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Review Article

Diseases of linseed (*Linum usitatissium* L.) in India and their management - A Review

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ABSTRACT

In India linseed (*Linum usitatissium* L.) is grown mainly for seeds which has high industrial, nutritive and medicinal value. Bast fibre obtained from straw which has an additive value. India ranks second in area but productivity of crop is very low. One of the major factors affecting its productivity is diseases. The important diseases damaging linseed crop in India are mainly fungal, causing crop losses upto 80-100 per cent under epidemic condition. Rust (*Melampsora lini* Erenb), wilt [*Fusarium oxysporum* f.sp. *lini* (Bolley) Snyder & Hansen], Alternaria blight (*Alternaria lini* Dey, *A. linicola* Groves and Skolko), powdery mildew [*Oidium lini* Skoric, *Leviellula taurica* (Lev.) Arnaud] are the major ones. The minor diseases are *Macrophomina/Rhizoctonica* wilt/collar rot, Sclerotium stem rot, Pythium damping-off, Botrytis rot, anthracnose and Dreschlera blight. The number of seed borne mycoflora are more. Bacterial disease has not been reported and only tobacco leaf curl virus has been reported. Few nematodes *viz., Rotylenchus reniformis, Tylenchorynchus brevidens, Meloidogyne incognita, M. hapla* and *Pratylenchus* are known to be associated with root. A phanerogamic parasite, cuscuta also become a serious problem in certain areas. The paper deals with the diseases occurring, crop losses and management of the commercially important oilseed crop in India.

Keywords: Diseases, Linseed, Management, Symptoms, Yield losses,

Linseed /flax (*Linum usitatissimum* L.) is a commercially important oilseed crop grown in several countries for its oil and fibre. It is second most important *rabi* (winter) oilseed crop in India. It has various industrial, edible and medicinal uses. However, the area under linseed cultivation is declining due to its low productivity. Among several abiotic and biotic stresses, diseases are the major one affecting its productivity. Sometimes, they cause 100 per cent losses. Some of the diseases of linseed have been the cause of temporary elimination of the crop in certain parts of the world. The crop suffers from several diseases (Table 1). Of them rust in hilly and cooler part of the country, Alternaria blight in Northern Indogangetic plains and wilt and powdery mildew in central and peninsular regions cause appreciable yield losses if they appear in epidemic form.

Rust, wilt and powdery mildew were the major disease problems in early and middle of the 20th century; therefore, the major research work during that period was focused on these diseases. However, in later part of the century Alternaria blight has been a major challenge, causing significant yield losses (Chauhan and Srivastava, 1975). This review includes the major diseases and research work related to these diseases in India.

Rust

Rust caused by *Melampsora lini* (Ehrenb.) Lev. is of wide occurrence and has been reported almost from all linseed growing countries. *M. lini* was first described by Persoon (1801) under the name *Uredo minuta*. Its occurrence was reported by Butler (1914) for the first time from the country. It was of common occurrence in United Provinces (now parts of Madhya Pradesh and Uttar Pradesh) (Prasada, 1940). The disease was in epiphytotic form in Indogangetic Plains in Northern India during 1961 (Hora *et al*., 1962). Rust is now a serious problem in Jammu & Kashmir, Northern hills of Himachal Pradesh and Uttar Pradesh, adjacent part of Punjab, Bihar, West Bengal, Odisha and certain parts of Uttar Pradesh (Gill, 1987).

Yield losses: The yield losses from rust have been estimated by several workers in the country under naturally infected as well as under artificial conditions (Chauhan and Srivastava, 1976; Misra, 1979; Shukla, 1992). The disease was found to cause 16-100 and 70-100 per cent yield losses in moderately to highly susceptible varieties but no loss in resistant varieties (Hora *et al.,* 1962). The disease not only causes the yield loss but also damages the fibre quality and oil contents. In light infection there is no loss in oil content but in heavy infection, it is reduced by 10-34% (Srinivasachar and Seetharaman, 1971). Singh *et al.* (1978) found 13.1 per cent loss in oil content in heavily infected linseed variety artificially infected with *M. lini*.

Symptoms: The rust attacks all the aerial parts of the plant. It appears in the form of bright yellow or orange

uredopustules on both surfaces of leaves, which may extend to stem and capsules (Vasudeva, 1962). The heavily affected leaves give a scorched appearance. The leaves get chlorotic and die out prematurely. In the later stage of infection the uredopustules are replaced by teleutopustules as brownish black encrustations on the stem. The teleutosori are not common on leaves as they shed off by the time of formation of teleutosori but they can be seen on remaining leaves. As a result the entire physiology of the plants gets disturbed, resulting into heavy yield losses, shrivelled seeds and poor fibre quality.

Perpetuation and epidemiology: Linseed rust is macrocyclic and euautoecious, surviving only on *Linum* species. In India the uredospores and teleutospores do not survive in plains due to unfavorable weather conditions (Vasudeva, 1962). However, they may survive in hills throughout the year (Mathur *et al*., 1961). Misra and Sethi (1962) and Prasada (1967) have shown actual role of teleutospores in initiating the disease in hilly areas of Northern India. The disease is initiated from pieces of linseed straws, bearing viable teleutospores in stored seeds, which is common in the hills, hence, clean seeds preferably after seed dressing was recommended. Rains in the hills during '*kharif*' almost obviate the possibility of self-sown linseed plants. However, there is no report, so far, of such plants in the offseason. Even highly susceptible wild species like *Linum mysporense* has not been found to harbour rust in nature. Prasada (1967) however, surmised the possibility of survival of self sown plants of linseed to harbour rust in the hills during off season.

The germination and survival of the uredospores dependent upon temperature, leaf wetness and light. The disease appears in seven days at temperatures between 13° to 21°C, while in 18 days at 0°-10°C. They are killed on exposure for 24h at 38-43°C and in 9h at 43-50°C. Teleutospores develop and germinate best at 18-24°C. The leaf wetness and light intensity also play a vital role in infections. Saharan (1978) reported that 4h leaf wetness and 15 to 25°C temperature were optimum for infection. At 0°C the maximum duration of leaf wetness required is 8 hour. Light affects the incubation period and it is twice in reduced light as compared to continuous light. Race and cultivar speciality with regards to effect of light intensity, incubation and latent period have been demonstrated by Saharan and Singh (1979). Analyses of simple correlation coefficient indicated that TEMP, MRH, RH and CD have a highly significant positive correlation with latent period whereas MTEMP had a highly significant negative correlation. Studies showed that mean temperature below 5ºC considerably influences rust development. For the progress of rust, the partial regression coefficients for TEMP are significant in TEMP, MRH and CD and TEMP and CD combinations. The multiple regression equation build up from different combination of variables for the progress of disease with R^2 values explained variation ranging from 39.9 to 61.5 per cent (Saharan and Singh, 1985).

M. lini is restricted only to *Linum* spp. From cross inoculation studies, the rust parasitizing common linseed/flax (*L. usitatissimum*) was found different from that on wild species. *M. lini* has been cultured on modified medium on host tissue (Truel and Ledingham, 1957), but there is no report of culture *in vitro* of *M. lini* from India.

Physiological specialization: Existence of physiological specialization in *M. lini* was observed for the first time from U.S.A. later from Argentina, Australia, Newzealand, Great Britain, India, Pakistan, Holland, Portugal, France and USSR. However, the systematic study initiated by Flor (1935) in USA is remarkable. In India Butler (1918) was first to predict the possibility of appearance of new races of *M. lini* through hybridization. Padwick (1940) indicated the possibility of existence of physiological races in linseed rust pathogen on the basis of two rust samples collected from Pusa and Karnal. Later, Prasada (1948) demonstrated the occurrence of five physiological races based on reaction on seven collections of 50 local linseed cultivars. Later aecidial stages were found in nature in Kangra valley (Misra and Sethi, 1962).

A total of 18 physiological races have been found to occur so far in India. These have been designated as I-1 to I-17 and I-43. They are all different from those given in Flor's key except for the last one (Saharan and Singh, 1978a). The races I-9 and I-14 were further split into sub races I-9a, I-9b, I-9c and I-14b, respectively on the basis of their reaction on cvs LC-95, K-2 and LC-256. Collectively these races were virulent on selections with resistance genes N, L^2, P, P^1, L5, L4, L10, L^1 and M^2.

The initial 7 Indian races were separated in two groups i.e., group A (I-1, I-3, I-4, and I-7) to which Bison was resistant and group B (I-2, I-5 and I-6) to which Bison was susceptible (Misra and Prasada, 1966). The race group A was predominant. Prasada *et al.* (1962) reported races I-1, I-2, I-3 and I-4 to be homozygous. However, Misra (1969) found heterozygocity in races I-1, I-4, I-5 and I-6. The F_2 cultures of crosses in races produced new races which extended virulence for genes L_{66} (in Birio), L_7 L_7 (in Barnes), M_{44} (in Victory) N_2 2(in Marshal), P_3P_3 (in Leona or Wells).

Host resistance: A large number of cultivars have been identified in India against different races or a mixture of races. Vasudeva (1962) found some of the NP lines eg. RR-5, RR-9, RR-10, RR-37, RR-38, RR-40, RR-45, RR-197, RR-204, RR-236, RR-262, RR-267 and RR-272 to be resistant against all existing races during that period. Misra (1964) tested 286 varieties and 12 species of Linum obtained from USA, Argentina, USSR, Afghanistan and that identified in India individually against six races.

Table 1 Diseases of linseed reported from India

Diseases	Causal organism	References
Fungal		
Rust	*Melampsora lini*	Butler (1914, 1918), Pearl (1923), Prasad (1940), Chowdhury (1951), Hora (1961)
Wilt	*Fusarium oxysporum* f.sp. *lini*	Butler (1918), Pearl (1924), Verma (1945), Merh and Kulkarni (1961), Sharma *et al*. (1971)
Powdery mildew	*Oidium lini*	Padwick (1941), Mundkur and Ahmed (1946), Patel *et al*. (1949), Porwal (1964), Pavgi and Singh (1965), Sandhu and Chandwani (1965), Shukla and Pathak (1967)
Alternaria blight	*Alternaria alternata* *A. lini*	Dey (1933), Arya and Prasad (1953), Kalia *et al.* (1965), Singh *et al.* (1974), Khanna and Chandra (1981), Kumar *et al.* (1985)
	Alternaria sp.	Mc Rae (1929)
	A. brassicae	Arya and Prasad (1953)
Cercospora leaf spot	*Cercospora lini*	Rathaiah and Pavgi (1971)
Macrophomina stem rot	*Macrophomina phaseolina*	Sunderraman (1931), Uppal (1935)
	Rhizoctonia sp.	Asthana (1957)
	Rhizoctonia bataticola	Bedi *et al*. (1961)
Stem/Collar rot	*Sclerotium rolfsii*	Khatti *et al.* (1980)
Viral		
Leaf curl	Tobacco leaf curl virus	Vasudeva (1958)
Nematodes		
Root knot	*Meloidogyne incognita* *M. hapla* *M. triticorhiza*	Prasad and Khan (1990) Chhabra (1992) Gaur and Sharma (1999)
Lesion nematodes	*Pratylenchus* sp. *Rhotylenchus reniformis* *Tylenchorhynchus brevidens*	Prasad and Khan (1990) Swarup *et al*.(1967), Nath *et al*.(1969) Sethi and Swarup (1968)
Phanerogamic Parasite		
Doddar	*Cuscuta epilinum* Weiche *C. hylina*	Rai (1982) Anon (1990-2004)
Biodeterioration	Nineteen genera	Kadian and Suryanarayana (1971), Saharan and Khosla (1971), Misra and Kanaujia (1973), Bhargava and Shukla (1980), Chandra *et al*. (1981 a&b), Mondal *et al*. (1981 and 1985), Kanwar and Khanna (1981), Dubey (1982), Prasada *et al*. (1983), Dubey *et al*. (1985), Sahay and Mathur (1985), Sahay *et al*. (1990), Chauhan *et al.* (1996), Kumar *et al.* (1997), Singh *et al.* (2001 a), Kumar *et al.* (2003)

Among species of *Linum* those resistant to all the races were *L.africanum, L.augustifolium, L.creptans, L.flocossum, L.gallicum, L.marginale, L.perenne, L.strictum, L.tenue* and *L.trigyna* while *L.mysorense* and *L.pallecum* were found susceptible. *L. alpinum, L. corymbiferum, L. grandiflorum, L. hispidum, L. hologynum, L. odoratum* and *L. tenke* were found resistant in addition to those reported earlier (Misra 1964), Kotasthane *et al.* (1973) found 29 varieties resistant against *M. lini*.

Prasad *et al.* (1988) found 24 lines free from both rust and powdery mildew and 38 free from rust. Cultivars like LC-216, LC-255 and LC-256 were recommended by Saharan (1988, 1991) as they were found to be resistant against all prevalent races. Twenty two germplasm including Hira, Mukta, Neelum and KL-1 (Surabhi) were found to be resistant to rust by Sinha *et al.* (1993). Kumar *et al.* (1999) tested 136 selected cultures under artificial conditions at two locations i.e., Kangra and Gurdaspur and found 11 cultivars to be free from rust and 47 as resistant. A number of cultivars *viz.,* 9X11, 5/47-2/1-10/10, Jabalpur local, JRF-2, JRF-4, H-36, H-40, H-45, H-49, ICAR-1, KL-1, KL-31, KL-178, LC-2021, LCK-9517, LCK -9436, LMH-43, Polf-30, Polf-31, RL-903, RL-906 and RLC-73 have been identified as promising germplasm in AICRP (Linseed) trials.

Genetics of host resistance: The genetical and inheritance studies were based on Flor's work. It was shown in his studies that resistance is dominant and that in same varieties in which virulence was conditioned by 1, 2 or 3 genes there were complementary genes for resistance. Thus on flax varieties with 1,2,3 or 4 genes for resistance to an avirulent race of rust, the ratio of avirulent to virulent cultures were 3:1, 15:1, 63:1 and 255:1, respectively (Flor, 1967). As many as 30 resistant genes were identified. They occur in five

series of closely linked or allelic genes at loci designated as K, L, M, N and P which contain 2, 13, 7, 3 and 5 genes, respectively. The resistance determined by all these genes were found dominant in all inheritance studies reported. Some of Flor's differentials were found to carry additional resistant genes in the five loci and some other *Linum* materials were found to carry sixth locus designated as D (Misra 1964b, 1966a, 1966b).

In India the resistance was found to be governed by 1, 2 or 3 dominant genes. Jeswani *et al.* (1963, 1964) found resistance to be controlled by one dominant gene in NP (RR) 262, two complementary genes in NP (RR) 45 and two dominant genes in NP (RR) 204. Misra (1964, 1966a, 1966b, 1969) found resistance governed by 1-3 resgenes in various differentials against 5 Indian races of *M. lini.* Saharan and Singh (1978b) found that resistance in LC - 255 to races I-8 and I-9b was due to single dominant gene but to race I-9c it was due to two dominant genes. In LC-216 also the resistance to races I-8 and I-9b was due to single dominant gene and for I-9b it was due to two dominant genes but these genes were different from those of LC-255. They had one parent i.e., EI-5643 in common and they were found resistant against most of the Indian races. Comprehensive screening of a large number of genotypes under artificial conditions against all prevalent races of *M. lini* indicated LC-216, LC-255, LC-256, Ottawa 770 B(L), Dacota (M), Victory A (M_4), Tower (L_8), Marshal (N_2) and pale blue crimed (L_3) as excellent sources of resistance (Saharan & Singh, 1978b & 1980b). Differences in reaction related to temperatures were considered partly responsible for differences in race prevalence (Saharan and Singh, 1983, 1987).

Misra and Shukla (1981) found that resistance to rust in EC-77959 and A-7-1-1 was governed by the same genes. BS-44 carried two resgenes, one of which was the same or allelic to that of EC-77959 and A-7-1-1. Hira carried two resgenes, different from others. EC-77959 was found an useful donor owing to its resistance to rust and powdery mildew both (Misra and Pandey, 1981). Goray *et al.* (1987a) using mixture of I -1 to I -7 races found inheritance of rust resistance in cultivars R-17, R-552, JLS (J) 1, R-556 and ILS-73-25 governed by a single dominant gene. Dominant duplicate gene was observed in Himalini, C-59 and Dhar local -2. Crosses between Dhar local - 2x Himalini and Dhar local -2x C-59 exhibited 255 R: 1S indicating operation of 4 diverse dominant genes.

Management: The varieties namely JLS (J)-1, Jawahar-7, Jawahar-17, J-23, R-552, LC-54, LC-185, K-2, Garima, Himalini, Janki, Kiran, Nagarkot, Surabhi (KL-1), Meera, Padmini, Rashmi, Sheela and Sweta have been recommended as resistant against rust (Saharan, 1991; Kerkhi *et al.,* 1999, Husain *et al.,* 2005 & 2010). The transmission of rust infection by means of seed and stem fragments could be avoided by using clean seeds. Storage of seeds in warmer regions is recommended (Agarwal and Kotasthane, 1973). Destruction of plant debris and growing resistant cultivars reduce the primary source of inoculum (Misra and Prasada, 1965; Saharan, 1988; Saharan and Chand, 1988). Excessive nitrogenous manures should be avoided. Early sowing helps in lower disease intensity (AICRP Report, 2004). Seed treatment with organo-mercurials has been found effective in reducing disease (Agarwal and Kotasthane, 1973). Sprays of fungicides like wettable sulphur, copper oxychloride, Cuman-L, Dithane M-45, (Agarwal and Kotasthane, 1973); Plantavax and Vitavax (Goel *et al.,* 1973); tridiomefon and tridemorph (Raut and Somani, 1988) reduced disease severity. Infection of rust was minimized by field treatment wih herbicide bentazoe (Ahmed, 1996). Indofil M-45 (mancozeb) and Rovral (iprodione) were found to reduce the rust disease. Resistant varieties managed 95 per cent disease but in susceptible cultivars early sowing, seed treatment with suitable fungicide and one spray of 0.25 per cent Indofil M-45 (mancozeb) reduced disease between 62-71 per cent

Wilt

The wilt caused by *Fusarium oxysporum* f.sp. *lini* (Bolley) Snyder & Hansen is one of the most serious diseases of linseed. The disease was first reported by Luggar (1890) from Minnesota, USA. He found that the disease was transmitted by water or old straw from infested to the noninfested fields. Bolley (1901) isolated the pathogen and proved pathogenicity. In India it was first reported from Central Province (Pearl, 1924; Mc Rae, 1926), Maharashtra (Verma, 1945) and Rajasthan (Sharma *et al*., 1971). The disease is now reported from all linseed growing areas of the country.

Yield losses: Regular cropping of linseed in same field causes soil sickness resulting into high disease percentage. The extent of damage depends largely upon the time of attack. About 80-87 per cent yield loss is reported under favourable conditions. Sharma *et al*. (1971) reported 36.7 per cent yield losses due to Fusarial wilt from Rajasthan.

Symptoms: The pathogen may attack plants at any stage from seedling to maturity. The young seedlings are affected almost three weeks after sowing when atmospheric temperature is generally high. The cotyledons of the affected seedlings turn dull coloured and the edges roll inward. The base of hypocotyl shows a constricted appearance. The young seedlings collapse from this point and ultimately die-off. In the older plants the disease appears as small ill defined dark green patches on the leaves. Later the leaves shrivel, branches droop and ultimately the plants die-off, although they remain standing. Sometimes partial wilting is

also observed in which the affected part turns brown while other side remains green. On splitting of infected basal stem and roots the brownish dicolouration of vascular tissues is observed. The mycelium of the pathogen is found in abundance in the infected dicoloured vascular tissues.

Perpetuation and epidemiology: *F. oxysporum* f.sp. *lini* is both seed and soil borne. In soil it is known to persist for several years even in the absence of host due to strong competitive saprophytic ability (Arora, 1980). In seeds the mycelium may persists on or inside seed coat. It may penetrate the soil upto 12 inches but more abundantly found at a depth not exceeding 5 inches. The chlarmydospores of the fungus present in soil/debris germinate in response to root exudates and the germ tubes penetrate plants through root hairs. Cortical parenchyma is the first tissue to be invaded by the pathogen. In roots of resistant varieties the entrance of the pathogen across vascular system is prevented by suberization of the cell wall and formation of cork layers in the cortex. Nair (1958) reported that invasion of resistant host is restricted to the cortex. It can also enter into the young seedlings through epidermal cells or stomata. In seeds it enters through macrophyle or wounds, subsequently invading cotyledons and redicals.

The soil type, temperature and moisture content influence the incidence of wilt. The temperature between 25-28°C is most suitable for the pathogen. At unfavourable temperature below 12°C and beyond 38°C even susceptible cultivars escaped from much damage (Kolte and Fitt, 1997). Incidence of wilt appeared to be more at soil pH 5.5 to 7.5 than at higher or lower levels (Nair, 1957). A temperature range between 25-30°C and pH 5-7.5 was found to be optimum for the growth of *F. oxysporum* f.sp. *lini in-vitro* (Souramma and Singh, 2004). Low moisture content (25 per cent) and sandy soils were favourable for wilt, while farmyard manure decreased the disease incidence (Goel and Swaroop, 1964). The nitrogen and potassium fertilization also reduced the wilt incidence while phosphorus was found to increase. Dastur and Bhatt (1964) found that the wilt resistant plants contained more potassium content than susceptible ones. Zinc also reduced the leaf symptoms caused by *F. oxysporum* f. *lini*, which was attributed to the inhibition of fusaric acid production in the pathogen. Singh (1999) found that increase in nitrogen (60 kg/ha) significantly reduced wilt while increase in phosphorous content increased disease incidence at Ujjain, Madhya Pradesh.

Variability/physiological specialization: From India two groups of isolates with distinct pathogenic behavior were identified from Uttar Pradesh. Bedi *et al.* (1961) identified two distinct races based on pathogenicity tested on ten selected varieties of linseed. Kulkarni *et al.* (1969) reported a new race from Nagpur, which differed from other races in cultural and pathogenic behaviour. Sharma and Mathur (1971) obtained monoconidial isolates from infected roots of linseed from Rajasthan and they established the prevalence of six distinct pathogenic races. Thakur and Husain (1972a) have demonstrated the existence of ten races (R_1 to R_{10}) from Uttar Pradesh based on their reaction on eight differentials and toxic metabolites.

Host resistance: In India a great deal of work has been done on identification of sources of resistance and breeding flax for resistance to wilt. A-4-3-1, BR-1, EC-544, NP(RR)65, T.N. Kangra (Chauhan and Muheet, 1975); LC-54, LC-2002, LC-2057, LCK-9119, DPL-19 and KL-187 (Pant *et al.* 2001). The genotypes 1_2 Jabalpur-1986, BR-1, EC-544, H-22, NP (RR) 65, RLC-6, RLC-29, RLC-46, R-552, T.N. Kangra were found as resistant sources. Twenty six germplasm *viz.,* Ayogi, BAU-9906, BAU-2K-04, BAU-2K-05, DPL-19, EC-41656, 12-JBP local, L-103, Kl-1, Kl-31, L-107, LC-2057, M-3, NL-14, No-7, NP(RR)65, RLC-46, JLS-9, Nagarkot, Padmini, Rashmi, R-552, Surabhi, Sweta and T-397 were found resistant (Kishore *et al.,* 2011). Singh and Singh (2011) found ten genotypes *viz.,* NP-19, NP(RR)-271, No.294, LC-2221, LMS-154-03, LMS-166-03, RLC-94, SLS-56, Ayogi and LMS-129-1 to be resistant against root rot - wilt complex in sick fields. Varieties Jawahar-23, Kiran, R-552, Surabhi and T-397 were moderately resistant.

Genetics of host resistance: The disease tolerance is reported to be governed by 1-2 dominant genes, single dominant genes in R-552 and R-556 and two dominant genes in G-194 unlinked to those of R-552 and R-556 (Goray *et al.,* 1987b, 1988). Agarwal *et al.* (1991) found wilt resistance to be determined by recessive allels. Conventionally the reactions to wilt in varieties and progeny of crosses have been recorded on the basis of percentage of plants killed versus survivals in the wilt sick plots. This has been quite workable, yet there was possibility of escapes. Hence, for genetic precision a technique was evolved where cent percent kill of susceptible plants could be ensured (Misra *et al.,* 1980). Misra *et al.* (1980) and Kamthan *et al.* (1981) disproved the hypotheses that resistance to wilt and rust were interdependent as shown in NPRR lines (Vasudeva, 1962). Misra *et al.* (1980) found EC9830 as monogenic for rust resistance and Neelum monogenic for wilt resistance and these were independent. Kamthan *et al.* (1981) showed wilt resistant varieties EC544(W), NP (RR) 65 and BRI to be monogenic against wilt in crosses with Norman while single pair of genes were responsible for rust resistance. Monogenic nature against wilt and rust was independently reflected. Later Kamthan *et al.* (1991a) demonstrated monogenic independent resistance against wilt and rust in Clay, Polk,

Abyssinian, Cass, Cortland, Birio, Min.R3083, Min R3115, EC41628, CI2796 and BRI while in Dakota bigenic for wilt and monogenic for rust. Kamthan *et al.* (1991c) further confirmed monogenic resistance against wilt in EC41628 and BRI and found that the two varieties carried different genes and perhaps the loci were different. Variety EC41628 also carried single independent gene for resistance to rust and powdery mildew.

Management: Cultivation of resistant variety is the most effective method for management of wilt disease. A number of resistant cultivars *viz.,* K2, LC-185, LC-54, Himalini, Jawahar -23, Jeevan, Kiran, Meera, Padmini, R-552, Rashmi, Sheela, Sheetal, Surabhi and T-397 have resistance against disease (Anonymous, 1986; Kerkhi *et al*., 1999; Husain *et al*., 2005; 2010). Early sowing and acidic soil should be avoided to reduce the disease. Application of farmyard manure and high doses of NPK reduces the wilt incidence. Crop rotation and flooding of fields reduced wilt inoculum. Since pathogen is known to survive in soil for a number of years in the absence of the host or on other hosts, a long crop rotation, may prove effective (Saharan, 1980; Saharan and Chand, 1988). Singh (1999) found that increase in nitrogen (upto 60 kg/ha) reduced wilt incidence significantly and increase in P increased wilt incidence. Dry heat treatment of linseed seed upto 50°C reduced wilt incidence upto 45.7 per cent (Singh and Souramma, 2005). Kishore *et al*. (2007) found presowing soil solarization for 4 weeks reduced fusarial wilt of linseed effectively (58.7%). Late sowing i.e., during 5-15 November resulted in lower disease percentage (Singh and Singh, 2011). *Trichoderma viride* and *T. harzianum* were able to reduce wilt incidence upto 65 per cent as compared to control plots (98%) at Raipur (Sharma *et al*., 2003). Singh and Kishore (2007) found that soil amended with *T. harzianum* and *T. viride* cultures reduced wilt percentage to the extent of 70.6 to 58.3 per cent, respectively. Farm-yard manure was also very effective in reducing wilt percentage (28 to 50% inhibition). Sawdust, Neem oil cake and mustard oil cake were also inhibitory but non-significant. Seed treatment with *T. viride* (4g/kg) and *T. harzianum* (4g/kg) singly or in combination within themselves or with Thiram significantly reduced the wilt percentage (Singh *et al*., 2008).

Seed borne inoculum of *F. oxysporum* f.sp. *lini* play a vital role in seedling infection, therefore, seed treatment with seed dressing fungicides have been found very effective in disease control. Seed treatment with systemic fungicides *viz*., Benlate, Chloroneb, Plantavax, Thiobendazole and Vitavax reduced Fusarium wilt *in vitro* (Haware, 1972; Desai and Siddaramaiah, 1980). Thiram, chlorothalonil and captan were also significanly effective. Kumar *et al*. (2003) found that carbendazim eliminated the entire pathogenic flora associated with linseed. Seed treatment with mancozeb (Indofil M-45), Bavistin, SAN 518W and BAS 38601 enhanced seed germination, emergence, vigour significantly and controlled most of pathogenic fungi. Kishore and Singh (2007) found Bavistin (carbendazim) and Benlate to completely check the growth of the pathogen *in vitro.* In field trials seed treatment with Bavistin was most effective followed by Benlate. Thiram+Bavistin, Thiophanate methyl, Thiram, Captan and Vitavax were also highly effective in reducing disease percentage over control. Seed treatment with *T. harzianum* (TH) was found most effective in reducing wilt percentage followed by TH+Thiram and TH+TV+Thiram at Kanpur, while minimum incidence of wilt was recorded in FYM amended plots followed by TV+TH, TH,TV+Thiram at Raipur centre (Anonymous, 2002).

Alternaria blight

The disease Alternaria blight or black bud in linseed is caused by three *Alternaria* spp. *viz., Alternaria lini* (Dey), *A. linicola* Groves and Skolko and *A. infectoria* anamorph *Lewia infectoria* or Alternaria state of *Pleospora infectoria* Fuckel. However, *A. lini* is predominant under Indian conditions (Singh, 2006). The disease was reported for the first time from Kanpur, then from Gorakhpur, Uttar Pradesh (Dey, 1933). Later it was reported from IARI, Delhi (Arya and Prasad, 1952), Punjab (Kalia *et al*., 1965) and Jabalpur (Singh *et al*., 1974). Now it is reported from all linseed growing areas. It is a serious disease in areas where high humidity persists especially in Indogangetic tracts.

Yield losses: The disease may cause losses ranging from 27.9 to 59.6 per cent. It is most harmful when buds and capsules are affected. The bud infection may cause loss upto 90 per cent (Chauhan and Srivastava, 1975) and there is significant negative correlation (r=0.7567) between the disease intensity and yield. Yield loss can be estimated through regression equation, Y=733.35-8.24X (Garg, 1982). The disease affects the seed weight, fibre quality and oil percentage. It is a serious disease in Uttar Pradesh causing upto 40.6 per cent yield losses (Singh *et al*., 2003 b).

Symptoms: The first symptom of the disease appears after 2-3 weeks of sowing on cotyledons. At later stage all the aerial parts of the plants get affected. The disease appears as small dark brown irregular spots on leaves, which enlarge and pass over to the stem and pedicel. In severe cases the entire leaves get blighted and dry up. On floral parts the dark brown spots appear near base of the calyx, which enlarge passing over to buds and pedicel. The buds fail to open and all floral parts including sepals, petals and other floral parts completely die and get converted into black powdery mass. When young buds are attacked, they do not open and dry-off.

The pedicel shrink at the base of buds and a dark circular ring is seen on the pedicel of diseased buds. The pedicel breaks from this point with any jerks. It is a distinguishing character from the buds infected with linseed bud fly (*Dasyneura lini* Barnes). The affected capsules give burnt appearance. If infection occurs during seed setting the seeds get shrivelled or not formed at all.

Perpetuation and epidemiology: The perpetuation of pathogen is known to be through contaminated debris and seeds, which cause primary infection. Secondary infection is through air borne conidia. The conidia, however fail to survive during summer months under field conditions. *A. lini* is both externally and internally seed-borne (Kumar *et al.,* 1985). The temperature between 26-33°C and high relative humidity (85-90 %) were found to be most favourable meteorological factors for the growth of the pathogen and disease severity. If relative humidity (RH) falls below 75 per cent then disease development restricts. Pandey *et al.* (2002) found temperature between 25-30°C and pH between 6-6.5 to be optimum for the growth of the pathogen. Singh *et al*. (2008) reported that most favourable period for disease development was between end of January to February, the maximum and minimum temperature ranged between 24.9-31.2°C and 8.4-15.4°C was most favourable.

Variability/physiological specialization: Very little work has been document from India on physiological specialization or pathogenic variability in Alternaria blight pathogens. The variability among eight strains of *Alternaria lini* isolated from different agroclimatic regions was studied on molecular level (Singh *et al.,* 2010). UPGMA analyses of RAPD data showed that eight isolates collected from geographically distinct regions of country, could be broadly clustered into four groups.

Host resistance: Identification of the sources of resistance against Alternaria leaf spot and bud blight in linseed remains the most challenging field as most of the recommended cultivars grown across the country are either susceptible or have partial resistance. Singh (2003) found six genotypes *viz.,* A-469(B), A-482, AKL-10, Alipur (Hamirpur) II, Polf-2 and Polf-15 resistant against Alternaria blight. Singh and Prasad (2005) found eight lines having field resistance and 18 were moderately resistant out of 200 germplasm evaluated. Kailash *et al.* (2007) reported 8 lines *viz.,* A-66, A-75, A-184, A-225B, A-226, A-232, A-361 having moderate resistance (12.67-25%). Ramakant *et al.* (2007) found six lines *viz.,* NDL-2004, NDL-2005-03-2, EC-22704, EC-41623, NDLS-4 and NDLS-169 having resistance to disease under artificial epiphytotic condition. The preliminary studies conducted to find biochemical basis of disease resistance revealed that high HCN, polyphenols and phosphorus content were associated with resistance as found in most of the highly tolerant cultivars *viz*., Ayogi and BAU-610 A (Singh and Vajpayee, 2002).

Genetics of host resistance: Kalia *et al.* (1965) found that resistance was conditioned by single pair of recessive alleles using K_2 and EI-5665 as sources of resistance against *A. lini.* Singh and Chauhan (1988) observed that resistance was governed by a single dominant gene in a study with ten crosses between resistant lines (SPS 77/23-10, Flake, R-552 and LHCK-222) and susceptible cultivars (T-397, CI-1889 and Neelum). Singh *et al.* (2006) reported that phenotypic characters like seed colour could not be the diagnostic character for resistance as reported earlier. The inheritance of resistanceto Alternaria blight in linseed was found to be reccessive as found in F_1 generationof 14 crosses involving seven resistant and four susceptible donors. The reaction appeared to be governed by single reccessive gene in twelve crosses and by two independent non-allelicgenes exhibiting complementary epistasis for susceptibility. With F_2 digenic ratio of 9:7 in the remaining two crosses (Ramjeet *et al*., 2012).

Management: The varieties like K_2, LC-54, Himalini, Jeevan, Gaurav, Meera, Nagarkot, Rashmi, Sheela, Shubhra, Sweta and Surabhi have been recommended for tolerance to this disease (Anonymous, 1986; Kerkhi *et al.,* 1999, Husain *et al.,* 2005, Husain *et al.,* 2010). Well drained high lying fields were recommended for cultivation. The disease severity is affected by the fertilizer doses and crop density. Excessive use of nitrogen fertilizers enhance the disease severity, therefore, it should be avoided (AICRP Report, 1992). The disease intensity is lower with lower seed rates (AICRP Report, 1999). Higher incidence of blight has been observed in early showing (middle to last week of October) therefore, sowing during first fortnight of November has been recommended (Singh *et al.,* 2000; Singh, 2003; Singh *et al.,* 2008c). Intercropping linseed with wheat (4:2 row ratio) reduced Alternaria infection in linseed (Singh *et al.,* 2005). Aqueous leaf extract of *Azadirachta indica, Lawsonia inermis, Datura metel, Calotropis procera, Lantana camara* and *Citrus* sp. reduced Alternaria blight infection in range of 42.3 to 19.0 per cent under field condition (Singh and Singh, 2007). Sprayimg of *T. viride* and *T. harzianum* were found effective in reducing Alternaria blight in linseed upto 25.0-36.0 per cent. Aqueous extract of neem leaf and linseed leaf also reduced disease severity significantly in range of 21.8 and 14.2 per cent, respectively (Singh and Kerkhi, 2007).

Seed treatment or foliar spray of Bordeaux mixture before flowering minimized the infection of Alternaria blight (Vasudeva, 1962). Seed treatment with dithiocarbamates *viz.,* mancozeb and zineb and blue copper reduced cotyledonary

blight caused by *A. linicola* (Singh *et al.,* 2001a & b). Spraying of mancozeb or tridemorph/calixin were found very effective against the disease. Spray of Iprodione or Indofil M-45 reduced the Alternaria blight disease significantly (Singh, 2002; Khan *et al.,* 2004; Singh *et al.,* 2004; Singh and Chandra, 2005; Singh *et al.,* 2007). Foliar spray of new fungicides *viz.,* propiconazole 25EC @ 0.1%, hexaconazole 25EC @ 0.12%, difenconazole 25 EC @ 0.05% and iprobenphos 48EC @ 0.1% managed disease economically. Spraying of combination fungicide, carbendazim 12%+mancozeb 63% WP75 @ 0.12% was most effective against Altrnaria blight with maximum benefit-cost ratio and seed yield. Resistance inducing chemicals namely, benzoic acid (0.1%), naphthalene acetic acid (NAA 0.1%), BION (acibenzolar-S-methyl 0.05%) were found effective in reducing Alternaria blight infection (Singh *et al.,* 2005; Singh and Singh, 2006). Singh (2007) found salicylic acid (1.0mM), phosphoric acid (0.1%) and BION (30g a.i./ha) reduced disease incidence.

Different IPM modules were tested against Alternaria blight. The module including tolerant variety sowing during first week of November with optimum dose of fertilizers, seeds treated with thiram or thiophanate methyl (2g/kg) and one or two sprays of 0.25 % Indofil M-45 significantly reduced disease intensity over module including farmer's traditional practice. The good monetary returns were recorded with this IPM package in disease prone areas (Singh *et al.,* 2003; Singh and Singh, 2006).

Powdery mildew

Occurrence of powdery mildew (*Oidium lini* Skoric) of linseed/flax was reported for the first time from Yugoslavia (Skoric, 1926). Since then four powdery mildew fungi *viz., Oidium lini, Erisiphe cichoracearum* DC, *E. polygoni* DC, *Spherotheca lini* Zvetk have been reported from world, and the first two reported from India (Sandhu and Chandwani, 1965; Pavgi and Singh, 1965; Shukla and Pathak, 1967). Powdery mildew of linseed is a disease of wide occurrence, but it is more serious in Central and Penincular regions of the country.

Yield losses: The disease causes heavy yield losses if appears at early growth stage. Heavy infection may cause shrivelling of grains (Gill, 1958). The yield losses in range of 2-10 per cent were reported from Jabalpur (Kushwah and Chand, 1971). Sharma and Khosla (1976) reported 63 per cent loss in unprotected plots. They found a negative correlation (-0.96) between seed yield and disease severity, the regression equation being Y=3.81-0.6. Yield losses ranging between 13.81 to 31.49 per cent was recorded in tolerant and susceptible varieties, respectively.

Symptoms: The first symptom of the disease is appearance of small white floury patches on the upper surface of leaves, which enlarge and cover the entire plant surface including stem, leaves and capsules. It results into increased respiration and decreased photosynthesis. The leaves covered with thick powdery masses show twisting and drooping symptoms, which ultimately dry up. Early infected plants remain small in size, produce less number of capsules and small sized seeds.

Disease perpetuation and epidemiology: The pathogen survives through formation of cleistothecia in diseased plant debris present in soil. The asci and ascospores are released in next crop season under favourable environmental conditions, initiating primary infection. The disease appears first in Central and Southern parts of the country, where the temperature rises early then it proceeds to the Northern regions. A temperature range between 20-25ºC, humidity less than 65 per cent and less rainfall are the predisposing factors (Saharan, 1988). Saharan and Saharan (1994a) found that powdery mildew severity was negatively correlated with temperature and relative humidity. Also the conidial germination of *O. lini* was initiated after 2 hours at 30ºC and 40 or 80 per cent RH and appresoria formation was highest (16.8%) after 14 hours at 70 per cent RH (Saharan and Saharan, 1994c).

Host resistance: A number of linseed cultivars have been identified as sources of resistance till now. Some of the powdery mildew resistant cultivars reported from the country are K-5835, EC-5663, EC-9832, EC-22587 and EC-22684 (Agarwal, 1975). Singh and Saharan (1979) found resistance in LC-216, LC-255, LC-256 and LC-269. Prasad *et al.* (1988b) reported 38 genotypes, free from powdery mildew and 24 having resistance to both powdery mildew and rust. The promising lines identified on multilocational testing under AICRP (Linseed) programme are A-125, A-127, 9X JBP1986, ILS-150, ILS-153, JRF-5, LC-2023, LC-2045, LC-2057, LCK-8605, LCK-8776, LCK-89512, LMH-43, LMH-16-3, Polf-23, RL-903, RL-906, RLC-34, RLC-35 (AICRP Reports, 1991-2004). Genotypes A-127, H-24, H-34, LC-2045, LH-1, Mahoba local, Mayurbhanj local, NP-27, NP-24, RR-55K, NP-100, NPHY-10,NP (RR)88R, RL-903, RLC-95, R-552, Nagarkot and Sheela were found free from disease even in delayed sowing (Sharan *et al*., 2008). Pandey *et al.* (1981) reported high HCN (Hydrocyanic acid) content in resistant genotypes like EC-77959 and EC-1456, whereas susceptible genotypes were low in HCN. In case of resistant variety Argan the peroxidase activity was found to be more as compared to the susceptible Mukta (Tomar *et al.,* 1985). Singh and Vajpeyi (2002) also reported high HCN and polyphenol contents in resistant cultivars *viz.,* KL-178 and Kiran as compared to susceptible cultivars, Chambal and Kanpur local.

Genetics of host resistance: The resistance of powdery mildew in linseed was found to be governed by a single

dominant gene (Kaushal and Shrivas, 1974; Singh and Saharan, 1979; Goray *et al.,* 1989; Singh *et al.,* 1989). Badwal (1975) had reported one partially dominant gene governing resistance in EI-5665. Misra and Pandey (1981) found monogenic resistance in EC77959 against rust as well as against powdery mildew. These genes had linkage value of 78 to 85 per cent, indicating to be potential donor for resistance to both the diseases. The resistance in cultivars LCK-8776, RL-33-4, RL-49-4-8-2 and DPL-20 was found to be conditioned by single dominant gene whereas RL-50-3 and Flake-1 was controlled by two dominant genes. Further the resistant genes were located on different loci (Saharan and Saharan, 1999).

Management: Tolerant/resistant varieties *viz.,* Himalini, J-23, Janki, Jeevan, Kiran, Meera, Nagarkot, Padmini, R-552, Rashmi, Sheela, Shikha, Surabhi have been recommended for different regions of the country (Kerkhi *et al.,* 1999; Husain *et al.,* 2005; 2010). Early sowing is the best cultural practice for disease escape. High fertilizer doses may be avoided as they enhance disease severity. Predatory role of adults and larvae of coccinallid, *Thea cincta* (*Psilobora cincta*) have been reported feeding and cleaning powdery mildew from infected parts of the plant (Prasad *et al*., 1988 a).

Powdery mildew was effectively controlled through the application of Elosal, Afugan and Morestan (Sharma and Khosla, 1979). Vyas *et al.* (1982) tested 6 fungicides against *O. lini* and carbendazim gave best control followed by Morocide (binapacryl) and tridemorph. Sprays of sulfex or wettable sulphur (0.3%) or Karathane (0.2%) at 15 days interval were found highly effective (Anon, 1988; Kerkhi *et al*., 1999). Two or three sprays of sulfex (wettable sulphur @ 0.3%) followed by Dithane-M45 reduced powdery mildew infection in linseed significantly (Singh, 2002).

Macrophomina stem rot

The stem rot caused by *Macrophomina phaseolina* was reported from India for the first time by Sunderaman (1931) and Uppal (1935). The sclerotial stage i.e., *Rhizoctonia bataticola* (Taub.) was reported by Asthana (1957) and later by Bedi *et al.* (1961), causing seed and seedling rot. *R. bataticola* (*M. phaseolina*) was found associated with linseed roots (Misra and Shukla, 1982). The disease has also been reported from Kanpur, Hoshangabad and Sagar. The disease may occur at any growth stage under suitable moisture and temperature conditions. It has been reported to cause upto 80 per cent wilting in linseed crop in Kangra district of Himachal Pradesh (Bedi *et al*., 1961).

Symptoms: The symptoms appear either at early growth stage marked by yellowing and withering of leaves, stunting and partial or entire discolouration of root system. At later stage dark brown discolouration of basal part of stem and roots is observed. The bark of the roots peel off and the small sclerotia are present on the bark, wood of the root and basal part of the stem. About 80 per cent wilting in linseed crop in Kangra district of Himanchal Pradesh due to *Rhizoctonia bataticola* (Taub.) Butler was reported by Bedi *et al*. (1961) and Paracer and Bedi (1962). Chandwani and Srivastava (1968) reported that Rhizoctonia does not attack the linseed plant directly as does *Fusarium lini* but makes its entry where root system has been damaged by either soil borne insect or physical injury.

Survival / disease perpetuation: Misra and Sinha (1982) found survival of the pathogen on six collateral hosts *viz., Sorghum halepense, Cynodon dactylin,* cowpea, pigeonpea, *Cyprus rotundus* and *Panicum atrosanguineum*, which were cross pathogenic.

Management: Among cultivars tested, NP-5 was found to be moderately resistant to macrophomina stem rot (Mishra and Sinha, 1982). Seed treatment with organamercurials reduced disease incidence (Kadian and Suryanarayana, 1971), seedling mortality was reduced by 0.25% by Captan (Mishra and Sinha, 1982).

Cercospora leaf spot

The disease was reported for the first time from India occuring in severe form in Varanasi area of the Uttar Pradesh (Rathaiah and Pavgi, 1971). The disease is caused by *Cercospora linicola* Pavgi and Rathaiah. The disease appears as small circular leaf spots on upper surface of leaves. The pathogen survives through mycelium in crop debris (Rathaiah and Pavgi, 1973a). The resistance of 24 linseed varieties to *C. linicola* was assessed but none was found resistant. However, BS 12-9 and KB 96/10 were found moderately resistant (Rathaiah and Pavgi, 1973b). Rathaiah and Pavgi (1970) evaluated some fungicides against some leaf spot diseases of oilseed crops including Cercospora leaf spot of linseed.

Root rot, Foot rot and Seedling blight

Root rot in linseed caused by *Sclerotium rolfsii* was reported from India by Khati *et al.* (1980). Root and stem rot caused by *S. rolfsii* was reported regularly from Raipur and other Chhattisgarh area. Nine fungicides were tested as soil and seed dressing against *Sclerotium rolfsii* on flax in pots. Maximum protection was obtained with soil drenches of Pancotine (guazatine), Captan and Brassicol (quintozene). Seedling stand and vigour was good with Pancotine and Brassicol. Thriam, Brassicol and Pancotine gave good control by either method (Siddaramaiah and Desai, 1980). *Trichoderma* sp. isolated from mushroom beds showed more than 50 per cent inhibition in *S. rolfsii.* The maximum

inhibition of test fungi was from *Trichoderma* sp. isolated from rice straw (Bhosale *et al*., 2007).

Other diseases

The intermitent occurrence of Sclerotinia stem rot disease was reported from Kangra (AICRP Reports 1996, 1997, 2001). Besides above mentioned fungal diseases Botrytis rot from Raipur (1994), Scorch (*Pythium* sp.) and leaf blight caused by *Dreschlera* sp. were reported (AICRP Report 2005).

SEED HEALTH

Several fungi are known to be associated with linseed seeds either externally or internally, causing seed or seedling rot, the seed borne diseases, bio deterioration and quality losses. The major seed borne diseases have already been discussed. Here only seed or seedling rot and biodeterioration are given as under:

Seed and seedling rots

The predominant fungi associated with linseed seeds causing seed and sedling rot are *Alternaria alternata, A. linicola, Aspergillus flavus, A. niger, Colletotrichum linicola, Curvularia lunata, Fusarium moniliforme, F. oxysporum* f. sp. *lini*, *Fusarium pallidoroseum, Rhizoctonia bataticola* (*Macrophomina phaseolina*), *R. solani, Phoma exiguea,* var. *linicola* were predominant (Kumar *et al.,* 1997). The number of field fungi decreased on storage while storage fungi increased and most of the field fungi disappeared after three years (Chandra *et al.,* 1981 b). These fungi affect seed germination as well as cause seedling mortality. Seed and seedling rot may cause complete loss in crop emergence but more commonly they result in reduced and patchy crop stand, causing significant yield losses. The plants, which survive are likely to be weak and susceptible to diseases. Seed and seedling rots caused by *Pythium* sp., *Rhizoctonia bataticola* and *R. solani* are more common.

Use of good quality seeds, stored in proper containers at low temperature are useful in maintaining the viability of seeds and avoid seed rotting. Saharan and Kaistha (1974) reported that if seeds are stored in polythene bottles at low temperature or after treatment with 0.2 per cent Captan or Ceresan or Dithane Z-78, then their viability is higher with no fungal infection. Seed treatment with carbendazim (2g/kg) was effective in eliminating seed borne fungi. Singh *et al.* (2001 b) reported seed treatment with Thiram and Captan to be most suitable resulting into 96.2, 93.4 and 92.1 per cent germination, respectively. Seed and seedling rot was found reduced with seed treatment with carbendazim, Dithane M-45, Captan @ 2g/kg (Kumar *et al*., 2003).

Biodeterioration

In addition to the seed borne fungi which cause disease in the seedling stage or at later stage, several other type of field and storage fungi grow and multiply on seeds as long as food and moisture are adequate, causing deterioration in quality and viability of seeds. Most commonly encountered are *Alternaria tenuis* (*alternata*), *A .linicola, Aspergillus niger, Penicillium chrysogenum* and *Stemphyllium* sp Other fungi which have been detected on linseed seeds are *Aspergillus aculeatus, A. flavus, A. fumigatus, A. ochraceous, A. repens, Cephalosporium* sp., *Cephalosporium irregularis, Chaetomium dilichotrichus, C. funicolum, C. indicum, Cladosporium epiphyllum, C. herbarum, Curvularia lunata, Drechslera tetramera, Epicoccum* sp., *Fumago* sp., *Fusarium culmorum, F. equiseti, F. moniliforme, Paecilomyces spiceria, Pyronellaea fumaginoides, P. nicotinae, Mucor* sp., *Rhizopus nigricans, Trichoderma viride, Torula alli* and *Trichothecium roseum* (Kanwar and Khanna, 1981;Chandra *et al.,* 1981a; Prasad *et al*., 1983; Dubey *et al.,* 1985).

Flax seeds have excellent keeping quality when stored in relatively dry state. If the moisture level increases from 10.5 per cent, the deterioration starts. Other factors which affect are temperature, oxygen supply, method of harvesting, stage of seed at time of harvest, length of storage, presence of moulds and insects, which influence the extent of biochemical changes which take place during storage (Kolte and Fitt, 1997). The seeds of certain varieties are more susceptible than others. The spontaneous heating of flax seed during storage is attributed to accelerate the rate of respiration due to infestation of storage fungi. These fungi influence germination also (Dubey, 1982). Some fungi *viz., Fusarium culmorum* and *F. eqiseti* caused reduction in oil content (Sahay and Mathur, 1985). Infestation with *Alternaria alternata, Aspergillus flavus* and *A. respens* reduced the iodine number, while it increased with *Cladosporium herbarum* and *Fusarium culmorum. Aspergillus flavus* was more destructive in reducing oil content and inducing hydrolytic rancidity (Dubey *et al*., 1985). Prasada *et al.* (1983) found maximum loss in caloric value with *Aspergillus* niger followed by *A. flavus* and *F. oxysporum.* The storage fungi also result in aflatoxin production (Sahay *et al.,* 1990; Chauhan *et al.,* 1996). Sahay *et al.* (1990) found *Aspergillus* sp., *Penicillium citrinum* and *Fusarium* sp. to be more frequent in stored seeds. The gunny bag storage system exhibited higher count of *A. flavus* than occurred in Kothi. Belladona (*Atropha belladona*) and sulphur 30 were the most preventive treatments as oilseeds treated with them produced no aflatoxin B_1. Post inoculation treatments were less effective than preinoculated (Srivastava and Attri, 1998).

Storage of seed in dry (moisture level below 10%) and cool places is most essential. The essential oil of mustard (0.1%) containing allyl isothiocynate and propionic acid (0.1%) have been found useful in post harvest preservation of linseed seeds (Mondal *et al*., 1985).

Viral diseases

Among the virus diseases of linseed only leaf curl caused by tobacco leaf curl virus has been reported from country (Vasudeva, 1958). Incidence of phyllody has been reported frequently from AICRP (Linseed) centers *viz.,* Kanpur, Berhampore and Faizabad (AICRP reports, 1998, 1999, 2002), which still needs confirmation through proper study.

Parasitic nematodes

In India *Rotylenchus reniformis* (Swarup *et al*., 1967; Nath *et al*., 1969) and *Tylenchorhynchus brevidens* (Sethi and Swarup, 1968) have been found to infect flax plants in the host range studies. The root knot nematodes *viz., Meloidogyne incognita* and *M. hapla* and lesion nematodes, *Pratylenchus* sp. are known to be associated with linseed (Prasad and Khan, 1990). *M. hapla* has been reported to increase the incidence of wilt fungus, *Fusarium oxysporum* f. sp. *lini* (Chhabra, 1992). Linseed is a good host of *M. triticoryzae* also (Gaur and Sharma, 1999).

Phanerogamic parasites

Doddar / Cuscuta: The dodder or cuscuta represented by two species *viz., Cuscuta epilinum* Weiche and *C. hylina* are flowering stem parasites of flax. It is a serious problem in Chattisgarh, Maharashtra and Orissa (Rai, 1982). Earlier it was an endemic problem in Chattisgarh area. However, it has now entered the new areas like Mauranipur and Kangra also. It is a very fast growing parasite causing so much damage that it has become a matter of quarantine. The doddar first appears as slender pale vine, yellowish leaf less stem, entwins around the flax plant. They draw nutrient from flax plant through haustoria. The infected plants show decline in vigour and gradually turn yellow like the colour of parasite. Such plants produce less seed. The parasite survives through seeds, which fall on soil or plant debris or as contaminant in seed.

Cultural practices *viz.,* use of clean seed, cuscuta seeds should be removed from linseed seed through sieve before sowing, harrowing between crop rows before it parasitises host plants, Avoiding the flow of irrigation water from infested field to noninfested fields, Movement of grazing cattles from infested to noninfested area should be avoided, cultural practices like tillage, rotation and intercropping reduce cuscuta infestation. Use of herbicides *viz.,* Pronamide (as pre-emergence or post emergence after 15-20 days of sowing @1.25-2 kg/ha); Pendimethalin (pre emergence @1.0 kg/ha); Fluchlorin (presowing @1.0-1.25 kg/ha) or Paraquat (spot treatment, spray of 1.0% solution) have been recommended against cuscuta/dodder (Mishra and Raghuwanshi, 2002).

Research gaps and future thrust

- The foremost need is to develop a proper mapping of major diseases with respect to their occurrence and severity in different agroclimatic regions. This is to be updated regularly to enable for resistance breeding and developing need based strategy for disease management.
- Identification of key factors which influence the outbreak of diseases or proper disease forecasting system.
- Easy and reliable screening techniques need to be developed.
- Studies related to identification of the races / biotypes of the major pathogens.
- Identification of race specific and multirace resistant donors
- Pyramiding genes for resistance to different races with respect to different pathogens.
- Exploration for secondary gene pool for resistance against disease like Alternaria blight for which there is no complete resistance in present germplasm collection.
- Application of biotechnology and genetic engineering in developing the resistant genotypes against major diseases like Alternaria blight.
- Practical application of IPM modules and improvement of IPM modules from time to time, which are location specific in view of significance and severity of the disease.

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Validation of SSR markers linked to oil content in groundnut (*Arachis hypogaea* L.)

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ABSTRACT

A set of 14 SSR markers that are specific for six QTLs for oil content reported by various authors was selected for validation in the present study. The $F_{4:5}$ mapping population of the cross ICGV 00440 x ICGV 03128 was used to validate the markers as well as QTLs specific for oil content. Among the selected markers, six markers were polymorphic for the parents ICGV 00440 and ICGV 03128. Among the six polymorphic SSR markers, two markers IPAHM103 and PGS16F10 revealed a strong association for oil content with a PVE of 15.3 and 19.6 respectively in single marker analysis. Validation of QTLs was also performed through composite interval mapping analysis. A QTL with flanking markers IPAHM103 and PM36 could be considered as a potential tool for marker assisted selection of the trait oil content in groundnut.

Keywords: Groundnut, Oil content, QTL, SSR markers, Validation

Groundnut (*Arachis hypogaea* L.) is a major oilseed crop worldwide and one of the most widely produced legume (Weiss, 2000; CGIAR, 2010). It is used as food (raw, roasted or boiled), animal feed (pods, seeds and plant material) and for industrial raw material. The products derived from groundnut include flour, oil, peanut butter, confectionary and paste. Chemical and epidemiological studies conducted by Dean *et al.* (2009) suggest that the nutritional properties of peanuts are favorable due to the fatty acid profiles, a high quality protein and a source of naturally occurring folate. Regular consumption of peanuts in diet has been confirmed to have positive impacts on human health by providing significant source of protein (20 to 36%), edible oil (45 to 50%) containing essential fatty acids, carbohydrates, fiber, folacin, phosphorus, magnesium, zinc, iron, potassium, calcium, vitamins (riboflavin, thiamine, niacin) and three forms of fat-soluble tocopherols (α, γ and δ), which are the most important lipid soluble antioxidants discovered by Herbert Evans in 1922 (Packer *et al*., 2001; Munné-Bosch, 2005; Mondal and Badigannavar, 2016; Ajay *et al*., 2016).

Groundnut occupies a unique position among oilseeds as it contains approximately 50 per cent oil which is comparably higher than other vegetable oil. About two-thirds of world groundnut production is crushed for cooking oil and the remaining one-third is used in the form of edible products. The production of groundnut is largely confined to Asian and African countries. Asia accounts for about 50 per cent of area and 60 per cent of world production of groundnut with largest share of India (>20%) in the groundnut coverage, followed by China (>18%). However, China accounts for highest share (37%) in the total production of groundnut in the world. As per 2013-14 (GOI, 2014), India being the second largest groundnut producing nation occupies an area of 52.6 lakh ha with a production of 96.73 lakh tonnes which accounts for a productivity of 1750 kg/ha. Generally, the groundnut yields are low in the developing countries where the focus is more on to get rid of the obstacles for improving yield. The present production and productivity has to increase at a much higher rate to meet the growing needs of the oil market. It is estimated that each one per cent increase in oil content would raise the processor's benefit by seven per cent (Liao and Holbrook, 2005).

Presently, groundnut breeding programme is faced with the challenge of improving oil content and enhancing yield. Yield and oil content is a complex trait, polygenic in nature with significant environmental influences. Though selection for yield is practiced in early generations, selection for oil content is practiced only in advanced breeding lines as biochemical estimation or through NIR/NMR in segregating populations is cumbersome and demands high resources, as well as time. It is possible to specifically target such traits for improvement, and also enhance the efficiency of overall breeding program through use of molecular breeding strategies.

Molecular markers have been used to identify genomic regions (QTL) involved in expressing seed oil content, through available molecular information/approaches and then attain the genetic gain by selection of QTL loci. SSR marker

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has been the most widely used at identifying molecular variation within the cultivated species of groundnut and considerable progress has been made in tagging economically important traits (Selvaraj *et al*., 2009; Ferguson *et al*., 2004). The markers associated with QTLs cannot be directly used in MAS as it requires validation in other population. An attempt was made to validate the markers and reported QTLs associated with oil content in the $F_{4:5}$ population of the cross ICGV 00440 x ICGV 03128.

MATERIALS AND METHODS

Plant material: The present study was done in the $F_{4:5}$ generation developed from cross ICGV 00440 x ICGV 03128. It consists of 103 RILs developed by single seed descent method at Department of Oilseeds, Tamil Nadu Agricultural University, Coimbatore. The parent ICGV 00440 is Spanish bunch type with an oil content of 42-45 per cent. Parent ICGV 03128 is Virginia bunch type with oil content of 52-55 per cent and drought tolerant (Table 1).

Experimental design and phenotyping: A total of 103 recombinant inbred lines (RILs) in their F_4 generation were sown along with their parents in 3m rows with spacing of 30 x 10 cm in progeny rows during rainy season, 2013. Single plants were tagged in each RIL. The DNA samples were collected from the tagged plants and used for genotyping. The tagged F_4 progenies along with three checks were raised in 3 m row following Augmented Block Design I to study the $F_{4:5}$ performance during rainy season 2014. Spacing adopted was 30 x 10 cm. Oil content in progeny row bulk seed samples were measured on a BRUKER Matrix-I NIR spectrometer in absorbance mode. The validation was carried out using 55 samples with known oil content as estimated using Sox-plus for oil content estimation. The samples were scanned in a wave number range of 6000 - 4000 cm^{-1} with a resolution of $2cm^{-1}$ (Sundaram *et al*., 2011). The sample was scanned 32 times. After validation with an R^2 value of 98.69 per cent, the analysis of oil content in 103 RILs, parents and checks was estimated.

Molecular marker analysis: For DNA extraction, leaves were collected from 103 RILs as well as from the two parents in two leaf stages. DNA extraction was performed according to the cetyl trimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1990). The extracted DNA content was measured using DNA standards in agarose gel (0.8 % w/v). The 10 µl PCR cocktail contained 20 ng of 2 µl DNA, 1 µl of 10XTaq buffer, 0.2 µl of 25 mM $Mgcl_2$, 1.0 µl of 0.2 mM of dNTP, 0.5 µl of 0.5 uM of each forward and reverse primer, 4.5 µl of sterile water and 0.3 µl of 0.03 IU Taq DNA polymerase. DNA amplification was performed in a 96-Well Fast Thermal Cycler (Applied Biosystems Inc., Foster city, CA). DNA samples were denatured initially at 94°C for 3 min, then subjected to the following 20 cycles: 94°C for 30 s, 63°C for 30 s with a decrement of 0.5°C per cycle, and 70°C for 1 min. This was followed by another 20 cycles of 94°C for 15 s, 55°C for 30 s, and 70°C for 1 min. A 10 min extension was performed at 72°C as the last step. Amplified products were analyzed using 6% polyacrylamide gel electrophoresis at 150 volts DC for 4 hrs and silver stained in accordance with the protocol described by (Benbouza *et al*., 2006). Fourteen SSR markers that account for six already reported QTLs (Table 2 and 3) were used to study the polymorphism between the two parents. Among these, six markers *viz*., IPAHM103, PGS16F10, PM 36, GM1890, GM1878 and GM1961 were found to be polymorphic. The F_4 generation was genotyped with the six polymorphic primers.

Marker trait association: Clear and unambiguous bands were scored for their presence or absence with the score 1 indicating their presence and 0 indicating their absence. The data matrix of binary codes thus obtained was subjected to further analysis *viz.,* single marker analysis and composite interval mapping analysis.

Single marker analysis: Phenotypic values of all the 103RILs were subjected to associate with corresponding marker score for its significance by using simple regression in SPSS software (version.16). Simple linear regression method (Haley and Knott, 1992) was used to identify significant marker trait association. The linear equation formed was as follows:

$$Y = \mu + f(\text{marker}) + \text{error}$$

where, Y = phenotypic trait value; μ = population mean and f (marker) = function of the molecular marker.

The potential relationship between the marker and trait was established considering the significance of the regression coefficient at 5 and 1 per cent probability. The phenotypic variance explained (PVE) was expressed in terms of adjusted R^2 values.

Composite interval mapping: QTL ICI mapping version 3.2 (Wang *et al*., 2012) was used to analyze the data for composite interval mapping. The reported map position of three QTLs by various authors (Pavithradevi, 2013; Sarvamangala *et al*., 2011; Pandey *et al*., 2014) was used to validate through inclusive composite interval mapping of additive and dominant (ICIM-ADD) method. The ICIM-ADD was performed with mapping parameters as scan interval of 1cM, 0.001 probability in step-wise regression and LOD threshold of 3.0.

RESULTS AND DISCUSSION

One of the most important use of QTL mapping is to apply them in marker-assisted selection (MAS) for genetic improvement of quantitative traits. The already identified QTLs has to be validated in a different population to use it further in marker assisted selection. Once the tightly linked markers have been identified, the traits can be selected indirectly using MAS. In the present study, phenotype data were obtained from F_5 population of ICGV 00440 x ICGV 03128 and genotypic data from F_4 population. The analysis of variance for the F_5 generation of the cross ICGV 00440 x ICGV 03128 revealed significant differences for oil content. Frequency distribution for the oil content revealed a typical normal distribution indicating their quantitative nature of inheritance (Fig. 1).Validation of reported SSR markers and known QTLs was performed in the $F_{4:5}$ generations. Microsatellites (SSRs) are markers of choice in groundnut as they are ubiquitous, co-dominant and multi-allelic in nature. A total of 14 markers that account for reported QTLs linked to oil content were selected, of which only six markers were polymorphic. The reason for low level polymorphism is due to the narrow genetic base between the two parents selected for mapping population. Young *et al.* (1996) reported that low level of genetic polymorphism in cultivated groundnut was attributed to its origin from a single polyploidization event that occurred relatively on an evolutionary time scale.

For single marker analysis, the markers were subjected to single factor regression analysis using the marker as independent and the respective phenotype as dependent. The result of the single marker analysis for the trait oil content is presented in Table 2. Among the six markers, only three markers IPAHM103, PGS16F10 and PM 36 revealed a strong association for oil content with a PVE of 15.3,19.6 and 3.8, respectively (Table 4). Persuasive results were reported by Anitha *et al*. (2014) for the marker IPAHM103. Sarvamangala *et al*. (2011) also reported similar results for the same marker for two different seasons. The marker PGS16F10 was reported by Pavithradevi (2013) in the QTL region PM36_PGS16F10 for the trait oil content. The marker PM36 recorded a comparatively low PVE of 3.8. This was also reported in the QTL region IPAHM103_ PM36 by Sarvamangala *et al*. (2011).

Three QTL region reported by previous workers (Pavithradevi, 2013; Sarvamangala *et al*., 2011; Pandey *et al*., 2014) for oil content were used for QTL validation. Among the three linkage groups, IPAHM103_PM36 recorded significant QTL with 3.07 LOD and 12.82% PVE (Table 5). This reiterates the findings of Sarvamangala *et al*. (2011). The distance between the flanking markers is 9.5cM.Thus these two markers can be of utmost importance in MAS for oil content. Inclusion of some more markers in this region will further improve the success of MAS.

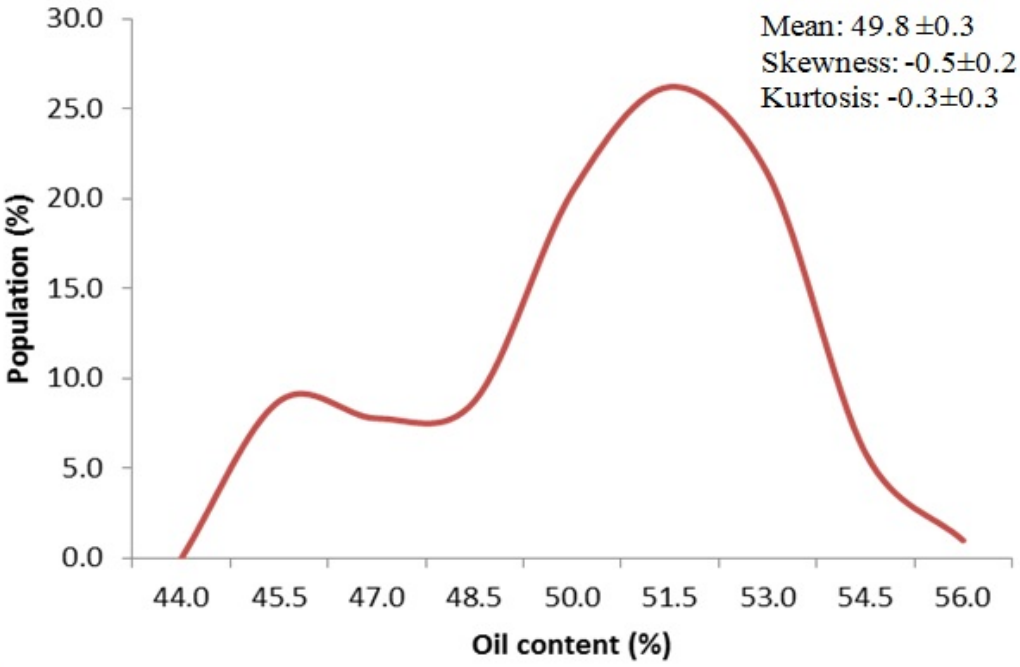

Fig. 1. Frequency distribution for oil content in F_5 progenies of the cross ICGV 00440 x ICGV 03128

Table 1 Details of parents used in the mapping population

Particulars	ICGV 00440	ICGV 03128
Pedigree	(ICGV 88386 x ASHFORD) x ICGV 95172	(ICGV 99160 x ICGV 99240)
Habit	Spanish bunch	Virginia bunch
Oil content (%)	42-45	52-55
Oleic (%)	45-50	35 -43
Linoleic (%)	28-30	40-57
Special features	Low oil and Confectionery line	High oil and Drought tolerant

Table 2 List of reported QTLs and markers linked to oil content and used for validation

Linkage group	Identified QTLs		Position (cM)	Interval (cM)	PVE (%)	Markers	Reference
	Left marker	Right marker					
2	AC2AO6	GM1907	120	126.1-165.6	23.7	AC2AO6,GM1907	Pavithradevi (2013)
2	PM36	PGS16F10	220	209.9-243.3	24.5	PM36, PGS16F10	
3	IPAHM103	PM36	28	25.5-34.0	10.2	IPAHM103,PM36	Sarvamangala *et al*. (2011)
5	GM1702	GM1878		0-23.8	10.23	GM1702,GM1878	Pandey *et al*. (2014)
5	GM1878	GM1890	28	25.8-31.8	25.52	GM1878-GM1890	
8	GM2690	IPAHM606	-	37.4-50.5	14.07	GM2690, IPAHM123 GM2689,GM1961, PM505,IPAHM606	

Table 3 List of fourteen SSR primers and sequences used for F_5 genotyping

SSR primer	Forward (5'-3')	Reverse (5'-3')
GM1702	GATTGGGAAGCAGCAAGAAG	CAACCAGCTCCTTCTCTACCC
GM1878	TCAGTGGTTCAGTGCATCAAG	GTCCCTTGGTCATCTTCGATT
GM2690-1	GACGCCGTGGTTTATGACTT	CAACCAGCTCCTTCTCTACCC
IPAHM123	CGGAGACAGAACACAAACCA	TACCCTGAGCCTCTCTCTCG
GM2689	GACGCCGTGGTTTATGACTT	CAACCAGCTCCTTCTCTACCC
GM1961	TGTATTCTCCCTGAAATGACGA	CTTCTTCCTCCATCCTCCCTA
PM505	TCCTCACATTGACGATGACC	CGGAGAACGAGAGGTTGAAG
IPAHM606	CCTAACTCAGCCTGCGAAAC	CAGAGGTGTTTGGAGAACTAGGA
GM1890	CTCTCCGATTATAGGCCAACC	TGGCTTCTCCGTGAAAATAAC
AC2A06	ATCATCTCGATCCATCCTTCTG	CTCCTTCTTCTCGCGTATTTGT
GM1907	CACTGTCCTCTTCCCTCACTCT	GGTGGACGAAGAAGAAGAAGAA
PM36	ACTCGCCATAGCCAACAAAC	CATTCCCACAACTCCCACAT
IPAHM103	GCATTCACCACCATAGTCCA	TCCTCTGACTTTCCTCCATCA
PGS16F10	TGGAGGGAAAAACATTTTGG	CCTGGAGGGGTGAGAGGT

Table 4 Single marker analysis for oil content in $F_{4:5}$ population of ICGV 00440 x ICGV 03128

Marker name	PVE	Significance
IPAHM103	15.3	**
PGS16F10	19.3	**
PM36	3.8	**
GM1890	2.4	not significant
GM1878	1.2	not significant
GM1961	0.9	not significant

Table 5 Details of QTL validated for oil content

Trait Name	Position cM	Left Marker	Right Marker	LOD	PVE (%)	Additive Effect
Oil content (%)	25.5	IPAHM103	PM36	3.07	12.82	ICGV 03128

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Oil quality of exotic safflower (*Carthamus tinctorius* L.) cultivars in India

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ABSTRACT

Improvement of oil content and quality are important goals in safflower breeding. In this study, a set of 10 high oil exotic safflower cultivars were evaluated at Hyderabad (India) for oil content and fatty acid composition in order to assess their suitability for Indian safflower breeding programmes. Oil content in the exotic varieties ranged from 35.3 per cent (Finch) to 41.4 per cent (Centennial). Overall, the exotic varieties showed about 5 to 10 per cent increase in oil content over the most popular Indian high seed yielding varieties A-1 and Bhima. Furthermore, six varieties showed high oleic acid content ranging from 70.0 per cent to 80.2 per cent. The exotic varietal sources reported in this study would be helpful to expedite breeding high oil-high oleic safflower cultivars in India.

Keywords: Safflower, Fatty acid composition, Exotic varieties, Genetic improvement, Safflower

Safflower (*Carthamus tinctorius* L.) originated in the Fertile Crescent region over 4000 years ago and was domesticated in the Far East, India, Pakistan, the Middle East, Egypt, Sudan, Ethiopia and Europe (Chapman and Burke, 2007). It is a traditional crop of India, primarily grown for extraction of edible oil from seeds. It also has variety of other uses including poultry feed, extraction of natural dye (carthamin) from petals and preparation of industrial and pharmaceutical products. Standard safflower oil is a healthy oil with high amount of polyunsaturated fatty acid (PUFA), linoleic acid (>70%) and high ratio between polyunsaturated and saturated fatty acids among edible oils (Kostik *et al*., 2012). Safflower is also a good source of monounsaturated fatty acid (MUFA), oleic acid (>70%) (monounsaturated, MUFA) (Knowles, 1968), which is more stable and preferred for deep frying applications in the food industry.

Despite the economic importance, safflower cultivation is declining in India, from 10 lakh ha in 1988 to 2.11 lakh ha in 2014 (FAOSTAT, 2014), which is perhaps due to low productivity and profitability. India has a long history of breeding programme in safflower, which has resulted in release of more than 30 cultivars. Oil content in the popular cultivars remains low (~30%), which is a concern for increasing profitability of safflower cultivation (Nimbkar, 2008). Therefore, improvement of oil content is an important goal of the Indian safflower breeding programmes. Availability of high oil sources is critical for this purpose. The United States Department of Agriculture (USDA) collection of safflower accessions, which includes released varieties has high level of variation for oil content (13% to 46%) and unsaturated fatty acids (oleic or linoleic) (~20% or ~80%) (Johnson *et al*., 1999). Safflower cultivars with high oil have also been reported from Mexico (41.9%) (Montoya Coronado, 2008), Australia (42%) (GRDC, 2010) and Argentina (43.4%) (Baümler *et al*., 2014). Among germplasm sources, exotic varieties with specific traits would be valuable for breeding efforts in different countries (Holland, 2004) because they can be deployed immediately in crossing programmes without much pre-breeding effort. Kadirvel *et al*. (2016) found that a subset of Mexican safflower varieties showed high oil content (~38%) and high oleic acid content (>70%) under Indian conditions, which have already been introduced in All India Coordinated Research Programme (AICRP) on safflower (IIOR, 2015). In this study, our aim was to evaluate a set of 10 exotic safflower varieties at Hyderabad (India) for oil content and quality in order to identify their potential for improvement of oil and oleic acid content in Indian cultivars.

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MATERIALS AND METHODS

Plant material: A set of 10 safflower cultivars (9 American and 1 Canadian) comprising of Centennial, Finch, Oker, Montola 2000, Oleic Leed, Lesaf, S-334, S-518, S-719 and CW-99 was used in this study. Seeds of Centennial, Finch, Oker, Montola-2000, Oleic Leed and Lesaf-496 were obtained from USDA and S-334, S-518, S-719 and CW-99 were obtained from Instituto Nacional de Investigaciones Forestales, Agricolasy Pecuarias (INIFAP), Mexico. Indian popular varieties namely A-1, Bhima and NARI-57 were used as local checks. Lesaf-496 (PI 603208) is a germplasm line with high oleic acid and high oil content, developed at the Agriculture and Agri-Food Canada Lethbridge (Alberta) Research Centre (Mündel and Braun, 1999). Details of the safflower varieties are provided in Table 1.

Field trial: Field evaluation of the cultivars was carried out in vertisol at a research farm of Indian Council of Agricultural Research (ICAR)-Indian Institute of Oilseeds Research, Hyderabad (India) during *rabi* season of 2013-14.

Randomized complete block design (RCBD) with three replications was followed. The trial plot consisted of 5 rows of 5 m length with the spacing of 45 cm (between rows) x 20 cm (between plants). The field was irrigated once by sprinkler after seed sowing to facilitate germination.

Estimation of oil content: The oil content (%) was measured by Nuclear Magnetic Resonance (NMR) Spectroscopy using 30 g of pooled seed samples from each plot as described by Yadav and Murthy (2016).

Estimation of fatty acid composition: Oil from seed was extracted in hexane on soxhlet apparatus (Extraction unit, E-816, Buchi). Methyl esters were obtained by a two-step catalytic process according to slightly modified method of Ghadge and Raheman (2005). In the first step, the oil (100-150 mg) was treated with 2% sulphuric acid in methanol (5 ml) for 2 hours at 60ºC. After the reaction, the mixture was allowed to settle for an hour and methanol-water mixture that separated at the top was removed. In the second step, product at the bottom was transesterified using 2 ml of 13 per cent methanolic KOH for 30 minutes at 55ºC. The organic phase was extracted with hexane and washed with water till it reaches neutral pH. The hexane was dried over anhydrous sodium sulphate and concentrated with nitrogen to get methyl esters.

Fatty acid composition was determined using an Agilent 7860A gas chromatograph (GC) equipped with a flame ionization detector (FID), a split injection port and an auto sampler. Peak separation was performed on a DB-225 fused silica capillary column (diameter-250 µm, length-30 meter, film thickness-0.25 µm) from Agilent Technologies. The samples (0.2 µl) were injected in split mode (split ratio 1:20). The initial oven temperature was set at 160ºC for 2 min, raised to 220ºC (at a rate of 6ºC/min) and held at 220ºC for 10 min. Both inlet and detector were set to 230ºC. The carrier gas was nitrogen set to a constant flow rate of 1.2 ml/min. Peak identification was performed by comparing the relative retention times with those of a commercial standard mixture of FAME (Supelco 37 Component FAME Mix). Fatty acid composition [palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2)] was determined by calculating relative peak areas percent by GC post run analysis EZChrom elite compact software.

Data analysis: Analysis of variance (ANOVA) and mean comparison (LSD at 5% level of significance) of the data were carried out using a statistical analysis package Plant Breeding Tools (PBTools) v 1.3 (IRRI, 2013).

RESULTS AND DISCUSSION

Oil content in the exotic varieties ranged from 35.3 per cent (Finch) to 41.4 per cent (Centennial) whereas Indian check varieties A-1, Bhima and NARI-57 recorded 26.3 per cent, 31.1 per cent and 37.1 per cent, respectively. The most popular Indian variety A-1 recorded the least oil content while Centennial recorded highest oil content. NARI-57, a recently released Indian variety had oil content similar with the exotic varieties. Centennial (77.1%), Finch (75.3%), Oker (76.4%) and S-719 (71.8%) recorded high linoleic acid content. High oleic acid content was recorded in Montola-2000 (80.2%), Oleic Leed (78.6%), Lesaf-496 (70.8%), S-334 (69.6%), S-518 (76.7%) and CW-99 (76.5%). In the Indian check varieties, high linoleic content ranging from 72.4 per cent to 74.7 per cent was recorded. Overall, the exotic varieties exhibited 5 per cent to 10 per cent increase in oil content over the most popular Indian high yielding varieties A-1 and Bhima. The results show that exotic varieties could be potential donors for improvement of oil content and oleic acid content of the Indian safflower cultivars.

Comparison of the oil content and fatty acid composition of the varieties, as observed under exotic conditions (Table 1) with the current data collected at Hyderabad (Table 2) indicates reduction in percentage of oil content in most of the varieties (except Finch, Oker and Montola-2000) at Hyderabad. Highest reduction was observed in S-334 (-6.5%) and the lowest reduction was observed in S-518 (-2.1%). The seed oil content is a typical quantitative trait, which is controlled by polygenes (Yermanos *et al*., 1967; Fernandez Martinez *et al*., 1986). Therefore, low heritability and influence of genotype x environment (G x E) interaction are expected. In an evaluation trial in Albania, Montola-2000 recorded 33.4 per cent (by Soxhlet method) (Vorpsi *et al*., 2010). In Turkey, Montola-2000 recorded 35.2 per cent whereas Centennial recorded only 29 per cent (Arslan, 2007). In another experiment under highland conditions in Turkey, Montola-2000 (20.76%), Centennial (27.45%) and Oleic Leed (27.59%) recorded low oil content under non-irrigated conditions (Öztürk *et al*., 2008). Interestingly, these varieties showed higher oil content at Hyderabad. These observations suggest the influence of G x E interaction effects on oil content in safflower in diverse environments. Nevertheless, the oil content of the exotic varieties was substantially higher (5%-10%) than the most popular Indian check varieties A-1 and Bhima at Hyderabad, which is encouraging for their utilization in Indian breeding programmes. Genotypes that show minimal G x E interaction for the target trait would be more desirable for breeding programmes.

Unlike oil content, the content of major fatty acids (oleic or linoleic) of most of the test varieties (except S-334) at Hyderabad was comparable with the data from other countries. These observations suggest that oleic or linoleic acid content of safflower are fairly stable across environments. This is expected because oleic or linoleic acid content in safflower is qualitatively inherited and highly heritable (Golkar *et al.,* 2011). High stability of oleic or

linoleic acid content could possibly be due to high temperature stability of the fatty acid desaturase (*FAD2*) enzyme in safflower, which is primarily responsible for oleate desaturation in oilseeds (Esteban *et al*., 2004). High oleic acid content in safflower is controlled by a mutation in this gene (*ol* locus, *FAD2-1*) (Knowles and Mutwakill, 1963; Hamdan *et al*., 2012), which is recessively inherited. However, range of high oleic acid content (70%-80.2%) indicate the role of modifier genes as postulated by Knowles (1972). Maximum of 87 per cent of oleic acid content has also been reported in safflower lines (Hamdan *et al*., 2009). Due to simple inheritance and larger allelic effect of *ol* locus, improvement of high oleic acid content in safflower cultivars has been highly successful by conventional breeding efforts (Knowles, 1968).

Table 1 Details of exotic cultivars of safflower used in the study

Cultivar	Seed source	Accession Identity Number	Oil content (%)	Fatty acid composition			Reference
				Oleic acid (%)	Linoleic acid (%)	Palmitic + Stearic (%)	
Centennial	USDA	EC 736516 (PI 538779)	44.1	10.8	79.6	7.7	Bergman *et al*. (2001)
Finch	USDA	EC 736519 (PI 525458)	37.0	11.0	78.2	8.7	Bergman *et al*. (1989)
Oker	USDA	EC 736521 (PI 601166)	39.0	-	-	-	Bergman *et al*. (1985)
Montola-2000	USDA	EC 736515 (PI 538025)	38.3	80.8	12.3	5.6	Bergman *et al*. (2000); Armah-Agyeman *et al*. (2002)
Oleic Leed	USDA	EC 736514 (PI 560177)	39.0	76.1	16	5.5	Urie *et al*. (1979); Fernandez Martinez *et al*. (1986)
Lesaf-496	USDA	EC 736517 (PI 603208)	42.0	75.0	18.7	6.3	Mündel and Braun (1999)
S-334	INIFAP, Mexico	EC 755660	42.6	81.0	-	-	Silveira Gramont *et al*. (2009)
S-518	INIFAP, Mexico	EC 755662	40.8	77.0	12.0	-	Montoya Coronado *et al*. (2008)
S-719	INIFAP, Mexico	EC 755684	40.0	-	-	-	Muñoz-Valenzuela *et al*. (2007)
CW-99	INIFAP, Mexico	EC 755664	41.0	76.0	-	-	Montoya Coronado (2010)
A-1 (Check)	India	-	26.5	15.6	76.4	8.0	Nagaraj (2001); Kadirvel *et al.* (2016)
Bhima (Check)	India	-	30.0	16.2	75.5	8.3	DOR (2006); Kadirvel *et al*. (2016)
NARI-57 (Check)	India	-	37.6	13.4	75.9	10.7	Kadirvel *et al*. (2016)

Table 2 Oil content and fatty acid composition of exotic safflower cultivars in an evaluation trial at Hyderabad, India

Cultivar	Oil content (%)	Fatty acid composition			
		Palmitic acid (%)	Stearic acid (%)	Oleic acid (%)	Linoleic acid (%)
Centennial	41.4	7.1	2.3	13.6	77.1
Finch	35.3	6.4	4.1	14.2	75.3
Oker	38.8	7.0	2.2	14.4	76.4
Montola-2000	39.1	4.8	2.0	80.2	13.1
Oleic Leed	36.1	3.6	1.3	78.6	16.5
Lesaf-496	38.4	5.3	2.1	70.8	21. 9
S-334	36.1	5.8	2.0	70.0	22.8
S-518	38.7	4.8	2.4	76.7	16.2
S-719	37.7	7.0	2.7	18.5	71.8
CW-99	37.3	5.0	2.0	76.5	16.5
A-1 (Check)	26.3	6.0	2.6	17.3	74.2
Bhima (Check)	31.1	5.7	2.7	17.1	74.7
NARI-57 (Check)	37.1	7.7	2.5	17.2	72.4
F-value	29.9**	17.7**	12.5**	204.7**	196.2**
$LSD_{0.05}$	2.0	0.78	0.51	6.12	5.97
CV (%)	3.4	8.2	13.1	8.6	7.6

Increase of oil content is a common breeding goal in safflower. To date, this has been successfully attempted by reducing the hull content (Mundel and Bergman, 2009) as both traits are negatively related (Rao *et al*., 1977). Various hull mutants (reduced hull, partial hull, striped hull and thin hull) with reduced seed coat have been developed in safflower, which have been exploited in breeding of commercial varieties (Mündel and Bergman, 2009). Nutrasaff, a variety with 50 per cent seed oil content has been developed in USA with reduced hull content (Bergman and Flynn, 2008). Rubis (2001) reported a new hull type with over 55 per cent seed oil content. Among the varieties tested in this study, Centennial, Oker, S-334, S-518, S-719 and CW-99 are striped hull types. Genetics of striped hull trait is simple and recessively inherited (Ebert and Knowles, 1966), which can easily be incorporated in the background of improved cultivars through backcrossing.

In conclusion, the most popular safflower cultivars currently grown in India are low oil and high linoleic types. Considering the market demand, new impetus is given to breed high oil yielding varieties with high oleic acid content. The exotic varietal sources reported in this study would be helpful to expedite such breeding efforts in India.

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Interspecific hybrid between silver leaf sunflower (*Helianthus argophyllus* T. & G.) and cultivated sunflower: Cytomorphological characterization of F_1 hybrid

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ABSTRACT

Hybrid plants were obtained by crossing cultivated sunflower (*Helianthus annuus* L., 2n=2x=34) line ARM-243B and a wild *Helianthus* species [*H. argophyllus;* 2n=2x=34; HEL-153/83 (PI-649865)], using the latter as pollen parent. The wild *Helianthus* accession was selected for this study because of its short duration and short plant height compared to other accessions of *H. argophyllus.* Morphological and cytological analyses were carried out to confirm the hybrid nature of the F_1 plants. The hybrids exhibited morphological features intermediate to both the parents for few attributes and more related to wild *Helianthus* species like leaf and stem pubescence, stem hairiness, flower colour, stem size, branching, disc floret pigmentation, plant height, seed size and seed shape etc. A reduction in pollen fertility (87.5%) was recorded in F_1 plants as compared to both the parents. Meiotic analysis revealed a mixture of univalents, bivalents, trivalents and quadrivalents in all the pollen mother cells (PMCs) analysed. In addition to bivalents and univalents, a trivalent was also observed in few PMCs, indicating segmental homology between chromosomes. Frequently observed chromosome configurations in diakinensis were 15 II + 1 IV and 13 II + 2 IV. The results suggested that the species *H. argophyllus* and *H. annuus* differ by 1-2 translocations and 1-2 inversions. Results show that the wild species is compatible with cultivated sunflower and using *H. argophyllus* cultivated sunflower can be improved for biotic (downy mildew) and abiotic stresses (drought and salinity).

Keywords: Cytomorphological characterization, *Helianthus argophyllus,* Interspecific Hybridization, Sunflower

Due to a bottleneck of gene-flow that occurred during domestication, cultivated sunflower lacks the genetic diversity to adapt to emerging biotic and abiotic stresses. For this reason, wild sunflower species are used extensively in sunflower breeding as a donor of favourable alleles for increasing genetic diversity and incorporation of agricultural traits (Seiler, 1992; Quresh *et al.,* 1993; Korell *et al.,* 1996; Quagliaro *et al.,* 2001; Tavoljanski *et al.,* 2002; Velasco *et al.,* 2007; Mallik *et al.,* 2016; Meena *et al.,* 2016a). Natural hybrids between *H. annuus* and *H. argophyllus* have been found in Texas (Heiser Jr., 1951). The species is used as an ornamental in Fiji and cultivated elsewhere (Smith, 1991). It has been known in cultivation as the 'silver leaf sunflower' since 1889 (Heiser Jr., 1951). Strong research interest in *H. argophyllus* has been developed because populations of this species contain dominant genes conferring resistance to all known races of downy mildew which have been incorporated into inbred lines of cultivated sunflower (Miller and Gulya, 1988; Seiler, 1991; Miller *et al.,* 2002; Dussle *et al.,* 2004; Jan and Gulya, 2006). Silver leaf sunflower is the closest relative of common sunflower (Schilling and Heiser, 1981), widely used in sunflower breeding as donor of disease resistance alleles (Heiser Jr, 1951; Miller and Gulya, 1991; Slabaugh *et al.,* 2003; Dussle *et al.,* 2004; Radwan *et al.,* 2004; Seiler *et al.,* 2007), fertility restoration to PET1 cytoplasm (Chepurnaya *et al.,* 2003) and cytoplasmic male sterility (Horn *et al.,* 2002). It was also reported as a source of favourable alleles for salt and drought tolerance (Rauf, 2008) and insect resistance (Rogers and Thompson, 1980; Rogers *et al.,* 1987; Sujatha and Lakshminarayana, 2007). This species also possess considerable variability for resistance to drought, diseases and parasitic plant which can be utilized for the improvement of cultivated sunflower (Jan *et al.,* 2008). This study aims to present the results of interspecific hybridization between cultivated sunflower and *H. argophyllus,* characterization of the F_1 hybrids through morphological and cytological features and present their potential useful for breeding and selection.

MATERIALS AND METHODS

An interspecific cross was derived by involving a cultivated sunflower ARM-243B genotype as female parent and a wild diploid species Accession No. HEL 153/83 (PI-649865) (*H. argophyllus*) as male parent. The investigation was carried out during the period 2014-2016. ARM-243B was used as female parent and hand emasculation was done to make the plant unisexual and covered with cloth bags. The crossing was effected at 9.00 AM by pollen collected from *H. argophyllus.* Observations on randomly selected five plants were recorded for various morphological characters on parents and the interspecific hybrids. Hypocotyl anthocyanin pigmentation, days to 50% flowering (days), leaf length (cm), leaf width (cm), leaf

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colour, stem colouration, stem girth (cm), stem hairiness, number of leaves per plant, disc floret colour, head diameter (cm), head shape, days to maturity (days), plant height (cm), plant type (branching/non branching), type of branching (no branch, basal branches, top branches, full branches), seed length (cm), test weight (g) and seed colour were recorded. For cytological studies flower buds were fixed at appropriate button stage in Cornoy's II fixative for 24 h and stored in 70% alcohol after proper processing. Cytological investigation was carried out by following acetocarmine method elaborated by of Georgieva-Todorova (1976) and also to determine chromosome number of pollen mother cells (PMCs). Chromosome behaviour was studied mainly in diakinesis, metaphase I, anaphase I and telophase II. Further, percent pollen fertility was determined by the method given by Alexander (1969). Fully stained round pollen grains were counted as fertile and the shrivelled unstained pollen grains were scored as sterile. The percentage of pollen fertility was worked out by using the formula.

$$\text{Pollen fertility (\%)} = \frac{\text{No. of fertile pollen grains}}{\text{No. of fertile pollen grains + sterile pollen grains}} \times 100$$

RESULTS AND DISCUSSION

Morphological observations: *H. annuus* and *H. argophyllus* differ significantly for most of the morphological traits such as hypocotyl anthocyanin pigmentation, days to 50% flowering, leaf size, leaf colour, leaf hairiness, stem colouration, stem girth, number of leaves per plant, disc floret colour, head diameter, head shape, days to maturity, plant height, plant type (branching/non-branching), number of branches, seed length, test weight and seed colour. Despite the differences in morphological characteristics in both the species, the species were easily crossable. Hybridization was successful as evident from the good seed set in the female parent. Interspecific hybrid plants exhibited intermediate characters of either parent with a completely new phenotype. This large genetic variability being created provides the basis necessary for successful plant breeding (Encheva and Christov, 2006). Twenty three morphological characters of F_1 hybrid and the parents recorded is presented in Table 1 and the biological characters are presented in Table 2.

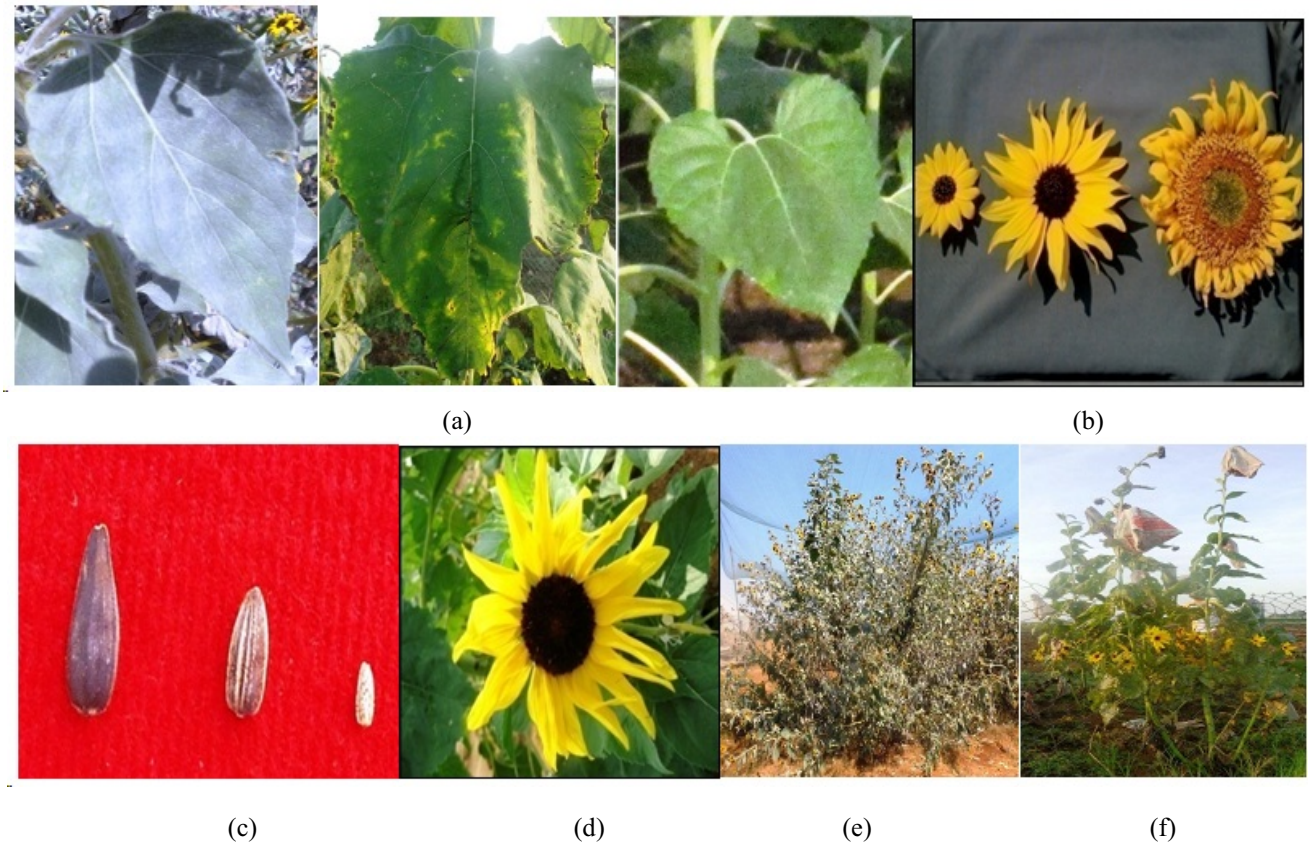

Fig. 1. Morphological characterization of the hybrid between cultivated sunflower and *H. argophyllus,*
a) difference in leaf shape, size and colour, b) difference in disc flower colour and size,
c) difference in seed shape, size and colour, d) disc floret colour,
e) branching in wild accession, and f) branching in F_1

The hypocotyl pigmentation differed in both the parents as it was absent in cultivated sunflower, and dark purple pigmentation was present in wild species. The interspecific cross showed the character as that of wild parent. There was considerable difference in days to 50% flowering. The cultivated sunflower was early in flowering by 48.0 days while the wild species took 68.0 days for flowering. The interspecific hybrid plants took more days to flower with mean of 59.4 days for 50% flowering. Nikolova and Christov (2004) reported intermediate 50% flowering in the F_1 hybrids. The leaf size was medium in case of ARM-243B, small in *H. argophyllus*, but the F_1 hybrid plants had medium sized leaves. Similar results were reported by Valkova and Christov (2004) in interspecific crosses obtained between wild *H. annuus* and cultivated sunflower. Encheva and Christov (2006) reported intermediacy with regard to the indices for leaf size in interspecific cross between *H. annuus* (hybrid Albena) × *H. salicifolius*. Hristova and Cherbadzi (2004) observed smaller leaf size than that of cultivated parent in interspecific crosses involving annual diploid *H. bolanderi, H. neglectus* and *H. petiolaris.*

Stem colouration was green in case of cultivated sunflower, light green in *H. argophyllus* with dark green stripes and the cross exhibited light green with dark green stripes as that of male parent which was dominantly from pollen parent. Valkova and Christov (2004) observed stem colour similar to that of wild parent in crossed plants involving wild *H. annuus* E-114, E-167 as male parent. The leaf colour of cultivated sunflower was dark green compared to *H. argophyllus* which was ashy green but the cross exhibited leaf colour which was more towards female parent with light green colour indicating intermediacy. Encheva and Christov (2006) reported that leaf colour was similar to female parent in interspecific cross between *H. annuus* (hybrid Albena) × *H. salicifolius.* In contrast, Valkova and Christov (2004) observed dark green leaves. The stem hairiness in ARM-243B was very sparse compared to *H. argophyllus* which had dense hairs on its stem. Intermediate and sparse amount of hairs were witnessed in the cross. Hristova and Cherbadzi (2004) reported that the interspecific crosses involving annual diploid *H. bolanderi, H. neglectus* and *H. petiolaris* had coarse hairs on the stem and these results are in contrast to results obtained in this study. Valkova and Christov (2004) observed stems covered with short, sharp hairs in their interspecific cross between cultivated and wild H. annuus E- 114, E-167.

The disc floret colour observed in cultivated sunflower was yellow and *H. argophyllus* was purple. While the disc floret observed in the interspecific cross exhibited purple colour similar to that of wild parent. Hristova and Cherbadzi (2004) reported similar type of results in interspecific crosses involving *H. bolanderi, H. neglectus* and *H. petiolaris.* Valkova and Christov (2004) observed that the disk flowers were yellow with strong anthocyanin colouration of the stigmas for crosses, derived from cultivated sunflower and wild *H. annuus* L. E-114, E-167. The head shape for *H. argophyllus* was flat and that of cultivated sunflower was convex, the interspecific cross inherited the female parent character showing convex head. This finding was in agreement with Nikolova and Christov (2004) for head shape.

Branching is a dominant character for most of the wild species. *H. argophyllus* showed dominant branching while ARM-243B was devoid of branching. In the interspecific hybrid, three types of branching were observed: basal, axil, and branching both at the base and the apical part of the stem. Similar observations were reported by Nikolova and Christov (2004), Saciperov (1961), Georgieva-Todorova (1976) and Christov (1988)in the interspecific hybrids involving different species. Results of Valkova and Christov (2004) using wild *H. annuus* E-114, E-167 indicated basal branching of plants from crosses 6075A x E-114 and 6075A x E-167 while other crosses were distinguished by full branching. There was significant difference in seed coat colour as well. Cultivated sunflower (ARM-243B) had black colour seeds with shiny texture and without stripes while *H. argophyllus* had brown colour seeds with rough texture with stripes present on it and the interspecific crossed seeds were light black in colour indicating the character was intermediate. In contrast, Hristova and Cherbadzi (2004) obtained pale gray-brownish to dark brown seed colour, in their interspecific crosses involving *H. bolanderi, H. neglectus* and *H. petiolaris.* The results from the investigation gave the reasons for confirming the statement already established by different authors (Georgieva-Todorova, 1976; Bohorova, 1983; Christov, 1988) that after crossing between wild *Helianthus* species and cultivated sunflower adequate diversity is created in the cross.

Leaf petiole pigmentation was absent in cultivated sunflower and very light in wild species. While in the interspecific hybrid, petiole pigmentation was dark and similar to male parent. Similar type of results was reported by Meena *et al.* (2016b). New types of characters were reported for leaf blistering, leaf serration, leaf base, ray floret shape and bract shape.

Quantitative traits of F_1 hybrid and parents are presented in Table 2. The test weight in ARM-243B was an average 5.8 g and in *H. argophyllus* it was 1.5 g. Interspecific cross had an average weight of 4.6 g. Seed length of *H. argophyllus* was very small (1.0 cm) while that of cultivated sunflower was medium (1.5 cm) and interspecific cross recorded intermediate size (1.3 cm) i.e. larger than *H. argophyllus* and smaller than ARM-243B. Encheva and Christov (2006) recorded intermediacy for seed length in interspecific cross between *H. annuus* (hybrid Albena) × *H. salicifolius.*

Hristova and Cherbadzi (2004) also reported intermediate seed length. The two species differed widely for plant height. *H. argophyllus* was taller (165.0 cm) than the cultivated sunflower (144.3 cm) and the interspecific cross recorded 262.1 cm. Encheva and Christov (2006) observed negative transgression for plant height in interspecific cross between *H. annuus* (hybrid Albena) × *H. salicifolius* compared to both the parents. Hristova and Cherbadzi (2004) reported that all plants reached a height of up to 150 to 160 cm in interspecific crosses involving annual diploid *H. bolanderi, H. neglectus* and *H. petiolaris,* while individual plants from the cross *H. neglectus* x *H. annuus* being as tall as 195 cm. Nikolova and Christov (2004) observed depression for plant height with respect to wild parent which was established in hybrid combinations *H. argophyllus* (E-007) x L.1234, and L.2607 x *H. argophyllus* (E-091) and L.HA-300 x *H. argophyllus* (E-091).

Table 1 Morphological characteristics of parents and F_1 interspecific hybrid

Character	*H. annuus*	*H. argophyllus*	*H. annuus* x *H. argophyllus*
Hypocotyl pigmentation	Absent	Present	Present
Leaf shape	Cordate	Cordate	Cordate
Leaf colour	Medium green	Ashy green	Light green
Leaf blistering	Very weak	Absent	Medium
Leaf serration	Very low	Fine	Medium
Leaf base	Cordate	Cordate	Auriculate
Leaf petiole pigmentation	Absent	Present (light)	Present (dark)
Stem hairiness	Very sparse	Dense	Medium sparse
Stem colouration	Green	Light green	Light green
Ray floret shape	Elongated	Ovate	Narrow ovate
Ray floret colour	Yellow	Lemon yellow	Orange
Disc floret colour	Yellow	Purple	Dark purple
Disc floret pigmentation	Absent	Present	Present
Bract shape	Rounded	Rounded	Elongated
Bract anthocyanin colouration	Absent	Present	Absent
Head shape	Concave	Convex	Convex
Type of branching	Absent	Full	Basal, axil and apical
Seed shape	Elongated	Broad ovoid	Ovoid elongate
Seed size	Medium	Small	Medium
Seed base colour	Black	Brown	Brown
Seed stripes	Absent	Absent	Present
Seed stripes on margin or between margin	-	On margin	Between margin
Seed stripe colour	-	Dark brown	Light brown
Seed mottling	Absent	Absent	Present

As regard to head diameter, ARM-243B produced larger capitula (14.6 cm) compared to *H. argophyllus* (2.8 cm). The interspecific cross showed an average of 6.3 cm head diameter indicating intermediate type. Similar type of result was obtained by Encheva and Christov (2006). While, Nikolova and Christov (2004) observed depression for head diameter with respect to wild parent which was established in hybrid combinations *H. argophyllus* (E-007) x L.1234, and L.2607 x *H. argophyllus* (E-091) and L.HA-300 x *H. argophyllus* (E-091), which are in agreement with the results in this study. There existed much difference in case of days to maturity in existed cultivated sunflower and *H. argophyllus.* The female parent ARM-243B matured in 96.0 days and male parent *H. argophyllus* took 101.5 days. But

interspecific cross took little more time in maturity (104.0 days) compared to the male parent. Nikolova and Christov (2004) reported similar results of days to maturity in interspecific cross between *H. annuus* L. line LHA- 300 x *H. argophyllus* (E-091).

Number of leaves per plant varied on an average of 28.0 leaves in case of cultivated sunflower, and 62.1 leaves in wild *H. argophyllus* and the F_1 exhibited an average of 57.2 leaves. Encheva and Christov (2006) observed that there was negative transgression for the number of leaves per plant, in cross between *H. annuus* (hybrid Albena) × *H. salicifolius.* Results of Valkova and Christov (2004) indicated number of leaves in the F_1 hybrid were intermediate of the parents. The stem girth in cultivated sunflower was lower (5.0 cm) as compared to that in *H. argophyllus* (8.0 cm). The cross showed mean stem girth of 8.2 cm and that the central stem girth was higher in the direct crosses, i.e., 7 to 19 cm was vigorous than both the parents. Hristova and Cherbadzi (2004) observed in interspecific crosses involving *H. bolanderi, H. neglectus* and *H. petiolaris.* Encheva and Christov (2006) reports were contradictory showing intermediacy with regard to stem girth in interspecific cross between *H. annuus* (hybrid Albena) × *H. salicifolius*.

Cytological observations: In both the parental species, pairing between the homologous chromosomes was normal at diakinesis and metaphase I with 17 bivalents formed. On the contrary, in the hybrid usually 15 II (bivalents) + 1 IV (quadrivalent) were observed. Meiotic analysis of the F_1 hybrids of *H. annuus* and *H. argophyllus* showed a mixture of univalents, bivalents, trivalents and quadrivalents in a total of 50 PMCs analysed. These results were in conformity with the findings of Narkhede *et al.* (1986) where the chromosome association of 13 II (bivalents) + 2 IV (quadrivalents) were occasionally observed (Table 3). The presence of single quadrivalent at diakinesis/metaphase I indicate that the genome of *H. argophyllus* differs from that of *H. annuus* by at least one reciprocal translocation. These results are in agreement with the findings of Vishnu *et al*. (2015) and Meena *et al.* (2016c).

Among the several bivalent shapes, ring, rod and open ring bivalents occurred more frequently than other configurations, like '8', 'V', loose chains, bracket, etc. Higher chromosome associations such as trivalents and quadrivalents during meiosis were reported in F_1 interspecific hybrids involving cultivated and wild diploid species of *Helianthus* (Whelan, 1979; Whelan and Dorrell, 1980; Chandler *et al.,* 1986). This led to the conclusion that the genomes of these species in the primary gene pool differ from each other by a limited number of reciprocal translocations. Exchange of unequal chromatin segments between the non-homologous chromosomes (translocation of chromosomes) may be a reason for the formation of quadrivalents (Manjula and Seetharam, 2000). Narkhede *et al*. (1986) reported a single quadrivalent in the interspecific hybrid between sunflower and *H. argophyllus.* Kulshreshta and Gupta (1979) and Meena *et al.* (2016c) also reported that the difference of one reciprocal translocation as the cause for the formation of single quadrivalent in *H. annuus* x *H. argophyllus* interspecific hybrids.

Table 2 Quantitative characteristics of F_1 hybrids and parents

Characteristics	*H. annuus*	*H. annuus* x *H. argophyllus*	*H. argophyllus*
Days to 50% flowering (days)	48.0	59.4	68.0
Days to maturity (days)	96.0	104.0	101.5
Plant height (cm)	144.3	165.0	262.1
No. of leaves/plant	28.0	57.2	62.1
Leaf length (cm)	18.6	14.9	32.0
Leaf width (cm)	17.8	14.6	29.0
Petiole length (cm)	20.6	16.5	25.0
Stem girth (cm)	5.0	8.2	8.0
Ray floret number	36.0	16.3	18.0
Ray floret length (cm)	7.2	3.8	5.6
Ray floret width (cm)	1.4	2.3	1.7
Head diameter (cm)	14.6	6.3	2.8
Seed length (cm)	2.6	1.0	1.3
Bract length (cm)	4.6	2.4	3.0
Bract width (cm)	2.4	1.0	1.2
100-seed weight (g)	5.8	1.5	4.6

Pollen fertility (%): Frequency of pollen fertility varied among parents and interspecific hybrids. The parental species showed pollen stainability as high as 97.6 per cent in *H. annuus* and 97.4 per cent in *H. argophyllus.* However, the F_1 hybrids showed reduction in pollen stainability, recording only 87.5 per cent. In the present study, pollen fertility in the interspecific hybrids was low because abnormalities like quadrivalents in earlier stages and abnormal disjunction in later stages of meiosis. Very recently, Vishnu *et al.* (2015), Meena *et al.* (2016a), Meena *et al.* (2016c) also observed 87.2 per cent, 89.9 per cent and 87.8 per cent pollen fertility in the *H. annuus* x *H. argophyllus* interspecific crosses, respectively. Other authors also reported reduced pollen viability in different F_1 hybrids (Georgieva-Todorova, 1976; 1984; 1990; Whelan, 1978; Christov, 1988; Narkhede *et al.,* 1986; Espinasse *et al.,* 1995; Manjula and Seetharam, 2000).

Today climate change is the major challenge for breeders and particularly for crops like sunflower confronting abiotic as well as biotic stress problems. Silver leaf sunflower is compatible with cultivated sunflower and positive traits available in wild *H. argophyllus* can be successfully transferred to broaden the genetic base of cultivated sunflower. Using Hel-153/83, cultivated sunflower can be improved for abiotic stress (like drought) and biotic stress (like downy mildew) through interspecific hybridization.

Table 3 Frequency of different meiotic configurations in *H. annuus, H. argophyllus* and their interspecific hybrid

Phase	Characteristics	*H. annuus* (ARM-243B)	*H. argophyllus* (HEL153/83)	Interspecific hybrid (F_1)
Diakinesis	% of meiocytes with	-	-	-
	Univalents	0	1.63	2.13
	Bivalents	100	98.7	94.78
	Trivalents	0	0	0.97
	Quadrivalents	0	0	2.34
	Hexavalents	0	0	0.13
	Multivalents	0	0	1.67
Metaphase I	Fast chromosomes	0	0	2.37
Anaphase I	Lagging chromosomes	0	1.23	1.26
	Chromosome bridges	0	0	1.42
Telophase II	Lagging chromosomes	0	0	0.78
	Pollen fertility	97.6	97.4	86.5-89.1 (87.5)

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Effect of preceding crops on growth and yield of zero-till *rabi* castor (*Ricinus communis* L.) under different nitrogen levels

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ABSTRACT

An experiment was conducted to find out the effect of preceding crops on growth and yield of zero-till *rabi* castor under different nitrogen levels during *kharif* and *rabi* seasons of 2010-11 and 2011-12. The results revealed that among different preceding *kharif* crops, greengram and bajra were found to have positive influence on growth parameters like plant height, dry matter production, number of leaves, LAI and seed yield (means yield of 3006 and 2832 kg/ha greengram and bajra, respectively) of castor compared to that of other preceding crops (groundnut and maize). Among nitrogen levels, with increase in nitrogen dose, there was significant increase in seed yield upto 120 kg N/ha (3221 kg/ha).

Keywords: Castor, Growth, Preceding crop, Yield, Zero-tillage

Castor has been a traditional oilseed crop grown under rainfed conditions in an area of 0.38 m ha in Andhra Pradesh. India annually earns foreign exchange of about ₹2500 crores through export of oil and its derivatives (Ramanjaneyulu, 2014). However, these days, most of the dry land farmers in Andhra Pradesh are shifting from castor to Bt cotton and maize during *kharif* season due to realisation of less yield (10-12 q/ha) and remunerative returns from castor due to incidence of *Botryotinia* gray rot disease and intermittent drought. However, there appears to be a lot of potential for castor cultivation during *rabi* season with assured irrigation as we can avoid *Botryotinia* gray rot disease and moisture stress. It is necessary to evolve suitable practices in a cropping system mode to make castor cultivation remunerative. Millets are short duration crops and can grow well in marginal soils under limited soil moisture and fit well in double cropping systems involving cultivation of castor during *rabi* season. The ameliorating effect of legumes on soil and their positive effect on succeeding crop growth and yield as well as in the present day context of energy crises and phenomenal increase in cost of fertilizers, these crops play an important role in cropping systems (Kumpawat and Rathore, 2003). The research work on these lines is meagre, hence, this experiment was proposed. Zero-tillage is an extreme form of minimum tillage, in which primary tillage is completely avoided and secondary tillage is restricted to seed bed preparation in row zone only and crop can be sown (second crop) immediately after the harvest of preceding (*kharif*) crop. In variance, under conventional tillage, primary and secondary tillage operations are done to prepare seed bed, which takes 5 to 7 days, eventually these 7 days period is advantageous in zero tillage practice. Zero-tillage improves soil quality, carbon, organic matter and protecting the soil from erosion, evaporation of water and structural breakdown. A reduction in tillage passes helps prevent the compaction of soil.

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MATERIALS AND METHODS

Field experiment was conducted at the College Farm, College of Agriculture, Rajendranagar, Hyderabad, on performance of zero-till *rabi* castor under the influence of different preceding crops and N levels during *kharif* and *rabi* seasons of 2010-11 and 2011-12. The soil of the experimental site was sandy clay loam in texture with pH of 7.2, organic carbon 0.59, available N 264 kg/ha (low), 34 kg/ha available P_2O_5 (medium) and 236 kg/ha available K_2O (high). The experiment was laid out in split plot design with four preceding crops (greengram, groundnut, bajra and maize) in main plots and five nitrogen levels in sub plots (0, 40, 80, 120 and 160 kg N/ha) replicated thrice. The *kharif* crops were grown at the onset of monsoon and they were raised with recommended package of practices. Castor was sown in zero-till plots by hand dibbling in the opened furrows immediately after harvest of *kharif* crops. Castor Hybrid (PCH-111) was sown at a spacing of 90 cm x 60 cm. Before sowing the seeds were treated with thiram @ 3g/kg seed as a prophylactic measure against seed borne diseases like alternaria leaf blight, seedling blight and wilt. Sowing and harvesting dates of each crop year wise were mentioned below (Table 1).

Table 1 Calendar of operations in different crops under zero-tillage

Kharif crops				*Rabi* castor					
Crop	DOS	DOH	Crop duration	DOS	DOH	Crop duration	1st Picking of castor	2nd Picking of castor	3rd Picking of castor
2010-11									
Greengram	22/7/2010	24/9/2010	64	27/9/2010	31/03/2011	185	12/2/2011	09/3/2011	31/3/2011
Bajra	22/7/2010	14/10/2010	84	16/10/2010	08/04/2011	175	26/2/2011	20/3/2011	08/4/2011
Groundnut	22/7/2010	01/11/2010	102	04/11/2010	20/04/2011	168	09/3/2011	31/3/2011	20/4/2011
Maize	22/7/2010	02/11/2010	103	04/11/2010	08/04/2011	156	03/3/2011	22/3/2011	08/4/2011
2011-12									
Greengram	08/7/2011	06/9/2011	60	08/9/2011	03/3/2012	182	17/1/2012	12/2/2012	03/3/2012
Bajra	08/7/2011	28/9/2011	82	30/9/2011	22/3/2012	175	06/2/2012	03/3/2012	22/3/2012
Groundnut	08/7/2011	16/10/2011	100	18/10/2011	30/3/2012	165	21/2/2012	12/3/2012	30/3/2012
Maize	08/7/2011	20/10/2011	104	22/10/2011	22/3/2012	153	18/2/2012	07/3/2012	22/3/2012

DOS: Date of sowing, DOH: Date of harvest

RESULTS AND DISCUSSION

Effect of preceding crops on castor: The results showed that, among different preceding crops evaluated, greengram markedly increased growth parameters like plant height, number of leaves, LAI, dry matter production of castor. The castor seed yield totalled over all the three pickings in greengram-castor system was at par with that of bajra-castor and was found superior to maize-castor system. At primary spike (6501, 6046 kg/ha) harvest stages, the dry matter production of zero-till *rabi* castor was significantly higher when greengram was the preceding crop compared to that of other crops in the system *viz*., bajra, groundnut and maize (Table 2). The difference in dry matter production of castor due to these three preceding crops was also found significant. The dry matter production of any crop is a product of photosynthetically active leaf surface and net assimilation rate. Higher dry matter accumulation of castor recorded in greengram-castor and bajra-castor systems was due to vacation of land in a short period of 62 and 83 days, respectively by *kharif* crops and sowing of *rabi* castor well in advance so that castor had put forth better growth compared to that of castor following groundnut and maize, which took more time for harvest (102 and 104 days, respectively). Similar results were reported by Patel *et al*. (2009), Anonymous (2006). Synergistic effect of temperature, relative humidity, less evaporation and optimum sunshine hours on castor with early sowing of crop have contributed to better growth and development, ultimately culminating in enhanced dry matter production of castor. More number of leaves in castor was recorded when it followed greengram/bajra, the LAI was significantly higher over that of following groundnut and maize.

Similarly, the greengram-castor system recorded higher castor seed yield (3137 and 2875 kg/ha) over bajra-castor (2949 and 2714 kg/ha), groundnut-castor (2630 and 2373 kg/ha) and maize-castor (2479 and 2256 kg/ha) systems. The increase in seed yield of castor grown after greengram was to the tune of 6.4, 19.3 and 26.6 per cent during 2010-11 and 5.9, 21.2 and 27.4 per cent during 2011-12, respectively over bajra-castor, groundnut-castor and maize-castor systems (Table 3). The present findings are in conformity with those reported by Patel *et al*. (2009), Madhu and Venkataramana (2017) and Anonymous (2006). However, the seed yield of former two systems was found at a par during both the years. The greater seed yield of castor in greengram-castor system could attributed to better growth and yield contributing parameters like LAI, dry matter production, spike length, number of spikes/plant, number of capsules/primary spike and total number of capsules/plant. These are physiologically important growth and yield attributes, which have a positive correlation with seed yield of castor. Stalk yield and harvest index followed the same trend.

Effect of N levels on castor: Among nitrogen levels, application of 160 kg N/ha and 120 kg N/ha, being at par, recorded taller plants, greater number of leaves/plant and higher dry matter production (7052 and 6511 kg/ha at 160 kg N/ha; 6789 and 6201 kg/ha at 120 kg N/ha) at all the stages of crop growth and superior LAI at 90 DAS and better yield components over lower N levels. Graded nitrogen levels (0, 40, 80, 120 and 160 kg N/ha) had a significant effect on dry matter accumulation of castor, however, application of 160 kg N/ha recorded significantly higher dry matter production at primary spike harvest over lower nitrogen levels. Similarly, the probable reason for such a positive response up to 120 kg N/ha was availability of nitrogen in synchrony with crop need which has resulted in good vegetative growth, better root development and efficient photosynthesis and finally accumulated more dry matter. Narkhede *et al*. (1984), Saradadevi *et al*. (2002), Patel *et al*. (2009) and Anonymous (2013) also observed similar response of *rabi* castor upto 120 kg N/ha. Similarly, the seed yield (3539 and 3144 kg/ha) and stalk yield (3713 and 3144 kg/ha) of castor at 160 kg N/ha was comparable to that of 120 kg N/ha 3396 and 3046 kg/ha seed yield; 3598 and 3484 kg/ha stalk yield, during 2010-11 and 2011-12, respectively and both were found superior to lower N levels during two years of study. The

more synchronous availability of 'N' as per crop need with the application of 120 kg N/ha might have been contributed for better growth and yield attributing characters which have eventually given higher seed yield at higher level of 'N'. These results are in conformity with the findings of Patel *et al*. (2009 and 2005), Madhu and Venkataramana (2017), Anonymous (2013), Mathukia and Modhwadia (1993) and Anonymous (2005).

Table1 2 Plant height (cm), dry matter production (kg/ha), number of leaves, and LAI at different growth stages of zero-till *rabi* castor as influenced by preceding crops and nitrogen levels

Treatment	Plant height (cm) at primary spike harvest			Dry matter production at primary spike harvest			Number of leaves at 90 DAS			Leaf area index (LAI) at 90 DAS		
	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean
Preceding crop												
Greengram	112.7	106.3	109.5	6501	6046	6274	18.8	18.2	18.5	2.24	2.12	2.18
Groundnut	91.9	82.5	87.2	5270	4786	5028	17.2	16.8	17.0	1.95	1.84	1.90
Bajra	106.4	100.1	103.3	5810	5313	5562	17.9	17.2	17.6	2.08	2.02	2.05
Maize	85.4	74.7	80.1	4982	4524	4753	16.8	15.9	16.4	1.88	1.81	1.84
SEd ±	2.2	2.3		118	115		0.5	0.6		0.02	0.01	
CD (P=0.05)	5.4	5.7		294	281		1.1	NS		0.05	0.02	
'N' levels (kg/ha)												
0	67.3	59.8	63.6	3215	2958	3087	13.2	14.1	13.7	1.21	1.15	1.18
40	87.8	80.0	83.9	5016	4606	4811	17.0	16.4	16.7	1.79	1.71	1.75
80	105.3	95.8	100.6	6132	5562	5847	18.6	17.7	18.2	2.14	2.10	2.12
120	115.8	107.8	111.8	6789	6201	6495	19.6	18.1	18.8	2.47	2.36	2.42
160	119.3	111.2	115.3	7052	6511	6782	20.2	18.8	19.5	2.57	2.42	2.49
SEd ±	1.9	1.9		96	93		0.5	0.6		0.02	0.03	
CD (P=0.05)	3.9	3.9		196	190		1.1	1.1		0.05	0.05	
Interaction	NS	NS		Sig.	Sig.		NS	NS		NS	NS	

Table 3 Seed yield, stalk yield (kg/ha) and harvest index (%) at different growth stages of zero-till *rabi* castor as influenced by preceding crops and nitrogen levels

Treatment	Seed yield			Chaff yield			Stalk yield			HI		
	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean	2010	2011	Mean
Preceding crop												
Greengram	3137	2875	3006	1885	1722	1804	3576	3472	3524	36.5	35.6	36.1
Groundnut	2630	2373	2502	1596	1433	1515	2889	2807	2848	37.0	36.0	36.5
Bajra	2949	2714	2832	1778	1635	1707	3285	3111	3198	36.9	36.4	36.6
Maize	2479	2256	2368	1497	1355	1426	2737	2620	2679	36.9	36.2	36.6
SEd ±	89	76		41	31		72	135		1.1	1.1	
CD (P=0.05)	218	182		100	76		176	331		NS	NS	
'N' levels (kg/ha)												
0	1598	1566	1582	986	943	965	2115	1858	1987	34.0	35.9	34.9
40	2441	2284	2362	1471	1361	1416	2833	2719	2776	36.2	35.9	36.0
80	3019	2735	2877	1831	1647	1739	3350	3235	3293	36.9	36.0	36.4
120	3396	3046	3221	2038	1834	1936	3598	3484	3541	37.6	36.4	37.0
160	3539	3144	3341	2119	1896	2008	3713	3716	3715	37.8	35.9	36.9
SEd ±	76	78		48	51		79	126		1.0	1.1	
CD (P=0.05)	155	158		98	104		161	257		2.0	NS	
Interaction	Sig.	Sig.		NS	NS		NS	NS		NS	NS	

The interaction effect of preceding crops and nitrogen levels on dry matter accumulation of castor was significant at primary spike harvest during both the years. The response of castor to nitrogen levels at primary spike harvest varied significantly upto 120 kg N/ha in all the systems except in maize-castor in second year where in response was noticed upto 160 kg N/ha. Among all treatment combinations greater values for dry matter production of castor were observed with greengram as preceding crop at 160 kg N/ha and 120 kg N/ha over other treatment combinations, while in groundnut-castor system such improvement was observed upto 80 kg N/ha. The effect of preceding crops at same or different levels of N on castor indicated that greater values for castor dry matter accumulation were observed at 120 and 160 kg N/ha when castor followed greengram and bajra, respectively. It clearly indicated that when a cereal preceded castor, it required higher N application. The interaction effect of preceding crops and nitrogen levels on total castor seed yield during 2010-11 showed that castor responded significantly upto 120 kg N/ha in all the systems, except in maize-castor system. The differences between N levels 80 and 120; and 120 and 160 were not significant. However, application of 160 kg N/ha was found superior to 80 kg N/ha. Similar trend was also observed in groundnut-castor system during second year of experimentation.

From the results of experiment, it could be concluded that, greengram and bajra were found to have positive influence on growth and yield of castor compared to that of other preceding crops (groundnut and maize). Application of 120 kg N/ha to *rabi* castor resulted in better crop growth and yield of *rabi* castor compared to lower N levels.

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Seed priming for improving germination of sunflower (*Helianthus annuus* L.) at low temperature

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ABSTRACT

Seeds of two sunflower hybrids, KBSH-44 and PSH-1962 were subjected to different treatment methods *viz.,* direct sowing, priming for 24 and 48 hours along with absolute control to enhance germination at low temperature. Differences were observed for germination at low temperature between hybrids and PSH-1962 showed higher germination at low temperature. Among the treatments, response to absolute control (25°C) and direct sowing was similar. Priming did not improve germination in any of the hybrids at 10 and 15°C. Increase in priming duration resulted in decreased germination. Inhibitory effect of priming was more in KBSH-44 than PSH-1962. None of the treatments studied were effective in enhancing germination at low temperature.

Keywords: Germination, Low temperature, Seed Priming, Sunflower

Spring sunflower area is expanding in India and the productivity is comparable to world average. Present practice is to take up sowing during mid February due to congenial temperatures at that time though the ideal time to get higher yields is mid January. Earlier sown crop suffers low temperature during germination and seedling stage while later sown crop is exposed to higher temperatures during later stages of crop like seed filling and maturity which limits achieving potential yield. Delaying sowing to March enhanced germination rate and vigour index (Dhillon and Sharma, 2016) while early spring sowing of sunflower increases yield (Sheoran *et al.,* 2014). In different genotypes, base temperature varied from 3.3 to 6.7°C, optimum temperature for germination from 23-26°C and maximum rate of germination occurred at 30.4 to 35.6°C (Khalifa *et al*., 2000). Suboptimal temperatures at sowing results in poor and delayed germination. Increasing the area under spring sunflower is limited by cold tolerance of crop especially at germination and early stages of crop. Both early and late flowering types respond similarly with respect to low temperature tolerance (Hewezi *et al*., 2006). Breeding specifically for cold tolerance has not been attempted in sunflower (Skoric, 2009). By using cold seed germination test, some tolerant varieties were identified in Russia. Treatment of pollen with low temperature has shown to increase the proportion of cold tolerance of genotypes (Lyakh and Totsky, 2014). For successful establishment of crop in this season, it is necessary to develop methods that ensure good germination. Priming is one such option (Afzal *et al*., 2008) found promising in several crops (Table 1) to improve germination at low temperatures. In field conditions, chlorophyll content and specific leaf area are genetically associated with cold tolerance (Allinne *et al*., 2009). Different chemicals used for priming to enhance germination at low temperature in other crops were tested in this study on sunflower to enhance germination at 10°C constant temperature. The main objective of this study was to identify suitable method, chemical and concentration that can enhance germination at low temperature.

MATERIALS AND METHODS

Two sunflower hybrids *viz.,* KBSH-44 released for all India and PSH-1962 developed at Punjab and released for the state for spring cultivation were tested for enhancing germination at 10°C constant temperature as the mean temperature were around that during 2nd fortnight of January and first fortnight of February. For this, experiment was initially conducted with 15ºC by growing them in Petri plates in three replications @ 20 seeds per replication and recording observation on 7th day. As good (≥ 80 %) germination was noticed at 15ºC, the study temperature was reduced to 10ºC. On 7th day, as the seeds were still germinating, the experiment was continued till 10 days. Different treatments tried include, direct sowing (benzyl adenine 20 ppm and acetyl salicylic acid 10 mM), where seeds were given the said treatment solution for germination and subsequently when ever required to keep the filter paper in moist condition and a set receiving distilled water (unprimed) served as control for these treatments. Priming for 24 h and 48 h (chitoran 0.25, 0.5, 0.75%, PEG 6000, -0.6 and -0.8 MPa; KNO_3, 200 and 400 mM; NaCl, 200 and 400 mM) where seeds were soaked in respective solutions maintained at 25°C for 24 or 48 hours depending on treatment and at the end of treatment, thoroughly washed with distilled water repeatedly and then kept for germination @ 20 seeds per replication in three replicates and the filter paper moistened with distilled water. Priming with distilled

water for 24 and 48 h served as control for the priming set. A set maintained at 25°C served as absolute control. Observations on germination (%) , root and shoot length were taken on 7th day for 15ºC and on 10th day for 10°C and seedling vigour was computed from germination per cent and seedling length. Analysis of variance (ANOVA) and comparison of means by least significant difference for factorial CRD was done using SAS V.9.3. Temperature was not considered as a factor and analysis for both temperatures was done separately as the day on which observations were recorded was different. It may be noted that ANOVA for germination (%) is based on arcsine transformed values.

Table 1 Chemicals used in different crops to enhance germination at low temperature

Crop	Chemical used	Temperature (∘C)	Reference
Pepper	Saturated and unsaturated fatty acids, KNO_3, water	15 and 25	Sachs *et al*., 1980
Soybean	GA_3 and Kinetin (0, 0.1, 0.2, 0.3, 0.4 mM)	10	Wang *et al*., 1996
Canola	Hydro Priming 24 h, PEG 6000 (-0.6 MPa), Matripring with Jute bag, Compost, pressmud	10	Afzal *et al*., 2004
Water melon	KNO_3 (0.25M) with methyl jasmonate (1, 3, 5 µM), Spermine (1, 3, 5 mM)	15	Korkmaz *et al*., 2004
Bottle gourd	KNO_3 (5%), NaCl (1%)	15 and 18	Kenanoglu *et al*., 2007
Maize	Chitosan (0.25, 0.5, 0.75%)	15	Guan *et al*., 2009
Cucumber	KNO_3 (0.1, 2.5, 5%) K_2HPO_4 (0.1, 2.5, 5%) NaCl (0.1, 2.5, 5%)	15 and 25	Piri *et al*., 2009
Sorghum	PEG(200, 300 400 g/litre), NaCl (2, 3, 4%), KNO_3 (2, 3, 4%), Boric acid (1, 2, 3%), Glycerol (10, 20, 30%), PEG 300g/litre + hormones	14	Tiryaki and Buyukcingil, 2009
Alfalfa	NaCl (2%), KNO_3 (2%), PEG 300 g/litre	15	Tiryaki *et al*., 2009
Egg plant	KNO_3 (3%) + Salicylic acid (0.05, 0.1, 0.5, 1.0 mM)	15	Zhang *et al*., 2011
Cotton	Distilled water, Hot water	18	Bolek *et al*., 2013
Maize	Moringa leaf extract, Sorghum water extract, Ascorbic acid, H_2O_2 & their combinations	10 and 25	Imran *et al*., 2013
Cumin	PEG 6000 (-0.8, -1.2 MPa)	10, 15 and 25	Rahimi, 2013
Sunflower	Gamete selection by Pollen storage at low temperature	3±1∘C for 7 days	Lyakh and Totsky, 2014
Castor	$CaCl_2$ (2%), Carbendazim + thiram, Trichoderma	25	Jamadar and Chandrasekhar, 2015
Sunflower	KNO_3(0.2%), GA (0.04%), distilled water	5	Lekic *et al*., 2015
Fennel (saunf)	Putrescine (polyamine) (10ppm, 20ppm)	15 and 20	Musafavi *et al*., 2015

RESULTS AND DISCUSSION

Among four methods of treatment, absolute control and direct sowing showed similar effect on germination at both the temperatures. However, priming treatments showed differential response at 10 and 15°C (Table 2). Hybrids did not differ in their response to treatment method. As there was no positive response to treatments, their effect was not discussed. Maximum germination (100%) in KBSH-44 was recorded in absolute control (25°C) but was affected at 15°C in direct sowing, whereas at 10°C, both absolute control and direct sowing control were at par as it was allowed to germinate till 10th day (Table 3). Higher germination percentage was recorded in PSH-1962 at 10°C compared to absolute control indicating its adaptive nature to low temperature. Genotypic differences for low temperature germination were reported earlier (Bolek *et al*., 2013).

Table 2 Effect of seed priming on germination, root, shoot, seedling length and seedling vigour

Method of treatment	10°C					15°C				
	Germi-nation (%)	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Seedling vigor	Germi-nation (%)	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Seedling vigor
Absolute control (25°C)	77 a*	5.5 a	5.0 a	10.6a	785a	79 a	2.4 a	3.6 a	5.9a	469a
Direct sowing	85 a	1.1 b	1.0 b	2.1b	181b	71 a	1.3 b	1.1 b	2.3b	160b
24 h Priming	54 b	1.3 b	0.9 b	2.1b	118c	43 b	1.1 b	0.8 c	1.9b	87c
48 h priming	44 c	1.2 b	0.8 b	2.0b	97c	35 b	1.1 b	0.7 c	1.7b	73c
LSD(P<0.05)	9.2	0.4	0.3	0.6	39	10.9	0.5	0.4	0.8	44
Hybrid x Treatment method										

*means in the same column followed by the same letter are not different (P<0.05)

Table 3. Response of sunflower hybrids to seed priming for germination and seedling growth

Treatment	10°C										15°C									
	KBSH-44					PSH-1962					KBSH-44					PSH-1962				
	Germi-nation (%)	Root length (cm)	Shoot length (cm)	Seed-ling length (cm)	Seed-ling vigor	Germi-nation (%)	Root length (cm)	Shoot length (cm)	Seed-ling length (cm)	Seed-ling vigor	Germi-nation (%)	Root length (cm)	Shoot length (cm)	Seed-ling length (cm)	Seed-ling vigor	Germi-nation (%)	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Seedling vigor
Absolute control (25°C)	87	4.5	3.9	8.5	737	67	6.5	6.1	12.6	833	100	3.1	3.4	6.5	647	57	1.6	3.7	5.3	290
Direct sowing																				
Control (no priming)	95	1.4	1.2	2.7	255	88	1.7	0.7	2.3	207	75	2.3	1.3	3.6	267	67	2.5	1.7	4.2	284
Benzyl Adenine 20 ppm	92	1.1	1.1	2.2	197	90	0.5	1.8	2.3	210	75	0.7	0.6	1.3	90	80	0.5	1.2	1.7	138
Acetyl salicylic acid 10 mM	82	1.1	0.4	1.5	121	63	0.9	0.6	1.5	95	80	0.8	0.7	1.4	112	45	0.6	0.9	1.5	70
Mean	89	1.2	0.9	2.1	191	81	1.0	1.0	2.1	170	77	1.3	0.9	2.1	156	64	1.2	1.3	2.5	164
24 h Priming																				
Control (24h)	32	1.4	0.9	2.4	74	83	1.8	1.0	2.9	241	38	1.4	0.7	2.1	83	70	1.3	0.8	2.1	152
Chitosan 0.25%	20	1.1	0.8	1.9	41	58	1.3	0.9	2.2	127	37	0.5	0.5	1.1	39	62	1.1	0.6	1.7	106
Chitosan 0.5%	28	1.0	1.0	1.9	52	73	1.5	0.9	2.4	175	34	0.7	0.7	1.4	48	48	1.1	0.6	1.7	82
Chitosan 0.75%	28	0.8	0.5	1.4	45	72	1.8	0.8	2.6	189	33	0.6	0.5	1.1	35	55	2.2	0.8	3.0	168
PEG -0.6 MPa	65	1.0	0.7	1.7	108	72	1.7	0.8	2.5	176	48	0.8	0.5	1.3	65	60	1.5	0.8	2.3	136
PEG - 0.8 MPa	37	0.9	0.5	1.4	52	80	1.3	0.7	2.0	158	35	0.7	0.5	1.2	51	53	1.0	0.6	1.6	85
KNO_3 100 mM	13	1.1	0.9	2.1	30	52	1.7	1.0	2.6	137	12	0.4	0.2	0.6	11	28	1.2	0.7	1.9	56
KNO_3 200 mM	7	1.0	0.2	1.2	10	38	1.6	1.0	2.5	98	17	0.7	0.6	1.3	21	10	2.2	1.5	3.7	28
KNO_3 400 mM	23	0.9	0.7	1.6	38	30	0.9	0.8	1.7	52	12	0.5	0.2	0.8	10	30	0.6	0.5	1.0	33
NaCl 200 mM	18	1.2	0.8	2.0	38	57	1.4	0.8	2.1	122	10	0.7	0.4	1.1	10	20	1.5	1.1	2.7	54
NaCl 400 mM	12	1.0	0.8	1.9	22	43	1.6	0.9	2.5	109	7	0.6	0.3	0.8	8	13	2.0	1.5	3.5	33
Mean	43	1.1	0.8	1.9	85	65	1.4	0.9	2.3	151	39	0.8	0.6	1.4	67	47	1.4	1.0	2.3	106
48 h Priming																				
Control (48h)	27	1.8	1.3	3.1	83	68	1.5	1.0	2.5	170	15	1.4	0.8	2.2	36	68	1.4	0.8	2.2	152
Chitosan 0.25%	8	0.6	1.0	1.6	20	72	1.5	1.0	2.4	173	35	0.6	0.6	1.2	42	55	2.1	0.7	2.8	150
Chitosan 0.5%	13	0.7	0.8	1.5	19	67	1.4	0.9	2.3	152	27	0.7	0.3	1.0	30	41	1.1	0.5	1.6	61
Chitosan 0.75%	17	1.3	0.8	2.1	31	58	1.1	0.9	2.0	117	25	0.7	0.3	1.1	30	60	1.7	1.4	3.2	194
PEG -0.6 MPa	25	1.2	0.5	1.7	45	60	1.5	0.7	2.2	130	22	0.8	0.4	1.2	26	40	1.7	0.7	2.4	101
PEG - 0.8 MPa	23	0.9	0.6	1.5	34	67	1.3	0.6	1.9	127	33	1.0	0.5	1.5	55	42	1.9	1.0	2.9	120
KNO_3 100 mM	5	0.4	0.8	1.3	11	30	1.4	1.3	2.7	82	0	0.0	0.0	0.0	0	18	1.0	0.9	1.9	40
KNO_3 200 mM	3	0.8	0.5	1.3	7	8	2.0	1.3	3.3	31	2	0.0	0.0	0.0	0	8	1.6	2.4	4.0	32
KNO_3 400 mM	2	0.2	0.0	0.2	1	8	0.3	1.0	1.4	14	0	0.0	0.0	0.0	0	5	0.1	0.4	0.4	2
NaCl 200 mM	3	0.4	0.2	0.6	6	40	2.2	1.2	3.5	139	2	0.0	0.0	0.0	0	23	1.2	1.1	2.3	48
NaCl 400 mM	0	0.2	0.0	0.2	0	37	1.3	0.9	2.2	75	0	0.0	0.0	0.0	0	13	1.3	1.0	2.3	16
Mean	30	1.0	0.7	1.7	60	58	1.4	0.9	2.3	134	29	0.7	0.4	1.1	48	41	1.4	1.0	2.3	97
Overall mean	29	1.1	0.8	1.9	80	57	1.6	1.1	2.7	159	30	0.8	0.5	1.3	66	41	1.4	1.1	2.5	101

Treatment with benzyl adenine (20 ppm) recorded higher germination at 15°C in PSH-1962 but the same treatment at 10°C was similar to control and therefore offer no added advantage. In both the hybrids at both the temperatures, priming for 48 hours recorded negligible germination with KNO_3. Between the two hybrids, germination in KBSH-44 was more affected by priming. Increase in the priming duration further affected germination. Though positive results were reported in many crops with priming, negative results were not uncommon. In cucumber and bottle gourd, priming did not show any positive effect (Kenanoglu *et al.,* 2007; Piri *et al.,* 2009).

No significant difference was observed for root shoot and seedling length at 10 and 15°C, among treatment methods *viz.,* direct sowing, priming for 24 or 48 hours. Significantly lower values were recorded with seed treatment irrespective of method when compared to absolute control (Table 2). However, seedling vigor showed differential response to treatment methods with significantly higher vigor in direct sowing method mainly due to high germination percentage in this method though growth parameters were at par with priming.

Lower temperatures severely affected growth and seedling vigor. KBSH-44 showed highest root, shoot and seedling length and vigor in absolute control followed by direct sowing control at 10 and 15°C (Table 3). Similar response was also observed in PSH-1962. Negative effect of low temperature on germination, root length were reported, but priming with distilled water neutralized such negative effect (Lekik *et al*., 2015). Highest seedling vigor was noticed in absolute control followed by direct sowing.

In conclusion, low temperatures affected growth more than the germination. Priming resulted in decreased germination per cent. Increase in priming duration resulted in further reduction in germination. BA treatment enhanced germination of PSH-1962 at 15°C but not at 10°C. None of the treatments tried were effective in improving germination under low temperatures. Further studies with new methods and combination treatments are needed for identifying promising combinations to improve germination at low temperature.

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Potentiality of native *Bacillus* species in enhancing sesame seed germination and their antagonism against *Macrophomina phaseolina* under *in vitro* conditions

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ABSTRACT

Native *Bacillus* species screened from rhizosphere of sesame (*Sesamum indicum* L.) were tested for their growth promoting and antagonistic effects. Based on 16S rRNA gene sequence based homology, the bacterial isolates were identified as *B. amyloliquefaciens, B. aryabhattai, B. cereus, B. flexus, B. megaterium, B. methylotrophicus, B. pumilus* and *B. subtilis.* Results on biochemical characterization of *Bacillus* species indicated that majority of the isolates were producing catalase and were found to be diazotrophs. Only few isolates were found to produce protease, cellulase and HCN. Only one isolate (*B. amyloliquefaciens*) was found to be phosphate solubilizer. Further, *in vitro* antagonistic assay revealed that *Bacillus subtilis* and *B. methylotrophicus* could inhibit the mycelial growth of *M. phaseolina* by 30.9 and 29.0 per cent, respectively. Majority of the *Bacillus* species tested have increased sesame seed germination, radicle and plumule length compared to control. Overall, our results suggested the scope and potentiality of *Bacillus* species in promoting sesame seed germination and growth promotion besides suppression of pathogen.

Keywords: *Bacillus,* Growth promotion, *Macrophomina,* PGPR, *Sesamum indicum*

India is one of the major producers of sesame (*Sesamum indicum*) in the world and is a rich source of edible oil (Sreedhar *et al*., 2016). India ranks first in sesame area and second in production in the world. In Andhra Pradesh, sesame is grown in an area of 0.85 lakh ha with a production of 0.26 lakh tonnes and productivity of 306 kg/ha (DAC, 2014). Due to intensive cultivation practices the crop is known to suffer from many diseases of which very important disease is stem and root rot caused by *Macrophomina phaseolina* (Maubl) Ashby results to low yield of sesame in Andhra Pradesh (Kumhar and Meena, 2016). Losses due to this disease were estimated to range from 5 to 100 per cent. Yu and Park (1980) reported that *M. phaseolina* causes severe reduction in seed germination and seedling stand. The seed infection results in seed rot, poor seedling stand, pre and post emergence damping off, reduced vigour, progressive wilting and premature drying (Khamari *et al*., 2016).

Plant growth promoting rhizobacteria (PGPR) are the soil bacteria inhabiting around/on the plant root surface and are directly or indirectly involved in promoting plant growth and development via production and secretion of various regulatory chemicals in the vicinity of rhizosphere. Generally, plant growth promoting rhizobacteria facilitate the plant growth directly by either assisting in resource acquisition (nitrogen, phosphorus and essential minerals) or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogens on plant growth and development in the forms of biocontrol agents (Ahmed and Kibret, 2014). Some examples of PGPR include *Azotobacter, Azospirillum, Bacillus, Burkholderia, Pseudomonas, Micrococcus,* etc (Bhattacharya and Jha, 2012). The present investigation was undertaken to study the antagonistic ability of *Bacillus* species against *M. phaseolina* and their potential for enhancing sesame seed germination.

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MATERIALS AND METHODS

Isolation of *Bacillus* species: Native *Bacillus* species were isolated from sesame rhizosphere (cv. YLM 66) on *Bacillus* Agar (Himedia) by serial dilution method. Soil samples (10 g) from rhizosphere of sesame were transferred to 90 ml sterile distilled water and mixed thoroughly by shaking the flask on a rotary shaker for 5 minutes. After serial dilution 0.1 ml suspension was spread over pre-sterilized and cooled down *Bacillus* agar plates in triplicate. The inoculated plates were incubated at 26±10°C for 48 hours. The *Bacillus* type colonies were selected based on morphology and maintained on Luria Bertani agar slants at 4°C for further use.

Isolation of pathogen: The pathogen, *Macrophomina phaseolina,* was isolated from roots of infected sesame plants on potato dextrose agar (PDA) using standard protocols. The culture was identified based on morphological characters and maintained on PDA slants at 4°C for further use.

Identification of *Bacillus* species: The identity of the bacteria was established through 16S rRNA gene sequence

based homology analysis. The genomic DNA was isolated according to Sambrook and Russel (2001). Amplification of 16S rRNA genes of bacterial isolates was carried out by PCR using universal primers, FGPS6-63-GGAGAGTTAGATCT TGGCTCAG and FGPL 132-38-CCCGGTTTCCCCATT CGG (Normand *et al.,* 1992). The thermocycler conditions as initial denaturation at 95°C for 3 min followed by 35 amplification cycles of 95°C for 1 min, 55°C for 1 min, 72°C for 2 min followed by final extension at 72°C for 3 min. The PCR products were analyzed in 1% agarose gel in 1x Tris-acetate EDTA, run for 90 min at 100 V, and the amplified products were excised and outsourced (Bioserve Biotechnologies India Pvt. Ltd., Hyderabad) for partial sequencing. Similarity of 16S rRNA gene sequence was aligned using BLAST Programme of GenBank database (NCBI).

In vitro* antagonism against *Macrophomina phaseolina: The bacterial isolates were tested for antagonistic potential against *M. phaseolina,* the causal agent of sesame stem and root rot by dual culture assay. An agar block (5 mm diameter) of 5-day-old culture of *M. phaseolina* was placed in the centre of Petri plates (90 mm diameter) containing PDA. A loopful of 24-h-old culture of bacteria test antagonist was streak inoculated at either sides of *M. phaseolina* disc at a distance of 2 cm apart. The pathogen culture inoculated centrally on PDA plates, without bacterial streak served as control. Each treatment was replicated thrice in completely randomized design and the inoculated plates were incubated at 28±1°C for 5 days and per cent inhibition was calculated (Dennis and Webster, 1971).

Protease activity: All bacterial isolates were screened for protease activity by inoculating on skim milk agar plates. Plates were incubated at 28±1°C for 72 hours and observed for proteolysis i.e., clear zone production around the inoculated bacterial disc.

Qualitative determination of phosphate solubilisation: Phosphate solubilisation ability of bacterial isolates was detected by inoculating them on Pikovaskaya's agar plates (Pikovaskaya, 1948). The inoculated plates were incubated at 28+1°C for 3 days and observed for appearance of clearing zone around the colonies.

Qualitative determination of nitrogen fixing ability: Nitrogen fixing ability of bacterial isolates was determined by spot inoculation of log phase culture on nitrogen free medium (Burk's medium) comprising: 10g dextrose, 0.41g KH_2PO_4, 0.52g K_2HPO_4, 0.05g Na_2SO_4, 0.2g $Cacl_2$, 0.1g $MgSO_4$, 0.005 g $FeSO_4$. $7H_2O$, 0.0025g $Na_2MoO_4.2H_2O$ and 20g agar (Wilson and Knight, 1952). The medium was adjusted to pH 7.0.

Catalase activity: The catalase activity was determined by adding few drops of 3% (v/v) H_2O_2 to 5 ml of 18 hours grown bacterial cultures in Luria-Bertani broth (Kannan, 2002).

Qualitative screening of *Bacillus* species for cellulolytic activity: Cellulolytic activity of *Bacillus* species was determined qualitatively by inoculating individual strains on to Bushnell Haas medium (BHM) agar plates amended with carboxy methyl cellulose (CMC) containing (g/L) CMC (10.0), K_2HPO_4 (1.0), KH_2PO_4 (1.0), $MgSO_4.7H_2O$ (0.2), NH_4NO_3 (1.0), $FeCl_3.6H_2O$ (0.05), $CaCl_2$ (0.02), and agar (20.0). For the secretion of cellulase enzyme CMC agar plates were incubated at 30±2°C for 5 days. After incubation, culture plates are flooded with 0.1% Congo red solution for 20 minutes. The stain was poured off, and the plates were washed with 1 M NaCl for 15 minutes. A clear zone formation around the bacterial colonies indicates the hydrolysis of CMC (Ruijssenaars and Hartmans, 2001).

HCN production: HCN production was determined by modified method of Bakker and Schippers (1987). Exponentially grown cultures of bacterial isolates were streaked on to Luria-Bertani agar plates supplemented with 4.4 g glycine L^{-1}. Simultaneously, a filter paper soaked in 0.5% picric acid in 1% Na_2CO_3 was placed in the upper lids of each Petri plate along with uninoculated control. The plates were sealed with parafilm and incubated at 28±1°C for 4 days and observed for colour change from yellow to brown for putative HCN production.

Evaluation of *Bacillus* species for enhancing sesame seed germination: A preliminary study was conducted to test the potential of *Bacillus* species in enhancing the sesame seed germination. Fifty seeds of sesame cultivar, YLM 66, were dipped in each test bacterial suspension (2 x 10^9 CFU/ml) for 1 hr and were sown in plastic containers with field soil mixed with sand (1:1 v/v). Germination percentage, radicle and plumule length were recorded 5 days after sowing and standard deviation was calculated.

RESULTS AND DISCUSSION

Isolation and identification of *Bacillus* species: Eight *Bacillus* species were isolated from sesame rhizosphere on *Bacillus* agar and purified on LB agar. The morphological characteristics of all eight isolates revealed them as Gram positive, rod shaped bacteria with opaque to white and irregular edged colonies on LB agar. These *Bacillus* species were characterized as *B. amyloliquefaciens, B. aryabhattai, B. cereus, B. flexus, B. megaterium, B. methylotrophicus, B. pumilus* and *B. subtilis* based on 16S rRNA sequence based homology.

Biochemical characterization: Results on biochemical characterization of *Bacillus* species indicated that majority of the isolates were producing catalase and found to be diazotrophs (Table 1). Only few isolates were found to produce protease, cellulase and HCN. Only one isolate was found to be phosphate solubilizer (*B. amyloliquefaciens*). Ramirez and Kloepper (2010) reported that the phosphate solubilisation by *B. amyloliquefaciens* strain FZB45 is modulated by the phytate content of the soil revealing the phytase activity as its major mechanism for growth promotion.

Table 1 Biochemical traits of *Bacillus* species isolated from sesame rhizosphere (cv. YLM 66)

Bacillus species	Protease	PSB	N-fixation	Catalase	Cellulase	HCN production
B. amyloliquefaciens	+	+	+	+	+	-
B. subtilis	-	-	+	+	-	-
B. pumilus	+	-	+	+	+	+
B. cereus	+	-	-	-	-	-
B. megaterium	+	-	+	+	-	+
B. aryabhattai	-	-	-	-	+	+
B. flexus	-	-	-	-	-	+
B. methylotrophicus	-	-	+	+	-	-

(+) Positive and (-) Negative reaction

Among the *Bacillus* species tested for protease production, four species (*B. amyloliquefaciens, B. pumilus, B. cereus* and *B. megaterium*) were found to solubilise protein and produced a clear zone around the bacterial colonies on skim milk agar (Table 1). However, only *B. amyloliquefaciens* could solubilise phosphate. Most of the *Bacillus* species (*B. amyloliquefaciens, B. subtilis, B. pumilus, B. megaterium, B. methylotrophicus*) were found to be diazotrophs and are catalase positive. Cellulose was found to be utilized by *B. amyloliquefaciens, B. pumilus* and *B. aryabhattai.* Four *Bacillus* species (*B. pumilus, B. megaterium, B. aryabhattai* and *B. flexus*) produced HCN as evidenced by the change in colour of filter paper from yellow to reddish brown.

Pankaj Kumar *et al*. (2012) reported that the strain BPR7, antagonistic to *Macrophomina phaseolina,* produced Indole acetic acid (IAA), siderophore, phytase, organic acid, ACC deaminase, cyanogens, lytic enzymes, oxalate oxidase, and solubilised various sources of organic and inorganic phosphates as well as potassium and zinc. In our study, *B. amyloliquefaciens* was found to be a diazotroph with ability to solubilise phosphate and it also produced enzymes like protease, cellulase and catalyse. However, it has limited antagonistic activity against *M. phaseolina*.

In vitro* antagonism against *Macrophomina phaseolina: *In vitro* assay revealed that *B. subtilis* and *B. methylotrophicus* could inhibit the mycelial growth of *M. phaseolina* by 30.92 and 29.07 per cent, respectively (Table 2). This is followed by *B. flexus* (22.04%), *B. megaterium* (22.55%) and *B. amyloliquefaciens* (20%) which are statistically on par with each other. *B. cereus* and *B. aryabhattai* have no antagonistic effect against *M. phaseolina.* Elewa *et al.* (2011) reported the efficacy of *B. subtilis* and *Trichoderma viride* in suppressing the hyphal growth of *M. phaseolina* and reducing the root-rot incidence of sesame plants under artificially inoculated conditions.

Table 2 *In vitro* antagonism of *Bacillus* species against *Macrophomina phaseolina*

Bacillus species	Inhibition over control (%)
B. amyloliquefaciens	20.00
B. subtilis	30.92
B. pumilus	16.48
B. cereus	0.56
B. megaterium	20.55
B. aryabhattai	0.93
B. flexus	22.04
B. methylotrophicus	29.07
CD (P=0.05)	2.84
SE (m)	0.94

Effect of seed bacterization on seed germination: Data obtained on seed germination assay (Table 3) exhibited significant differences among the applied treatments. Seed germination was enhanced when the seed is soaked in a suspension of *Bacillus* species for 1 hour prior to sowing. Highest germination percentage (94%) was observed in *B. methylotrophicus* treated seeds, followed by *B. megaterium* (80%) and *B. pumilus* (78%), when compared to control (38%). Ranganathan and Thavaranjit (2015) also reported the promotion of *Vigna sinensis* seed germination when soaked in a suspension of *Bacillus* sp. for 20 minutes. They also observed that there is a significant difference in mean length of germ tubes of soaked seeds compared to control.

Effect of seed bacterization on seedling growth: Soaking the seeds in bacterial suspension for 1 hour prior to sowing augmented the radicle and plumule length compared to control (Table 3). Radicle length of the sesame seedling was more when the seed is treated with *B. cereus* (Avg. 7.0±0.43), *B. megaterium* (6.82±0.96), *B. metbylotrophicus* (5.38±1.07) and *B. pumilus* (5.24±0.98) compared to control (3.64±0.73). *B. amyloliquefaciens* treated seeds recorded higher plumule length (Avg. 4.66±0.82) followed by *B. subtilis* (4.48±0.63) and *B. aryabhattai* (4.46±0.44) when compared to control (3.18±0.86). However, seed bacterization with *B. aryabhattai* reduced sesame seed germination (8%) and radicle length (2.54±1.09).

The results indicate that per cent seed germination and seedling growth was enhanced on seed bacterization with selected *Bacillus* species when compared with control. The significant growth increase caused by *Bacillus* species, *B. methylotrophicus, B. cereus* and *B. amyloliquefaciens,* qualifies them as PGPR. Plant growth promotion by *Bacillus* species was related to the production of phytohormones like auxins (Asghar *et al.,* 2002), gibberellins (Joo *et al.,* 2005) and cytokinins (García de Salamone *et al.,* 2001).

Ortiz-Castro *et al.* (2008) studied the role of cytokinin signalling in plant growth promotion by *Bacillus meaterium* in *Arabidopsis thaliana* and *Phaseolus vulgaris* using single and double cytokinin mutants and reported that cytokinin receptors play a complimentary role in plant growth promotion by *B. megaterium.* Radish seeds inoculated with *Bacillus subtilis* were found to enhance the fresh and dry masses of roots and leaves, photosynthetic pigments content, phytohormones content and mineral uptake in saline soils (Mohamed and Gomaa, 2012).

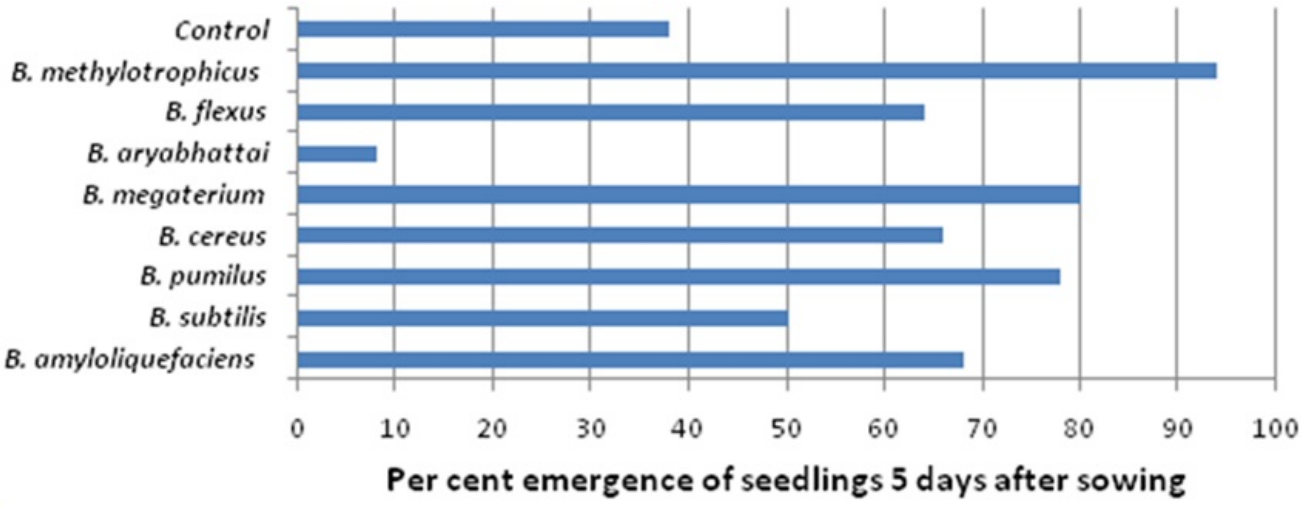

Fig. 1. Effect of seed bacterization with *Bacillus* species on sesame seed germination

Table 3 Effect of seed bacterization with *Bacillus* spp. on seed germination and seedling growth (5 days after sowing)

Bacillus species	Radicle length in cm (Mean ± SD)	Plumule length in cm (Mean ± SD)	Seed germination percentage (%)
B. amyloliquefaciens	4.72±1.42	4.66±0.82	68
B. subtilis	4.14±1.14	4.48±0.63	50
B. pumilus	5.24±0.98	4.12±0.63	78
B. cereus	7.0±0.43	3.96±0.32	66
B. megaterium	6.82±0.96	3.70±0.46	80
B. aryabhattai	2.54±1.09	4.46±0.44	8.0
B. flexus	2.86±1.04	3.92±0.27	64
B. methylotrophicus	5.38±1.07	3.96±0.17	94
Control	3.64±0.73	3.18±0.86	38

Overall, our results suggested the scope and potentiality of these *Bacillus* spp. for enhancing sesame seed germination and reducing mycelial growth of *M. phaseolina* under *in vitro* conditions. In conclusion, seed soaking in a suspension of *Bacillus* spp., notably with *B. methylotrophicus,* for 60 min prior to sowing can enhance sesame seed germination and promote seedling growth. In the present investigation, though some *Bacillus* species were found antagonistic to *M. phaseolina* under *in vitro* conditions, further studies need to be conducted to identify an elite strain antagonistic to *M. phaseolina* with plant growth promoting and disease suppressing ability under greenhouse and field conditions.

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Molecular characterization of insecticide resistance in larval population of *Spodoptera litura* (Fab.)

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ABSTRACT

Acetylcholinesterase gene activity in populations of *Spodoptera litura* (Fab.) collected from groundnut fields of different regions of Andhra Pradesh and Telangana was compared using α- and β-napthyl acetate as substrate for enzyme reaction. Among insecticide pre exposed *S. litura* population analyzed, nine out of ten populations showed elevated activities of acetylcholinesterase gene. A specific amplification product at 600 bp region indicating the presence of acetylcholinesterase gene in resistant strains of *S. litura,* whereas in susceptible base line strain of *S. litura* it was not found due to non amplification of specific gene at the same loci (600 bp) a phenomenon due to low copy number of gene. RAPD-PCR analysis of resistant and susceptible *S. litura* population revealed the existence of polymorphism. Specific amplification for acetylcholinesterase gene using custom made primers produced amplified product in resistant *S. litura* populations.

Keywords: Acetylcholinesterase gene, Groundnut, Insecticide Resistance, *Spodoptera litura*

Groundnut (*Arachis hypogaea* L.) is an important edible oilseed crop grown in many countries under diverse agro-climatic condition. India accounts for nearly 65 and 54 per cent of world's groundnut area and production, respectively (NRCG, 2014). However, among several pests on groundnut tobacco caterpillar, *Spodoptera litura* (Fab.) is one of the most devastating agricultural pest worldwide as its effects the yield of a broad range of agricultural, fiber, vegetable and ornamental crops (Rathi *et al.,* 1982; Manju *et al.,* 2016). The evaluation of pesticide resistance has been identified worldwide as the most serious threat to the development of sustainable integrated pest management practices (Labbe *et al.,* 2005). The development of resistance is an evolutionary and multi-disciplinary process, which is influenced by several interacting factor, such as the initial resistance allele frequency, inheritance of resistance, relative fitness of the various genotypes, management practices, among other factors (Georghiou and Taylor, 1977). Worldwide, in many agricultural crops high level of resistance *S. litura* has been reported for many synthetic pyrethroids, organophosphate and carbamate insecticides (Reddy and Reddy, 1984). Integration of genetic diversity component of individual S populations and the DNA markers in devising IPM and IRM strategies is required. PCR based Random Amplified Polymorphic DNA (RAPD) is widely being adopted to detect the polymorphism among populations/individuals including insects ever since its discovery. The RAPD method of genome analysis is fast and allows the examination of genomic variation without prior knowledge of DNA sequences (Williams *et al.,* 1990). Random amplified polymorphic DNA-Polymerase chain reaction technique has been used previously for population genetic studies of a number of insects including aphids (Black *et al.,* 1992), grasshoppers (Chapco *et al.,* 1992) and *S. litura* (Stevens and Wall, 1995). In spite of the work done on various insecticides, information on molecular diagnostic techniques for monitoring commonly used insecticide resistance in *S. litura* population is lacking. In view of the above, attempts have been made to identify genetic variability in insecticide resistant and susceptible population of *S. litura* associated with groundnut crop from different geographic locations in Andhra Pradesh and Telangana.

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MATERIALS AND METHODS

Insects collection: The egg-masses of *S. litura* were collected from Anantapuramu, Chittoor, Kurnool and Kadapa districts of Rayalaseema region, Nellore and Guntur districts of Coastal region, Mahaboobnagar, Warangal and Karimnagar districts of Telangana region which were pre exposed to chemicals in the groundnut field. Insects emerging from these eggs (F_1 generation) were reared in the laboratory up to F_2 generation and third instar larvae from F_2 generation were used in the bioassay studies. Similarly, baseline *S. litura* population maintained under laboratory conditions up to 21 generations without exposing to insecticides were also collected from Division of Entomology, Indian Agricultural Research Institute, New Delhi for bioassay. Both the cultures were maintained under controlled conditions of 27±2°C, 65 to 70 per cent relative humidity on fresh groundnut leaves.

Acetylcholinesterase analysis: Approximately a 20 mg of larvae heads were homogenized in 1 ml phosphate buffer (0.05 M, pH 7.2) containing 0.05 ml (0.5%) triton X-100 and 0,074 g (2mM) EDTA. The homogenized sample were collected in a 2 ml glass tube and centrifuged at 10,000 rpm in 4°C for 20 min and the supernatant was used for Acetylcholinesterase enzyme assay. The protein concentrations in the supernatant from various populations were estimated by the method of Lowry *et al.* (1951) using Bovine Serum Albumin as standard.

Extraction of genomic DNA: The excised midgut (single individual) from body of insecticide treated larvae showing high and low level of acetylcholineesterase activity was pooled separately. The genomic DNA was extracted from the midgut tissues with Tris-EDTA phosphate buffer (50 mM Tris; 10 mM EDTA; pH 8.0) followed by phenol:chloroform extraction and absolute ethanol for DNA precipitation (Ballinger and Crabtree, 1992). The DNA pellet was resuspended in 100 µl of Tris-EDTA buffer and 2 µl (50 ng genomic DNA) of the dilution was used for RAPD-PCR analysis. Random Amplified Polymorphic DNA (RAPD) analysis of genomic DNA of all the populations using 11 different random decamer primers (5 primers from OPA, 3 primers from OPC and 3 primers from OPE series, Operon technology inc., USA) were carried out. The composition of 25 µl of reaction mixture contained 20 mM Tris HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM of each dNTPs, 15- Picomole Operon primers, 0.3 units/µl of hot start Taq DNA polymerase, 50 ng of Template DNA and 15.375 µl of RNase free water. Amplification was performed in PCR machine by following temperature profile i.e., initial denaturizing at 95ºC for 2 min followed by 45 cycles of denaturizing at 92ºC for 1 min; annealing at 37 ºC for 1 min; extension at 72ºC for 2 min with final elongation of 72ºC for 5 min (Pascal *et al.* 2000) was followed. The amplified DNA products were fractionated on 1.0 per cent agarose in 1x TBE buffer and visualized on transilluminator under UV light after staining with ethidium bromide.

Specific amplification of acetylcholinesterase gene in selected test insect *S. litura* along with least susceptible baseline population was carried out. An earlier reported primer sequence for esterase gene (Forward-5'-TGAATTGCTCGGTGACACCTC-3'; Reverse: 5'ATCTCCTTCGACCG GCATTATTTC-3') (Vontas *et al*., 2000) was synthesized and used. PCR reaction was carried as per the protocol mentioned below with following modifications: Initial denaturation for 2 min at 95ºC, 30 cycles having initial denaturation for 1 min at 94ºC, annealing for 1 min at 52ºC and extension for 1 min at 72ºC and final elongation for 7 min at 72 ºC. The amplified PCR products were analyzed by 1.5 per cent agarose gel electrophoresis in 1x TBE buffer and visualized by staining with ethidium bromide.

RESULTS AND DISCUSSION

The mean enzymatic activity of acetylcholinesterase (AchE) in insecticides treated third instar larvae of *S. litura* indicated that, significantly highest AchE activity in Guntur (0.75±0.26µmoles/min/ml), Karimnagar (0.56±0.22µmoles/min/ml) and Kurnool (0.58± 0.24µmoles/min/ml) strains and which are on par with each other (Table 1). This was followed by Mahaboobnagar (0.49±0.23µmoles/min/ml), Nellore (0.47±0.23µmoles/min/ml) and Chittoor (0.44±0.19µmoles/min/ml) strains, where as Anantapuramu (0.33 ± 0.27µmoles/min/ml), Kadapa (0.40 ± 0.23µmoles/min/ml) and Warangal (0.46 ± 0.18µmoles/min/ml) strains recorded relatively higher AchE activity than susceptible baseline population (0.11 ± 0.04 µmoles/min/ml). On the basis of acetylcholinesterase (AchE) as biochemical marker (enzyme), the order of resistance showed by various populations was Warangal > Kadapa > Anantapuramu > Chittoor > Nellore > Mahaboobnagar > Karimnagar > Guntur.

In the present investigations, DNA from insecticide resistant strains was used as template for the amplification. However, due to several limitations in morphological data, the traditional method of DNA profiling were largely used for identification of genetic relations in different strains. Eleven arbitrary primers were used for RAPD analysis and detected a total of 128 fragments with an average of 11.63 fragments per primer and 95.61 per cent fragments with polymorphism (Table 2). Among the 11 primers, OPA-05 generated distinguishable amplification products and consistent band (600 bp) in cypermethrin, quinalphos, chlorpyriphos and acephate resistant strains of *S. litura* which was not found in susceptible baseline strain (Fig. 1). Repeated amplifications using individual resistant and susceptible baseline strains also confirmed the existence of this polymorphism among different *S. litura* populations. The potential benefits of a marker (enzymes) assisted selection have been discussed earlier by various workers (Janarthanan *et al*., 2003; Muthusamy *et al*., 2013; Field *et al*., 1996; Goh *et al*., 1995; Srinivas *et al*., 2004). The phylogenetic relations among cypermethrin, quinalphos, chlorpyriphos and acephate resistant strains of *S. litura* were studied by constructing dendrogram through UPGMA method and it indicated that, Guntur, Karimnagar and Kurnool strains formed a cluster followed by Mahaboobnagar, Warangal and Nellore in another cluster. Rest of the strains from Chittoor, Kadapa and Anantapuramu formed a third group. However, susceptible base line did not show any resemblance in phylogenetic relations with resistant strains of *S. litura*.

Further, to tag the acetylcholinesterase gene, specific amplifications were carried out using custom made primers and DNA samples derived from resistant populations *viz.,* Guntur, Karimnagar, Kurnool, Mahaboobnagar, Warangal,

Nellore, Chittoor, Kadapa and Anantapuramu. A sample from highly susceptible baseline was used as control. Earlier studies have suggested that, insecticide resistance is caused by synthesis of more copies of genes (DNA sequence per haploid genome above the level that is characteristic for an organism or amplification) that code for esterases. In these cases extra copies of structural genes apparently could cause increased production of enzymes that metabolize or sequester insecticides, thereby increasing the insect ability to insecticide resistance (Tabashnik, 1991; Field *et al.,* 1988). The results of present study showed a specific amplification product at 600 bp region indicating the presence of acetylcholinesterase gene in resistant strains of *S. litura,* whereas in susceptible base line strain of *S. litura* it was not found due to non amplification of specific gene at the same loci (600 bp) a phenomenon due to low copy number of gene (Fig. 2). Janarthanan *et al.* (2002) reported similar results while identifying insecticide resistant and susceptible genes of esterase in *S. litura.* In conclusion, the present study demonstrates the presence of acetylcholinesterase gene in *S. litura,* and that insecticide modifies the level of activity of AchE in resistant and susceptible population by interfering with mediated detoxification reactions.

Table 1 Activity level of acetyl cholinesterase (µmoles/min/ml) in insecticides treated different population of *S. litura*

Insecticides	Ananta-puramu	Chittoor	Kadapa	Kurnool	Nellore	Karimnagar	Mahaboob-nagar	Warangal	Guntur	Baseline susceptible	Mean
T_1	0.52 ± 0.17	0.78 ± 0.16	0.72 ± 0.21	0.90 ± 0.18	0.79 ± 0.18	0.90 ± 0.23	0.83 ± 0.21	0.76 ± 0.19	0.98 ± 0.20	0.23 ± 0.03	0.741
T_2	0.39 ± 0.20	0.59 ± 0.21	0.57 ± 0.26	0.83 ± 0.10	0.66 ± 0.26	0.79 ± 0.25	0.70 ± 0.21	0.60 ± 0.19	0.84 ± 0.28	0.14 ± 0.07	0.611
T_3	0.43 ± 0.29	0.68 ± 0.12	0.60 ± 0.29	0.81 ± 0.08	0.70 ± 0.15	0.84 ± 0.26	0.73 ± 0.09	0.65 ± 0.23	0.92 ± 0.38	0.13 ± 0.06	0.649
T_4	0.38 ± 0.23	0.50 ± 0.26	0.38 ± 0.26	0.71 ± 0.13	0.51 ± 0.18	0.69 ± 0.21	0.55 ± 0.23	0.52 ± 0.25	0.88 ± 0.18	0.10 ± 0.01	0.522
T_5	0.16 ± 0.14	0.17 ± 0.08	0.16 ± 0.11	0.24 ± 0.15	0.14 ± 0.09	0.19 ± 0.11	0.16 ± 0.09	0.21 ± 0.11	0.45 ± 0.04	0.04 ± 0.01	0.168
T_6	0.37 ± 0.24	0.63 ± 0.23	0.47 ± 0.32	0.81 ± 0.10	0.68 ± 0.35	0.73 ± 0.31	0.69 ± 0.23	0.57 ± 0.32	0.88 ± 0.37	0.12 ± 0.06	0.595
T_7	0.38 ± 0.16	0.52 ± 0.24	0.52 ± 0.33	0.79 ± 0.10	0.50 ± 0.22	0.67 ± 0.34	0.49 ± 0.17	0.55 ± 0.25	0.86 ± 0.29	0.12 ± 0.06	0.540
T_8	0.37 ± 0.27	0.45 ± 0.24	0.42 ± 0.21	0.75 ± 0.23	0.54 ± 0.31	0.70 ± 0.33	0.68 ± 0.20	0.54 ± 0.26	0.88 ± 0.27	0.10 ± 0.01	0.543
T_9	0.51 ± 0.13	0.75 ± 0.09	0.71 ± 0.25	0.88 ± 0.12	0.77 ± 0.22	0.84 ± 0.23	0.79 ± 0.31	0.70 ± 0.28	0.96 ± 0.22	0.16 ± 0.08	0.707
T_{10}	0.39 ± 0.25	0.31 ± 0.19	0.24 ± 0.15	0.37 ± 0.23	0.46 ± 0.29	0.48 ± 0.20	0.34 ± 0.29	0.33 ± 0.17	0.54 ± 0.23	0.10 ± 0.01	0.356
T_{11}	0.12 ± 0.08	0.12 ± 0.10	0.13 ± 0.02	0.15 ± 0.11	0.12 ± 0.10	0.13 ± 0.11	0.16 ± 0.12	0.19 ± 0.10	0.51 ± 0.12	0.05 ± 0.01	0.192
T_{12}	0.12 ± 0.10	0.10 ± 0.02	0.11 ± 0.02	0.19 ± 0.12	0.12 ± 0.10	0.15 ± 0.12	0.16 ± 0.12	0.18 ± 0.11	0.53 ± 0.14	0.06 ± 0.01	0.177
T_{13}	0.13 ± 0.03	0.13 ± 0.12	0.13 ± 0.10	0.17 ± 0.11	0.11 ± 0.02	0.16 ± 0.01	0.15 ± 0.03	0.18 ± 0.13	0.57 ± 0.18	0.04 ± 0.01	0.172
Average	0.33 ± 0.27	0.44 ± 0.19	0.40 ± 0.23	0.58 ± 0.24	0.47 ± 0.23	0.56 ± 0.22	0.49 ± 0.23	0.46 ± 0.18	0.75 ± 0.26	0.11 ± 0.04	
NFHAOSB	3.00	4.00	3.64	5.27	4.27	5.09	4.45	4.18	6.82		

Figures indicate mean value ± standard deviation of three replications

T_1 : Control; T_2 : Flubendiamide 480SC; T_3 : Emamectin benzoate 5SG; T_4 : Rynaxypyr 18.5SC; T_5 : Thiodicarb 75WP; T_6 : Novaluron10EC; T_7 : Lambdacyhalothrin 2.5EC; T_8 : Indoxacarb 14.5SC; T_9 : Cypermethrin 10EC; T_{10} : Spinosad 45SC; T_{11} : Quinalphos 25EC; T_{12}:Chlorpyriphos 20EC; T_{13}:Acephate 75WP; NFHAOSB: No. of folds higher AchE over baseline susceptible.

Table 2 Primer survey for determination of polymorphism in *S. litura* populations

RAPD-Primers	Sequence (5'-3')	Cypermethrin			Quinalphos			Chlorpyriphos			Acephate		
		Total Bands	Polymorphic bands	Per cent polymo-rphism	Total Bands	Polymorphic bands	Per cent poly mo-rphism	Total Bands	Polymorphic bands	Per cent polymo-rphism	Total Bands	Polymorphic bands	Per cent polymo-rphism
OPA-01	CAGGCCCTTC	5	5	100	6	5	83	5	5	100	4	3	75
OPA-05	AGGGGTCTTG	12	12	100	8	8	100	13	13	100	11	11	100
OPA-07	GAAACGGGTC	11	11	100	11	11	100	11	11	100	12	12	100
OPA-08	GTGACGTAGG	17	17	100	18	18	100	16	15	94	14	14	100
OPA-13	CAGCACCCAC	10	10	100	10	10	100	10	10	100	10	10	100
OPC-02	GTGAGGCGTC	11	10	90	12	10	83	11	10	91	11	10	91
OPC-08	TGGACCGGTG	15	15	100	15	15	100	15	15	100	15	15	100
OPC-15	GACGGATCAG	10	9	90	10	9	90	10	10	100	10	9	90
OPE-04	GTGACATGCC	15	15	100	15	14	93	13	13	100	15	15	100
OPE-08	TCACCACGGT	6	5	83	5	5	100	6	5	83	6	5	83
OPE-15	ACGCACAACC	16	14	88	13	13	100	15	14	93	17	16	94
Total		128	123		123	118		125	121		125	120	
Average		11.63	11.18	95.61	11.18	10.72	95.45	11.36	11.00	96.48	11.36	10.90	93.94

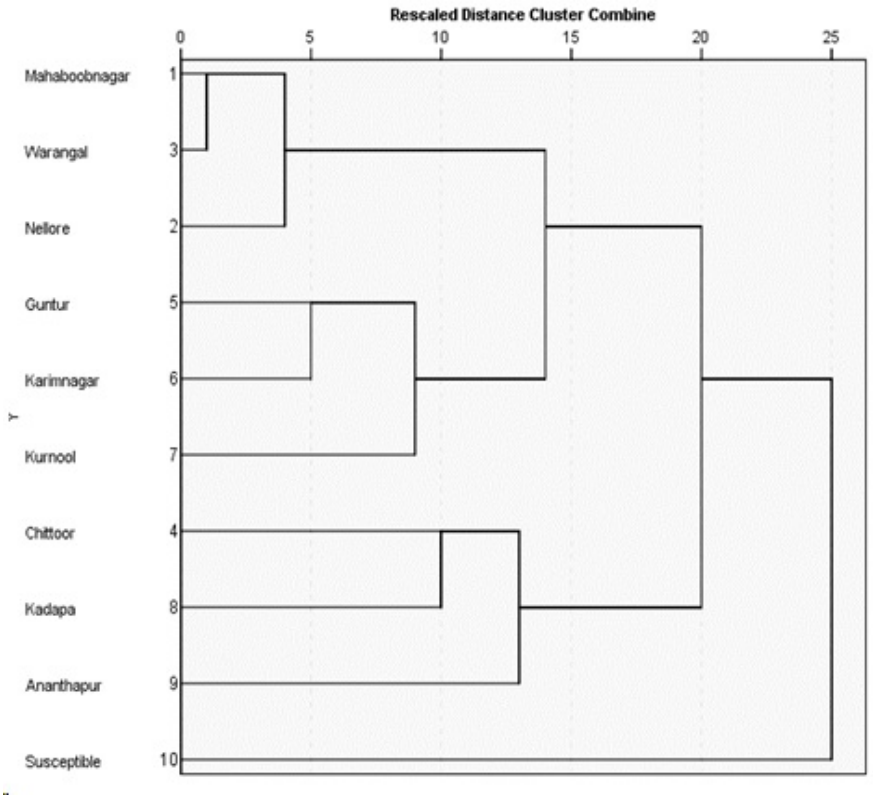
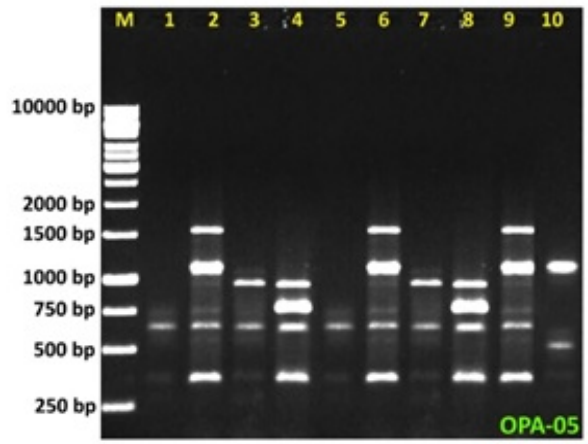


Fig. 1. Dendrogram depicting variation among different insecticide resistant larval populations of *S.litura* based on RAPD analysis



RAPD profiles of quinalphos resistant strains of *S. litura* generated by random primer OPA-05 in 1 per cent agarose gel

M : 1 kb DNA Ladder
1 : Mahaboobnagar
2 : Nellore
3 : Warangal
4 : Chittoor
5 : Guntur
6 : Karimnagar
7 : Kurnool
8 : Kadapa
9 : Anantapuramu
10 : Base line

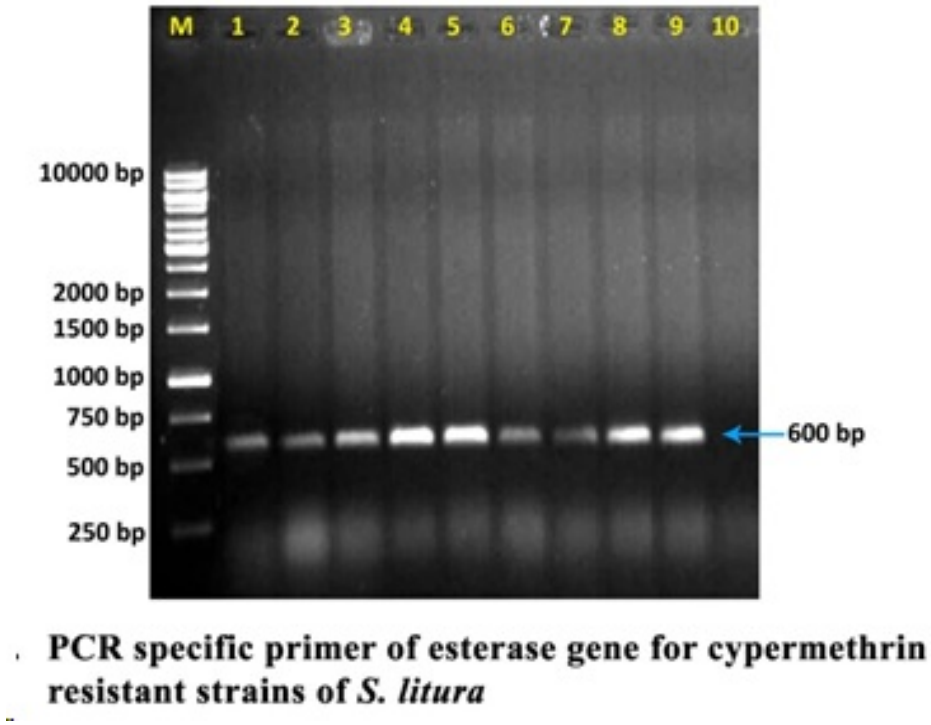


Fig. 2. RAPD profiles of quinalphos resistant strains of *S. litura* generated by random primer OPA-05 and PCR specific primer of esterase gene for cypermethrin resistant strains of *S. litura*

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Study of gene action for seed yield, oil and quality components in linseed (*Linum usitatissimum* L.)

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ABSTRACT

An experiment was conducted to study the gene action of seed yield and quality components in linseed. The analysis of variance showed highly significant differences among the genotypes for all the quality traits. Graphical analysis exhibited non additive gene action playing major role for most of the attributes *viz.,* seed yield per plant, harvest index, moisture content, oil content, protein content of seed, iodine value, palmitic acid, stearic acid and omega-3 content. Components of variance revealed the presence of additive components for oleic acid content and omega-6. Average degree of dominance ($\hat{H}_1 / \tilde{D}^{0.5}$ showed over dominance for all the traits except oleic acid content and omega-6. The value of $\hat{H}_2/4\hat{H}_1$ indicated asymmetrical distribution of positive and negative genes showing dominance for all the characters. Utilization of biparental mating and recurrent selection schemes which has the virtue of effectively exploiting both additive and non-additive components was suggested for obtaining high yielding genotypes with better quality and high percentage of omega-3 fatty acid.

Keywords: Fatty acid contents, Gene action, Linseed, Omega-3, Protein content

Linseed is unique among oilseeds for its technical grade vegetable oil producing ability and fibre production (Oomah, 2004; Biradar *et al.,* 2016). Of late, its value addition has paved the way for its diverse field uses in nutraceutical and medicinal purposes. Its seeds are laden with complete protein (all 8 essential amino acids), water soluble fibre fraction, higher order of linolenic acid (an essential poly unsaturated omega-3 fatty acid), complex carbohydrates, vitamins and minerals (Warrand *et al*., 2005). It is the best herbal source of omega-3 and omega-6 fatty acids. Omega-3 fatty acid imparts in cholesterol lowering, cardiovascular benefits by affecting prostaglandins and leukotrienes related to blood clotting and inflammatory disorder like rheumatoid arthritis. The fundamental objective of researcher breeding is to evolve varieties that combine productivity with quality having wide adaptability with stable performance. The present investigation was undertaken with the objective to evolve genotypes having high seed yield coupled with better quality and high percentage of omega-3 fatty acid.

The materials for the present investigation comprised of 11 parents and their 55 F_1s grown in a randomized block design with three replications at Oilseed Research Farm, C.S. Azad University of Agriculture and Technology, Kanpur during *rabi* 2010-11. Each genotype was sown in two rows of three metre length with row to row and plant to plant spacing of 40 cm and 10 cm, respectively. Data were recorded on ten randomly selected plants in parents and F_1s in each replication for eleven quality traits *viz.,* seed yield/plant (g), harvest index (%), moisture content (%), oil content (%) protein content of seed (%), iodine value, palmitic acid content (%), stearic acid content (%), oleic acid content, omege-6 fatty acid and omege-3 fatty acid. Data on various variables were analyzed by analysis of variance. Oil content was determined by Soxhlet method, moisture content of seed by air-oven method, protein content by Kjeldahl method and iodine value by Wijs method. Methyl esters of fatty acids for determining fatty acid composition of oil were prepared by "Transesterification procedure" described by Jamieson and Reid (1965). Methyl esters were subjected to Gas-Liquid Chromatographic analysis for determination of fatty acid composition. Graphic analysis was done as per Haymen (1954). The analysis was based on variance and covariance (Vr, Wr) graph. A diallel table was prepared for each trait to calculate Vr, Wr and Vp. Related parameters were determined on the basis of the formula suggested by Haymen (1954). The significance of the components was tested by "t^2 test". A general approach to this method based on second degree of statistics the concepts of D, H, components of variation was suggested by Yates (1947).

Significant variability was present among the parents and their F_1 progenies with respect to all the traits under study (Table 1). Non significant value of 't^2' in all the traits except moisture content, protein content of seed, stearic acid content and omege-3 content of oil, supported the validity of the hypothesis. However, 't^2'value were significant in moisture and protein content of seed, stearic acid and omege-3 content of oil indicating failure of one or more assumptions in these cases (Table 2). The graphical analysis displayed that the slope of regression line in Vr - Wr graph for seed yield per plant, harvest index, moisture content, oil content, protein content, iodine value, palmitic acid, stearic acid and omege-3 acid was found to deviate significantly from unity indicating non allelic interaction of genes playing

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major role in expression of these attributes. The slope of regression line for remaining traits *viz.,* oleic acid and omege-6 acid content did not deviate significantly from unity but deviated significantly from zero revealing the preponderance of additive type of gene action.

The estimates of additive variance (D) was highly significant for all the traits except moisture content where it was found to be non-significant (Table 3). Graphical analysis (Table 3) displayed importance of partial dominance for harvest index, oil content, protein content of seed, omega-6 and omege-3 contents and over dominance for seed yield per plant, moisture content, iodine value, palmitic acid, stearic acid and oleic acid content. Component analysis revealed that the average degree of dominance $(\dot{H}_1 / \bar{D}^{0.5}$ was more than unity for most of the traits except oleic acid and omege-6 acid content suggesting manifestation of over dominance for these traits. The ratio $(\dot{H}_1 / \bar{D})^{0.5}$ was less than unity for omega-6 content displaying partial dominance. However, oleic acid content showed complete dominance (Table 4). It may be explained that estimates obtained through formula $(\dot{H}_1 / \bar{D})^{0.5}$ gave an appropriate value of the degree of dominance. Hayman (1954) suggested that in particular combinations positive, negative and complementary type of gene action and simply correlated gene distribution may seriously inflate the mean degree of dominance and convert partial dominance in to apparent over dominance. Comstock and Robinson (1952) emphasized that the degree of dominance might be biased upward either by linkage or epistasis or both. Both graphical and component analysis revealed over dominance for seed yield per plant, moisture content, iodine value, palmitic acid and stearic acid content. It might be a real one and could be utilized practically; however, it could be done where loci having over dominance effect tight linkage.

The estimates of '$\bar{F}$' value were positive for all the traits except palmitic acid content but the significant value was observed for harvest index, protein content of seed and oleic acid content of oil. Significant and positive estimates of h^2 were examined for seed yield per plant, harvest index, moisture content, protein content, palmitic acid content, oleic acid content and omega-3 acid content. Significant value of $\bar{F}$ and $\bar{h}^2$ in positive direction confirmed the higher frequency of dominant genes playing a significant role in controlling these attributes.

Dominance components ($\bar{H}_1$ and $\bar{H}_2$) were also recorded to be highly significant for all the traits. The third measure of dominance effect i.e. h^2 was highly significant for seed yield per plant, harvest index, moisture content, protein content, palmitic acid, oleic acid content and omegea-3 acid content exhibiting that there may be higher concentration of dominant alleles in parents which lead to high mean performance. Similar findings were observed by Singh and Tewari (2014). The magnitude of dominance component ($\bar{H}_1$) was higher than additive component ($\bar{D}$) for seed yield per plant, harvest index, moisture content, oil content, protein content of seed, iodine value, palmitic acid, stearic acid and omega-3 acid contents indicating that these characters are largely governed by non-additive gene action. Therefore, it would be difficult to improve these traits through selection breeding. The additive type of gene action was most prevalent in the expression of these attributes. Such traits may be improved following pedigree method.

Table 1 Analysis of variance for seed yield, oil and quality components of seed and oil in linseed

Source	df	Seed yield / plant (g)	Harvest index (%)	Moisture content (%)	Oil content (%)	Protein content (%)	Iodine value	Palmatic acid content (%)	Stearic acid content (%)	Oleic acid content (%)	Omega-6 fatty acid (%)	Omega-3 fatty acid (%)
Replications	2	0.054	5.410	8.611	0.820	3.558	26.750	0.462	0.732	3.605	0.368	5.050
Treatments	65	43.097**	61.385**	0.887**	11.669**	4.202**	105.665**	0.201**	1.869**	18.128**	5.014**	27.709**
Parents (P)	10	17.151**	108.581**	0.272**	14.161**	4.858**	133.746**	0.174**	1.268**	29.906**	9.425**	35.186**
Crosses	54	21.687**	49.640**	0.891**	10.964**	2.076**	100.550**	0.191**	1.979**	14.900**	4.284**	25.537**
Parent Vs. Crosses	1	1458.692**	223.640**	6.865**	24.880**	112.410**	102.300**	1.034**	1.929**	74.610**	0.324	70.450**
Errors	130	1.079	0.677	0.164	0.560	0.750	0.912	0.033	0.061	0.375	0.137	0.543

** - Significant at 1% level

The value of $H_2/4H_1$ was observed to be less than theoretical value (0.25) for all the traits indicating asymmetrical distribution of positive and negative genes analysis also supported asymmetrical distribution of positive and negative genes at loci showing dominance. Graphical analysis also supported asymmetrical distribution of dominant and recessive alleles in the parents. The proportion between dominant and recessive alleles $(4DH_1)^{0.5}+F/(4DH_1)^{0.5}-F$ being more than unity for all the traits except palmitic acid content suggested excess of dominant genes. The ratio h^2/H_2 was found more than unity for seed yield per plant and protein contents suggesting that

more than one major group of genes were responsible for the expression of these traits. The ratio h^2/H_2 was close to one for moisture content, palmitic acid and oleic acid contents indicating that these traits were governed by one major block of genes. For rest of the attributes the ration h^2/H_2 was less than unity probably due to gene interaction. The significant negative correlation between parental order of dominance and parental measurement for seed yield per plant, moisture content, protein content and oleic acid content suggested association of dominant genes for the expression of these traits. The negative but non-significant value of correlation coefficient for harvest index, omega-6 acid and omega-3 acid contents did not confirm the association of dominance alleles with these attributes.

Table 2 Estimates of b, sb, (b-o/sb) and (b-1/sb) and t^2 for seed yield, oil and quality components of seed and oil in linseed

Characters	Statistics				t^2 value
	b	sb	(b-0/sb)	(b-1/sb)	
Seed yield/ plant	0.466	0.141	3.305**	-3.787**	4.584
Harvest index	0.649	0.123	5.276**	-2.854**	3.240
Moisture content	0.243	0.114	2.132	-6.640**	13.22**
Oil content	-0.175	0.155	-1.129	-7.581**	5.073
Protein content of seed	0.092	0.134	0.687	-6.776**	9.591*
Iodine value	0.686	0.157	4.369**	-2.003*	0.965
Palmitic acid	0.526	0.144	3.653**	-3.292**	3.498
Stearic acid	0.173	0.141	1.227	-5.865**	7.818*
Oleic acid	1.196	0.128	9.343**	1.531	5.090
Omega-6	0.972	0.125	7.776**	-0.224	0.120
Omega-3	0.647	0.085	7.612**	-4.153**	9.173*

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

Table 3 Comparative evaluation of gene action and average degree of dominance for seed yield, oil and quality components of seed and oil in linseed

Characters	Component analysis	Graphic at gene action	Average Wr ,Vr	degree of dominance $(\hat{H}_1/D)^{0.5}$
Seed yield/plant $\bar{D}\,\bar{H}_1$	Gene action	NA	OD	OD
Harvest index	HS < HS NA	NA	PD	OD
Moisture content	NS < HS NA	NA	OD	OD
Oil content	HS < HS NA	NA	PD	OD
Protein content of seed	HS < HS NA	NA	PD	OD
Iodine value	HS < HS NA	NA	OD	OD
Palmitic acid	HS < HS NA	NA	OD	OD
Stearic acid	S < HS NA	NA	OD	OD
Oleic acid	HS < HS A	A	OD	CD
Omega- 6	HS < HS A	A	PD	PD
Omega-3	HS < HS NA	NA	PD	OD

S - Significant; HS - Highly significant; NS - Non- significant; A - Additive; NA - Non-additive; PD - Partial dominance; CD - Complete dominance; OD - Over dominance

Table 4 Estimation of genetic parameters and related statistics for seed yield, oil and quality components of seed and oil in linseed

Character	Genetic parameters and related statistics										
	$\bar{D}$	$\bar{H}_1$	$\bar{H}_2$	$\bar{F}$	$\bar{h}_n$	$\bar{E}$	$(\bar{H}_1/\bar{D})(\bar{H}_1/4\bar{H}_1)$		$(4\bar{D}\bar{H}_1)^{0.5}+\bar{F}/(4\bar{D}\bar{H}_1)-F$	$\bar{h}_n/\bar{H}_2$	r
Seed yield/ plant	5.36** ±2.39	53.09** ±4.94	46.48** ±4.15	10.75 ±5.48	175.23** ±2.78	0.36 ±0.69	3.14	0.22	1.94	3.77	-0.70** ±0.24
Harvest index	35.97** ±4.29	48.46** ±8.85	30.45** ±7.44	28.92** ±9.82	26.80** ±4.98	0.22 ±1.24	1.16	0.15	2.06	0.88	-0.50 ±0.28
Moisture content	0.04 ±0.10	1.04** ±0.21	0.77** ±0.17	0.12 ±0.23	0.81** ±0.12	0.06 ±0.03	5.37	0.18	1.84	1.05	-0.70** ±0.24
Oil content	4.53** ±1.56	12.36** ±3.21	11.25** ±2.70	3.29 ±3.56	2.93 ±1.81	0.19 ±0.45	1.65	0.23	1.56	0.26	0.25 ±0.32
Protein content of seed	1.37** ±0.30	3.94** ±0.62	3.15* ±0.52	1.63** ±0.69	13.43** ±0.35	0.25* *±0.09	1.70	0.20	2.08	4.26	-0.69** ±0.24
Iodine value	44.28** ±6.68	87.34** ±13.78	73.97** ±11.58	20.34 ±15.28	12.21 ±7.75	0.30 ±1.93	1.40	0.21	1.39	0.16	0.66** ±0.24
Palmitic acid	0.04* ±0.01	0.13** ±0.03	0.12** ±0.02	-0.01 ±0.03	0.12** ±0.01	0.01 ±0.00	1.70	0.21	0.94	1.00	0.76** ±0.21
Stearic acid	0.40* ±0.19	2.25** ±0.39	1.74** ±0.33	0.42 ±0.44	0.22 ±0.22	0.02 ±0.05	2.37	0.19	1.58	0.12	0.46 ±0.29
Oleic acid	9.84** ±0.55	9.72** ±1.14	8.51** ±0.96	3.40** ±1.26	8.92** ±0.64	0.12 ±0.16	0.99	0.21	1.42	1.04	-0.76** ±0.21
Omega-6	3.09** ±0.04	0.46** ±0.09	0.42** ±0.07	0.02 ±0.10	0.02 ±0.05	0.04** ±0.01	0.38	0.22	1.02	0.05	-0.16 ±0.32
Omega-3	11.55** ±1.34	18.21** ±2.76	17.17** ±2.32	2.07 ±3.07	8.41** ±1.55	0.18 ±0.38	1.25	0.23	1.15	0.49	-0.10 ±0.33

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Effect of salicylic acid and potassium dihydrogen phosphate on heat stress induced changes in mustard [*Brassica juncea* (L.) Czern. & Coss.]

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ABSTRACT

The present investigation was carried out during *rabi* season of 2010-11 at Main Castor and Mustard Research Station, Sardarkrushinagar Dantiwada Agricultural University, Gujarat to study the chemical based management of heat stress in mustard. The foliar spray of chemicals was applied at 60 DAS and 75 DAS. Results revealed that foliar application of 75 ppm salicylic acid with first date of sowing 20th October, 2010 when compared with the control were found superior for plant height (183 cm), number of primary branches per plant (4.38), number of secondary branches per plant (10.25), number of siliqua per plant (403), length of siliqua (5.98 cm), number of seed per plant siliqua (12.63), biological yield (70), harvest index (19.58%), seed yield (13.71g) and oil content (39.65%).

Keywords: Dihydrogen phosphate, Heat Stress, Potassium mustard, Salicylic acid

Mustard [*Brassica juncea* (L.) Czern. & Coss.] is one of the most ancient oilseed crop belongs to the family cruciferous and genus *Brassica*. Mustard is an important edible oil yielding crop accounting for about 80 per cent of the cultivated area in North-Western parts of India (Singh *et al.,* 2014; Singh *et al.,* 2016). Mustard being a cool season crop requires optimum temperature range 6-26°C for better growth and development. High temperature in *Brassica* inhibits plant development and caused flower abortion with appreciable loss in seed yield. Flowering stage have a strong influence on seed yield with rise of 30°C of maximum daily temperature, the seed yield decline (Nuttall *et al.,* 1992). Chemical like salicylic acid and potassium di hydrogen phosphate can relieve high temperature effect from the mustard by increasing activity of antioxidant enzymes. But adequate and effective chemical management is not significantly worked out for achieving high productivity of this crop in the state. Hence the present investigation was undertaken to study the effect of salicylic acid and potassium dihydrogen phosphate on heat stress induced changes in mustard under agro-climatic conditions of North Gujarat region.

A field experiment was conducted during *rabi* season of 2010-11 at the Main Castor and Mustard Research Station, Sardarkrushinagar Dantiwada Agricultural University, Gujarat. The experiment was laid out in split plot design with 4 replications. All possible 12 treatment combinations consisting of two dates of sowing *viz.,* D_1-20th October and D_2-20th November as main plot treatments, six spray levels *viz.,* F_1-Control, F_2-Foliar spray of water, F_3-Foliar spray of salicylic acid 50 ppm, F_4-Foliar spray of salicylic acid 75 ppm, F_5-Foliar spray of potassium di-hydrogen phosphate 50 ppm, F_6-Foliar spray of potassium di-hydrogen phosphate 75 ppm as sub plot treatments. The spray solution of 50 ppm and 75 ppm of salicylic acid and potassium di-hydrogen phosphate was prepared by dissolving 50 mg and 75 mg of salicylic acid and potassium di-hydrogen phosphate in one litre of water. The foliar spray of chemicals was applied at 60 DAS and 75 DAS. Mustard variety GM 3 was sown in rows at 30 cm apart on 20th October and 20th November. A common irrigation was given immediately after sowing for satisfactory seed germination and proper establishment of crop. Other agronomical operations were followed as per recommendations made for the mustard in the region. Five plants were randomly selected from each net plot. Each selected plant was labeled for easy identification. The same five plants were harvested separately for post-harvest study. Another five plants selected for destructive parameters. The mean of five observations were used for calculating sampling values of growth parameters, yield attributes and yield. The experimental data were statistically analyzed for level of significance and pooled analysis for both the dates of sowing.

The plant height was measured in centimeter (cm) from ground level to the tip of the main branch at the time of maturity. Tagged five plants were used for recording observations of number of primary and secondary branches per plant, number of siliqua, their average worked out. The siliqua length, numbers of seed per siliqua of the plant was measured in centimeter (cm) at physiological maturity. Randomly selected tagged plants were collected from plot and weighed in grams for biological yield. A random sample of seeds weighing approximately 12 gm was taken from bulk seeds harvested from five randomly selected plants of each genotype and oven dried. Oil content of each sample was determined in percentage by using Nuclear Magnetic Resonance Technique (NMR) at the Main Castor and Mustard Research Station, Sardarkrushinagar Agricultural University, Sardarkrushinagar.

Plant height, number of primary branches per plant, number of secondary branches per plant, length of siliqua, number of seed per siliqua, biological yield per plant, harvest index were not found significant due to the effect of different

chemical application except number of siliqua per plant showed significant value (Table 1). Foliar spraying of salicylic acid 75 ppm produced maximum number of siliqua per plant followed by the foliar application of 75 ppm potassium dihydrogen phosphate. While in both environmental conditions it seemed that environmental condition first i.e. 20th October 2010 recorded significant superior value as compared to second environmental condition i.e. 20th November 2010. During second date of sowing mean temperature was increased due to that reduction on siliqua production occurred. It may be due to floral sterility caused by high temperature exposure as this was indicated in *Brassica napus* by Morrison and Stewart (2002). Foliar spray of salicylic acid as well as potassium dihydrogen phosphate mitigate heat stress effect by inducing flowering, flower life, retard senescence and increase cell metabolic rate (Metwally *et al.,* 2003); Bhuiyan *et al.* (2008).

The mean seed yield of first date of sowing. i.e. 20th October (D_1) were recorded maximum value to second date of sowing 20th November (D_2). Increase in yield under first date of sowing might be due to the fact that during the first date of sowing high temperature stress was not occurred. The optimum temperatures resulted in improvement in number of siliqua per plant, seed yield per plant as well as increased plant height, branches per plant resulted in more efficient partitioning of dry matter to the yield attributing parts of the plant. Late sowing reduced the growth phase leading to lower photosynthesis and dry matter accumulation. The findings are in close agreement with the results obtained by Shivani *et al.* (2002), Panda *et al.* (2004) and Loyal *et al.* (2006).

Foliar spraying of salicylic acid 75 ppm produced maximum seed yield. Seed yield obtained with foliar spraying of potassium dihydrogen phosphate 75 ppm, potassium dihydrogen phosphate 50 ppm, salicylic acid 50 ppm were comparable to those obtained with application of salicylic acid 75 ppm. The variations in growth parameters and yield attributing characters were not much appreciable among different chemical management, which resulted in the production of identical seed yields. However distinct superiority in number of siliqua per plant, spraying of salicylic acid 75 ppm increased utmost seed yields. It could be stated that the beneficial effect of salicylic acid and potassium dihydrogen phosphate on improving seed yield might be due to enhanced photosynthetic efficiency and translocation of more assimilates from source to sink there by increased seed yield. These results were close conformity with the findings of He *et al.* (2005) and Kaur *et al*. (2009).

Different date of sowing schedules significantly affected the oil content which were observed in first date of sowing as against in second date of sowing. During second date of sowing high temperature stress occurred due to that reduction was registered. Oil content is mainly directly related with seed yield. The oil content of seeds did not differ much due to varying chemical management, but seed yields showed variations between them. Hence oil content under different chemical management followed almost same order as to seed yields. Oil content was significantly increased with 75 ppm salicylic acid over rest of the treatments. Higher concentration of nutrients in seed along with higher seed yield under foliar spray of salicylic acid resulted in higher uptake of nutrients. The increment in oil content might be due to the increase in vegetative growth and monoterpins biosynthesis. The results are in conformity with the findings of Singh *et al.* (2002) and Bala *et al.* (2011) in mustard.

Table 1 Effect of date of sowing and chemical treatments on growth parameters, yield attributes of mustard

Treatments	Plant height (cm)	No. of primary branches/plant	No. of secondary branches/plant	No. of siliqua/ plant	Length of silique (cm)	No. of seed/ siliqua	Biological yield/plant (g)	Harvest index (%)	Seed yield/ plant (g)	Oil content (%)
A. Main Plot (Sowing Date)										
20th October (D_1)	179	3.86	10.04	383	5.54	12.14	63.24	19.81	12.53	40.64
20th November (D_1)	172	2.89	8.68	328	4.89	10.82	61.22	17.13	10.49	36.76
SEm ±	1.41	0.07	0.26	4.57	0.06	0.16	0.13	0.23	0.12	0.40
C.D at 5 %	4.27	0.21	0.80	13.78	0.18	0.49	0.39	0.68	0.36	1.24
B. Sub Plot (Chemical Treatment)										
Control (F_1)	165	3.13	8.25	322	4.70	11.00	59.00	17.13	10.11	35.48
Foliar spray of water (F_2)	167	3.75	8.50	333	4.92	11.00	60.00	18.65	11.23	37.11
Foliar spray of Salicylic acid 50 ppm (F_3)	179	3.88	8.88	358	5.37	11.13	65.00	19.04	12.38	38.04
Foliar spray of Salicylic acid 75 ppm (F_4)	183	4.38	10.25	403	5.98	12.63	70.00	19.58	13.71	39.65
Foliar spray of KH_2PO_4 50 ppm (F_5)	178	4.00	9.25	376	5.28	11.88	66.00	18.75	12.38	38.11
Foliar spray of KH_2PO_4 75 ppm	182	4.25	10.63	377	5.37	11.75	71.00	19.26	13.68	39.23
SEm ±	2.74	0.22	0.46	7.67	0.14	0.31	0.37	0.73	0.16	0.69
C.D at 5 %	8.22	0.63	1.37	22.03	0.41	0.94	1.11	2.18	0.47	2.07
Interaction effects of Date of Sowing × Chemicals										
SEm (±)	3.87	0.31	0.65	10.85	0.20	0.45	0.53	1.04	0.28	0.98
CD (P=0.05)	NS	NS	NS	31.16	NS	NS	NS	NS	1.12	3.2

Table 2 Interaction effect of date of sowing and chemical treatments on growth parameters, yield attributes of Mustard

Levels of Date of sowing/ Chemical treatments	No. of siliqua per plant		Seed yield per plant (g)		Oil content (%)	
	20th Oct 2010 (D_1)	20th Nov 2010 (D_2)	20th Oct 2010 (D_1)	20th Nov 2010 (D_2)	20th Oct 2010 (D_1)	20th Nov 2010 (D_2)
Control (F_1)	36	31	11.32	10.42	37.23	33.72
Foliar spray of water (F_2)	40	35	11.37	11.00	38.12	34.09
Foliar spray of Salicylic acid 50 ppm (F_3)	41	40	12.65	12.12	39.32	36.75
Foliar spray of Salicylic acid 75 ppm (F_4)	43	41	14.12	13.03	42.08	37.21
Foliar spray of KH_2PO_4 50 ppm (F_5)	41	39	12.65	12.07	38.35	37.86
Foliar spray of KH_2PO_4 75 ppm (F_6)	43	40	14.02	13.32	39.79	38.66
SEm (±)	1.47		0.48		1.80	
CD (P=0.05)	4.24		1.12		5.4	

Growth components, seed yield, oil content were recorded significantly higher when the crop was sown on 20th October, 2010 (D_1) along with the application of 75 ppm salicylic acid (F_4) i.e. ($D_1 \times F_4$). Treatment combination $D_1 \times F_6$ recorded significantly higher seed yield then the rest of the combinations, but it remained at par with $D_1 \times F_4$ combination (Table 2). Application of salicylic acid potentiates the generation of ROS (Reactive Oxygen Species) in photosynthetic tissues. It could also promote heat tolerance by alleviating the glutathione content there by increasing the physiological processes during terminal heat stress. The results are in close agreement of the results obtained by Afroz *et al.* (2011) and Orabi (2011).

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Management of linseed powdery mildew through soil and foliar application of sulphur

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ABSTRACT

A field experiment was conducted during *rabi* 2013-14 and 2014-15 to test the efficacy of soil and foliar application of sulphur against powdery mildew in linseed. The pooled results showed that two sprays of wettable sulphur (0.4%) recorded minimum disease of 13.5 per cent with maximum yield (555 kg/ha) and BC ratio (2.68). It was followed by soil application of sulphur (30 kg/ha) through gypsum at the time of sowing followed by two sprays of 0.3 per cent wettable sulphur found promising and recorded less disease, seed yield and BC ratio of 18.5 per cent, 526 kg/ha and 2.44, respectively.

Keywords: Linseed, Management, Powdery mildew, Wettable sulphur

Linseed or flax (*Linum usitatissimum* L.) is an important oil and quality fiber-bearing crop of sub-tropical and temperate regions. In India, linseed is growing in an area of about 2.86 lakh ha producing 1.55 lakh tonnes of seed. It represents 11.58 per cent and 7.91 per cent of the global linseed acreage and production, respectively (Anonymous, 2016; Biradar *et al.,* 2016). This crop is prone to number of diseases and powdery mildew caused by *Leveillula taurica* (Lev.) is currently the most common and widespread foliar disease of linseed in India. Over the last decade, the importance of this disease has increased due to the occurrence and rapid distribution of the pathogen capable of attacking the previously existing resistant cultivars (Ajithkumar *et al.,* 2013; Anjum *et al.,* 2016). Even though, the resistance is available in the commercially grown linseed cultivars, due to rapid distribution and variability in races, pathogens are able to overcome the resistance of the cultivars. Therefore, over the years when environmental conditions are favourable for the development of disease, foliar as well as soil application of sulphur has become the only practice for the management of disease (Aly *et al.,* 2012).

A field experiment was conducted at Main Agricultural Research Station, UAS, Raichur, Karnataka during *rabi* 2013-14 and 2014-15 to test the efficacy of soil and foliar application of sulphur on linseed powdery mildew. The trial was laid in randomized block design with eight treatments including untreated control and replicated thrice. The crop (cv. T-397) was sown during second fortnight of October during both the seasons with a plot size of 3.9 m x 5 m and spacing of 30 and 5 cm between rows and plants, respectively. The crop was grown under rainfed conditions by adopting all the agronomic practices as per recommendations. The crop was protected from insect pests such as *Spodoptera litura* at initial stages and from pod fly at flowering stage through blanket sprays of selective insecticides in all the experimental plots uniformly to avoid the yield losses due to insect pests. The soil application of sulphur through gypsum and foliar spray of wettable sulphur (Sulphex) was given according to the specification of treatments. The scheduled spraying was given with knap-sack sprayer at the rate of 600 liters of spray fluid per hectare for thorough coverage of foliage with spray fluid. The disease severity of powdery mildew was recorded one day before the first spray and finally seven days after the second spray by using 0-5 scale (Anonymous, 2012) during both the seasons and per cent disease index (PDI) was calculated using the formula given by Wheeler (1969). The yield was recorded from each net plot excluding border rows and computed to seed yield in kg/ha. The data was subjected to statistical analysis after using suitable transformations. Economics of all the treatments were worked out by considering the price of products, cost of fungicides and labour charges. Benefit cost ratio was worked out to compare the economics of fungicidal treatments.

The severity of powdery mildew was high during *rabi* 2013-14 when compared to *rabi* 2014-15 in all the experimental plots, which was mainly attributed to the favorable environmental conditions like average relative humidity and average day temperatures during December and January months of 2013-14. The per cent disease index was ranged from 12.33 to 92.00 during *rabi* 2014-15, while it was 14.67 to 97.33 during *rabi* 2013-14 in different experimental plots (Table 1). The results of pooled data of the field experiment clearly indicated that the disease incidence was significantly low in all the treated plots compared to the untreated control plots. Among all the treatments, spraying of 0.4 per cent wettable sulphur two times recorded lowest disease severity of 13.5 which was significantly superior over rest of the treatments. The next best treatment was soil application of sulphur (30 kg/ha)

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through gypsum at the time of sowing followed by two sprays of 0.3 per cent wettable sulphur (PDI-18.5). The incidence of powdery mildew was very high in untreated control with disease severity of more than 92 per cent during both the seasons. The results obtained from the present study were in conformity with many of the earlier workers. Rahman and Bhattiprolu (2005) reported that sulphex (0.3%) was also very effective in managing the powdery mildew in okra with maximum yield. Dhruj *et al.* (2000) reported that propiconazole, penconazole, hexaconazole, triadimefon, tridemorph, sulphur and dinocap significantly reduced powdery mildew in fenugreek. Sulphex (sulphur 80 WP) at 0.25 per cent was effective in managing the powdery mildew of mango (Thind *et al.,* 2005). Gogoi *et al.* (2013) observed that all the sulphur fungicides such as nano sulphur, sulphur 80 WP and Merck sulphur were effectively inhibited conidial germination of *Erysiphe cichoracearum*, powdery mildew fungus.

All the treatments recorded significantly increased yield over untreated control. However, highest seed yield of 555 kg/ha was recorded from the plots sprayed with 0.4 per cent wettable sulphur which was significantly superior over remaining treatments with highest BC ratio of 2.68. Soil application of sulphur (30 kg/ha) through gypsum at the time of sowing followed by two sprays of 0.3 per cent wettable sulphur also recorded more seed yield (526 kg/ha) with BC ratio of 2.44 (Table 2).

Sulphur is the oldest fungicide, never developed resistance by the pathogen despite centuries of use. Sulphur is cheaper than most other fungicides and works better if sprayed well. It has both direct contact and volatile action, the effect of volatile activity is more at temperatures more than 20°C and the contact activity occurs at any temperature but it needs good coverage of upper and lower surfaces (Magarey, 1999). Hence, two sprays of wettable sulphur (0.4%) or soil application of sulphur (30 kg/ha) through gypsum at the time of sowing followed by two sprays of 0.3 per cent wettable sulphur can be used for effective management of powdery mildew disease in linseed.

Table 1 Efficacy of sulphur for the management of linseed powdery mildew

Tr. No.	Treatments	Disease Severity (%)			Yield (kg/ha)		
		2013-14	2014-15	Pooled	2013-14	2014-15	Pooled
T_1	Soil application @ 30 kg S ha^{-1}. through gypsum at the time of sowing	86.3 (68.3)	84.3 (66.6)*	85.33 (67.4)	266	232	249
T_2	T_1 + one foliar spray of sulphur @ 0.3 % at 45 DAS	69.3 (56.3)	69.0 (56.1)	69.1 (56.2)	373	345	359
T_3	Foliar spray of sulphur at 45 and 60 DAS @ 0.3 %	31.3 (34.0)	26.6 (31.0)	29.0 (32.5)	460	460	460
T_4	Foliar spray of sulphur at 45 and 60 DAS @ 0.4 %	14.6 (22.5)	12.3 (20.5)	13.5 (21.5)	569	541	555
T_5	T_1 + one foliar spray of sulphur @ 0.3 % at 45 DAS + one spray at initiation of disease	19.3 (26.0)	17.6 (24.8)	18.5 (25.4)	536	516	526
T_6	Foliar spray of sulphur at 45 DAS @ 0.3 % + one spray at initiation of disease	21.3 (27.5)	20.3 (26.8)	20.8 (27.1)	529	496	513
T_7	T_1 + Foliar spray of sulphur at 45 and 60 DAS @ 0.3 %	24.0 (29.3)	23.3 (28.8)	23.6 (29.1)	516	474	495
T_8	Control	97.3 (80.6)	92.0 (73.5)	94.6 (76.6)	198	190	194
	SEm (±)	1.09	1.29	1.04	7.29	9.33	6.94
	CD (P=0.05)	3.23	3.9	3.15	22.09	28.31	21.06

***** Figures in the parenthesis are arcsine transformed values

Table 2 Computation of BC ratio for the management of linseed powdery mildew through sulphur as foliar and soil application

Tr. No.	Treatments	Disease Severity (%)	Yield (kg/ha)	Chemical treatment cost	Other cost	Total cost of cultivation	Gross returns	Net returns	BC Ratio
T_1	Soil application @ 30 kg S/ha through gypsum at the time of sowing	85.33	249	539	8395	8934	11205	2271	1.25
T_2	T_1 + one foliar spray of sulphur @ 0.3 % at 45 DAS	69.17	359	923	8395	9318	16163	6845	1.73
T_3	Foliar spray of sulphur at 45 and 60 DAS @ 0.3 %	29	460	768	8395	9163	20715	11552	2.26
T_4	Foliar spray of sulphur at 45 and 60 DAS @ 0.4 %	13.5	555	924	8395	9319	24960	15641	2.68
T_5	T_1 + one foliar spray of sulphur @ 0.3 % at 45 DAS + one spray at initiation of disease.	18.5	526	1307	8395	9702	23670	13968	2.44
T_6	Foliar spray of sulphur at 45 DAS @ 0.3 % + one spray at initiation of disease.	20.83	513	768	8395	9163	23063	13900	2.52
T_7	T_1 + Foliar spray of sulphur at 45 and 60 DAS @ 0.3 %	23.67	495	1307	8395	9702	22275	12573	2.30
T_8	Control	94.67	194	0	8395	8395	8745	350	1.04

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