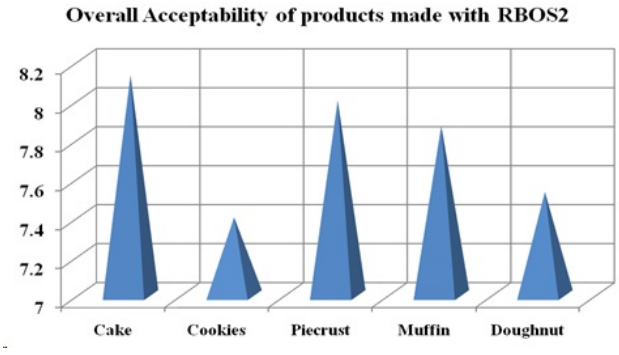

Fig. 1. Overall acceptability of baked products prepared with HF -Hydrogenated Fat; RBO - Rice Bran Oil; RBOS1 - Rice Bran Oil Spread 1; RBOS2 - Rice Bran Oil Spread

Lipids play an important role in the majority of baked products (Rios, 2014). When choosing a lipid source for baking, the nutritional characteristics of lipid and the ability of the lipid to impart a desired physical quality to the finished product need to be considered. One of the key dietary recommendations is to consume less than 10 percent of calories from saturated fatty acids by replacing them with monounsaturated and polyunsaturated fatty acids, and to reduce TFA intake as much as possible (Cheong *et al.,* 2011). RBOS2 being a fat spread with less amount of saturated fat can be considered as a healthy fat compared to HF and it is important to consider the nutritional characteristics of the lipid sources included in food production.

The role of fat in manufactured bakery products in general is very important both from the technological point of view and the sensory point of view. Many bakery products require a relatively high fat content, as reported by Sowmya *et al.* (2009). According to Zhou *et al.* (2011), shortenings have numerous functions in bakery products; among them are texture, softness, structure integrity, mouthfeel, lubrication, air entrapment, heat transfer, and extended shelf life. In addition to the nutritional profiles, lipids should be selected according to their specific performance in the finished product and the study proves that RBOS2 fat spread can give the desired sensory characteristics in baked products like cakes, cookies, piecrust, muffins and doughnuts.

As per the results of sensory evaluation of cookies, cakes, piecrusts, muffins and doughnuts, it was observed that all the baked products made with RBOS2 were given significantly higher scores compared to products made with other fat sources like hydrogenated fat, Rice bran oil and RBOS1. The results indicate that Rice Bran Oil Spread (RBOS) can be used as a potential fat for preparation and production of baked products like cakes, cookies, muffins, pie crusts and doughnuts with desired sensory and quality characteristics such as tenderness, moistness, sponginess, flavour, texture and overall acceptability. Apart from physical quality characteristics, RBOS is plant source oil derived from a mix of rice bran oil and hydrocolloids, which is healthier compared to hydrogenated fats or other animal fats.

REFERENCES

Bouchon P, Aguilera J M and Pyle D L 2003. Structure oil-absorption relationships during deep-fat frying. *Journal of Food Science*, **68**(9): 2711-2716.

Cheong L Z, Tan C P, Long K and Idris N A 2011. Baking performance of palm diacylglycerol bakery fats and sensory evaluation of baked products. *European Journal of Lipid Science and Technology*, **113**: 253-261.

Dhaka V, Gulia N, Ahlawat K S and Khatkar B S 2011. Trans fats-sources, health risks and alternative approach - a review. *Journal of Food Science and Technology*, **48**: 534-541.

Dogan I S, Javidipour I and Akan T 2007. Effect of interesterified palm and cottonseed oil blends on cake quality. *International Journal of Food Science and Technology*, **42**: 157-164.

Eady S, Wallace A, Willis J, Scott R and Frampton C 2011. Consumption of a plant sterol based spread derived from rice bran oil is effective at reducing plasma lipid levels in mildly hypercholesterolaemic individuals. *The British Journal of Nutrition*, **105**: 1808-1818.

FSSAI 2006. *Food Safety and Standards Authority of India* (FSSAI). Food Safety and Standards Authority of India, Government of India. Retrieved 2 April 2012.

Ghotra B S, Dyal S D and Narine S S 2002. Lipid shortenings: a review. *Food Research International*, **35**: 1015-1048.

Gisslen W 2008. *Professional Baking.* 6th edition, John Wiley & Sons.

Gómez M, Ronda F, Caballero P A, Blanco C A and Rosell C M 2007. Functionality of different hydrocolloids on the quality and shelf-life of yellow layer cakes. *Food Hydrocolloids*, 167-173.

Jacob J and Leelavathi K 2007. Effect of fat-type on cookie dough and cookie quality. *Journal of Food Engineering*, **79**: 299-305.

Jeyarani T, Khan I M and Khatoon S 2009. Trans-free plastic shortenings from coconuts stearin and palm stearin blends. *Food Chemistry*, **114**: 270-275.

Juliano C, Cossu M, Alamanni M C and Piu L 2005. Antioxidant activity of gamma-oryzanol: Mechanism of action and its effect on oxidative stability of pharmaceutical oils. *International Journal of Pharmaceutics*, **299**: 146-154.

Lee J W, Lee S W, Kim M K and Rhee C 2005. Beneficial effect of the unsaponifiable matter from rice bran on oxidative stress *in vitro* compared with a α-tocopherol. *Journal of the Science of Food and Agriculture*, **85**: 493-498.

Lee K Y, Park S Y and Lee H G 2011. Effect of oat β-glucan and of its oxidized derivative on the quality characteristics of sponge cakes. *International Journal of Food Science and Technology*, **46**: 2663-2668.

Matsakidou A, Blekas G and Paraskevopoulou A 2010. Aroma and physic characteristics of cakes prepared by replacing margarine with extra virgen olive oil. *Food Science and Technology*, **43**: 949-957.

Meilgaard M, Civille G V and Carr B T 1999. *Sensory Evaluation Techniques*. 3rd edition, CRC Press, Boca Raton, Florida.

Miller A and Engel K H 2006. Content of g-oryzanol and composition of steryl ferulates in brown rice (*Oryza sativa* L.) of European origin. *Journal of Agricultural and Food Chemistry*, **54**: 8127-8133.

Musavi A, Tekin A and Erinc H 2011. Formulation of trans-free margarines using hydrogenated and interesterified palm olein. *Journal of Oil Palm Research*, **23**: 1153-1158.

Patient D 1994. The chemistry of pastry products. *Nutrition and Food Science*, **94**: 33-35.

Pyler E J and Merriam K S 1988. Sosland Publishing Company. *Baking Science and Technology*, **2**(3): 984-987.

Psimouli V and Oreopoulou V 2011. The effect of alternative sweeteners on batter rheology and cake properties. *Journal of Science Food Agricultural*, **92**(1): 99-105.

Rios R V, Pessanha M D, Ferreira A, Poliana F, Clara V L and Lannes S C 2014. Application of fats in some food products. *Food Science and Technology*, **34**(1): 3-15.

Rogers D 2004. Functions of fats and oils in bakery products. *American Oil Chemists Society*, **15**: 572-574.

Sahri M M and Dian N L H M 2011. Formulation of trans-free and low saturated margarine. *Journal of Oil Palm Research*, **23**: 958-967.

Sen C K, Khanna S and Roy S 2006. Tocotrienols: Vitamin E beyond tocopherols. *Life Science*, **78**: 2088-2098.

Sen C K, Khanna S and Roy S 2007. Tocotrienols in health and disease: The other half of the natural vitamin E family. *Molecular Aspects of Medicine*, **28**: 692-728.

Sharma A R 2002. Edible rice bran oil - consumer awareness programme. Rice bran oil promotion committee, Solvent Extractors Association of India, Mumbai.

Sowmya M, Jeyarani T, Jyostna R and Indrani D 2009. Effect of replacement of fat with sesame oil and additives on rheological, microstructural, quality characteristics and fatty acid profile of cakes. *Food Hydrocolloids*, **23**(7): 1827-1836.

Stauffer C E 1998. Fats and oils in bakery products. *Cereal Food World*, **43**: 120-126.

Tan K J and Mittal G S 2006. Physiochemical properties changes of donuts during vacuum frying. *International Journal of Food Properties*, **9**: 85-98.

Wassell P and Young N W G 2007. Food applications of Trans fatty acid substitutes. *International Journal of Food Science and Technology*, **42**: 503-517.

Wilderjans E, Luyts A, Brijs K and Delcour J A 2013. Ingredient functionality in batter type cake making. *Trends in Food Science and Technology*, **30**(1): 6-15.

Yokoyama W H 2004. Plasma LDL cholesterol lowering by plant phytosterols in a hamster model. *Trends in Food Science and Technology*, **15**: 528-531.

Zambrano F, Hikage A, Ormenese R C C, Montenegro F M and Rauen-Miguel A M 2005. Effect of guar and xanthan gums as fat substitutes in industrially produced cakes. *Brazilian Journal of Food Technology*, **8**(1): 63-71.

Zhou J, Jon M F and Chuck E W 2011. Evaluation of different types of fats for use in high ratio layer cakes. *Food Science and Technology*, **44**: 1802-1808.

Oilseeds production and yield forecasting using ARIMA-ANN modelling

S RAVICHANDRAN*, B S YASHAVANTH AND K KAREEMULLA

ICAR-National Academy of Agricultural Research Management, Rajendranagar, Hyderabad - 500 030, Telangana

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ABSTRACT

Agriculture, the backbone of Indian economy, lot of time series data on various parameters and these time series data generated from agriculture can be effectively modelled using various time-series modelling techniques such as Box-Jenkins ARIMA modelling technique, State-Space modelling technique, Structural Time Series modelling methodology and various other time series modelling methodologies depending upon the availability and properties of the given datasets. Modelling and forecasting of time-series data on all-India production and productivity of nine oilseed crops from 1950-51 to 2015-16 is carried out in this study. Modelling and subsequent forecasting for the datasets under consideration is performed using Autoregressive Moving Average (ARIMA), Artificial Neural Network (ANN) and ARIMA-ANN hybrid modelling methodologies. It is important to note that ARIMA is a linear modelling methodology whereas ANN is a non-linear modelling methodology. ARIMA-ANN is a hybrid of these two methodologies which can efficiently capture both linear and non-linear structures present in the dataset under consideration. For efficient performance of ARIMA modelling, the data under consideration needs to satisfy stationarity criterion. i.e. mean and variance should constant over a period of time. First, the dataset on oilseeds production and productivity are tested for stationarity and subsequent to non-stationarity of the original data, first order differenced series are considered for modeling using the Box-Jenkins approach. Various ARIMA models are developed and among them ARIMA (1,1,0) and ARIMA (1,1,1) are found suitable for the production and productivity data, respectively, based on the Information Criteria such as Akaike Information Criterion (AIC) and Schwarz-Bayesian Criterion (SBC). Among the developed ANN models, the Neural Network Autoregression (NNAR) of order NNAR (2,2) is found to be suitable for both the variables under study. Later, models using ANN-ARIMA hybrid methodology are developed and the ARIMA (1,1,0)-NNAR(1,1) and ARIMA (1,1,1)-NNAR (1,1) for production and productivity are found to be suitable, respectively. All the three models are tested for their forecast accuracy using Mean Absolute Percentage Error (MAPE) and Root Mean Square Error (RMSE). Accordingly, the ARIMA-ANN hybrid methodology is found to be superior to the individual ARIMA and ANN methodologies. Based on the efficient developed model *viz.,* hybrid ARIMA-ANN model, forecasting annual all-India oilseeds production for the year 2022 is carried out and is found to be 35.6 (±3.2) million tonnes with a productivity of 1178 (±94.6) kg/hectare.

Keywords: ANN, ARIMA, ARIMA-ANN, Forecasting, Oilseeds, Time series

Indian agriculture has made substantial progress, particularly with respect to food crops like wheat and rice. But, the performance has not been that good in case of other crops like oilseeds, pulses, and coarse cereals. On the global oilseeds map, India occupies a prominent position in both acreage as well as production. A wide range of oilseed crops is produced in different agro-climatic regions of the country. Three main oilseeds namely, groundnut, soybean, and rapeseed-mustard accounted for over 88 per cent of total oilseeds output during 2011-12. The other important oilseed crops are sunflower, sesame, niger, linseed, safflower and castor. Except castor, all other oilseed crops along with palm oil are collectively called as edible oil crops. Edible oil constitutes an important part of our daily diet. Besides being source of energy, they are also a source for fatty acids like linoleic and oleic acids, amino acids like lysine, leusine, histidine, tryptophan etc., which are essential for our growth. Domestic consumption of edible oils has increased substantially over the years and has touched the level of 19.82 million tons in 2012-13 (Nov-Oct) and is likely to increase further with enhancement in income and population. With this background, an attempt is made to study and forecast the production and yield of these nine oilseed crops using time series modelling techniques.

Several time series analysis techniques are available in the literature and their optimum employment for modelling and forecasting the data depends upon the characteristics of the data. For example, when the data series is linear, the Auto-Regressive Integrated Moving Average (ARIMA) can be efficiently employed most of the times. But, under non-linear situations, the ARIMA models become inefficient due to their inability to capture non-linear structures in the data. Under such situations, one can opt for non-linear time series modelling techniques. ARIMA models (Box *et al.* 1994) have been appreciated for crop yield or any other agricultural production forecasting. Sarika *et al.* (2011) employed, ARIMA model for modeling and forecasting India's pigeon pea production data. Suresh *et al.* (2011)

*Corresponding author: srgmravi@yahoo.com

employed ARIMA model for forecasting sugarcane area, production and productivity of Tamil Nadu state of India. Recent research indicates that combining different models enhances the accuracy of forecasting as compared to individual model. The hybrid methodology given by Zhang (2003) is one of the most popular hybrid technique which combines ARIMA and ANN models. Naveena *et al.* (2017) used the ARIMA-ANN hybrid model to forecast the price of washed coffee. Mrinmoy *et al.* (2016) proposed the ARIMA-WNN model to forecast wheat yield.

MATERIALS AND METHODS

The time series data used for this study comprises of production and yield of nine oilseed crops for the period of 1950-51 to 2015-16 since data for 2016-17 is not available and hence not used. Out of the total available 66 years' data, the first 61 years' data are used for training the model and the last 5 years' data are used for model validation. The data is obtained from the Government of India publication: Agricultural Statistics at a glance, 2016. The most popular time series model used for short term forecasting is the ARIMA models, which are an extension to the ARMA models and comprise the most general class of time series models useful in modelling and forecasting non-stationary time series. In an ARIMA model, it is assumed that the time series under study is a linear function of the past values and random shocks.

In general, an ARIMA model, represented as ARIMA (*p, d, q*), comprises three components: (i) p, the order of Auto-Regression (AR); (ii) d, the order of integration (differencing) to achieve stationarity; and (iii) the order of Moving Average (MA). ARIMA technique is a parsimonious approach representing both stationary and non-stationary processes.

Consider an ARMA (p, q) process defined by Equation

$$y_t = \mu + \phi_1 y_{t-1} + \phi_2 y_{t-2} + \ldots + \phi_p y_{t-p} + \varepsilon_t - \theta_1 \varepsilon_{t-1} - \theta_2 \varepsilon_{t-2} - \ldots - \theta_q \varepsilon_{t-q} \quad (1)$$

where, y_t are the actual value and ε_t are random shocks at time period t.ϕ_i (i=1, 2,……,p) and θ_i (j=1, 2,……,q) are the model parameters. The random errors, ?t are assumed to be independently and identically distributed with a mean of zero and a constant variance of σ^2. The first and most important requirement in ARIMA modeling is to ensure that the series under study is stationary since the estimation procedure is available only for a stationary series. A series is regarded stationary if its statistical characteristics such as the mean and the autocorrelation structures do not change over time. The stationarity of a time series can be confirmed either by a time plot, which requires some experience or by using unit root tests. If a series is found to be non-stationary based on these tests, it can be made stationary by differencing. The number of times a series is differenced to achieve stationarity is referred to as the order of integration, d. Sometimes, other appropriate transformations like logarithmic transformation are used to achieve stationarity. Once the stationarity of the series is established, the Box-Jenkins approach, which goes in four steps *viz*., (i) identification (ii) estimation (iii) diagnostic checking and (iv) forecasting is employed. In the identification stage, multiple ARMA models with different values for q (MA terms) and p (AR terms) are chosen based on Auto-Correlation Function (ACF) and Partial Auto-Correlation Function (PACF), respectively. This is followed by the estimation stage, where parameters of the tentative models are estimated by employing any of the non-linear optimization procedures such that the overall measure of errors is minimized or the likelihood function is maximized. Among all the candidate models, the best suited ARIMA model is selected by using Information criteria. The model which has the smallest Akaike Information Criterion (AIC) or Schwarz- Bayesian Criterion (SBC) value is chosen as the best suited model for the data under study. In the diagnostic checking stage, the residuals are checked for the normality and adequacy of the model. In the final forecasting stage, the future values are forecasted using the chosen model.

Though ARIMA models are widely used, they are best suited only for linear models as they fail to capture the non-linear structures. A wide variety of ANN models are used in such cases where non-linear structures are to be captured. The popular Multi-Layer Perceptron (MLP) networks with two layers, one hidden and one output layer connected acyclically are very often used for non-linear time series modelling. In the MLP networks, the relation between the output x_t and inputs $x_{t-1}, x_{t-2}, \ldots, x_{t-p}$ is as below:

$$x_t = \alpha_0 + \sum_{j=1}^{q} \alpha_j \cdot g(\beta_{0j} + \sum_{i=1}^{p} \beta_{ij} x_{t-i}) + \varepsilon_t \quad (2)$$

where α's and β's are the model parameters, p is the number of input nodes, q is the number of hidden nodes and g is the transfer function. In case of ANN autoregressive models (ANNAR), the lagged variables x_{t-i} (i=1,2,…,p) are the inputs. Such an ANNAR model is represented as NNAR (p,q). Among several transfer functions, the logistic function given is most often used.

According to Zhang (2003), a time series is composed of a linear autocorrelation structure and nonlinear component as

$$x_t = L_t + N_t \quad (3)$$

where L_t and N_t denote the linear and non-linear components, respectively. Hence, a hybrid model which can capture both linear and non-linear components may perform better than the individual models. Building a hybrid ARIMA-ANN model consists of two steps. In the first step, the linear

component L_t is modelled by using ARIMA. The residuals of the ARIMA model,

$$e_t = x_t + \hat{L}_t \qquad (4)$$

contain information on the non-linearity of the series. In the second step, the residuals are modelled through an ANN in order to capture the non-linear relation of the series using p input nodes:

$$e_t = f(e_{t-1}, e_{t-2}, ..., e_{t-p}) + \varepsilon_t \qquad (5)$$

where f is a non-linear determined by the neural network and ε_t is the random error. The prediction of the residual e_t is denoted through the ANN model as $\hat{N}_t$. Thus the combined forecast provided by the hybrid model is given by

$$\hat{x}_t = \hat{L}_t + \hat{N}_t \qquad (6)$$

Forecast evaluation methods: The ability of different models to forecast the time series values is assessed by using two common performance measures, *viz.,* the root mean squared error (RMSE) and the mean absolute percentage error (MAPE). The RMSE measures the overall performance of a model and is given by Equation (7)

$$\text{RMSE} = \sqrt{\frac{1}{n}\sum_{t=1}^{n}\left(\hat{y}_t - y_t\right)^2} \qquad (7)$$

where, y_t is the actual value for time t, $\hat{y}_t$ is the predicted value for time t, and n is the number of predictions. The second criterion, the mean absolute percentage error is a measure of average error for each point forecast and is given by Equation (8)

$$\text{MAPE} = \frac{1}{n}\sum_{t=1}^{n}\left|\frac{\hat{y}_t - y_t}{y_t}\right| \times 100 \qquad (8)$$

where the symbols have the same meaning as above. The model with least RMSE and MAPE values is considered as the best model for the data.

RESULTS AND DISCUSSION

The foremost step in time series analysis is to plot the data under study to have a visual inspection over its behaviour. Figure 1 shows the time series plot of annual production and yield of oilseeds in India for the periods 1950-51 to 2015-16.

A perusal of Figure 1 indicates a positive trend in both production as well as yield indicating nonstationary of both the series. The Augmented Dickey-Fuller (ADF) test is performed to confirm the presence of non-stationarity in the original data, results of which are given the Table 1. The Table also includes the results of ADF test performed after first differencing. The values clearly indicate the non-stationarity of both original series. Hence, the series were differenced and tested for stationarity using ADF test, again. The ADF test results indicated that the differenced series are stationary and no further differencing is needed. Figure 2 gives the plot of annual production of rice and wheat after first differencing.

After obtaining the stationary series, the candidate ARIMA models were identified based on the Autocorrelation and Partial Autocorrelation functions. The autocorrelation and partial autocorrelation plots (Fig. 3) indicate that the maximum order AR is 2 and for MA is 1, since autocorrelation and partial autocorrelation coefficients are not significant for higher orders. Accordingly, 5 candidate ARIMA models are identified. Among these candidate models, the appropriate model is chosen based on the Information Criteria. The candidate models and their respective AIC and SBC values are given in table 2. From the table, it is pertinent that the ARIMA (1,1,0) model is best suited for modelling production and ARIMA (1,1,1) is best suited for modelling yield. Subsequently, both the series were modelled using neural networks. The NNETAR (2,2) model was found best for modelling both production yield.

The ARIMA models are successful in capturing the linear structures whereas the neural network models are successful in capturing the non-linear structures in the series. To capture both linear and non-linear effects, the ARIMA-ANN hybrid models are tried out where in the residuals obtained from the ARIMA models are once again modelled using the neural networks. The NNETAR (1,1) model was found suitable for modelling both the residual series. Later, both ARIMA model for the data series and the NNETAR models for the residual series are combined and used to forecast the production and yields, respectively. The performance of all the three models, *viz.,* ARIMA, ANN and ARIMA-ANN hybrid models is evaluated using forecast evaluation measures, namely, RMSE and MAPE (Table 3 and 4). The results indicated that the ARIMA-ANN hybrid models are best suited for forecasting the production and yield since it was successful in capturing both the linear and non-linear structures. Using the most efficient model *viz.,* hybrid ARIMA-ANN model, forecasting annual all-India oilseeds production for the year 2022 is carried out and is found to be 35.6 (±3.2) million tonnes with a productivity of 1178 (±94.6) kg/ha.

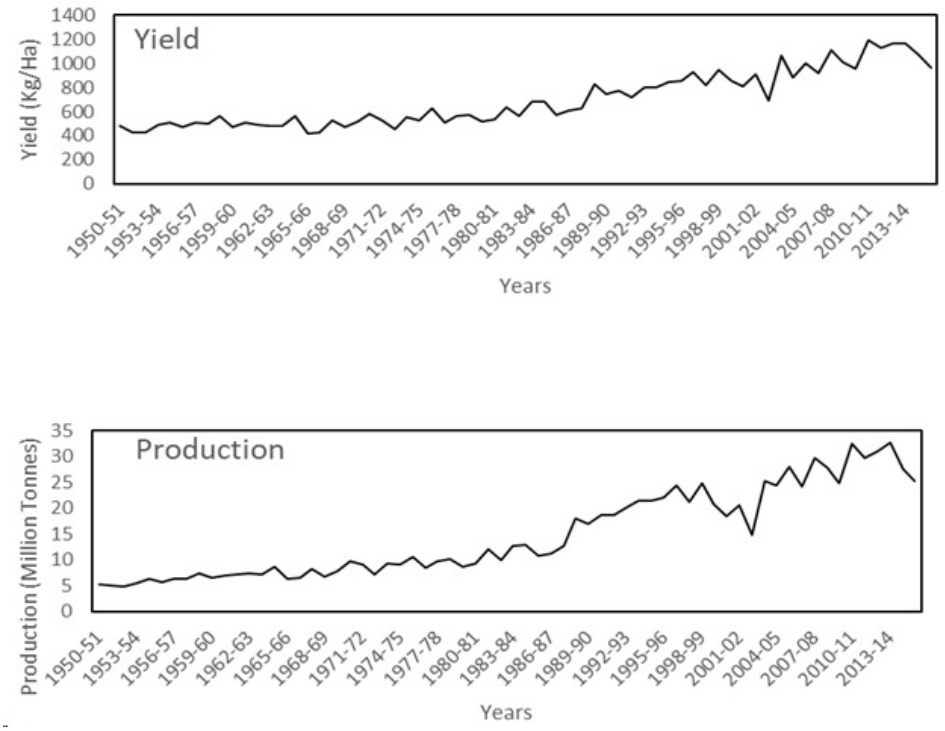

Fig. 1. Time plots of yield and production of nine oilseed crops

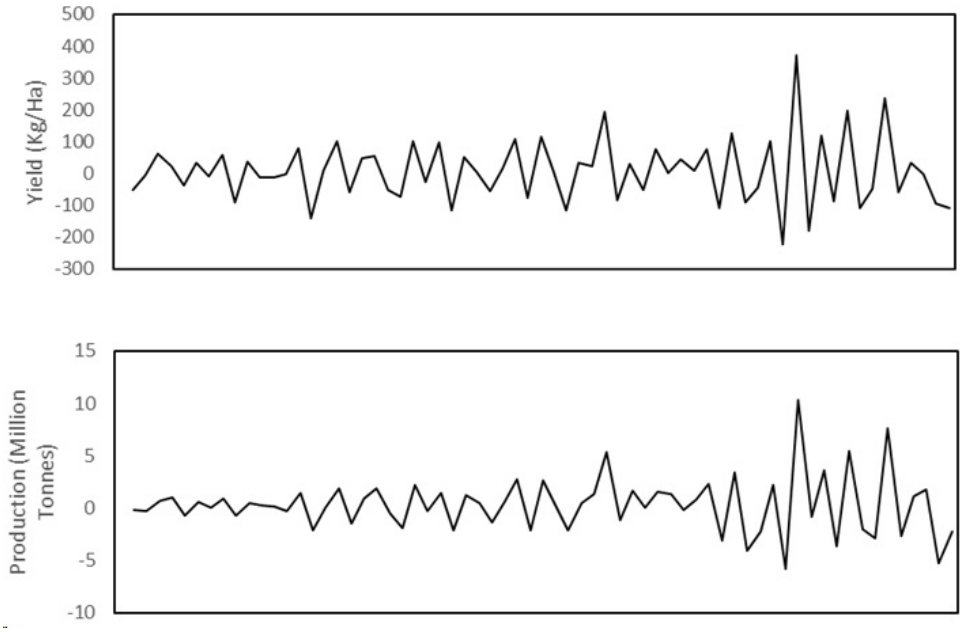

Fig. 2. Time plots of yield and production of nine oilseed crops after first differencing

Table 1 Results of augmented Dickey-Fuller test for stationarity

Series	Type	Before differencing		After differencing	
		Rho	Pr < Rho	Rho	Pr < Rho
Production	Zero mean	1.17	0.931	-81.66	<0.001
	Single Mean	-0.27	0.939	-98.86	<0.001
	Trend	-10.87	0.3416	-103.05	<0.001
Yield	Zero mean	0.69	0.848	-140.38	<0.001
	Single Mean	-0.86	0.894	-163.75	<0.001
	Trend	-16.13	0.114	-170.83	<0.001

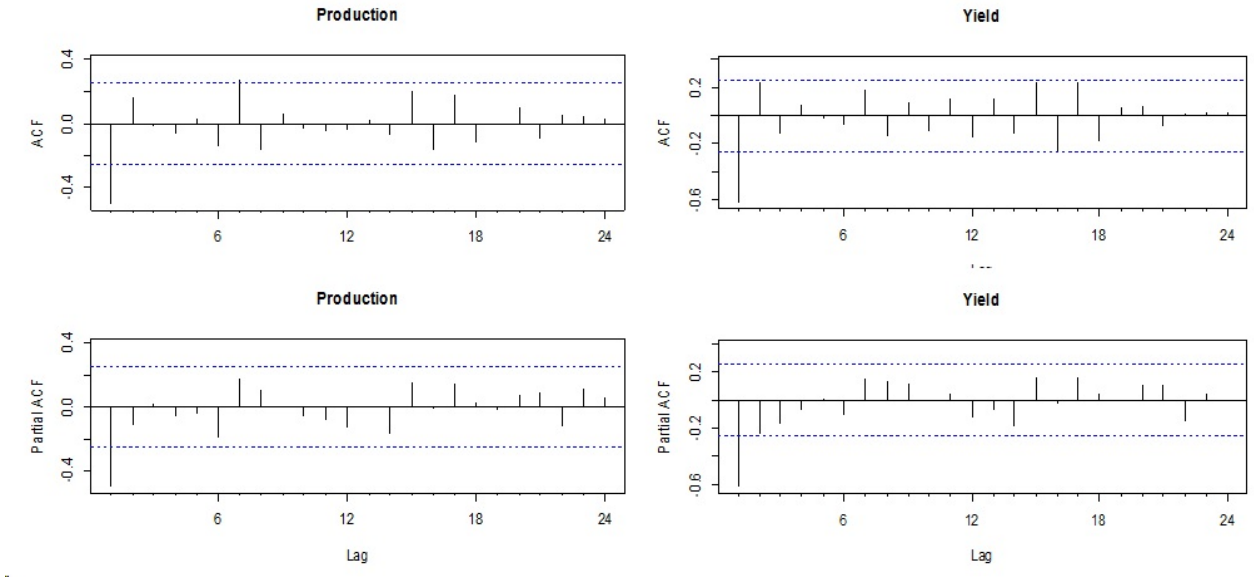

Fig. 3. Autocorrelation and partial autocorrelation plots

Table 2 Candidate ARIMA models and corresponding information coefficients

Production			Yield		
Model	AIC	SBC	Model	AIC	SBC
ARIMA (0,1,1)	261.737	265.890	ARIMA (0,1,1)	675.932	680.087
ARIMA (1,1,0)	259.818	263.973	ARIMA (1,1,0)	678.963	683.118
ARIMA (1,1,1)	261.617	267.850	ARIMA (1,1,1)	673.370	679.603
ARIMA (2,1,0)	261.616	267.848	ARIMA (2,1,0)	677.325	683.557
ARIMA (2,1,1)	261.213	269.523	ARIMA (2,1,1)	675.078	683.388

Table 3 Forecast evaluation measures for the model fitting data

Model	Production		Yield	
(Model Fitting)	RMSE	MAPE	RMSE	MAPE
ARIMA	2.45	12.07	66.83	8.34
ANN	2.02	11.60	66.63	8.33
ARIMA-ANN Hybrid	1.82	3.46	60.22	3.77

Table 4 Forecast evaluation measures for the model validation data

Model	Production		Yield	
(Model Validation)	RMSE	MAPE	RMSE	MAPE
ARIMA	10.63	32.96	239.72	14.11
ANN	2.92	8.99	116.72	8.77
ARIMA-ANN Hybrid	2.90	8.94	116.63	8.76

Time series analysis of agricultural data plays an important role in making policy decisions. It helps in fore-seeing, well in advance, the repercussions that may happen in due course of time and thus helps in being prepared to face the situations. There are many time series analysis methodologies available in the literature. Most used among them is the ARIMA methodology which owes its popularity to the Box-Jenkins model building process. But ARIMA model can capture only linear structures present in the data. To capture non-linear structures, one has to adopt non-linear methodologies like ANN. Hence, in the present study, the ARIMA-ANN hybrid methodology, which is a combination of both linear ARIMA and non-linear ANN methods, is used to model and forecast the all-India production and yield of nine oilseed crops. The importance of oilseed crops, which are a part of edible oils group is very well established by looking at the huge amount spent in importing them. Keeping all these points under consideration, the ARIMA- ANN hybrid methodology is used to forecast the production and productivity of oilseed crops for 2022 is found to be 35.6 (±3.2) million tonnes with a productivity of 1178 (±94.6) kg/hectare. The performance of the ARIMA-ANN methodology is also compared with the individual ARIMA and ANN methods using forecast accuracy measures, where in the hybrid methodology outperformed.

REFERENCES

Box G E P, Jenkins G M and Reinsel G C 1994. *Time Series Analysis, Forecasting and Control*. 3rd edition, Prentice Hall, Englewood Clifs.

Naveena K, Subedar Singh, Santosha Rathod and Abhishek Singh 2017. Hybrid ARIMA-ANN modelling for forecasting the price of Robusta coffee in India. *International Journal of Current Microbiology and Applied Sciences*, **6**(7): 1721-1726.

Ray M, Rai A, Ramasubramanian V and Singh K N 2016. ARIMA-WNN hybrid model for forecasting wheat yield time-series data. *Journal of the Indian Society of Agricultural Statistics*, **70**(1): 63-70.

Sarika, Iquebal M A and Chattopadhyay 2011. Modelling and forecasting of pigeonpea (*Cajanus cajan*) production using autoregressive integrated moving average methodology. *Indian Journal of Agricultural Sciences*, **81**(6): 520-523

Suresh K K and Krishna Priya S R 2011. Forecasting sugarcane yield of Tamil Nadu using ARIMA Models. *Sugar Tech*, **13**(1): 23-26

Zhang G 2003. Time series forecasting using a hybrid ARIMA and neural network model. *Neurocomputing*, **50**: 159- 175.

Effect of different levels of nitrogen and phosphorus on nutrient uptake and quality of soybean [*Glycine max* (L.) Merrill]

RAGHUVEER AND J A HOSMATH

College of Agriculture, University of Agricultural Sciences, Dharwad-580 005, Karnataka

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ABSTRACT

A field experiment was conducted to study the effect of different levels of nitrogen and phosphorus on nutrient uptake and quality of soybean during *kharif* 2015. There were twelve treatment combinations consisted of three levels of nitrogen (20, 40 and 60 kg N/ha) and four levels of phosphorus (40, 60, 80 and 100 kg P_2O_5/ha). Significantly higher soybean seed yield (25.77 q/ha), haulm yield (31.32 q/ha), uptake of nitrogen (209.16 kg/ha), phosphorus (28.09 kg/ha), potassium (114.47 kg/ha) and protein yield (8.81 q/ha) recorded with combined application of nitrogen @ 60 kg/ha and phosphorus @ 80 kg/ha compared to other treatments and it was on par with application of nitrogen @ 60 kg/ha and phosphorus @ 100 kg/ha. Application of nitrogen @ 60 kg, phosphorus @ 80 kg and potash @ 25 kg per hectare found optimum to obtain substantial soybean seed yield.

Keywords: Nitrogen, Nutrient uptake and quality, Phosphorus, Soybean

Soybean [*Glycine max* (L.) Merrill], is an introduced and commercially exploited crop in India. The crop is also called as "Golden Bean" or "Miracle crop" of the 21st century on account of its multiple uses. It has highest protein 40 per cent, oil 20 40 per cent, rich in lysine and vitamins A, B and D and also rich in mineral salts. Among the nutrients; nitrogen is a major essential plant nutrient element. It has the quickest and most pronounced effect on plant growth and yield of crops. It tends primarily to encourage above ground vegetative growth and to impart deep green colour to the leaves. In all plants, nitrogen governs a considerable degree of utilization of potassium, phosphorus and other nutrients. Plants receiving insufficient nitrogen are stunted in growth with restricted root systems (Penas and Wiese, 1987). The leaves turn yellow or yellowish green and tend to drop off. Phosphorus stimulates rhizobial activity, nodule formation and thus helps in N_2-fixation. It increases the water use efficiency, improves storage quality and hardiness of the bean seed coat. As phosphorus plays a role in photosynthesis, respiration, energy storage and transfer, cell division and enlargement, it has been shown to be important for growth, development and yield of soybean (Kakar *et al.,* 2002). It helps in uptake of more nutrients and balances the nitrogen deficiency in soil and assists in seed maturation. Thus, it is needed to find out proper amount of nitrogen and phosphorus required for achieving better yield of soybean. Hence, in order to verify and workout the optimum nitrogen and phosphorus dose the present investigation was undertaken.

The field experiment was carried out at Main Agricultural Research Station, Dharwad, during *kharif* 2015. The experiment was replicated thrice in Randomized Complete Block Design in factorial concept. There were twelve treatment combinations consisted of three nitrogen levels (20, 40 and 60 kg N/ha) and four phosphorus levels (40, 60, 80 and 100 kg P_2O_5/ha). One of the treatment combinations comprised the recommended dose of 40 kg N, 80 kg P_2O_5 and 25 kg K_2O/ha. The soil was medium deep black with pH 7.10. The available N, P_2O_5 and K_2O contents were 252, 32.5 and 292.8 kg/ha, respectively. FYM @ 5 t/ha was applied 15 days before sowing of the crop. The gross plot size was 5.0 m × 3.6 m and net plot size was 4.8 m × 3.0 m. Seeds were treated using Rhizobium and Phosphorus solubilizing bacteria @ 1250 g/ha. Two seeds per hill were dibbled 5 cm deep in furrows at a spacing of 30 cm x 10 cm. Recommended dose of K_2O @ 25 kg/ha was applied at the time of sowing. N and P_2O_5 were applied as basal as per the treatments. The crop was harvested at its physiological maturity. The data was statistically analysed as per the procedure given by Gomez and Gomez (1984).

Application of nitrogen @ 60 kg/ha recorded significantly higher seed yield (24.44 q/ ha) and haulm yield (29.75 q/ha) compared to 20 (17.45 and 21.40 q/ha, respectively) and 40 (22.42 and 27.35 q/ha, respectively) kg N/ha. Among the phosphorus levels, application of phosphorus @ 80 kg/ha recorded significantly higher seed yield (22.57 q/ha) and haulm yield (27.52 q/ha) compared to 60 (20.73 and 25.32 q/ha, respectively) and 40 (19.47 and 23.81 q/ha, respectively) kg P_2O_5/ha, however, it was on par with 100 (22.97 and 28.00 q/ha, respectively) kg P_2O_5/ha. In combined application of nitrogen @ 60 kg/ha and phosphorus @ 80 kg/ha recorded significantly higher seed yield (25.77 q/ha) and haulm yield (31.32 q/ha) compared to other treatment combinations however, it was on par with application of nitrogen @ 60 kg/ha and phosphorus @ 100 kg/ha (26.07 and 31.67 q/ha) (Table 1). With respect to harvest index. non-significant difference among treatment

E-mail: ragu5362@gmail.com

combinations noticed. It is mainly attributed to application of nitrogen and phosphorus accelerated the photosynthetic rate leading to more production of carbohydrates, it involved in nodulation and being the constituent of ATP which regulate vital metabolic processes in the plant, helping in root formation and nitrogen fixation results in positive effect on photosynthesis which in turn favors better growth and yield of the crop. These results are in line with the findings of Yadav and Chandel (2010), Sohrabi *et al.* (2012), Bhattacharjee *et al.* (2013) and Dhage *et al.* (2014).

Table 1 Seed yield and haulm yield of soybean as influenced by different levels of nitrogen and phosphorus

Phosphorus (kg/ha)	Seed yield (q/ha)				Haulm yield (q/ha)				Harvest index (%)			
	Nitrogen (kg/ha)											
	20	40	60	Mean	20	40	60	Mean	20	40	60	Mean
40	16.55	19.59	22.26	19.47	20.33	23.95	27.15	23.81	44.88	44.99	45.05	44.97
60	17.32	21.20	23.66	20.73	21.25	25.87	28.85	25.32	44.91	45.04	45.06	45.00
80	17.70	24.23	25.77	22.57	21.69	29.57	31.32	27.52	44.94	45.04	45.14	45.05
100	18.22	24.63	26.07	22.97	22.31	30.02	31.67	28.00	44.95	45.07	45.15	45.07
Mean	17.45	22.42	24.44		21.40	27.35	29.75		44.91	45.05	45.10	
	SEm±		CD at 5 %		SEm±		CD at 5 %		SEm±		CD at 5 %	
Nitrogen	0.15		0.45		0.16		0.48		0.11		NS	
Phosphorus	0.18		0.51		0.19		0.55		0.12		NS	
Interaction	0.30		0.89		0.33		0.96		0.21		NS	

Table 2 Nitrogen, phosphorus and potassium uptake (kg/ha) of soybean as influenced by different levels of nitrogen and phosphorus

Phosphorus (kg/ha)	Nitrogen				Phosphorus				Potassium			
	Nitrogen (kg/ha)											
	20	40	60	Mean	20	40	60	Mean	20	40	60	Mean
40	133.81	157.09	176.49	155.80	17.06	19.45	22.60	19.70	70.27	84.99	97.61	84.29
60	139.94	170.67	189.72	166.78	17.36	21.54	24.30	21.07	73.90	92.05	104.73	90.23
80	143.80	195.49	209.16	182.82	18.25	25.60	28.09	23.98	75.88	106.92	114.47	99.09
100	148.48	199.95	211.84	186.76	19.05	26.31	28.60	24.65	78.67	108.96	116.17	101.27
Mean	141.51	180.80	196.80		17.93	23.22	25.90		74.68	98.23	108.24	
	SEm±		CD at 5 %		SEm±		CD at 5 %		SEm±		CD at 5 %	
Nitrogen	1.29		3.77		0.25		0.72		0.61		1.80	
Phosphorus	1.49		4.36		0.28		0.83		0.71		2.08	
Interaction	2.57		7.54		0.49		1.44		1.23		3.60	

Table 3 Available nutrient in soil after harvest of soybean as influenced by different levels of nitrogen and phosphorus

Phosphorus (kg/ha)	Nitrogen (kg/ha)				Phosphorus (kg/ha)				Potassium (kg/ha)			
	Nitrogen (kg/ha)											
	20	40	60	Mean	20	40	60	Mean	20	40	60	Mean
40	212.8	225.9	235.2	224.6	29.7	30.7	31.1	30.5	259.6	261.4	263.0	261.4
60	216.5	226.8	238.9	227.4	30.2	29.8	31.3	30.4	260.1	262.6	263.5	262.1
80	218.4	229.6	240.8	229.6	31.1	32.2	32.5	32.0	262.6	262.5	264.2	263.1
100	220.3	231.5	242.7	231.5	33.0	33.7	34.1	33.6	263.5	262.7	264.4	263.5
Mean	217.0	228.4	239.4		31.0	31.6	32.3		261.5	262.3	263.8	
	SEm±		CD at 5 %		SEm±		CD at 5 %		SEm±		CD at 5 %	
Nitrogen	1.6		4.8		0.4		NS		0.9		NS	
Phosphorus	1.9		NS		0.3		0.9		1.1		NS	
Interaction	3.3		NS		0.5		NS		1.8		NS	

Table 4 Protein content and protein yield of soybean as influenced by different levels of nitrogen and phosphorus

Phosphorus (kg/ha)	Protein content (%)				Protein yield (q/ha)			
	Nitrogen (kg/ha)							
	20	40	60	Mean	20	40	60	Mean
40	33.02	33.34	33.51	33.29	5.46	6.53	7.46	6.48
60	33.10	33.60	33.80	33.50	5.73	7.12	8.00	6.95
80	33.19	33.83	34.18	33.74	5.87	8.21	8.81	7.63
100	33.28	33.98	34.36	33.87	6.06	8.38	8.96	7.80
Mean	33.15	33.69	33.96		5.78	7.56	8.30	
	SEm±		CD at 5 %		SEm±		CD at 5 %	
Nitrogen	0.06		0.18		0.05		0.16	
Phosphorus	0.07		0.20		0.06		0.18	
Interaction	0.12		NS		0.11		0.31	

Application of nitrogen @ 60 kg/ha recorded significantly higher uptake of nitrogen, phosphorus and potassium (196.80, 25.90 and 108.24 kg/ha, respectively) compared to 20 (141.51, 17.93 and 74.68 kg/ha, respectively) and 40 (180.80, 23.22 and 98.23 kg/ha, respectively) kg N/ha. Among the phosphorus levels, application of phosphorus @ 80 kg/ha recorded significantly higher uptake of nitrogen, phosphorus and potassium (182.82, 23.98 and 99.09 kg/ha, respectively) compared to 60 (166.78, 21.07 and 90.23 kg/ha, respectively) and 40 (155.80, 19.70 and 84.29 kg/ha, respectively) kg P_2O_5/ha however, it was on par with 100 (186.76, 24.65 and 101.27 kg/ha, respectively) kg P_2O_5/ha. In combined application of nitrogen @ 60 kg/ha and phosphorus @ 80 kg/ha recorded significantly higher uptake of nitrogen, phosphorus and potassium (209.16, 28.09 and 114.47 kg/ha, respectively) compared to other treatment combinations however, it was on with application of nitrogen @ 60 kg/ha and phosphorus @ 100 kg/ha (211.84, 28.60 and 116.17 kg/ha, respectively) (Table 2). The higher uptake attributed to increased availability of nutrients in the soil, which inturn led to higher production of dry matter, yield components of soybean. These results are in line with the findings of Rathod *et al.* (2012), Mere *et al.* (2013), Gharpinde *et al.* (2014) and Yadravi and Angadi (2015).

Application of nitrogen @ 60 kg/ha recorded significantly higher nitrogen content in soil (239.4 kg/ha) compared to 20 (217.0 kg/ha) and 40 N kg/ha (228.4 kg/ha). Among the different levels of phosphorus, application of phosphorus @ 100 kg/ha recorded significantly higher phosphorus content in soil after harvest (33.6 kg/ha) compared to 40 (30.5 kg/ha), 60 (30.4 kg/ha) and 80 (32.0 kg/ha) kg P_2O_5/ha. In combined application of nitrogen and phosphorus did not show any significant difference with respect to available nitrogen, phosphorus and potassium in soil (Table 3).These results are in agreements with the work of Geeta and Radder (2015), Gharpinde *et al.* (2014) and Yadravi and Angadi (2015).

Application of nitrogen @ 60 kg/ha recorded significantly higher protein content (33.96 %) and protein yield (8.30 q/ha) compared to 20 and 40 kg N/ha. Among the phosphorus levels, application of phosphorus @ 80 kg/ha recorded significantly higher protein content (33.74 %) and protein yield (7.63 q/ha) compared to 60 and 40 kg P_2O_5/ha however, it was on par with 100 kg P_2O_5/ha. In combined application of nitrogen @ 60 kg/ha and phosphorus @ 80 kg/ha recorded significantly higher protein yield (8.81 q/ha) compared to other treatment combinations however, it was on with application of nitrogen @ 60 kg/ha and phosphorus @ 100 kg/ha. No significant difference was observed with respect to protein content (Table 4). These results are in conformity with the findings of Peric *et al.* (2009), Devi *et al.* (2012) and Geeta and Radder (2015). The investigation revealed that application of nitrogen @ 60 kg/ha and phosphorus @ 80 kg/ha and potassium @ 25 kg/ha found optimum to achieve higher soybean seed yield with improved protein quality.

REFERENCES

Bhattacharjee S, Singh A K, Kumar M and Sharma S K 2013, Phosphorus, sulfur and cobalt fertilization effect on yield and quality of soybean (*Glycine max* L. Merrill) in acidic soils of northeast India. *Indian Journal of Hill Farming*, **26**(2): 63-66.

Devi K N, Singh L N K, Devi T S, Devi H N, Singh K B, Singh K K and Singh M W 2012. Response of soybean [*Glycine max* L. Merrill] to sources and levels of phosphorus. *Australian Journal of Crop Science*, **7**(9): 1407-1415.

Dhage S J, Patil V D and Patange M J 2014. Effect of various levels of phosphorus and sulphur on yield, plant nutrient content, uptake and availability of nutrients at harvest stages of soybean (*Glycine max* L. Merrill). *International Journal of Current Microbiology and Applied Sciences*, **3**(12): 833-844.

Geetha G P and Radder B M 2015, Effect of phosphorus cured with FYM and application of biofertilizers on productivity of soybean (*Glycine max* L. Merrill.) and phosphorus transformation in soil. *Karnataka Journal of Agricultural Sciences*, **28**(3): 414-415.

Gharpinde B, Gabhane V V, Nagdeve M B, Sonune B A and Ganvir M M 2014. Effect of integrated nutrient management on soil fertility, nutrient balance, productivity and economics of soybean in an inceptisol of semi-arid region of Maharashtra. *Karnataka Journal of Agricultural Sciences*, **27**(3): 303-307.

Gomez K A and Gomez A A 1984. *Statistical Procedure for Agriculture Research*, 2nd Ed., John Willey and Sons, New York, pp. 680.

Kakar K M, Muhammad T, Fazal H and Khalid N 2002. Phosphorus use efficiency of soybean as effected by phosphorus application and inoculation. *Pakistan Journal of Agronomy*, **1**(1): 49-50.

Mere V, Singh A K, Singh M, Jamir Z and Gupta R C 2013. Effect of nutritional schedule on productivity and quality of soybean varieties and soil fertility. *Legume Research*, **36**(6): 528-534.

Penas E J and Wiese R A 1987. *Fertilizer suggestions for soybeans.* Neb Guide G87-859-A, University of Nebraska, Cooperative Extention, Lincoln, NE. pp. 58-65.

Peric V, Srebric M, Jankuloski L, Jankulovska M, Zilic S and Kandic V 2009. The effects of nitrogen on protein, oil and trypsin inhibitor content of soybean. *Genetica*, **4**(2): 137-144.

Rathod K S, Pannu R K and Dahiya S S 2012. Effect of nitrogen management on soil fertility and NPK uptake in soybean. *Journal of Food Legumes*, **25**(2): 153-155.

Sohrabi Y, Habibi A, Mohammadi K, Sohrabi M, Heidari G, Khalesro S and Khalvandi M 2012. Effect of nitrogen (N) fertilizer and foliar-applied iron (Fe) fertilizer at various reproductive stages on yield, yield component and chemical composition of soybean (*Glycine max* L. Merrill) seed. *African Journal of Biotechnology*, **11**(40): 9599-9605.

Yadav R and Chandel A S 2010. Effect of nitrogen and phosphorus nutrition at different physiological stages of soybean on yield and grain quality in Uttarakhand hills. *Progressive Agriculture*, **10**(2): 256-259.

Yadravi M and Angadi V V 2015. Effect of time and method of application of varied levels of nitrogen in soybean. M.Sc.(Agri) Thesis, University of Agricultural Sciences, Dharwad. Karnataka, India.

Weed management in broadcast sesame (*Sesamum indicum* L.) through sequential application of herbicides

K SATHISH BABU AND D SUBRAMANYAM

S.V. Agricultural College, Acharya N.G. Ranga Agricultural University, Tirupati-517 502, Andhra Pradesh

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ABSTRACT

A field experiment was conducted to study the effect of sequntial application of pre-and post-emergence herbicides on growth, nutrient uptake and yield of broadcast sesame on sandy loam soils. Eleven weed management practices consisting of pre-emergence application of pendimethalin @ 750 g/ha, oxyfluorfen @ 75g/ha and oxadiargyl @ 75g/ha alone and sequential application of these pre-emergence herbicides followed by post-emergence application of quizalofop @ 50g/ha and propaquizafop @ 60g/ha including two hand weedings at 20 and 40 DAS and unweeded check were compared in randomized block design with three replications. Results revealed that the lowest density and dry weight of weeds with lesser weed index were recorded in the plots treated with pre-emergence application of oxyfluorfen @ 75g/ha followed by quizalofop @ 50 g/ha applied at 20 DAS. The same weed management practice recorded significantly higher stature of growth parameters and yield attributes. The increase in seed yield of sesame was 38.0 per cent with sequential application of oxyfluorfen @ 75g/ha followed by quizalofop @ 50 g/ha applied at 20 DAS compared to unweeded check. Heavy weed infestation in unweeded check plots resulted in incresed weed nutrient uptake upto 44.6, 5.6 and 34.0 kg of N, P and K/ha, respectively. Oxadiargyl and oxyfluorfen @ 75 g/ha each showed phytotoxicity score of '4' and '3' respectively in 0 to 10 scale. Pre-emergence application of oxyfluorfen @ 75 g/ha followed by propaquizafop @ 60g/ha applied at 20 DAS recorded the highest benefit-cost ratio.

Keywords: Phytotoxicity, Pre-and post-emergence herbicides, Sesame, Weed management

Sesame (*Sesamum indicum* L.) is the oldest oilseed crop used by man since antiquity and the country ranks first in area and production in the world. It is an important oilseed crop grown in all the seasons in south and central India. In general, broadcasted sesame had heavy weed infestation than line sown crop as the weeds and crop emerges simultaneously and both of them are competing with each other for growth resources like nutrients, space and solar radiation. Further, sesame cotyledons are small and tender when they emerge and slow initial growth compared to other crops resulted in increased weed infestation. In broadcasted sesame, the selectivity of pre-emergence herbicides is quite different as the seeds are placed at shallow depth and damaged due to phytotoxicity of soil applied herbicides. The magnitude of phytotoxicity of the commonly used herbicides may vary with its dose, type of soil, soil moisture condition, method and depth of sowing. The soil applied herbicide molecules may come in contact with the absorption sites of sesame and causes phytotoxicity to germinating sesame seedlings (Grichar *et al.,* 2001). The weeds emerging at later growth stages of the crop escape the toxic effect of pre-emergence herbicides. Aryloxyphenoxypropionic group of herbicides like quizalofop and propaquizafop are used as post-emergence herbicides to control the grassy weeds in broad leaved crops. Though, the conventional methods of hand weeding is more effective, but due to high wages and non-availability of labour at appropriate stages for weeding and unfavourable weather conditions failed to control the weeds effectively. Most of the pre-emergence herbicides cause phytotoxicity at normal recommended doses might be due to difference in leaching behaviour in soil and shallow depth of sowing. Information regarding chemical weed management in broadcast sesame is lacking. In view of the above rationale, an attempt has been made to identify the effective weed management strategy based on sequential application of herbicides in broadcast sesame in sandy loam soils under irrigated conditions in Sothern Agroclimatic Zone of Andhra Pradesh.

A field experiment was conducted at S.V. Agricultural College, Tirupati campus of Acharya N.G. Ranga Agricultural University, Andhra Pradesh, India with eleven weed management practices in a randomized block design with three replications during summer, 2015. The soil of the experimental field was sandy loam in texture, slightly acidic in soil reaction (6.4 pH), low in organic carbon (0.45 %), available nitrogen (194 kg/ha) and available phosphorus (24 kg/ha) and medium in available potassium (175 kg/ha). The sesame cultivar 'YLM-66' was broadcasted @ 7 kg/ha by mixing the required quantity of seed with sand (1:3).The eleven weed management practices consisted of pre-emergence application of pendimethalin @ 750 g/ha, oxyfluorfen @ 75 g/ha, oxadiargyl @ 75 g/ha alone and sequential application these pre-emergence herbicides with quizalofop@ 50 g/ha and propaquizafop @ 60 g/ha at 20

days after sowing (DAS) including two hand weedings at 20 and 40 DAS and unweeded check. The pre-and post-emergence herbicides were applied at one and 20 DAS, respectively with the help of knapsack sprayer fitted with flat fan nozzle by using spray fluid @ 500 l/ha.The recommended level of N, P and K at 60, 20 and 20 kg/ha, respectively was applied. The entire dose of P, K and half the dose of N was applied as basal and the remaining half of the dose of N was applied at 30 DAS. Totally, five irrigations were given and need based plant protection measures were taken for growth and development of the crop. Dry weight of weeds were recorded from 1.0 m2 area after drying at 60°C for 72 hours. Phytotoxicity rating was registered on the sesame crop at 10 days after application of pre-and post-emergence herbicides in 0 to 10 scale where 0=no injury to crop and 10=complete crop damage. Plant population/m2 was recorded before thinning at 15 DAS and expressed in No./m^2. Nutrient uptake by weeds and crop was estimated by following the standard procedures. Plant height (cm) was measured from the base of the plant to the tip of the growing point. Leaf area (cm^2/plant) was recorded with LI-COR model, LT-300 leaf area meter at harvest. Yield components *viz.,* number of capsules/plant, number of seeds/capsule and 1000-seed weight were recorded from five randomely selected plants from the net plot area of respective treatments at harvest. The oil yield was computed by multiflying the seed yield with oil per cent of the corresponding treatments. The reduction in yield due to weed infestation in respective treatments interms of weed index was caluculated by considering two hand weedings is the best in maintaining weed free condition compared to rest of the treatments (Gill and Vijaya Kumar, 1969).The weed flora identified in the experimental field was *Cyperus rotundus* L., *Commelina benghalensis* L., *Cleome viscosa* L., *Boerhavia diffusa* L. and *Phyllanthus niruri* L.

Weed growth: Different pre-and post-emergence herbicides were significantly influenced the weed density and dry weight of weeds in broadcasted sesame (Table 1). Sequential application of oxyfluorfen @ 75 g/ha as pre-emergence followed by propaquizafop @ 60 g/ha or quizalofop 50 g/ha applied at 20 DAS were resulted in lowest density and dry weight of weeds compared to rest of the herbicides applied sequentially due to its broadspecrtum and seson long weed control. Post-emergence application of quizalofop or propaquizafop applied at 20 DAS were equally effective in reducing the density and dry weight of grassy weeds at later stage of the crop due to inhibition of acetyl CoA carboxylasekinase (EC 2.7.11.27),a key enzyme responsible forsynthesis of fatty acids in target plants resulted in yellowing and formation of necrotic patches followed by wilting. These results are in accordance with the findings of Dawale *et al.* (2009). Pre-emergence application of oxadiargyl @ 75 g/ha followed by post-emergence application of propaquizafop @ 60 g/ha or quizalofop @ 50 g/ha were not effective to supress the weed growth due to poor performance of oxadiargyl @ 75 g/ha as pre-emergence on weed flora associated with sesame.

Phytotoxicity: Among the pre-emergence herbicides applied, oxadiargyl and oxyfluorfen @ 75 g/ha each and pendimethalin @ 750 g/ha showed phytotoxicity rating of '4' '3' and '2' respectively on broadcast sesame in 0 to 10 scale where 0=no injury to crop and 10=complete crop damage. Bleaching of leaves and stunted growth of sesame seedlings were observed with pre-emergence application of oxadiargyl @ 75 g/ha and recovered within 20 days after herbicide application. The lowest plant population of sesame at 15 DAS was noticed with pre-emergence application of oxadiargyl @ 75 g/ha might be due to its more phytotoxicity on germinating sesame seeds compared to pendimethalin @ 750 g/ha.The plant population of broadcasted sesame was reduced by 26.56, 18.87 and 13.75 per cent with pre-emergence application of oxadiargyl, oxyfluorfen and pendimethalin, respectively compared to unweeded check (control). Venkatakrishnan and Gnanamurthy (1998) also reported that oxyfluorfen @ 100 g/ha caused moderate crop injury to sesame on sandy loam soils. Post-emergence application of herbicides quizalofop 50g/ha and propaquizafop 60 g/ha did not show any phytotoxicity effect at 10 days after its application on sesame seedlings.

Nutrient uptake: The highest uptake of N, P and K by weeds was obtained with unweeded check plot upto 44.6, 5.6 and 34.0 kg N, P and K/ha, respectively due to increased weed dry weight as a result of heavy weed infestation. Among the herbicidal treatments, pre-emergence application of oxyfluorfen @ 75 g/ha followed by quizalofop @ 50 g/ha at 20 DAS resulted in reduced nutrient uptake by weeds. The nutrient uptake by the sesame crop under two hand weedings was the highest i.e. 60.4, 20.4 and 58.4 kg N, P and K/ha. Among the chemical weed management practices, the highest uptake of nutrients by crop was estimated with sequential application of oxyfluorfen @ 75 g/haas pre-emergence followed byquizalofop @ 50 g/haapplied at 20 DAS, which was statistically at par with sequential application of oxyfluorfen @ 75 g/ha followed by propaquizafop @ 60 g/ha. It was evident that whenever effective control of weeds was observed, significant loss of nutrients could be avoided and there by increased the availability of nutrients to crop. Kavimani *et al.* (2001) also concluded that pre-emergence application of pendimethalin @ 0.75 kg/ha supplemented with hand weeding at 30 DAS recorded significantly higher and lower nutrient uptake by sesame and and its associated weeds, respectively in sandy loam soils.

Growth, yield components and seed yield: Among the weed management practices, growth parameters *viz.,* plant height, leaf area/plant, number of branches/plant and dry matter production were significantly higher with two hand weedings (Table 2). Sequential application of oxyfluorfen @ 75 g/ha followed by quizalofop @ 50 g/ha or propaquizafop @ 60 g/ha applied at 20 DAS were resulted in the highest growth parameters, which were comparable with each other due to season long and broad spectrum weed control in these weed management practices might have enhanced the growth parameters because of more availability of growth resources to crop. These results are in agreement with those of Dhaka *et al.* (2013).

The same weed management practices produced significantly higher yield components *viz.,* number of capsules/plant, number of seeds/capsuleand 1000-seed weight compared to rest of the weed management practices. The highest seed and oil yield of broadcasted sesame was obtained with pre-emergence application of oxyfluorfen @ 75 g/ha followed by quizalofop @ 50 g/ha applied at 20 DAS and the same weed management practice increased the seed and oil yield by 61.3 and 73.4 per cent, respectively compared to unweeded check. Oxadiargyl @ 75 g/ha as pre-emergence followed by quizalofop or propaquizafop applied at 20 DAS was not effective in incresing the growth, yield attributes and yield of sesame, but significantly superior to pre-emergence herbicides application alone. All the sequential application of pre-and post-emergence herbicides recorded significantly higher benefit-cost ratios than two hand weedings due to lesser cost of weeding. Pre-emergence application of oxyfluorfen @ 75 g/ha followed by propaquizafop @ 60 g/ha applied at 20 DAS recorded the highest benefit-cost ratio followed by sequential application of oxyfluorfen @ 75 g/ha followed by quizalofop @ 50 g/ha applied at 20 DAS, among the weed management practices tested.

Threfore, sequential application of oxyfluorfen @ 75 g/ha as pre-emergence followed by quizalofop @ 50g/ha or propaquizafop 60@ g/ha applied at 20 DAS is considered as the best chemical weed management practice for obtaining broadspectrum weed management, enhancing the productivity and profitability of broadcasted sesame on sandy loam soils.

Table 1 Effect of pre-and post-emergence herbicides on weed growth, phytotoxicity and nutrient uptake of broadcast sesame

Treatments	Dose (g/ha)	Time of application (DAS)	Weed density* (No./m^2)	Weed dry weight* (g/m^2)	Phyto-toxicity rating	Plant population (No./m^2)	Dry matter production (kg/ha)	Nutrient uptake by weeds (kg/ha)			Nutrient uptake by crop (kg/ha)		
								N	P	K	N	P	K
Pendimethalin	750	1	209.61 (14.54)	94.54 (9.77)	2.0	69.4	2964	24.4	2.6	9.8	40.2	14.0	44.6
Oxyfluorfen	75	1	199.23 (14.18)	94.38 (9.76)	3.0	65.2	3123	20.6	2.2	9.2	44.8	14.8	46.2
Oxadiargyl	75	1	229.10 (15.20)	98.74 (9.98)	4.0	59.3	3071	34.8	3.2	11.4	37.4	13.5	43.2
Pendimethalin + quizalofop	750 +50	1 + 20	129.61 (11.44)	72.15 (8.54)	2.0	68.8	4199	12.6	0.9	7.2	53.8	17.5	54.6
Oxyfluorfen + quizalofop	75 + 50	1 + 20	105.86 (10.34)	62.00 (7.92)	3.0	64.6	4567	8.2	0.6	6.6	58.4	18.4	56.8
Oxadiargyl + quizalofop	75 + 50	1 + 20	146.51 (12.16)	80.16 (9.00)	4.0	58.4	3293	16.2	1.6	8.0	48.8	16.2	51.2
Pendimethalin + propaquizafop	750 + 60	1 + 20	130.39 (11.48)	74.93 (8.71)	2.0	67.1	4060	14.8	1.2	7.4	52.4	17.1	54.0
Oxyfluorfen + propaquizafop	75 + 60	1 + 20	101.01 (10.11)	64.00 (8.05)	3.0	63.2	4421	9.4	0.7	6.9	56.9	18.0	56.2
Oxadiargyl + propaquizafop	75 + 60	1 + 20	150.12 (12.31)	83.02 (9.16)	4.0	57.0	3274	18.4	1.9	8.4	47.4	15.9	50.1
Two hand weedings	-	20 + 40	76.31 (8.79)	16.59 (4.14)	0.0	78.5	5059	4.2	0.3	4.6	60.4	20.4	58.4
Unweeded check(Control)	-	-	297.44 (17.31)	141.12 (11.93)	0.0	79.3	2813	44.6	5.6	34.0	34.5	12.4	40.6
CD (P=0.05)			0.91	0.57	-	8.6	148	1.02	0.13	0.69	1.49	0.32	0.83

*Figures in parentheses are the square root transformed (√X+0.5) values

Table 2 Effect of different pre-and post-emergence herbicideson growth and yield components and yield of broadcast sesame

Treatments	Dose (g/ha)	Time of application (DAS)	Plant height (cm)	Leaf area/ plant (cm^2)	No. of branches /plant	No. of capsules/ plant	No. of seeds/ capsule	1000-seed weight (g)	Seed yield (kg/ha)	Oil yield (kg/ha)	Weed index (%)	B:C ratio
Pendimethalin	750	1	89.6	815.4	5.9	30.6	43.2	2.80	554	268	33.49	1.67
Oxyfluorfen	75	1	90.0	860.5	6.2	31.9	45.4	2.82	582	285	30.13	1.88
Oxadiargyl	75	1	82.7	721.8	5.6	28.9	41.0	2.78	527	255	36.73	1.70
Pendimethalin + quizalofop	750 +50	1 + 20	93.5	995.6	7.2	35.2	52.5	2.90	752	379	9.72	2.04
Oxyfluorfen + quizalofop	75 + 50	1 + 20	97.5	1085.4	8.1	37.2	59.0	2.93	784	404	5.88	2.25
Oxadiargyl + quizalofop	75 + 50	1 + 20	92.2	935.8	6.9	33.6	48.4	2.85	677	334	18.72	1.95
Pendimethalin + propaquizafop	750 + 60	1 + 20	93.0	974.6	7.1	34.8	51.2	2.89	751	375	9.84	2.06
Oxyfluorfen + propaquizafop	75 + 60	1 + 20	96.7	1055.6	8.0	36.8	57.4	2.92	779	396	6.48	2.27
Oxadiargyl + propaquizafop	75 + 60	1 + 20	91.8	924.6	6.8	33.1	47.2	2.84	666	327	20.04	1.94
Two hand weedings	-	20 + 40	100.2	1120.5	8.4	40.1	61.4	2.98	833	432	-	1.97
Unweeded check	-	-	79.4	585.7	5.3	25.4	38.9	2.76	486	233	41.65	1.65
CD (P=0.05)			0.84	30.43	0.13	0.53	1.65	0.05	25.0	13.8		0.03

REFERENCES

Dawale J S, Zalate P Y, Padmani D R and Nikam D R 2009. Effect of weed population on yield, yield attributing characters and oil content of sesamum (*Sesamum indicum* L.). *Bioinfolet*, **6**: 331-333.

Dhaka M S, Yadav S S, Shivran A C, Choudary G L and Prajapat K 2013. Effect of weed managementpractices on performance of sesame under varying levels of nitrogen. *Annals of Agricultural Research*, **34**: 179-184.

Gill G S andVijay Kumar K 1969. Weed index-a new method for reporting weed control traits. *Indian Journal of Agronomy*, **14**: 96-78.

Grichar W J, Sestak D C, Brewer K D, Besler B A, Stichler C R and Smith D T 2001. Sesame (*Sesamum indicum* L.) tolerance and weed control with soil applied herbicides. *Crop Protection*, **20**: 389-384.

Kavimani R, Vijaybaskaran S and Annadurai K 2000.Nutrient uptake by sesame and associated weeds under different methods of sowing. *Madras Agricultural Journal*, **87**: 709-711.

Venkatakrishanan A S and Gnanamurthy P 1998. A preliminary evaluation of herbicides on the control of weeds in irrigated sesame (*Sesamum indicum* L.). *Sesame and Safflower NewsLetter*, **13**: 77-79.

Influence of varying seed rate and fertilizer levels on yield and quality of linseed (*Linum usitassimum* L.)

S CHANNABASAVANAGOUDA, S A BIRADAR, B M CHITTAPUR, SHAMRAO KULKARNI AND S R BALANGOUDAR

College of Agriculture, University of Agricultural Sciences, Raichur-584 104, Karnataka

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ABSTRACT

A field experiment was conducted during *rabi* 2016-17 to study the influence of varying seed rate and fertilizer levels on yield and quality of linseed. The result of this experiment revealed that crop sown with seed rate @ 30 kg/ha recorded significantly higher seed yield (909 kg/ha), straw yield (1699 kg/ha), harvest index (0.35), fibre yield (1247 kg/ha), fibre percentage (74 %) and oil yield (331 kg/ha) as compared to seed rate @ 20 kg/ha. While application of 150 per cent recommended dose of fertilizer (RDF) recorded significantly higher seed yield (861 kg/ha), straw yield (1624 kg/ha), harvest index (0.35), fibre yield (1111 kg/ha), fibre percentage (68 %) and oil yield (313.5 kg/ha). Combination of seed rate @ 30 kg/ha with 150 per cent RDF was recorded significantly higher seed yield (923 kg/ha), straw yield (1727 kg/ha), harvest index (0.35), fibre yield (1273 kg/ha), fibre percentage (74 %) and oil yield (335 kg/ha).

Keywords: Fertilizer levels, Quality, Linseed, Seed rate, Yield

Linseed (*Linum usitatissimum* L.) is considered as the most important industrial oilseed crop of India stands next to rapeseed-mustard in *rabi* oilseed in terms of area and production. It is grown either for oil extracted from seed or fiber from the stem. The oil content of linseed varies from 37-43 per cent and very part of the plant is utilized commercially either directly or after processing. Most of the oil is used in the industry for manufacturing of paints, varnishes, ink, soaps and small fraction of used for edible purposes. To sustain the linseed production, there is need to develop appropriate agronomic practices to obtain the higher crop yield. Fertilization especially nitrogen, phosphorus, potassium and sulphur are the key factors that determines the crop yield and quality (Singh *et al.,* 2007). The seed rate has profound influence on cultivation of field crops including oilseeds also. To obtain the maximum yield, optimum plant population is the pre-requisite that can be maintained only by sowing of appropriate seed rates. This is an important constituent for determining the extent of growth, development and finally yields of the crop. Therefore, the fertility levels and seed rates are the major factors for increasing the productivity and profitability of linseed. Hence, the present study was carried out to evaluate the effect of fertility levels and seeding rates on productivity and profitability of linseed in irrigated condition.

The field experiment was carried out at Main Agricultural Research Station, University of Agricultural Sciences, Raichur during *rabi* season of 2016-17 which is situated between 16° 12' North latitude and 77° 20' East longitude with an altitude of 389 meters above the mean sea level and is considered as North Eastern Dry Zone (Zone 2) of Karnataka in black soil. The experiment was laid out in factorial randomized complete block design with three replications. There were nine treatment combinations consisted of three levels of seed rate (20, 25 and 30 kg/ha) and three fertilizer levels (100, 125 and 150 per cent RDF kg/ha). Healthy seeds of linseed (cv. NL-115) were used for sowing. Shallow furrows were opened manually 5 cm away from the fertilizer line with the help of a hand-drawn marker. The seeds were hand sown uniformly in the furrows and the furrows were covered with moist soil immediately after placing seeds. The sowing was done on 28th October, 2016. Recommended dose of fertilizer for linseed (40:20:20:10 kg of N:P_2O_5:K and S/ha) were applied in different dosage of (100, 125 and 150 per cent RDF) at the time of sowing. Nitrogen and phosphorous potassium were applied in the form of Urea, DAP, Muriate of Potash (MOP) and Sulphur applied in the form of gypsum.

Crop sown with seed rate @ 30 kg/ha recorded significantly higher seed yield (909 kg/ha) straw yield (1699 kg/ha), harvest index (0.35) compared to seed rate @ 20 kg/ha. It was on par with seed rate @ 25 kg/ha. Among fertilizer levels, application of 150 per cent RDF recorded significantly higher seed yield (861 kg/ha), straw yield (1624 kg/ha) compared to100 per cent RDF and it was on par with 125 per cent RDF. Interaction effects of seed rate @ 30 kg/ha with 150 per cent RDF recorded significantly higher seed yield (923 kg/ha), straw yield (1727 kg/ha) as compared to seed rate @ 20 kg/ha with 100 per cent RDF but it was on par with seed rate @ 30 kg/ha with 125 per cent RDF, seed rate @ 30 kg/ha with 100 per cent RDF, seed rate @ 25 kg/ha with 150 per cent RDF and seed rate @ 25 kg/ha with

125 per cent RDF. However, harvest index show non-significant difference among treatment combinations. The higher seed yield was due to outcome of significantly higher number of plants/population, higher harvest index, better utilization of soil moisture especially at flowering and early capsule formation and application of higher dose of fertilizers increased the fertility levels, enhanced the cell division cell elongation and tissue differentiation and also overall improvement in plant vigour and production of sufficient photosynthesis owing to higher availability of NPK and S, resulting in better yield attributes ultimately increases the yield. These results are in compliance with the findings of Gunnels *et al.* (1977), Kandil *et al.* (2009), Meena *et al.* (2011), Asraf *et al.* (2002) and Sune *et al.* (2006).

Crop sown with seed rate @ 30 kg/ha recorded significantly higher oil yield (331 kg/ha) fibre yield (1247 kg/ha), fibre percentage (74 %) compared to seed rate @ 20 kg/ha but it was on par with Seed rate @ 25 kg/ha. Among fertilizer levels, application of 150 per cent RDF recorded significantly higher oil yield (313 kg/ha), fibre yield (1111 kg/ha), fibre percentage (68%) compared to 100 per cent RDF but it was on par with 125 per cent RDF. Crop sown with combination of seed rate @ 30 kg/ha with 150 per cent RDF recorded significantly higher oil yield (335 kg/ha), fibre yield (1273 kg/ha) and fibre percentage (74%) compared seed rate @ 20 kg/ha with 100 per cent RDF and it was on par with seed rate @ 30 kg/ha with 125 per cent RDF, seed rate @ 30 kg/ha with 100 per cent RDF, seed rate @ 25 kg/ha with 150 per cent RDF, seed rate @ 25 kg/ha with 125 per cent RDF. However, oil content show non-significant difference among treatment combinations. This was due to higher plant population per unit area and higher fertilizer dose applied to the crop increases the seed yield and straw yield ultimately it increases the oil yield, fiber yield, as well as fibre percentage. These results also in line with the findings of Ashraf *et al.* (2013), Salah and Mohamed (2015), Singh (1994), Leilah *et al.* (2003), Berti *et al.* (2009) and Choudhary *et al.* (2016). Based on the results obtained, it may be concluded that crop sown with seed rate @ 30 kg/ha or seed rate @ 25 kg/ha and application 150 per cent RDF was optimum to achieve higher seed yield and improve quality of linseed.

Table 1 Seed yield, straw yield and harvest index of linseed as influenced by varying seed rate and fertilizer levels

Treatments	Seed yield (kg/ha)				Straw yield (kg/ha)				Harvest index			
	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean
S_1	630	697	777	701	1380	1477	1507	1454	0.31	0.32	0.34	0.32
S_2	863	880	883	876	1550	1608	1638	1599	0.36	0.35	0.35	0.35
S_3	893	910	923	909	1672	1700	1727	1699	0.35	0.35	0.35	0.35
Mean	796	829	861		1534	1595	1624		0.34	0.34	0.35	
	SEm±		CD @ 5%		SEm±		CD @ 5%		SEm±		CD (P=0.05)	
S	11		33		7		20		0.004		0.011	
F	11		33		7		20		0.004		NS	
S x F	19		57		12		35		0.006		NS	

NS-Non-significant; Factor A:Seed rate S_1: 20 kg/ha, S_2: 25 kg/ha, S_3: 30 kg/ha; Factor B: Fertilizer level, F_1: 100% RDF, F_2: 125% RDF, F_3: 150% RDF

Table 2 Fibre yield (kg/ha) and fibre percentage of linseed as influenced by varying seed rate and fertilizer levels

Treatments	Fibre yield (kg/ha)				Fibre percentage			
	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean
S_1	773	853	890	839	56	58	59	58
S_2	963	1120	1170	1084	62	69	71	68
S_3	1220	1247	1273	1247	73	74	74	74
Mean	986	1073	1111		64	67	68	
	SEm±		CD (P=0.05)		SEm±		CD (P=0.05)	
S	14		41		0.80		2.40	
F	14		41		0.80		2.40	
S x F	24		71		1.39		4.15	

NS-Non-significant; Factor A: Seed rate, S_1: 20 kg/ha, S_2: 25 kg/ha, S_3: 30 kg/ha; Factor B: Fertilizer level, F_1:100% RDF, F_2:125% RDF, F_3:150% RDF

Table 3 Oil content and oil yield of linseed as influenced by varying seed rate and fertilizer levels

Treatments	Oil content (%)				Oil yield (Kg/ha)			
	F_1	F_2	F_3	Mean	F_1	F_2	F_3	Mean
S_1	36.50	36.10	36.14	36.25	224.4	253	281	253
S_2	36.72	36.62	36.68	36.67	317.2	322	323	321
S_3	36.62	36.62	36.96	36.73	326.9	333	335	331
Mean	36.61	36.45	36.60		289.5	303	313	
	SEm±		CD (P=0.05)		SEm±		CD (P=0.05)	
S	0.37		NS		5		14	
F	0.37		NS		5		14	
S x F	0.65		NS		8		24	

NS-Non-significant; Factor A: Seed rate, S_1:20 kg/ha, S_2:25 kg/ha, S_3:30 kg/ha; Factor B:Fertilizer level, F_1:100% RDF, F_2:125% RDF, F_3:150% RDF

REFERENCES

Ashraf A, El-Mohsen A, Amany M, Abdallah and Mahmoud O 2013. Optimizing and describing the influence of planting dates and seeding rates on flax cultivars under Middle Egypt region conditions. *Science Research Review Journal*, **1**(4): 142-152.

Berti M, Fischer S, Wilckens R and Hevia F 2009. Flax seed response to N, P and K fertilization in south central Chile. *Chilean Journal of Agriculture Research*, **69**(2): 145-153.

Choudhary A A, Nikam R R and Patil S S 2016. Effect of phosphorus and sulphur on oil, nutrient uptake and yield of Linseed. *International Journal of Life Science*, **6**: 33-36.

Kandil A A, Hob Allah A A, El-Hariri D M, Zeidan M S and Bakri A B 2009. Effect of seeding rate on yield and quality traits of some flax varieties (*Linum usitatissimum* L.) grown in newly reclaimed sandy soils. *Agronomy Department Faculty of Agriculture, Cairo University Egypt*, **1**(1): 15-21.

Leilah A A, Kassaby A T, Hindi M H and Abuzaid T A 2003. Requirements of some flax cultivars from NPK fertilizer. *Scientific Journal of King Faisal University*, **4**(2): 14-24.

Meena R L, Singh T K, Rakesh K, Amitesh K S and Hari Om 2011. Production performance of linseed to fertility levels and seed rates in dryland conditions of eastern Uttar Pradesh. *Indian Journal of Soil Conservation*, **39**(3): 230-235.

Salah M E and Mohamed D H D 2015. Seeding rates and phosphorus source effects on straw, seed and oil yields of flax (*Linum usitatissimum* L.) grown in newly-reclaimed soils. *Department Faculty of Agriculture, Fayoum University Egypt*, **6**(3): 35-38.

Singh R 1994. Studies on the influence of crop management and retting methods on productivity and fibre quality of dual purpose linseed. M.Sc. (Agri) Thesis, Himachal Pradesh Krishi Vishvavidyalaya, Palampur.

Sune S V, Deshpande R M, Khawale V S and Baviskar P K 2006. Effect of phosphorus and sulphur application on growth and yield of linseed. *Journal of Soil Crops*, **16**(1): 217-221.

Production potential and profitability of soybean [*Glycine max* (L.) Merill] as influenced by different production factors

A S KARLE, R V GITE, G S KHAZI AND D N GOKHALE

Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani-431 402, Maharashtra

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ABSTRACT

A field experiment was conducted during *kharif* season 2015 to prioritize production factors and also suggest cost effective agronomic operation in soybean production. Adoption of whole package (T_1) to soybean was significantly superior to all other treatments and produced highest gross monetary returns of ₹ 95104/ha and was at par with treatment T_4 (whole package-hoeing) (₹ 89689/ha) and T_5 (whole package-plant protection) (₹ 84455/ha). Lowest gross monetary returns (₹ 38442/ha) of soybean was obtained in control treatment (T_{12}).

Keywords: Fertilizer, Herbicide, Hoeing, Plant protection, Production, Soybean

Soybean is important crop in human and animal nutrition, due to major source of edible vegetable oil and high protein feed as well as food in the world. It is an excellent health food and contains 40-42 per cent quality protein, 23 per cent carbohydrates and 20 per cent cholesterol free oil. Soybean protein is rich in valuable amino acid, lysine (5%) which is deficient in most of the cereals. In India area, production and productivity of soybean during 2015 is 111 lakh ha, 86.4 lakh million tonnes and 781 kg/ha, respectively, in Maharashtra area, production and productivity of soybean during 2015 is 35.8 lakh ha, 27.8 lakh million tonnes and 776 kg/ha, respectively. Area, production and productivity of soybean in Marathwada during 2015 is 13.0 lakh ha, 6.91 lakh million tonnes and 670 kg/ha, respectively. Fertilizer is one of the most important inputs for successful crop production. A sustainable increase in production can be obtained by using balanced fertilizers. More scientific efforts are needed to increase the productivity of soybean per unit area and per unit time with soil moisture conservation and optimum fertilizer dose. Among the various factors responsible for the low yield of soybean, weeds have been considered to be of prime importance Application of herbicide is one of the best option. In India, the tobacco leaf eating caterpillar, *Spodoptera litura* (F.) has been widely recorded as serious polyphagous pest, which damages soybean crop also. Hence, a field experiment was conducted during kharif season 2015 to prioritize production factors and also suggest cost effective agronomic operation in soybean production.

A field experiment was conducted during *kharif* 2015 at College Farm, Department of Agronomy, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra. The soil of experimental plot was deep black in colour with good drainage. The trial was conducted with 12 treatments in a randomized block design with three replications using soybean variety MAUS-71. The treatments include T_1: Whole Package (WP), T_2:WP-Fertilizer, T_3: WP-Herbicide, T_4:WP-Hoeing, T_5:WP-Plant Protection, T_6:WP-Fertilizer + Herbicide, T_7:WP-Fertilizer + Hoeing, T_8:WP-Fertilizer + Plant Protection, T_9:WP-Herbicide + Hoeing, T_{10}:WP-Herbicide + Plant Protection, T_{11}:WP-Hoeing + Plant Protection and T_{12}:Control plot. The recommended dose of fertilizer was 30:60:30 NPK kg/ha for soybean. Each experimental unit was 5.4 m x 6.0 m and 3.6 m x 5.0 m in gross and net plot size, respectively. In treatments for weed control, herbicides pendimethalin @ 1.0 kg a.i./ha as pre emergence spray and imazethapyr @ 0.10 kg a.i./ha and quizalofop ethyl @ 0.075 kg a.i./ha are applied as post emergence spray. In plant protection factor, triazophos 0.06% and spinosad 0.01% was applied against soybean leaf eating caterpillars (semiloopers and *Spodoptera litura*). Five plants from each net plot were randomly selected and labeled for taking biometric observations at different growth stages. The same plants were harvested separately for post harvest studies. Data obtained at various variables were analyzed by analysis of variance method (Panse and Sukhatme, 1967). The gross monetary returns was calculated based on prevailing market prices of economic product, by product and crop residues. The net monetary returns of each treatment was worked out by deducting the mean cost of cultivation of each treatment from the gross monetary returns. The benefit: cost ratio of each treatment was calculated by dividing the gross monetary returns by cost of cultivation of the respective treatments.

It was observed from the data presented in Table 1 that adoption of whole package (T_1) to soybean crop recorded higher mean number of pods/plant (34.53) than rest of treatments but it was at par with T_4 (WP-Hoeing) and T_5 (WP-Plant protection). Significantly higher yield attributing character such as pod yield/plant (g), seed yield/plant(g),

number of seeds/plant, seed index, number of seeds/pod, seed yield kg/ha, straw yield kg/ha, biological yield, and harvest index were recorded in treatment whole package (T_1) than rest of the treatments. Herbicide spray had controlled the weeds to minimum level. Effect of plant protection showed that adoption of whole package (T_1) to soybean crop recorded significantly higher seed yield (2523 kg/ha) as compared to treatment T_5 (WP-plant protection), T_8 (WP-Hoeing) and T_{11} (WP-Plant protection). Lowest seed yield (1018 kg/ha) was observed in control (T_{12}).

Data pertaining to the gross monetary returns as influenced by different treatments are presented in Table 1. The mean gross monetary returns recorded were 66751/ha. The data in Table 1 showed that the highest gross monetary returns (₹95104/ha) were recorded by adoption of whole package (T_1) to soybean crop over rest of treatments and found statistically at par with T_4 and T_5. Lowest gross monetary returns (₹38442/ha) were recorded by control (T_{12}). Similar result was found by Deore *et al.* (2008) and Chaudhary *et al.* (2012).

Data pertaining to net monetary returns (₹/ha) as influenced by various treatments is presented in Table 1. The mean net monetary return recorded was ₹36087/ha. The data in Table 1 showed that the highest net monetary returns (₹60898/ha) was obtained with adoption of whole package (T_1) to soybean crop over rest of treatments however, it was found statistically at par with T_4 and T_5. The Lowest net monetary return was received by control (T_{12}). These results are in conformity with Jadhav *et al.* (2012) and Kothawade *et al.* (2007).

Data pertaining to B:C ratio as influenced by various treatments is presented in Table 1. The highest benefit: cost ratio (2.8) was recorded by the treatment T_1 (whole package) over rest of treatments but found statistically at par with T_2 (WP-Fertilizer), T_4 (WP-Hoeing) and T_5 (WP-Plant protection). The lowest B:C ratio was recorded by control (T_{12}). Similar results were observed by Prachands *et al.* (2014) and Umale *et al.* (2005) and Dhaker *et al.* (2010). The data in Table 2 showed that the highest weed control efficiency was observed in treatment T_1 (Whole package). The lowest weed control efficiency was observed in control (T_{12}). Lower is weed index believes higher is the efficiency of treatment. Lower weed index was observed where adaption of whole package is given to soybean crop.

Table 1 Yield attributes, yield and economics of soybean as influenced by different treatments

Treatment	No. of pods/plant	Pod weight/ plant (g)	No. of seeds/ plant	Seed yield/plant (g)	Seed yield (kg/ha)	Straw yield (kg/ha)	Biological yield (kg/ha)	Harvest index (%)	Gross monetary returns (₹/ha)	Net monetary returns (₹/ha)	B:C ratio
T_1: Whole package (WP)	34.53	13.13	96.35	7.74	2523	3482	6005	42	95104	60898	2.8
T_2: WP-Fertilizer	28.70	10.27	77.64	6.18	2097	3104	5201	40	79142	47936	2.5
T_3: WP-Herbicide	28.03	9.80	74.96	5.92	2052	3057	5109	40	77447	45741	2.4
T_4: WP-Hoeing	33.17	12.00	89.73	7.21	2379	3307	5687	42	89689	56483	2.7
T_5: WP-Plant protection	32.20	11.67	86.83	6.95	2240	3136	5376	42	84455	52249	2.6
T_6: WP-(Fertilizer + Herbicide)	16.87	6.00	44.89	3.55	1205	1843	3048	39	45501	16795	1.6
T_7: WP-(Fertilizer+Hoeing)	25.53	8.80	66.68	5.27	1648	2389	4037	41	62166	31960	2.1
T_8: WP-(Fertilizer+Plant protection)	23.30	8.13	60.58	4.79	1509	2203	3713	41	56944	27738	1.9
T_9: WP-(Herbicide+Hoeing)	22.33	7.27	58.29	4.62	1431	2117	3548	40	53993	23287	1.8
T_{10}: WP-(Herbicide + Plant protection)	21.13	7.00	54.46	4.30	1317	1962	3278	40	49698	19992	1.7
T_{11}: WP-(Hoeing + Plant protection)	26.70	9.00	72.07	5.64	1815	2577	4392	41	68436	37230	2.2
T_{12}: Control plot	15.70	5.33	40.68	3.18	1018	1577	2595	39	38442	12736	1.5
SEm±	1.55	0.70	4.00	0.31	99	145	244	-	3745	3745	0.12
CD (P=0.05)	4.57	2.05	11.73	0.93	291	424	715	-	10984	10984	0.35

Table 2 Weed control efficiency and weed index

Treatment	30 DAS		60 DAS		At harvest		Weed index
	Monocot	Dicot	Monocot	Dicot	Monocot	Dicot	
T_1: Whole package (WP)	66.30	70.00	67.20	60.87	66.22	61.10	0.00
T_2: WP-Fertilizer	62.92	65.00	66.67	56.52	66.29	56.16	16.88
T_3: WP-Herbicide	24.55	28.33	19.70	21.74	20.71	22.74	18.67
T_4: WP-Hoeing	40.98	46.67	41.67	33.33	43.57	34.25	5.71
T_5: WP-Plant protection	52.05	55.00	50.00	47.83	50.14	47.26	11.22
T_6: WP-(Fertilizer + Herbicide)	34.78	48.00	36.36	39.13	36.43	38.36	52.24
T_7: WP-(Fertilizer + Hoeing)	42.46	55.00	45.45	43.48	44.29	42.47	34.68
T_8: WP-(Fertilizer+ Plant protection)	53.96	60.00	60.61	52.17	57.86	51.37	40.19
T_9: WP-(Herbicide + Hoeing)	15.60	28.33	18.18	17.39	17.86	19.18	43.28
T_{10}: WP-(Herbicide + Plant protection)	44.57	40.00	31.82	30.43	32.14	30.14	47.80
T_{11}: WP-(Hoeing + Plant protection)	46.80	50.00	40.91	39.13	40.00	37.67	28.06
T_{12}: Control plot	-	-	-	-	-	-	59.65

REFERENCES

Chaudhary H R and Meghlal H P 2012. Bioefficiency of rynaxypyr and spinosad against green semilooper, *Chrysodexis actua* Walker on soybean. *Indian Journal of Entomology*, **74**(4): 323-325.

Deore N R, Shete B T and Tambe A D 2008. Effect of pre and post emergence herbicides on weed control and productivity of soybean. *Journal of Maharashtra Agricultural Universities*, **33**(2): 266-267.

Dhaker S C, Mundra S L and Nepaliya V 2010. Effect of weed management and sulphur nutrition on productivity of soybean. *Indian Journal of Weed Science*, **42**(3&4): 232-234.

Jadhav S G, Kalegore N K, Chavan D A, Shaikh F G and Dadgale P R 2012. Production of soybean in relation to cultivars and graded levels of nutrients. *Journal of Oilseeds Research*, **29**(1): 104-105.

Kothawade T R, Londhe T B, Sinare B T and Tambe A D 2007. Efficacy of different herbicide for weed control in soybean. *Journal of Maharashtra Agricultural Universities*, **32**(2): 273-274.

Prachand S, Kubde K J and Bankar S 2014. Effect of chemical weed control on weed parameter, growth, yield attributes and economics in soybean. *American-Eurasian Journal of Agricultural & Environmental Sciences*, **14**(8): 698-701.

Umale V S, Apotikar V A, Kakade S U, Nemade S U and Kulkarni U S 2005. Studies on integrated weed control in soybean [*Glycine max* (L.) Merrill]. *Crop Research Hisar*, **29**(3): 416-420.