VALIDATION OF MOLECULAR MARKERS LINKED TO QUALITY AND QUANTITATIVE TRAITS IN ADVANCED MUTANT POPULATION OF LINSEED (*Linum usitatissimum* L.)

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SEPTEMBER, 2017

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Thesis submitted to the
University of Agricultural Sciences, Dharwad
in partial fulfillment of the requirements for the
Degree of

Master of Science (Agriculture)

in

MOLECULAR BIOLOGY AND BIOTECHNOLOGY

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CERTIFICATE

This is to certify that the thesis entitled **"VALIDATION OF MOLECULAR MARKERS LINKED TO QUALITY AND QUANTITATIVE TRAITS IN ADVANCED MUTANT POPULATION OF LINSEED (*Linum usitatissimum* L.)".** submitted by **Ms. CHARITHA J.,** for the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **MOLECULAR BIOLOGY AND BIOTECHNOLOGY** to the University of Agricultural Sciences, Dharwad is a record of research work done by her during the period of her study in this University under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

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Acknowledgement

A journey is easier when you travel together. Interdependence is certainly more valuable than independence. This thesis will remain incomplete if I fail to acknowledge all those people who have there to support me some way or the other during my course. It gives me immense pleasure to place on records my profound gratitude and sincere appreciation to each and every one of those who have helped me in this endeavor.

I feel inadequacy of words to express my deep sense of gratitude and profound indebtedness to **Dr. SUMA MOGALI,** Scientist (Plant Breeding), AICRP on MULLaRP, Main Agricultural Research Station, University of Agricultural Sciences, Dharwad. She played an amazing role in my research as the esteemed Chairperson of my Advisory Committee with constant encouragement, critical suggestion, valuable advice, keen interest, constructive criticism and everlasting patience throughout the endeavor.

I have boundless pleasure in expressing my gratitude to the members of my advisory committee **Dr. YASHODA HEGDE** Professor, Department of plant pathology, University of Agricultural Sciences, Dharwad and **Dr. HANAMARATTI N. G**, Deputy Director of Research, UAS, Dharwad, for their affection, valuable suggestions and help extended in completing the task.

It is rather difficult to express in words my sincere and heartfelt gratitude to Dr. I. S. KATAGERI, Professor and Head, Department of Biotechnology, for his keen interest, eager involvement, enlightening discussions through meaningful feedback, constructive suggestions and encouragement during my research period.

I also acknowledge the help extended by **Dr. RAMESH BHAT, Dr. SUMANGALA BHAT, Dr. S. K. PRASHANTHI, Dr. NARAYAN MOGER**, **Dr. BASAVARAJ BAGEWADI**, **Dr. CHANNARAYAPPA**, **Dr. SPOORTHI,** professors, Department of Biotechnology, University of Agricultural Sciences, Dharwad, for their support and valuable suggestions during the study.

I greatly owe my deep sense of honor and gratitude to my beloved mother **Smt. Yashodha,** father **Sri. Jagadeesh**, brother **Madhu,** sister **Veena**, brother-in-law

Chandrashekar and my cousins for their affection, encouragement and blessings shown to me during the course of my study.

This thesis would have remained an impossible task without the persistent help and encouragement in each and every step of my research from my well-wisher and my best friends Bhavya, Chaitra, Leelavathi, Prathibha, Shivakumar. I convey my heartfelt thanks to them.

Above all, my success would have been an illusion without the persistent encouragement, flawless love and emotional support of all my dearest friends Gayathri, Laxmi hugar, Nayana, Pooja, Ranjitha K. M, Ranjitha H. N, Ragashree H. M, Shivaranjini, Sonam, Ashish, Pushpesh, Rajesh and my seniors Lalitha, prasanna, who encouraged me in each and every step of my post-graduation and they deserve a more personal note of gratitude**.**

I extend my sincere thanks to lab assisstants (IABT) and workers of MULLaRP (Ishwar, Pathrappa, Gangadar, Virupaksh, Jayashree, Saraswati, Laxmi, Madevi, Parvathi) for their cooperation during the investigation and providing the necessary materials during my research work.

I extend my heartfelt thanks to **Mr. Kalmesh and Mr. Arjun (Arjun Computers)** Dharwad for their co-operation in the preparation and neat printing of this manuscript.

……any omission in this small manuscript doesn't mean lack of gratitude.

DHARWAD

SEPTEMBER, 2017 **(CHARITHA J.)**

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1. INTRODUCTION

Linseed or flax (*Linum usitatissimum* L.) is a self pollinating annual crop species, with $2n = 2x = 30$ grown commercially for fiber and seed. Flax or linseed is among the oldest crop plants cultivated for the purpose of oil and fiber for more than 6000 years, and it is among the first plants to be domesticated. The botanical name, *Linum usitatissimum* was given by Linnaeus in his book "Species Plantarum" (Linnaeus, 1857). Flax is a dual purpose crop grown for its stem fiber (fiber flax) and oil (linseed, oilseed flax or flaxseed). The linseed and fiber types belong to the same species but are morphologically, anatomically, physiologically and agronomically distinct as a result of divergent selection (Diederichsen and Ulrich, 2009).

Flaxseed is one of the oldest crops, have been cultivated since the beginning of civilization. It was already cultivated in ancient Egypt and Samaria 10,000 years ago (Zohary and Hopf, 2000) to provide both fiber and oil. Recently, 30,000-year old processed and colored flax fiber was found, indicating that early humans made fabric or threads from the flax (Kvavadze *et al.*, 2009). In ancient Egypt, linen was used for wrapping the royal mummies and additionally linseed oil was used to embalm the bodies of deceased Pharaohs (Dewilde, 1983). It is believed to have originated in India or the Middle East (Vavilov, 1951) and spread throughout Asia and Europe, prior to its introduction into the New World (Green *et al*., 2008).

Flaxseed (*Linum usitatissimmum*), popularly known as Alsi, Jawas, Aksebija in Indian languages, is a blue flowering *rabi* crop. It is the third largest natural fiber crop and one of the major oilseed crops of the world, with production of 2.65 million tonnes from 2.3 million hectare of land. Annual production of flax is 2.65 million tonnes in the world and Canada is the world's largest producer of flax with 33 per cent of the global total along with Kazakhstan, China, Russia, United States and India. India is the sixth largest producer of linseed, with an area of 284 thousand hectares, with a production of 153 thousand tonnes per annum and productivity of 539 kg/ha. In Karnataka, it is grown over an area of five thousand hectares with the production of two thousand tonnes per annum, with a low productivity of 400 kg/ha as compared to major linseed growing states like Rajasthan and Uttar Pradesh which have productivity of 1285 and 560 kg/ha respectively (Anon, 2015).

Flaxseed is the richest plant source of the ω-3 fatty acid *i.e.* α- linolenic acid (ALA) (Gebauer *et al.*, 2006) short chain polyunsaturated fatty acids (PUFA), soluble and insoluble fibers, phytoestrogenic lignans (secoisolariciresinol diglycoside-SDG), proteins and an array of antioxidants (Ivanova *et al.*, 2011; Singh *et al.*, 2011; Oomah 2001; Alhassane and Xu, 2010).

Flaxseed oil is low in saturated fatty acids (9 %), moderate in monosaturated fatty acids (18 %), and rich in polyunsaturated fatty acid (73 %) (Cunnane *et al*., 1993). Of all lipids in flaxseed oil, α- linolenic acid is the major fatty acid ranging from 39.00 to 60.42 per cent followed by oleic, linoleic, palmitic and stearic acids, which provides an excellent ω-6:ω-3 fatty acid ratio of approximately 0.3:1 (Pellizzon *et al.*, 2007). Although flaxseed oil is naturally high in anti-oxidant like tocopherols and betacarotene, traditional flaxseed oil gets easily oxidized after being extracted and purified (Holstun and Zetocha, 1994).

Linseed is very rich source of lignan. While lignans are found in many plants, flaxseed is the richest source of SDG. SDG is a phytoestrogen, with several pharmacological properties including cardio-protective and anti breast cancer properties and also possess chemopreventive properties in animals and humans**,** including the potential to prevent hormone sensitive cancers (e.g. breast, prostate and colon cancer), hypercholesterolemic atherosclerosis and diabetes.

Omega-3 fatty acids are essential fatty acids. Modern diet is very deficient in this vital nutrient. Increase in incidence and severity of many degenerative diseases including cardiovascular diseases, diabetes, cancer, arthritis and mental disorders *etc* are attributed to this deficiency and more particularly to the omega-6 to omega-3 imbalance. To remedy this situation the global cry has been 'BRING BACK OMEGA-3 FATTY ACID INTO FOOD CHAIN'. It is no wonder therefore that Omega-3 Fatty acids are the most sought after nutrient today. Its growing popularity is due to health imparting benefits in reducing cardiovascular diseases, decreased risk of cancer, particularly of the mammary and prostate gland, anti-inflammatory activity, laxative effect, and alleviation of menopausal symptoms and osteoporosis.

Assessment of genetic variability is the first step in any crop improvement programme. It may be created through hybridization or induced mutation. In this crop

strong negative association between some of the yield components due to linkage and plieotropy is a bottleneck in realizing the predicted genetic advance under selection expected on the basis of performance of early generations in a hybridization programme. Therefore, induction of mutation and their utilization through effective selection sieves in the altered linkage situations appeared to be a reasonable approach in improving the yield potential of this crop.

However, the lack of high yielding linseed varieties is a challenge, a top priority is given to flax that exhibits higher yield and better quality fiber or oilseed. Because marker- assisted selection (MAS) has helped to achieve similar goals efficiently for other crops, MAS should facilitate marker-assisted breeding of flax, as well as for flax germplasm identification through analyzing variability (Wu *et al.*, 2017). To date, several molecular markers have been identified in flax using isozyme analysis (Krulickova *et al*., 2002; Yurenkova *et al*., 2005), RAPD (Fu, 2006), AFLP (Spielmeyer *et al.*, 1998; Everaert *et al*., 2001), and inter-simple sequence repeat (ISSR) analysis (Wiesner and Wiesnerová, 2003, 2004; Rajwade *et al*., 2010). Many important agronomic traits such as oil content and fiber quality are complex and polygenic in nature, controlled by several quantitative trait loci (QTL). Although traditional breeding continues to play an important role in enhancing the yield and quality of crop plants, it is hindered by the long selection cycle and the enormous resources required for a successful outcome.

More recently, a better approach utilizing simple sequence repeats (SSRs), or microsatellite DNA, has been developed. SSR markers are short, tandemly repeating nucleotide motifs (1–6 bp long) that are widely distributed in genomes of eukaryotic organisms including flax (Tautz, 1989; Temnykh *et al*., 2001). These PCR-based, genetically co-dominate markers are robust, reproducible, hyper variable, abundant, and uniformly dispersed in plant genomes (Powell *et al*, 1996). The abundance, highly polymorphic nature, heritability, distribution, reproducibility and generally co-dominant nature of SSR markers make them highly suitable for MAS and genetic diversity studies (Wiesner *et al*., 2001; Cloutier *et al*., 2011, 2012b; Soto-Cerda *et al.*, 2011a; Kumar *et al*., 2015; Kessuwan *et al*., 2016).

Marker-assisted selection (MAS) has the potential to reduce the time required for the development of new and improved varieties. However, the basic requirement of MAS is the availability of a large number of molecular markers with reasonable levels of polymorphism to allow construction of saturated genetic maps. A wide range of molecular markers have been developed and used over the past two decades. Among these, microsatellites or simple sequence repeats (SSR) are widely used because of their co-dominant, multiallelic, highly polymorphic nature and ease of genotyping (Weber and May 1989; Kale *et al*., 2012).

However, care should be taken when they are used in MAS whether the developed and reported marker will remain in different gene pools or in different experiments (da Silva *et al*., 2007). Marker development is influenced by several factors such as the phenotypic assessment accuracy, genotyping errors, lost data and environmental effects (Collard *et al.*, 2005; Francia *et al*., 2005). Thus, for application in breeding, the already published markers should be validated in independent populations (Fasoula *et al*., 2004), that is, the efficacy of these markers should be tested in determining the target phenotypic type in independent populations and in different gene pools (Fasoula *et al.*, 2004; Francia *et al.*, 2005).

Thus, validation of markers will speed up the process of introgression of yield attributing tarits and oil content gene(s) into preferred linseed genotypes through its planned deployment in molecular breeding programme. Integration of genomic tools like markers and marker assisted selection (MAS) with conventional breeding approaches might enhance the precision and speed of developing linseed varieties with improved yield, oil and alpha linolenic acid (ALA) percentage.

Realizing the importance and need for such a comprehensive study in linseed, the present investigation was undertaken with the following objectives:

a) To phenotype the mutant population of linseed (M_5) for yield and quality parameters.

b) Validation of reported markers for yield components and quality parameters.

2. REVIEW OF LITERATURE

Crop improvement is concerned with the creation, identification, isolation, multiplication and management of genetic variability towards the development of improved cultivars. Mutation breeding has been identified as a method for the creation of genetic variability for further selection and hybridization. Mutation breeding which has attained importance in recent years has yielded increasing number of desirable cultivars in different crops. Variability is the most important requirement for success in plant breeding programme. In nature, although the occurrence of natural mutations is less, mutations are the main source of variability. Ionizing radiations and chemical mutagens provide an opportunity to the breeder to enhance the mutation frequency.

Genetic variability is the gift of nature and its effective utilization in any crop species requires systematic collection, evaluation, description and grouping based on economic descriptors. The relevant literature pertaining to these studies in respect of linseed and other crops are reviewed in this chapter under following headings:

2.1 Centre of origin

2.2 Induced mutation

2.3 Induced mutation for quality characteristics in oilseeds

2.4 Genetic variability parameters

2.5 Molecular markers

2.6 Validation of molecular markers

2.1 Centre of origin

The centre of origin of flax (*Linum usitatissimum* L.) is uncertain. It is considered that *L. bienne* as the progenitor of small seeded flax, originating from Kurdistan and Iran, whereas it is also sometimes considered that *L. angustifolium* containing high oil content and seed weight, as progenitor, originating from the Mediterranean region (Zeven and de Wet, 1975). Others suggest that *L. bienne* and *L. Angustifolium* are the same species, and are widely distributed over Western Europe,

the Mediterranean basin, North Africa, the Near East, Iran and Caucasus (Zohary and Hopf, 1993), that has been known to mankind since the Paleolithic era (30,000 years ago) (Zohary 1999) with 6,000–7,000 years planting history.

The species is also believed to have originated in either the Middle East or Indian regions (Vavilov, 1951) and spread throughout Asia and Europe, prior to its introduction into the New World (Green *et al*., 2008). Divergent selection applied over thousands of years has resulted in fiber and linseed types which are the same species but differ considerably in morphology, anatomy, physiology and agronomic performance (Diederichsen and Ulrich, 2009). Fiber flax cultivars are taller and less branched and are grown in the cool-temperate regions of China, the Russian Federation and Western Europe. Linseed cultivars are shorter, more branched, larger seeded and are grown over a wider area in continental climate regions of Canada, India, China, the United States and Argentina (Green *et al*., 2008).

2.2 Induced mutation

Induced mutation either by physical or chemical mutagen is one way of creating variation in crop plants. The available literature on oilseeds indicates about the increased variability due to induced mutagenesis for different quantitative traits.

Linseed has been one of the most important oilseed crop. Breeding work is still limited in this crop. As it is a self pollinated crop, mutation breeding is one of the important approach to induce variation in this crop. Various attempts have been done in this direction by different scientists to determine the most effective mutagenic treatment for induction of desired trait in linseed (Badere and Choudhary, 2007).

Rowland (1991) treated flax (*Linum usitatissimum* L.) seed with ethyl methane sulphonate (EMS) and the resulting M_1, M_2, M_3 and M_4 progeny were screened for linolenic acid mutants, using the half-seed technique. A stable low-linolenic acid mutant was found in the M_4. The low linolenic character is controlled by recessive alleles at two independent loci, apparently the result of a rare double mutation.

Tolba (2000) has made crosses between Reina (fibre flax), Giza 4 and C.77/9 (dual purpose fibre/oil flaxes) genotypes. Seeds of parents and F_1 hybrids were irradiated (7 kr) and sown with those of non-irradiated controls. Simple correlations, regressions and path coefficients were studied to determine the main components of seed yield and their contributions to seed yield. Seed yield per plant was significant and positively correlated with fruiting zone length, number of fruiting branches, number of capsules per plant, number of seeds per capsule and 1,000-seed weight for all populations(non-irradiated and irradiated). It was concluded that number of capsules per plant is the most important selection criterion for flax seed yield improvement, followed by 1,000-seed weight.

Baculis (2001) treated linseed at different concentration of nitrosoethyl urea, nitrosomethyl urea, ethylene imine, dimetyl sulphonate and various doses of gamma rays. Variability of flax varieties Svetoch and Vaizgants were tested the frequency and spectrum of mutations as well as number of useful mutant types were determined in generation M_2. In later generation mutant lines, distinguishing themselves by valuable qualities, were selected and some were involved in breeding process.

Kumar and Sinha (2006) irradiated lentil genotypes whose seeds includes bold-seeds (Precoz) and small-seeds (Pat. L 96-7) with different doses of gamma rays and different concentrations of EMS (Ethyl methane sulphonate) and with the combinations of two to study the induced genetic variation in lentil. In the M_2 generation, it was observed that range had widened and the coefficients of variation increased in the treated populations in both the genotypes. Both positive and negative shifts in mean values were noticed. However, cumulative effects of physical and chemical mutagens were not observed in enlarging the variability.

Singh *et al.* (2006) reported the significant differences for polygenic variability in M_2 and M_3 generations for number of nodules, number of pods, grain yield per plant and 100-grain weight (M_3 only) in lentil. High dose of mutagenic treatments showed high reduction in grain yield per plant in combination treatments.

Badere and Choudhary (2007) followed induced mutation in two linseed varieties NNL-97 and RLC-6 to improve the yield and quality. As a result two mutants

with increased meal protein content and six mutants with increased test weight were isolated in M_3 population.

Nadaf *et al.* (2009) treated two cultivars of peanut, 'GPBD-4' and 'TPG-41' with γ-radiation (200 Gy & 300 Gy) and ethyl methane sulphonate (EMS- 0.5 %). The mutagenized populations showed significantly higher variability in the M_2 generation. Mutant lines showing higher yield per plant than the respective parents and checks were isolated in M_2 and subsequent generation. The evaluation of 10 superior mutants isolated in M_4 over three successive generation yielded few mutants performing better over the parents and checks. In both the genotypes, superior mutants were isolated from 200 Gy treatment, indicating effectiveness of the mutagen in obtaining the desired trait.

Khan and Goyal (2009) induced mutation in two varieties of mungbean, K-851 and PS-16 using EMS and gamma rays as mutagens. Mean values in traits increased significantly over the controls and genetic parameters were recorded higher for the mutants isolated in M_5 generation. High values of heritability and genetic advance for the mutants indicate that further improvement could be made in next generations**.**

Senapati and Misra (2009) used three doses each of gamma – rays, EMS, NG, MH and in combination to treat blackgram varieties, PU-30 and Sarala. In M_2 generation most treatment populations exhibited reduction in mean and increase in variance for all six quantitative traits and among the mutagens NG and combined treatments induced more variability in both the varieties.

Siddiqui *et al.* (2009) induced Genetic variability by gamma rays (750 Gy, 1,000 Gy) and ethylmethane sulphonate (EMS) (0.75 %, 1.00 % solution) alone and in combination (750 Gy + 0.75 % EMS, 750 Gy + 1.00 % EMS, 1,000 Gy + 0.75 % EMS and 1,000 Gy + 1.00 % EMS) in rapeseed (Brassica napus L.) cv. Waster and evaluated for important economic character. Comparison with control revealed that the mutagen treatments shifted the mean values in positive as well as negative directions. There was a considerable increase in variance for all the traits under study.

Burako (2010) carried out the experiment with sixty-four linseed genotypes, consisting of six exotic, one standard check and 47 single plant selections from different crosses, and evaluated by simple lattice design with two replications for their tolerance

to acidic soil at Bedi Trial Site in Central Ethiopia during 2009-2010 cropping season. They concluded that there is a significant differences among the genotypes for root length, days to flowering, days to maturity, plant height, seed yield per plot, seed yield per plant, tillers per plant, number of primary branches per plant, 1000 seed weight, oil yield and oil content. But they found that the numbers of capsules per plant and secondary branches per plant were non-significant. High phenotypic and genotypic coefficients of variations were recorded for seed yield per plant, seed yield per plot, oil yield per plot, number of primary branches and tillers per plant. Conversely, oil content, 1000 seed weight, days to flowering and days to maturity showed low phenotypic and genotypic coefficient of variations. Heritability in the broad sense was adequately high.

Chatterjee *et al.* (2010) induced genetic variability in opium poppy (*Papaver somniferum* L.) through mutation breeding using physical doses (gamma radiation of Kr 10 to Kr 50 at an interval of Kr10), chemical doses (EMS of 0.2, 0.4, 0.6 and 0.8 %) and combined doses (gamma and EMS) of mutagen and to evaluate the plants advance generations for different traits as well as for specific alkaloids, especially thebaine and codeine.. The Kr 10 + 0.4 % EMS dose created positive mutations for high thebaine and codeine content and low morphine content, while the Kr 40 + 0.6 % EMS dose did the same for narcotine.

Patil and Wakode (2011) studied the genetic variation inducing by gamma rays and chemical (EMS) mutagens in two cultivars *i.e.* PKV-1 and JS-335 of soybean. Results indicated that genotypic coefficient of variation, phenotypic coefficient of variation and heritability were significantly high for different characters studied. All the mutagenic treatments were effective in inducing genetic variability in both the varieties.

Emrani *et al.* (2012) induced variability in two canola cultivars (‘RGS003’ and ‘Sarigol’) by treating with 0, 800, 1,000, 1200 Gy of gamma rays. Results of analysis of variance indicated highly significant effect of mutagenic doses, genotypes and genotype × dose interaction on the traits, indicating the differential response of genotypes to mutagenic treatments in terms of inducing genetic variations.

Malek *et al.* (2012) irradiated mustard seeds of variety BARIsarisha-11 with gamma ray using 60Co gamma cells. Irradiated seeds were grown as M_1 and Selection was made from M_2 generation during 2005-06. Desirable mutants were confirmed in M_4 generation during 2007-08 and ten true breeding mutants having higher seed yield per plant with desirable morphological characters and yield attributes were selected. Selected mutants were evaluated along with the initial variety BARIsarisha-11 to select the most desirable ones. Results showed that two mutants, MM-10-04 and MM-08-04 selected from 700 Gy produced higher seed yield than BARIsarisha-11 with 2043 and 1893 kg ha per ha respectively, which was 23 (%) and 14 (%) higher than BARIsarisha-11 (initial variety). Mutants MM-10-04 and MM-08-04 also had the higher number of siliqua per plant, 1000-seed weight and oil content than BARIsarisha-11.

Bashir *et al.* (2013) studied an effect of different dose/concentration of gamma rays, ethyl methane sulphonate (EMS) and sodium azide (SA) on various biological parameters in M_1 generation and spectrum and frequency of macro-mutations induced in the M_2 generation of fenugreek. Mutagenic effectiveness decreased with the increase in dose/concentration of the mutagen where as the efficiency of mutagens showed variable trend depending on the criteria selected for its calculation. The lower or intermediate treatments of all the mutagens were found more efficient in causing less biological damage and inducing maximum macro-mutations. The order of mutagenic efficiency was EMS > SA > γ- rays.

Thagana *et al.* (2013) irradiated three rapeseed varieties Karat, Regent and Topaz at 0 Gy, 600 Gy, 800 Gy and 1,000 Gy. M_2 plants were selected at random and data taken on plant height and pods per plant for each level of irradiation. M_3 lines selected based on earliness were planted and data was taken on various characters. Observations were also made on M_6 lines. There were significant ($P<0.01$) differences between the levels of irradiation in the number of pods per plant and significant interaction ($P<0.01$) between varieties and levels of irradiation for pods per plant

Rai *et al.* (2014) conducted an experiment on two varieties of linseed *viz.*, T-397 and Shekhar by exposing them to different doses of gamma rays 40, 50 and 60 kR and found that there was significant differences between the mean values recorded for different population of mutagenic treatment and the control for all the characters studied

as well as, mean sum of squares due to treatments were also significant. The magnitude of induced genetic variability in terms of PCV and GCV was recorded greater in the mutagenic treatments were as compared to their respective control both in T-397 and Shekhar varieties in M_2 generation.

Kavera and Nadaf (2017) followed induced mutation using EMS and gamma rays to improve yield of two groundnut cultivars (GPBD- 4 and TPG-41). One hundred true breeding mutants were isolated at M_3 and were evaluated for yield at M_4/M_5. Thirteen superior mutants showing consistent performance across the generations were further evaluated for economic traits at M_6. Greater magnitude of induced variability was found for number of pods per plant (13.65-52.65; 11.83-34.62), pod yield per plant (10.40-49.71; 16.40-41.28) and 100 seed weight (30.18-52.20 g; 37.36-87.65 g) in GPBD-4 and TPG-41 mutant populations respectively in M_4 generation. Mutants G2-214 (34.51 Q/ha) and TE-147 (31.75 Q/ha) recorded 27.53 and 31.75 per cent increase in pod yield over the respective parents GPBD-4 (27.06 Q/ha) and TPG-41 (23.80 Q/ha). Most of the superior mutants were associated with increased 100-seed weight. In addition to this, mutant G2-214 which recorded highest pod yield (34.51 Q/ha) was also accompanied with increased O/L ratio (3.23) over parent GPBD-4 (1.76).

2.3 Induced mutations for quality characteristics in oilseeds

Soldatov (1971) observed increased genetic variability for oil content in M_3 generation in sunflower following treatments with chemical mutagens *viz.*, NMU, NEU and DMS. He isolated plants with 1-2 per cent higher oil content compared to control.

Srinivasachar *et al.* (1972) isolated mutant with high iodine value in addition to increased oil content in linseed following gamma irradiation and EMS treatment.

Vranceanu and Stoenescu (1982) found altered oil composition in sunflower by mutation. They found that irradiation with high energy radiations were effective to alter the oil content.

Osorio *et al.* (1995) screened single M_2 and M_3 seeds from plants obtained from mutaginized seeds utilizing ethyl methane sulphonate, sodium azide and x-ray and isolated the mutant lines, CAS-5,which has oil with a fivefold increase (252 g kg^{-1}) in

palmitic acid (16:0) and the appearance of palmitoleic acid (16:1.37 g kg^{-1}) and CAS-3, CAS-4 and CAS-8, which have two to six times (99-260 g kg^{-1}) the stearic acid (18:0) content.

Nothdurft *et al.* (1998) treated seeds of the German *C. sativa* cultivar Lindo with ethyl methane sulphonate (EMS) in order to modify the fatty acid composition in the seed oil and to select mutants with either reduced or increased linolenic acid (C18:3) content, respectively. The M_2-generation was evaluated for fatty acid composition by gas chromatography and shown that variation in fatty acid composition has been broadened in comparison to the controls. The analysis of single plant progenies again showed in some cases a higher linolenic acid content in the M_3-generation, *i.e.* 39.1 per cent linolenic acid (average of cv. Lindo 29.9 %).

Schnurbusch *et al.* (2000) studied an induced mutation in European winter oilseed rape. Increased palmitic acid content was phenotypically characterized and genetically analysed. The mutant showed a palmitic acid content of 9.2 per cent compared with 4.5 per cent in the parental cultivar. The oleic acid content decreased from 61.6 to 44.2 per cent, whereas the linoleic and linolenic acid contents increased.

Nichterlein (2006) conducted an experiment in linseed cultivars 'Raulinus' and 'Bionda' by treating with ethyl methane sulphonate (EMS) in order to broaden the variation of fatty acid composition in the seed oil. The M_2 generation was evaluated for fatty acid composition of the oil. Genetic variation for unsaturated fatty acids was demonstrated to be extended in the M_2 generation as compared to the controls. Finally in the M_5 generation of cv. 'Raulinus', lines with reduced C18:3 (< 40 % vs. 55 %) and correspondingly increased linoleic or oleic acid contents, respectively, could be selected. These mutants proved to be genetically stable as demonstrated by parent-offspring (M_4/M_5) regression.

Patil *et al*. (2007) carried out mutation breeding for identifying stable soybean mutants with altered fatty acid composition for improved oxidative stability and nutritional quality of soybean. They found that both gamma-radiation and EMS were effective in increasing the variability for the fatty acid content in soybean oil. The variability was skewed towards high levels of oleic (35–42 %) and low levels of

linolenic acid (3.77–5.00 %). M_3 and M_4 generations of desirable variants were analyzed for the stability of the mutated trait. A significant positive correlation between oxidative stability index (OSI) and oleic acid content (P\0.001) indicated improved oxidative stability of the oil while retaining nutritional quality.

Kavera *et al.* (2013) altered fatty acid composition through induced mutation in two groundnut cultivars (GPBD-4 and TPG-41) using EMS and gamma rays. Six hundred true breeding mutants were isolated in M_3 and were characterized for fatty acid profile at M_4/M_5 generations. Greater magnitude of induced variability was found for oleic acid (37.40-75.16 %), linoleic acid (9.01-40.30 %) and oleic to linoleic acid ratio (O/L) (0.95-8.34) in mutant populations at M_5. Mutant, GE-113 recorded the highest increase in oleic acid (74.48 %), lowest reduction in linoleic acid (9.17 %) and highest increase in O/L ratio (8.12) compared to parent GPBD-4.

2.4 Genetic variability parameters

Green and Marshall (1981) analysed 214 *Linum usitatissimum* accessions, for variability with respect to seed weight, oil content and fatty acid composition. They reported the significant variation for all characters. Further based on parent-offspring correlation analysis, they reported that at least a proportion of the variation within several varieties was due to genetic heterogeneity. They also concluded that Oleic acid and linolenic acid varied between 13.3 to 25.2 per cent and 45.5 to 64.2 per cent respectively, and were strongly negatively correlated within and between all varieties tested.

Ioan (2001) studied morphological variability for yield components in relation to yield potential in 35 linseed genotypes. Most of the genotype showed presence of high phenotypic variability for mean number of capsules per plant and seed weight per plant. The different environmental condition had a great influence on capsules per plant and seed weight per plant.

Akbar *et al.* (2003) conducted variability studies in eighteen varieties of *Brassica juncea* L. for plant height, number of branches per plant, number of siliquas per plant, 1000 seed weight and seed yield per plant. They tested for GCV, PCV, h^2, GA, correlation and path coefficient analysis. Number of siliquas per plant found strong

parameter followed by number of branches and plant height for seed yield improvement. Siliquas per plant had highest GCV, PCV, h^2, GA, highly significant positive correlation and maximum direct contribution for seed yield followed by number of branches per plant and plant height.

Diederichsen and Raney (2006) conducted a study to investigate variation and relationships among seed color, seed weight and seed oil content in cultivated flax. Seed from 2934 flax gene bank accessions were grown at Saskatoon, SK, Canada, originating from 72 countries was used to describe the variation of the seed characters. They observed that the world collection exceeded the range of variation of seed weight and oil concentration found in 52 North American cultivars and found that there was a weak, positive association of higher oil concentration with higher seed weight.

Kailashram *et al.* (2008) carried out the experiment to evaluate the collection of 440 elite germplasm lines of linseed from Project Coordination Unit (linseed), Kanpur for nine quantitative characters. They observed highest variability for number of capsules per plant and negative correlation of seed yield with pest and disease incidence on leaves. They found that there were significant positive associations of seed yield with days to maturity, plant height, number of branches per plant, number of capsules per plant and number of seeds per capsule.

Parameshwarappa *et al.* (2009) evaluated one hundred fifty one sesame genotypes for genetic variability in respect of nine quantitative characters. Analysis of variance revealed significant difference among genotypes for all the nine character studied. They observed high GCV and PCV for seed yield per plant, number of capsules per plant, number of primary branches per plant, number seeds per capsule and plant height. High heritability and genetic advance as per cent mean was observed for seed yield, number of primary branches per plant, number of capsules per plant, number of seeds per capsule, plant height and days to 50 % flowering. Seed/plant showed significant and positive association with number of primary branches/plant, number of seeds per capsule and capsule length.

Bayrak *et al.* (2010) assessed the levels of variation in oil content and fatty acid composition among the linseed accessions of different origins grown in Turkey. The oil content ranged from 23.28 to 40.36 per cent in the experimental materials. Three

saturated (C16:0, C18:0 and C20:0) and four unsaturated (C18:1, C18:2, C18:3 and C20:1) fatty acids, identified by GC represented 10.02 per cent and 89.91 per cent of the total oil, respectively. The C18:3fatty acid content ranged from 48.08 per cent to 57.58 per cent. The contents of the C18:1, C18:2, C16:0 and C18:0 fatty acids ranged 15.81 to 27.99, 11.18 to 16.13, 4.07 to 7.02 and 3.21 to 6.70 per cent respectively. The C20:0 and C20:1fatty acids were minor fatty acids in these materials and ranged from 0.11 to 0.24 and 0.10 to 0.24 per cent, respectively.

Dandigadasar *et al.* (2011) evaluated 79 linseed genotypes. Wide range of PCV and GCV were observed for number of capsules per plant, number of seeds per capsule and seed yield per plant. The heritability estimates were ranging between 30.20 per cent (number of seeds per capsule) to 99.7 per cent (days to maturity) and per cent mean genetic advance was high for number of branches per plant, seed yield per plant, days to flowering and number of capsules per plant.

Golkar *et al.* (2011) evaluated sixteen safflower genotypes for genetic diversity. They studied for eight different seed quality-related traits including fatty acid composition of seed oil (stearic acid, palmitic acid, oleic acid and linoleic acid), the contents of oil, protein, fiber and ash in its seeds. Analysis of variance showed significant variation in genotypes for the seed quality-related traits.

Wani, *et al.* (2011) evaluated seven accessions of *Jatropha curcas* germplasm which indicated a wide range of variability in vegetative growth and other qualitative attributes which can be harnessed in future improvement programme of *Jatropha curcas*. Seed yield per plant had a positive and significant correlation with number of branches per plant, oil yield, plant spread (r=0.806, 0.802, 0.782), plant spread had a highest correlation with plant height (r=0.840). The seeds analyzed for proximate composition, fatty acid and physiochemical characteristics revealed that fiber and ash content in seed flour were high (16.5 % and 4.35 %). Oil content varied from 24.5 (%) to 37.9 (%). The lower value of the viscosity suggests it as diesel oil. Accession JC006 could be an alternative source of linoleic acid (51 %) while the accession JC001 could be a source for oleic acid (48 %) and linoleic acid (42.4 %). Stearic acid was highest in accession JC003 (42.9 %).

Sivaraj *et al.* (2012) studied variability in eighty-four accessions of linseed germplasm collections from peninsular India for seed traits and fatty acid composition. The oil content of the linseed germplasm ranged between 29.4 per cent and 42.6 per cent. Linolenic acid was ranging from 39.5 per cent to 57.1 per cent. Linseed germplasm with high oleic acid content was identified recording the maximum of 32 per cent.

Abideen *et al.* (2013) studied the genetic variability and correlation among different traits in *Brassica napus* L. Results revealed highly significant differences among the genotypes for most of the traits. Non-significant differences were, however, observed among the genotypes for primary branches per plant and pods per plant. Genotype 1 was found superior for most of the traits that is maximum oleic acid content etc.

Gidey *et al*. (2013) tested genetic variability of eighty one sesame genotypes for fifteen characters. Analysis of variance revealed that there was highly significant ($p<0.001$) difference among all genotypes for all the fifteen characters studied. High Genotype and Phenotypic Coefficient of Variance was recorded for harvest index, seed yield per ha, height to first capsule, biomass per ha, number of capsules per ha, number of primary branches per ha, number of seeds per capsule and plant height. Harvest index showed positive significant phenotypic and genotypic correlation with grain yield.

Lodhi *et al*. (2013) evaluated ninety diverse genotypes of Indian mustard for fifteen quantitative traits. Both phenotypic and genotypic coefficients of variation were higher for number of secondary branches per plant, seed yield per plant, and 1000 seed weight. High heritability in conjunction with high genetic advance were observed for seed yield per plant, number of secondary branches per plant, 1000- seed weight, number of seeds per siliqua, primary branch angle, number of primary branches per plant, siliqua angle, siliqua on main shoot, and siliqua length suggesting predominant role of additive gene action for expression of these traits. Seed yield per plant was found to be positively and significantly correlated with number of primary branches per plant, number of secondary branches per plant, primary branch angle, main shoot length, siliqua length, and number of seeds per siliqua, seed yield per plant had negative association with oil content.

Begum and Dasgupta (2014) induced mutation in sesame genotypes Rama, SI 1666, and IC 21706 by 200 Gy, 400 Gy and 600 Gy doses of γ rays as well as by 0.5, 1.0, 1.5 and 2.0 per cent concentrations of ethyl methane sulphonate (EMS) separately. Mutant generations from M_1 to M_2 were assessed for the extent of variability, heritability, and genetic advance for yield and important yield components in mutant populations. The chemical mutagen (EMS) was much more effective than the physical mutagen (γ rays) in producing polygenic variability. The genotype IC 21706 and the treatment using 0.5 % concentration of EMS appeared to be the best for engendering variability, highlighting their potentiality for selecting higher yielding plants in early generations. All genotypes showed a promising increase in genotypic variability, heritability and genetic advance for all traits, implying that these characters can be transmitted to future generations.

Synrem *et al*. (2014) studied the genetic variability, heritability and genetic advance as percentage of mean for nine quantitative characters in intra specific crosses of Indian mustard *Brassica juncea* (L.). Analysis of variance showed significant differences among the crosses for all characters under study except for length of siliqua. Maximum genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) was exhibited by number of secondary branches per plant followed by seed yield per plant and number of primary branches per plant. High estimates of heritability coupled with high genetic advance as per cent of mean was observed for number of secondary branches per plant followed by seed yield per plant and number of primary branches per plant.

Yadlapalli (2014) evaluated groundnut genotypes for different yield components. Results revealed highly significant variations among the genotypes for all the characters studied. The highest genetic coefficient of variation was observed for number of pods per plant. The highest heritability was observed for 100 seed weight (98.0 %). while high values of genetic advance were obtained in all the characters except plant height and days to 50 per cent flowering. Pod yield exhibited significant and positive genotypic correlations with all the characters except with plant height. Number of pods per plant showed positive direct effect on pod yield per plant followed by 100 seed weight, number of branches per plant and days to 50 per cent flowering. Selection for characters showing high heritability with high genetic advance, positive

and high significant correlation and showing high direct effects will be helpful in the improvement of yield in the groundnut.

Singh *et al.* (2015) studied variability, heritability and genetic advance in 53 germplasm of linseed, estimates of genotypic and phenotypic coefficients of variability indicated significant variability for all the quantitative traits. High heritability estimates were reported for number of capsule per plant, plant height, biological yield per plant, seed yield per plant, harvest index, number of branches, test weight, days to maturity and number of seeds per capsule. These characters, therefore, may respond effectively to phenotypic selection. Further positive correlation of number of capsules per plant, test weight, number of seeds per capsules, biological yield per plant and plant height with grain yield proved that the genetic worth of these characters in the indirect selection of breeding for linseed improvement.

Tariq *et al.* (2014) reported that grain yield had significant and positive association with plant height, number of primary branches per plant, number of capsule per plant, number of grains per capsule and 1000 grain weight both at genotypic and phenotypic levels. Path analysis demonstrated that plant height, number of primary branches per plant, number of capsule per plant, number of grains per capsule and 1000 grain weight had the positive direct effect in determining the grain yield in linseed.

Tadesse *et al.* (2010) evaluated 81 geographically diverse Ethiopian accessions of linseed at two locations, Agricultural Research Centre, Sinana, and on farmers field at Robe in the highlands of bale, Ethiopia during 2001-2002 cropping season. The accessions differed significantly for all the traits under study at both locations. However, the genotypic differences were non-significant for number of primary branches, number of capsules per plant, number of seeds per capsule, seed yield per plant, biomass and per cent oil content in the combined analysis over locations. Estimates of genotypic and phenotypic coefficients of variability at the two locations indicated significant variability for most of the traits under study. At Sinana, heritability estimates were high for most of the traits. Whereas moderate estimates of heritability were observed for plant height, per cent oil content and biomass. Moderate estimates of heritability were observed for plant height, per cent oil content and biomass. At Robe, heritability estimates were high for seed yield per plant, days to flowering, number of

secondary branches, seed yield per plot, harvest index, number of primary branches, number of capsules per plant, thousand seed weight, days to maturity, tillers per plants and number of seeds per capsule. These characters, therefore, may respond effectively to phenotypic selection.

2.5 Molecular markers

The importance and value of the crop have been the justification for the development of genetic resources such as the first whole genome shotgun (WGS) sequence (Wang *et al*., 2012), expressed sequence tags (ESTs) (Venglat *et al*., 2011), a simple sequence repeat (SSR) consensus genetic map (Cloutier *et al*., 2012), a physical map (Ragupathy *et al*., 2011) and thousands of single nucleotide polymorphic (SNP) markers (Kumar *et al*., 2012). The ~370 Mb flax genome is estimated to have undergone whole genome duplication approximately, 5–9 million years ago (Kumar *et al*., 2015).

A wide range of molecular markers have been developed and used over the past two decades. Among these, microsatellites or simple sequence repeats (SSR) are widely used because of their co-dominant, multiallelic, highly polymorphic nature and ease of genotyping (Weber and May, 1989). However, generation of SSR markers is technically demanding due to the primary requirement of their *de novo* development by construction and sequencing of genomic libraries, there are very few (189) genomic SSRs reported to date for linseed (Cloutier *et al*., 2009; Deng *et al*., 2010; Roose-Amsaleg *et al*., 2006; Soto-Cerda *et al*., 2011a; Wiesner *et al*., 2001; Kale *et al*., 2012).

Molecular markers have several advantages over the morphological markers. This however, requires identification of molecular markers tightly linked to the trait of interest. Tanksley *et al*. (1989) have discussed the relative advantages of molecular markers over morphological markers for most genetic and breeding applications.

Different molecular markers have been used to study the molecular diversity of linseed germplasm including RAPD, AFLP, SSR and ISSR. However, there are only few reports where these markers are being used for dissecting the complex agronomic

and quality traits of linseed which will aid in the crop improvement programmes. New developments are taking place in this direction where many research groups are developing reliable molecular markers in linseed such as simple sequence repeats.

Hasan *et al.* (2008) identified potentially gene-linked markers for seed glucosinolate loci via structure-based allele-trait association studies in genetically diverse *B. napus* genotypes. Markers linked to homoeologous loci of the genes involved in biosynthesis of indole, aliphatic and aromatic glucosinolates in the paleopolyploid *B. napus* genome were found to be associated with a significant effect on the seed glucosinolate content. This example shows the potential of *Arabidopsis- Brassica* comparative genome analysis for synteny-based identification of gene-linked SSR markers that can potentially be used in marker-assisted selection for an important trait in oilseed rape.

Cloutier *et al.* (2009) generated a set of 146,611 expressed sequence tags (ESTs) from 10 flax cDNA libraries. After assembly, a total of 11,166 contigs and 11,896 singletons were mined for the presence of putative simple sequence repeats (SSRs) and yielded 806 (3.5 %) non-redundant sequences which contained 851 putative SSRs. This is equivalent to one EST-SSR per 16.5 kb of sequence. Trinucleotide motifs were the most abundant (76.9 %), followed by dinucleotides (13.9 %). Tetra-, penta- and hexanucleotide motifs represented <10 per cent of the SSRs identified. A total of 83 SSR motifs were identified. Motif (TTC/GAA)*n* was the most abundant (10.2 %) followed by (CTT/AAG)*n* (8.7 %) (TCT/AGA)*n* (8.6 %) (CT/AG)*n* (6.7 %) and (TC/GA)*n* (5.3 %). A total of 662 primer pairs were designed, of which 610 primer pairs yielded amplicons in a set of 23 flax accessions. Polymorphism between the accessions was found for 248 primer pairs which detected a total of 275 EST-SSR loci.

Zhang *et al.* (2009) obtained 222 SSR markers from 7941 ESTs in NCBI database representing 2.73 per cent of total ESTs. Trinucleotide repeats, accounting for 72.1 per cent of EST-SSRs, are dominant. Di-and Tri-nucleotide repeats were similar with frequency, accounting for 14.4 per cent and 13.5 per cent of SSRs respectively. AGAA was the most frequent motif, about 67.67 per cent in tetranucleotide repeats. 18

pairs from 21 designed primer pairs of EST-SSRs showed the amplification under a suitable PCR system in 10 flax germplasm. fourteen primers amplified clear bands with high SSR polymorphism. Based on SSR markers, dendrogram analysis could divide ten flax germplasm into three groups.

Deng *et al.* (2010) isolated thirty-five microsatellite loci and characterized in *Linum usitatissimum* L. using enriched genomic libraries. Those loci were screened in eight cultivars from different countries and regions and were found to be polymorphic, with the number of alleles per locus ranging from two to six, and observed and expected heterozygosities ranging from 0.125 to 0.375 (mean 0.013) and from 0.233 to 0.842 (mean 0.601), respectively. These polymorphic new microsatellite loci will be useful for genetic linkage map construction, germplasm classification and identification, gene identification and quantitative trait loci mapping, and marker-assisted selection in breeding in *L. usitatissimum.*

Fu *et al.* (2010) developed a set of 100 expressed sequence tag-derived simple sequence repeat (EST-SSR) primer pairs in linseed. They were characterized on 35 linseed accessions representing 17 linseed species for their transferability to other linseed species. Ninety-nine primer pairs displayed scorable polymorphisms across 35 linseed samples and generated 627 bands likely from 121 loci. About 50 per cent of the detected bands occurred only in three or fewer samples. A total of 393 bands, likely from 116 loci, were detected by 97 primer pairs in *Linum bienne* Mill. samples, but only up to 60 bands, likely from up to 39 loci, were revealed by 6 to 37 primer pairs in the samples of the other 15 linseed species. The *L. bienne* samples displayed 23.7 per cent more EST-SSR variation than the *L. usitatissimum* samples.

Soto-Cerda *et al.* (2011a) surveyed a total of 3,242 *Linum usitatissimum* genomic sequences for the identification of SSRs. Among them, 118 non-redundant sequences containing repeats were selected for designing primers. The most abundant motifs were tri- (72.4 %) and dinudeotide (16.6 %), within which AGG/CCT and AG/CT were predominant. Primers were tested for polymorphism in 60 *L. usitatissimum* cultivars/accessions including 57 linseed and three fiber flax. Eighty-eight pairs gave amplifications within the expected size range while 60 pairs were found

to be polymorphic. The mean number of alleles amplified per primer was 3.0 (range, 2–8; 180 total alleles). The mean polymorphism information content (PIC) value was 0.39 (range, 0.06–0.87), and the highest average PIC was observed in dinucleotide SSRs (0.41).

Soto-Cerda *et al.* (2011b) evaluated fifty expressed sequence tag-derived microsatellite markers (EST-SSRs) for polymorphism and transferability in 50 *Linum usitatissimum* cultivars/accessions and 11 *Linum* species. Among them 23 EST-SSRs were polymorphic in *L. usitatissimum*, while 2-4 alleles were detected (average 2.26 per locus).

Kale *et al.* (2012) developed SSR markers for linseed. They used three microsatellite isolation methods, *viz.*, PCR Isolation of Microsatellite Arrays (PIMA), 5'- anchored PCR method, and Fast Isolation by AFLP of Sequences containing repeats (FIASCO). They obtained contigs and the singlets containing 1,842 microsatellite motifs, with dinucleotide motifs as the most abundant repeat type (54 %) followed by trinucleotide motifs (44 %). As a result 290 SSR markers were designed, 52 of which were evaluated using a panel of 27 diverse linseed genotypes. Among the three enrichment methods, the 50-anchored PCR method was most efficient for isolation of microsatellites, while FIASCO was most efficient for developing SSR markers.

Soto-Cerda *et al.* (2012) carried out genotyping of Canadian flax core collection of 390 accessions with 464 simple sequence repeat markers, and phenotypic data for nine agronomic traits from up to eight environments was used for association mapping, 12 significant marker-trait associations for six agronomic traits were identified. Most of the associations were stable across environments as revealed by multivariate analysis. Their results confirmed the complex genetic architecture of yield-related traits and the inherent difficulties associated with their identification while illustrating the potential for improvement through marker-assisted selection.

Singh *et al.* (2012) used SSR markers to analyze the genetic diversity of 30 mutant lines along with its parent PUSA- 9072. A total of 8 primers were used for STMS analyses and 9 alleles were generated and the number of alleles per SSR primer ranged from 1 to 2 with an average of 1.15 per primer. The size of the amplification products varied in case of each primer and the range was 50 bp to 250 bp. The

dendrogram constructed based on SSR data using average linkage grouped the mutant lines into two different clusters having all the mutants in one cluster and the parent in other. Clustering pattern based on SSR marker data indicated that there is a narrow genetic base of mungbean mutant lines. The absence of polymorphism indicated that the mutation were not on the tandem repeat region. The results indicate the usefulness of SSR in the assessment of genetic diversity in plants.

Shalaby and El-Bann (2013) developed an *In vitro* technique suitable for mutation induction on tomato and characterized them by RAPD and SSR markers as well as horticultural characteristics. The influence of various concentrations (0, 0.07, 0.14 and 0.25 %) of the chemical mutagen, Ethyl Methane Sulfonate (EMS), on the *in vitro* shoot formation from cotyledon explants of two tomato cultivars was studied. The percentage of responding explants ranged from 45.2 to 95 % in dependence on genotype and EMS concentrations. Two PCR-based techniques, RAPD and SSR, were used for analysis of genetic variations in regenerated plants from *in vitro* cultures combined with EMS treatment (0.25 %). The percentage of polymorphism detected by RAPD and SSR primers reached 25.64 %. Grouping of the original cultivar and their mutants indicated the genetic distinctness as they are placed in different clusters/groups far from each other.

Pali *et al.* (2014) validated the utility of the genotype specific SSR markers in seed genetic purity assessments. They used, certified seed lots of Kartika, Deepika, Indira Alsi 32 and RLC 92 for assessing their genetic purity using both SSR marker analysis and 18 morphological characters in a grow-out test (GOT). The impurities detected in the SSR marker analysis were 2-3 per cent higher as compared to those detected based on morphological characters in GOT. The results indicated practical utility of the SSR markers in assessing the genetic purity of the flax cultivars.

Qin *et al.* (2015) detected a total of 333 markers, representing 733 polymorphic loci. Forty-six markers associated with the number of bolls per plant (NB), boll weight (BW), lint percentage (LP), fiber length (FL), fiber strength (FS) and fiber micornaire value (FM) were repeatedly detected in at least two environments. Of 46 associated markers, 32 were identified as new association markers, and 14 had been previously reported in the literature. Nine association markers were near QTLs that had been

previously described. These results provided new useful markers for marker-assisted selection in breeding programs.

2.6 Validation of molecular markers.

Fondevilla *et al*. (2008) identified the molecular marker linked to Er3 gene resistant to powdery mildew in wheat. A segregating F_2 population derived from the cross between a breeding line carrying the Er3 gene, and the susceptible cultivar 'Messire' was developed and genotyped. Bulk Segregant Analysis (BSA) was used to identify Random Amplified Polymorphic DNA (RAPD) markers linked to Er3. Four RAPD markers linked in coupling phase (OPW04_637, OPC04_640, OPF14_1103, and OPAH06_539) and two in repulsion phase (OPAB01_874 and OPAG05_1240), were identified. Two of these, flanking Er3, were converted to Sequence Characterized Amplified Region (SCAR) markers. The SCAR marker SCW4637 co-segregated with the resistant gene, allowing the detection of all the resistant individuals. Validation of these markers for polymorphism in different genetic backgrounds and advanced breeding material confirmed the utility of both markers in marker-assisted selection.

Tsilo *et al*. (2008) identified and validated the molecular markers linked to resistant gene *Sr6* that can be used for the detection of this gene in wheat breeding programs. A mapping population of 136 F_2 plants and their $F_{2:3}$ families derived from a cross between near-isogenic lines, 'Chinese Spring' and ISr6-Ra, were screened for stem rust reaction in the seedling stage. Bulk segregant analysis (BSA) based on seedling test was used to screen 418 SSR markers that covered the entire genome of wheat. Four markers, *Xwmc453, Xcfd43, Xcfd77,* and *Xgwm484,* were mapped within a chromosome region that spanned 9.7 cM from *Sr6*. The closest markers, *Xwmc453* and *Xcfd43,* were linked to *Sr6* at a distance of 1.1 and 1.5 cM respectively. Markers *Xwmc453* and *Xcfd43* amplified *Sr6*-specific marker alleles that were diagnostic for *Sr6* in a diverse set of 46 wheat accessions and breeding lines developed. These markers can now be used for marker-assisted selection of *Sr6* and for pyramiding it with other stem rust resistance genes.

Yi *et al*. (2008) employed Bulk segregant analysis (BSA) to identify SRAP (sequence related amplified polymorphism), sequence tagged site (STS) and simple

sequence repeat (SSR) markers linked to the Pm4b gene, which confers good resistance to powdery mildew in wheat. Out of 240 SRAP primer combinations tested, primer combinations Me8/Em7 and Me12/Em7 yielded 220-bp and 205-bp band respectively, each of them associated with Pm4b. STS-241 also linked to Pm4b with a genetic distance of 4.9 cM. Among the eight SSR markers located on wheat chromosome 2AL, Xgwm382 was found to be polymorphic and linked to Pm4b with a genetic distance of 11.8 cM. Further analysis was carried out using the four markers to investigate marker validation for marker-assisted selection (MAS). The results showed that a combination of the linked markers STS, Me8/Em7 and Xgwm382 could be used for marker-assisted selection of the resistance gene Pm4b in wheat breeding programmes

Sheeba *et al*. (2009) carried out the experiment to validate the molecular markers, which had been previously reported to be linked to fertility restorer (Rf) gene(s) for WA-CMS lines of rice. Two mapping populations involving fertility restorer lines for WA-cytoplasm, *viz*. (i) an F_2 population derived from the cross IR58025A/ KMR3R consisting of 347 plants and (ii) a BC_1F_1 population derived from the cross IR62829A/IR10198R//IR62829A consisting of 130 plants were analyzed. Eight SSR markers (RM6100, RM228, RM171, RM216, RM474, RM311, MRG4456 and pRf1&2) showed polymorphism between the parents of the F_2 population, while the SSR markers RM6100 and RM474 showed polymorphism between the parents of both the F_2 and BC_1F_1 populations. Only one CAPS marker, RG146FL/RL was polymorphic between the parents of the BC_1F_1 population.

Anita *et al*. (2011) investigation was carried out to validate the SSR markers (Satt228 and Satt409) reportedly linked to Ti locus in an Indian soybean population generated from the cross between soybean cultivar LSb1 (TiTi) and PI542044 (titi). Parental polymorphism was surveyed using Satt409, Satt228 and 5 SSR markers in the neighbouring genomic region of Ti locus. The SSR marker Satt228 reported to be tightly linked with Ti locus was not found to be polymorphic for the parents used in this study. Satt409 was found to be linked with Ti locus at 4.7 cM. Besides, a new marker Satt538 was found to be linked with Ti locus at a distance of 17.8 cM.

Acuna *et al*. (2012) aimed to develop and characterize functional molecular markers for population genetic analyses and molecular breeding in this *Eucalyptus*

species. Public expressed sequence tag (EST) databases were screened for non-redundant sequences to predict putative gene functions and to discover simple sequence repeats (EST-SSRs), which were then validated in E. globulus and six other *Eucalyptus* species. From a set of 56 random primer pairs, 37 could be validated in eight E. globulus genotypes and were also tested for cross transferability to other six *Eucalyptus* species. As a result, six highly informative markers were proposed for genetic diversity analyses, fingerprinting, and comparative population studies, between different species of E. globules.

Kaur *et al.* (2012) identified simple sequence repeat (SSR) containing expressed sequence tags (ESTs) from consensus sequences, and totals of 2,397 and 802 primer pairs were designed for field pea and faba bean. Subsets of 96 EST-SSR markers were screened for validation across modest panels of field pea and faba bean cultivars, as well as related non-domesticated species. For field pea, 86 primer pairs successfully obtained amplification products from one or more template genotypes, of which 59 percent revealed polymorphism between 6 genotypes. In the case of faba bean, 81 primer pairs displayed successful amplification, of which 48 % detected polymorphism.

Gajjar *et al*. (2014) selected thirty wild *Arachis* species (07, 08 and 15 - resistant to LLS, rust and rust + LLS respectively) for marker validation as they represented different peanut genomes *i.e.* 'A' and 'B'-genome. Extensive variation for morphological and physiological traits has been observed in both wild and cultivated species of peanut. Only 14 of the 30 wild-species validated at least one marker and, a maximum of 03 markers each were validated in 03 species (*A. appressipila, A. hagenbeckii* and *A. pintoi*). In addition, out of the 22 markers tested, only 10 SSRs gave the desired amplification in any of the wild-species tested.

Sukruth *et al*. (2015) employed recombinant inbred lines (RILs) from four populations involving cultivated varieties, and backcross lines from three populations involving cultivated varieties and synthetic tetraploids (developed from wild diploids) for validating late leaf spot (LLS) and rust resistance-linked markers in peanut.

GM2009, GM2301, GM2079, GM1536, GM1954 and IPAHM103 markers showed significant association with rust resistance. They were successfully validated in a new RIL (TG 19 9 GPBD 4) and two backcross (DH 86 9 ISATGR 278-18 and DH 86 9 ISATGR 5) populations and GM1954, GM1009 and GM1573 markers showed significant association with LLS resistance. TAG 19 9 GPBD 4 and ICGS 76 9 ISATGR 278-18 populations showed strong co-segregation of LLS linked markers with the phenotype.

3. MATERIAL AND METHODS

The present study aimed at evaluation of mutant lines of linseed for variability and to validate the markers associated with important nutraceutical traits which are reported in earlier studies. The material and methods used, biochemical and statistical procedures followed in the experiment are described in this chapter under following headings:

3.1 Experimental site

3.2 Experimental material

3.3 Layout of experiment

3.4 Crop management

3.5 Description of the observation

3.6 Molecular marker analysis

3.7 Statistical analysis

3.1 Experimental site

The present experiment was conducted in *rabi*, 2016-17 at AICRP on MULLaRP, Main Agricultural Research Station (MARS), University of Agricultural Sciences, Dharwad, which is located at latitude 15°26' N, longitude of 75°07' and situated at an altitude of 678 m above mean sea level, which falls under agro-climatic zone-8 (Northern Transitional Zone) of Karnataka.

3.2 Experimental material

The M_4 mutant population of linseed was generated at AICRP on MULLaRP by Dr. Suma Mogali. Here two varietal seeds (Indira Alsi and NL-115) were treated with chemical mutagen, ethyl methane sulphonate (EMS) at five different doses 0.1, 0.2, 0.3, 0.4 and 0.5 per cent EMS. The EMS solution was prepared in 0.1 M phosphate buffer

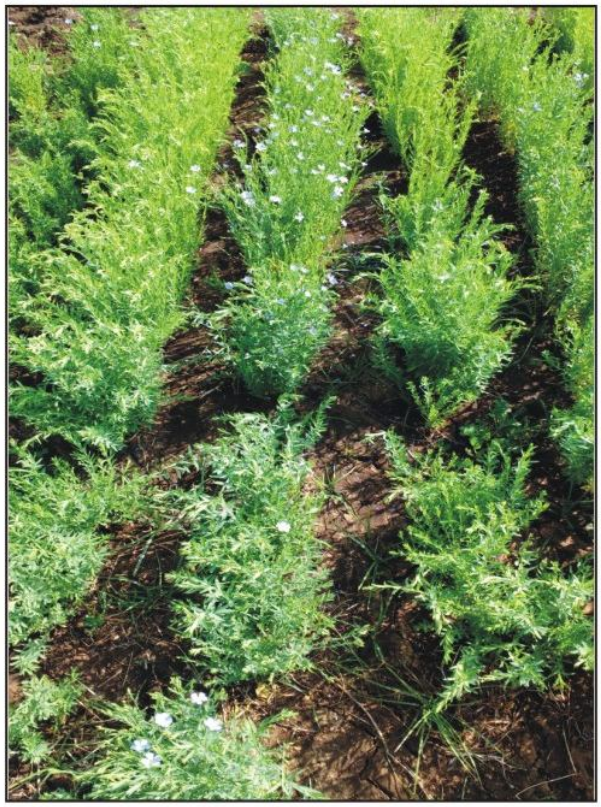


Plate 1: General view of experimental field

(pH-7.0). The pre hydrated seeds (8 hours) were soaked in EMS solution of respective concentrations for 18 hours and later washed in running water to remove chemical residue and shade dried before sowing. The treated seeds of both the varieties were grown in *rabi* 2012-13 along with respective control to raise M_1 generation. The surviving plants were harvested separately to raise M_2 generation.

In *rabi* 2013-14, the two EMS treated varieties were advanced to M_2 generation. The individual progeny row seeds harvested from M_1 generation were utilized to raise M_2 generation. However, no progenies were advanced from 0.3 per cent EMS treatment in Indira Alsi and 0.5 per cent EMS treatment in NL-115, respectively as no desirable progenies were observed. Approximately 200 M_2 plants of each treatment were maintained in the field.

A total of 106 mutants were selected from M_2 generation and were raised in *rabi* 2014-2015 to advance it to M_3 generation as plant to progeny rows along with respective untreated parents, which consisted of 41 plants selected from mutants of Indira Alsi and 65 plants selected from mutants of NL-115.

In M_3, 240 mutants (105 from Indira Alsi and 135 from NL-115 mutants) were selected. These 240 mutants were grown in an augmented design along with 4 checks and evaluated for growth and yield parameters in M_4 during *rabi*-2015-16 at MARS, Dharwad.

In the subsequent year 2016-17 during *rabi,* selected 50 mutants from M_4 were advanced to M_5 generation in an augmented design along with 4 checks and evaluated for yield and quality parameters at MARS Dharwad. Table 1 represents the characteristics of parents Indira Alsi and NL-115 used for mutation.

3.3 Layout of experiment

The experiment was laid out in an augmented design with single replication of each accession. It consisted of five blocks and four checks repeated in each block. The experiment was conducted in medium black soils under rain fed conditions with protective irrigation during *rabi* 2016 at the MARS, Dharwad, Karnataka.

Each mutant line and checks in each block were sown with a spacing of 30cm between the rows and 4cm between the plants in a row was maintained. All recommended agronomic practices were followed during the crop growth period.

3.4 Crop management

Seeds were sown directly in field at the spacing of 30 cm between rows and 10 cm between plants, recommended cultural practices were carried out to ensure uniform crop stand as per the package of practice. Crop was harvested at its physiological maturity. The observations were recorded on 5 random plants from each row of mutants except days to 50 per cent flowering and days to maturity which was on line bases.

3.5 Description of observation

The observations were recorded on five random plants from each row of mutants except days to 50 per cent flowering and days to maturity which was on line basis.

3.5.1 Days to 50 per cent flowering

For each mutant line, number of days taken from the day of sowing to the day on which 50 per cent of the plants showed flowering was recorded and expressed as the number of days taken for 50 per cent flowering.

3.5.2 Days to maturity

Days to maturity was taken from date of sowing to stage when 50 per cent of main branches had matured capsules, when the capsule color changed from green to brown.

3.5.3 Plant height at maturity

The height of plant from the base to the tip of the main stem was recorded in centimeters (cm).

3.5.4 Number of primary branches per plant

The number of branches arising from the main stem were recorded and expressed in number.

Table 1: Characteristics of parents used for mutation

Characters	Indira Alsi	NL-115
Days to flowering	4250	49-53
Days to maturity	94106	99-105
Plant height	45-55	45-55
Number of primary branches per plant	24	4-6
Number of secondary branches per plant	1020	15-25
Number of capsules per plant	2044	50-70
Number of seeds per capsule	57	68
1000 seed weight (g)	5.5-7.5	6.2-7.5
Average yield (kg per ha)	735	840
Oil content	36.54	36.55
ALA	52.2	53.4

3.5.5 Number of secondary branches per plant

The number of branches arising from primary branches in selected plants of each mutant line were recorded and expressed in number.

3.5.6 Number of capsules per plant

The total number of seed bearing capsules on the plant were counted and recorded.

3.5.7 Number of seeds per capsule

The seeds of five capsules at various heights were taken from each mutant and the mean number of seeds per capsule was recorded.

3.5.8 Test weight (1000 seed weight in grams)

The weight of 1,000 well-developed grains from each mutant line was recorded and expressed in grams (g).

3.5.9 Seed yield per plant

The seed yield of plants from each mutant line was recorded and expressed in grams (g).

3.5.10 Oil content

The oil content of selected mutants was determined by Soxhlet method at Department of Biochemistry, UAS, Dharwad and expressed in percentage (%).

3.5.11 Fatty acid composition

Fifty mutants along with standard checks were analyzed for fatty acid profiling at Biochem Research and Testing Laboratory, Dharwad.

3.6 Molecular marker analysis

The molecular analysis was carried out by screening mutants by 13 SSR markers linked to various traits as reported earlier.

3.6.1 Buffers and solutions used for DNA extraction

I. 1M TRIS HCI pH 8.0

1. 121.1 g of TRIS [Tris-(Hydroximytheyl) Aminomythel] was dissolved in about 650 ml of Millipore water.
2. pH was brought down to 8.0 by adding concentrated HCl.
3. Millipore water was added to bring the total volume to 1 litre.
4. Stock solution was sterilized by autoclaving.

II. 0.5 M EDTA

1. 186.12 g of EDTA [Ethylene Diamine Tetra Acetic acid] was added to about 650 ml of Millipore water.
2. pH was adjusted to 8.0 by adding 16-18 g of NaOH pellets.
3. Total volume was made up to 1 litre with Millipore water.
4. Stock solution was sterilized by autoclaving.

III. 5M NaCl

1. 292.2 g of NaCl was dissolved in 650 ml of millipore water.
2. Total volume was made up to 1 litre with Millipore water.
3. Stock solution was sterilized by autoclaving.

IV. TE buffer

1. 10 ml of 1M TRIS HCL pH 8.0
2. 2 ml of 0.5 M EDTA
3. Total volume was made up to 1 litre with Millipore water.

V. DNA extraction CTAB working buffer

1. 100 ml of 1M TRIS HCL pH 8.0
2. 40 ml of 0.5 M EDTA
3. 280 ml of 5 M NaCl
4. 2 % of CTAB (N, N, N, N-cetyl Trimethyl Ammonium Bromide)
5. 0.2 % β-mercaptaethanol (added just before use)
6. 1 g of PVP (Polyvinyl Pyrrolidone)
7. Total volume was made up to 1 litre with Millipore water.

3.6.2 Isolation of genomic DNA

The genomic DNA was isolated from fresh leaves of linseed mutants following modified C-TAB procedure (Prasanna, 2014) as explained below.

1. The 2-3 g fresh leaf sample was taken and ground with liquid nitrogen to make fine powder.
2. This was added to pre-warmed (65 ^{o}C) extraction buffer (1:10 ratio) in a centrifuge tube.
3. Tubes were incubated in water bath at 65^{o}C for 30 min with intermittent mixing.
4. Samples were cooled to room temperature.
5. For removing organic contaminants, sample was extracted with an equal volume of 24:1 chloroform/isoamyl alcohol (CI) and mixed thoroughly for five minutes to form an emulsion.
6. The samples were then centrifuged at 12,000 rpm for 10 minutes at room temperature.
7. Upper aqueous supernatant was transferred to a fresh tube carefully.

8. This step was repeated one more time with equal volume of Chloroform-isoamyl alcohol mixture.

9. Two third volume of pre-chilled Iso-propanol was added and mixed well by inverting the tubes and allowed for precipitation at –20 ^{o}C for overnight.

10. Samples were centrifuged at 15,000 rpm for 10 minutes to spin down the nucleic acids.

11. DNA pellet was air dried and re-suspended in 1M NaCl buffer.

12. The suspended extract was treated with DNAse free RNase (10mg/ml) and incubated for 20 min at 37 ^{o}C.

13. RNase contamination was removed by adding an equal volume of Phenol:Chloroform and the aqueous phase was collected in fresh microfuge tube after centrifugation at 12,000 rpm for 5 minutes at room temperature.

14. DNA was precipitated by adding a double volume of absolute ethanol and mixed gently by inverting the tubes.

15. It was centrifuged at 16,000 rpm for 10 minutes at room temperature to get the DNA precipitate.

16. Pellet was washed with 70 % ethanol, air dried and finally dissolved in a 100µl of TE (Tris 10 mM, EDTA 1 mM) buffer and stored at – 20 ^{o}C.

3.6.3 Quality-check for DNA

The yield of the extracted DNA was quantified by taking the readings at 260 nm and 280 nm in the Nano Drop (UV technologies, USA).

1) Initialization of the instrument was done with Nano pure water.

2) The instrument was set blank with help of 2µl $T_{10}E_{1.}$

3) The quantity of DNA was measured by loading 1µl DNA sample on Nano drop spectrophotometer pedestal.

4) The DNA quantity in ng/µl and OD value for each sample was noted.

DNA concentration and purity was also checked by running the samples on 0.8 % agarose gel with known concentration of uncut λ-DNA of 50ng/µl, 100ng/µl and 200ng/µl. The DNA stocks of the samples were diluted accordingly to make it to required concentration of 10.0ng/µl.

3.6.4 Selection of SSR markers

The thirteen SSR markers were choosen based on previous reports (Prasanna, 2014) and used to validate the markers in M_5 generation. The primer pairs were synthesized from Xcelris Genomics, Ahmedabad. Primers were diluted by giving a brief spin to collect the amorphous primer stock at the bottom of tubes supplied by the company and then HPLC grade water was added to prepare stock solution of 100 pM and the tubes were incubated at 37^0 C for 30 minutes. Then the working solution of 5 pM concentration was prepared. The details of primers are presented in Table 2.

3.6.5 PCR setup and conditions for the amplification of SSRs

Amplification reaction mixture for SSR markers was prepared in 0.2ml thin walled polypropylene PCR tube strips (Axygen, USA) containing following components in a total volume of 20 µl.

Components	Concentration^{-1} (µl)	PCR Reaction (20 µl)
Nuclease free H_2O	-	13.0 µl
Taq Buffer with Mg^{2+} (NEB)	10 X	2.0 µl
dNTP's (GeNei^{TM})	2 mM	2.0 µl
Primers (Forward + Reverse)	10 pM	(0.5 + 0.5) µl
Taq DNA polymerase (NEB)	5 U	0.5 µl
DNA Template	50 ng	1.5 µl

3.6.6 PCR cycling

PCR was carried out using Master Cycler Gradient 5331 (Eppendorf, Germany). The cycler was programmed as follows.

Step	Temperature (°C)	Duration (min)	No of cycles
Initial denaturation	94	5	1
Denaturation	94	1	35
Primer annealing	42-62	1	
Primer extension	72	2	
Complete primer extension	72	5	1
Hold	4	until removed	

The annealing temperature was standardized for each primer by keeping gradient PCR for each primer pair separately. Based on the amplification we obtained in the gradient PCR, annealing temperature was accustomed for each primer pair in PCR amplification. After the completion of PCR, the products were stored at 4^0C until the gel electrophoresis was done.

3.6.7 Visualization of amplified products

Agarose gel electrophoresis was done for visualizing SSR markers. Agarose gel was cast (3.5 %) in TBE buffer (1X) on a horizontal gel frame (Chromus Biotech). Amplified products were visualized by incorporating 1 µl (10 mg/ml) ethidium bromide per 10 ml of gel solution and viewed in a gel documentation system (Alpha Innotech, USA). The procedure followed for gel electrophoresis was as follows:

1. The casting tray and comb were thoroughly cleaned with 70 per cent alcohol using tissue paper.

2. The ends of tray were sealed and comb was inserted.

3. Agarose gel (3.5 %) was prepared by adding 3.5 g agarose to 100 ml of TBE (1X) buffer.

Table 2: List of primers used in present investigation

Sl. No.	Primer name	Forward primer (5' – 3')	Reverse primer (3' – 5')
1	Lub11	CCATGGGATGAAAATTCGAG	CTTCACGAGGGGGAATGTTA
2	Lua133	ATCGCTCCTCTCTCCCTCTC	GCTTTCTCAAGGGTGGAACA
3	NCL_4	CACACGACTGTGAAATTTGGTT	GCATTTCCATGGGGTTTTAGTA
4	Lu236	ACAAATACGCCAACATCAGC	CTTCTCGGCATTCGACTTGT
5	Lu144a	AAAAGCCCACTCAATTTTCG	AGCTGACCAGGAGTTCATGC
6	Lu151	CCAGACGAGCATGGACTTTC	CTAGCCAATCCCAGTTCCAG
7	Lu138	AACCTGAACCAGACGAGCAT	CATGGTTTTGAAGGGGTGAT
8	Lua49B	GCCAAAAACACCAATTCCTT	GGGGAAAAACAGAGTAAGATTCC
9	Lua60	GCTTCGTCGGGAAATGAAG	GCGGAGAGGAGATGAATGAG
10	Lua125a	GCCTTTGGAGGGCTTAACTT	ACAATCCCAACATTCCCAAA
11	Lua68	CCTCCTAATGGCAAAGAAGC	GGAAGGCAAGAGAATCCCTAA
12	Lu143	CACCCTTTTGCATCATCTCA	AAGAACGGGAGGCTAGCAAT
13	Lub14	CTAAGGGTCGTTTGGCTTTG	CTCAACCGTTCGATTCAACA

4. The solution was boiled by putting the flask in microwave oven and allowed to cool to 60 °C.

5. Ethidium bromide (10 µl of conc. 10 mg/ml) was added to the gel and mixed gently.

6. The gel was poured into the tray and air bubbles, if any were removed by using pipette. When the gel was completely set, tape was removed and the gel was placed into the electrophoresis tank.

7. Approximately 500 ml of TBE (1X) buffer was poured into the electrophoresis tank, enough to cover the gel to a depth of 5 mm.

8. Comb was removed carefully.

9. About $1/6^{th}$ volume of loading dye (6X) bromophenol blue dye was added to DNA samples and mixed by gentle tapping.

10. DNA samples were loaded onto the wells and the power supply constant 70 V was provided to run the gel.

11. The power supply was switched off when loading dye was about 2 cm from positive end, and the gel was removed from the gel apparatus.

12. The gel was viewed and photographed by using gel documentation system (Alpha Innotech, USA).

The amplified products were visually scored for further analysis. During scoring, only intense and clearly resolved amplification products were considered for association analysis. The gels were scored as A and B for parent type and mutant type of bands in each accession respectively and used in single marker analysis with phenotypic data of different traits recorded on 50 linseed mutants.

3.7 Statistical analysis

Statistical analysis of the data was carried out using statistical package Windostat Version 8.1 and SPSS Version 16.0 available at Department of Genetics and

Plant Breeding, University of Agricultural Sciences, Dharwad. Different statistical methods employed for the analysis are presented below.

3.7.1 Analysis of variance (ANOVA)

The data recorded on the mean values of five randomly selected plants in each mutant. The characters were subjected to statistical analysis as per Federer (1977) was recorded in order to assess the variability among the mutants.

ANOVA for Augmented RBD

Source of variation	Degrees of freedom	Sum of squares	M.S.S	F ratio
Blocks	(b-1)	bSS	**bMSS**	**bMSS/EMS**
Entries	(e-1)	eSS	eMSS	eMSS/EMS
Checks	(c-1)	cSS	cMSS	cMSS/EMS
Varieties	(v-1)	vSS	vMSS	vMSS/EMS
Checks vs. Varieties	1	cvSS	cvMSS	cvMSS/EMS
Error	(c-1) (b-1)	ESS	EMSS	
Total	N-1	TSS		

Where,

b = Number of blocks,

v = Number of mutant families,

e = Number of entries,

c = Number of checks

3.7.2 Estimation of genetic variability parameters

1. Mean

Mean value of each character was worked out by dividing the sum total by the corresponding number of observations.

$$\text{Mean } (x) = \frac{\sum x}{N}$$

Where,

$\sum x$ = sum of all observation for each character

N = Corresponding number of observations

2. Range

It was taken as the difference between the highest and lowest mean value for each character

$$\text{Range} = X_n - X_1$$

Where,

X_n = highest mean value of character

X_1 = lowest mean value of the character

3. Co-efficient of variability

Both phenotypic and genotypic co-efficient of variability for all the characters were estimated using the formulae of Burton and De Vane (1953).

Genotypic Co-efficient of Variability (GCV):

$$\text{GCV per cent} = \frac{\sqrt{\text{Genotypic variance}}}{\text{Grand mean}} \times 100$$

Phenotypic Co- efficient of Variability (PCV):

$$\text{PCV per cent} = \frac{\sqrt{\text{Phenotypic variance}}}{\text{Grand mean}} \times 100$$

PCV and GCV were classified as per Sivasubramanian and Menon (1973) as shown below:

0-10 % - Low

10 -20 %- Moderate

>20 % - High

4. Heritability in broad sense (h^2_{bs})

Heritability (broad sense) was estimated for all the characters as the ratio of genotypic variance to the total variance as suggested Lush (1949) and Hanson *et al.* (1956).

$$h^2_{bs} = \frac{\sigma_g^2}{\sigma_p^2} \times 100$$

According to Robinson *et al.* (1949) heritability estimates in cultivated plants can be placed in following categories,

0-30 %- Low

30-60 %- Moderate

>60 %- High

5. Genetic Advance (GA)

Genetic advance for each character was estimated by using the following formula of Johnson *et al.* (1955)

$$GA = h^2 \times K \times \sigma_p$$

Where,

h^2 = Heritability estimate

K = Selection differential which is equal to 2.06 at 5 per cent intensity of selection.

σ_p = Phenotypic standard deviation

6. Genetic advance over mean (GAM)

Genetic advance as per cent of mean (GAM) was computed by using the following formula,

$$GAM = \frac{GA}{\text{Grand mean}} \times 100$$

Genetic advance as per cent of mean was categorized according to Johnson *et al.* (1955), as given below,

0 -10 % = Low; 10 - 20 % = Moderate; >20 % = High

3.7.3 Correlation coefficients

To determine the degree of association of characters with yield and also among the yield components, the correlation co-efficients were calculated.

Phenotypic co-efficients of correlation between two characters were determined by using variance and covariance components as suggested by Al-Jibourie *et al.* (1958).

$$r_{xy}(p) = \frac{COV_{xy}(p)}{\sqrt{V_x(p) \times V_y(p)}} \times 100$$

Where,

r_p = Phenotypic correlation

Cov_p (XY) = phenotypic covariance between characters x and y

Vx (X) = phenotypic variance of character x.

Vy (Y) = phenotypic variance of character y.

Estimates of correlation coefficients were compared against r-values given in Fisher and Yates (1963) table at (n-2) df at the probability levels of 0.05 and 0.01 to test their significance.

3.7.4 Molecular Data analysis

Genotyping data available as A and B (A- parent type and B- mutant type) are considered for molecular analysis along with phenotypic data.

3.7.4.1 The association of SSR markers with morphological traits

Single marker analysis was performed to find the contribution of the markers towards the different traits by one way ANOVA using MS-EXCEL programme. The coefficient of determination of (R^2) which explains the per cent of phenotypic variance explained by the polymorphic marker was calculated.

4. EXPERIMENTAL RESULTS

The field and laboratory experiments comprising of 50 linseed mutants (M_5) were carried out during 2015-16. The extent of variability in morphological characters and association between yield and yield components as well as with molecular markers were studied. The results of the present investigation are presented under the following subheadings:

4.1 Analysis of variance

4.2 Genetic variability studies

4.3 Association studies

4.4 Mean performance of mutants and parents

4.5 Selection of promising mutants for yield and alpha linolenic acid

4.6 Marker trait association

4.1 Analysis of variance

The analysis of variance for all the fifteen characters *viz.*, days to fifty percent flowering, days to maturity, plant height, number of primary branches per plant, number of secondary branches per plant, number of capsules per plant, number of seeds per capsule, seed yield per plant, 1000 seed weight (g), oil content, palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid are presented in the Table 3 and 4.

Population exhibited significant variation for all the characters except number of seeds per capsules and stearic acid content. More significant variation was recorded for number of capsules per plant.

4.2 Genetic variability studies

The values of mean, range, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (h^2), genetic advance (GA) and genetic advance over mean (GAM) for fifteen characters studied in present study are presented

Table 3: ANOVA for yield and yield contributing traits of linseed mutants

Source of variation	df	Days to fifty per cent flowering	Days to maturity	Plant height	Primary branches per plant	Secondary branches per plant	Capsules per plant	Seeds per capsule	Seed yield per plant	Test weight
Block (eliminating Checks + mutants)	4	4.167*	5.166**	0.920	0.077	1.327	3.583	1.110	0.068	0.076
Entries(ignoring Blocks)	53	19.587**	25.299**	37.213**	1.857**	17.773**	1161.705**	1.270	3.097**	0.647**
Checks	3	55.167**	151.500**	93.572**	0.572*	5.239*	431.724**	0.790	1.309*	0.381**
Mutants	49	17.573**	16.489**	34.00**	1.584**	17.912**	1211.234**	1.287	3.210**	0.677**
Checks vs Mutants	1	7.521**	60.750**	19.089**	18.513**	48.824**	1023.790**	1.944	3.174**	0.065
Error	12	0.667	0.667	1.237	0.039	1.110	49.673	1.223	0.244	0.043
C.D. (5%)	1	1.306	1.306	1.779	0.316	1.685	11.274	1.769	0.791	0.332
C.V. (%)	1	2.309	2.309	3.145	0.558	2.979	12.929	3.128	1.398	0.587

* and ** indicates Significant at 1 percent and Significant at 5 percent level of probability

Table 4: ANOVA for oil and fatty acid profile of advanced linseed mutants

Source of variation	Df	Oil	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Alpha linolenic acid
Block(eliminating Checks+mutants)	4	1.373	0.011	0.038	0.022	0.084	0.384
Entries(ignoring Blocks)	53	35.121**	0.378**	0.124	2.549**	0.711**	3.987**
Checks	3	83.907*	0.214	0.241*	1.045**	0.663*	0.597
Mutants	49	28.316**	0.383**	0.095	1.366**	0.466*	2.044**
Checks vs Mutants	1	208.595**	0.642*	1.172**	62.678**	12.403**	105.465**
Error	12	0.841	0.073	0.049	0.145	0.129	0.188
C.D. (5%)	1	1.467	0.432	0.355	0.610	0.574	0.694
C.V. (%)	1	2.594	0.763	0.627	1.079	1.014	1.227

* and ** indicates Significant at 1 percent and Significant at 5 percent level of probability

in Table 5. Graphical representation of GCV and PCV of different traits is shown in Fig. 1 and Fig. 2 represents the graphical representation of heritability and GAM for all the traits.

4.2.1 Days to 50 per cent flowering

Overall mean for days to fifty per cent flowering in the mutants was 60.59 days with a wide range of variation from as early as 48 days to as late as 70 days. The genotypic (5.99 %) and phenotypic (6.14 %) co-efficient of variation were low accompanied with high heritability of 95.20 per cent and low genetic advance of 7.31 as per cent of mean and with fairly moderate values of genetic advance over mean (12.03)

4.2.2 Days to maturity

Maturity duration varied from as low as 106 days to 125 days with a mean of 115.19 days. The phenotypic (3.12 %) and genotypic (3.04 %) co-efficient of variation were low and the trait showed a very high heritability of 94.9 per cent and with low values of expected genetic advance (7.06) and per cent mean genetic advance of 6.10 per cent (Table 5).

4.1.3 Plant height (cm)

The population exhibited a wide range of variability for this trait with overall mean of 42.22 cm. The range in the mutant population for plant height varied from 31.8 cm to 52.4 cm. The per cent of genotypic and phenotypic coefficients of variations for this trait were moderate with 10.64 and 11.52 respectively (Table 5). This trait exhibited a high heritability (85.83) with expected genetic advance of 9.92 and with high per cent mean genetic advance as 23.50 per cent.

4.2.4 Primary branches per plant

The number of primary branches exhibited moderate variation from 2.8 to 8 in the mutants with overall mean of 4.89 primary branches per plant. The GCV and PCV for this trait were moderately high with 27.58 and 29.68 per cent respectively. This trait showed a high heritability of 86.34 per cent and low expected genetic advance of 2.23 with a high per cent mean genetic advance (45.60 %).

4.2.5 Secondary branches per plant

Population displayed a wide range of variation for this character ranging from as low as 4.2 to as high as 23.2 secondary branches per plant (Table 5). Overall mean number of secondary branches per plant was 9.71. The GCV and PCV were high with 35.53 and 37 per cent respectively. This trait exhibited a very high heritability (92.2 %) with a low expected genetic advance (7.18) and high per cent mean genetic advance (70.29 %).

4.2.6 Number of capsules per plant

Number of capsules per plant in these mutants varied with a wide range of variation from 9.4 to 152.2 with an overall mean of 61.54 capsules per plant (Table 5). The GCV and PCV were recorded high with 47.24 and 48.51 per cent respectively. This trait exhibited a very high heritability (94.8 %) with a high expected genetic advance (60.51) and moderately higher per cent mean genetic advance (94.77 %).

4.2.7 Number of seeds per capsules

The mutant lines recorded on an average of 6.82 seeds per capsule with a range varying from 4.6 to 10 seeds per capsule. The genotypic and phenotypic coefficients of variations were 14.81 and 15.98 per cent respectively. This trait exhibited a heritability of 85.89 per cent with a very low expected genetic advance (2.00) and high per cent mean genetic advance (29.33 %).

4.2.8 Seed yield per plant (g)

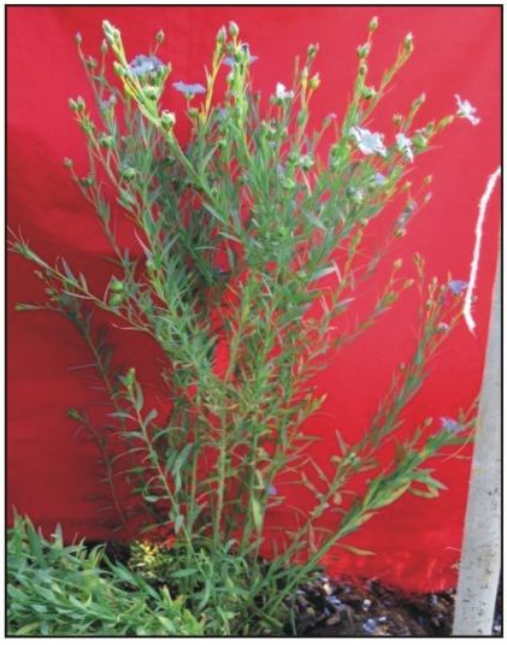
The mutant lines exhibited a wide amount of variation varying from 0.19 to 8.83 g per plant. The overall mean was 2.78 g. The genotypic and phenotypic coefficients of variations were high with 32.31 and 35.82 per cent respectively. This character displayed a high estimate of heritability (85.62) with a very low expected genetic advance (1.68) while per cent mean genetic advance (60.43 %) was high.

4.2.9 Test weight (1000 seed weight in grams)

An overall mean for 1,000 seed weight was 5.14 g with a range varying from 3.10 to 6.60 g (Table 5). Moderate GCV and PCV were accounted with 13.65 and 14.23

a. 90-3

b. 85-1



c. Indira Alsi (parent)

d. 75-1

Plate 2: Variation for number of secondary branches of Indira Alsi mutants

a. 57-2

b. Nl115 (parent)

Plate 3: Variation for number of secondary branches of NL 115 mutant

per cent respectively. This trait exhibited a high heritability estimate (92.02 %) and expected genetic advance was low with 1.39 and per cent mean genetic advance was high as 26.98 per cent respectively.

4.2.10 Oil content

The oil content ranged from 21.74 to 49.4 per cent with overall mean of 34.64 per cent. This character had 13.81 and 14.08 per cent of moderate GCV and PCV respectively. It showed high estimate of heritability (96.24 %) and expected genetic advance (9.38) was low and high per cent mean genetic advance (27.90 %).

4.2.11 Palmitic acid

Palmitic acid content in seeds had a narrow range between 4.30 per cent and 8.50 per cent with a mean of 5.08 per cent. The genotypic and phenotypic coefficients of variations were 9.59 and 10.94 per cent respectively. This character displayed a high estimate of heritability (76.90 %) with a very low expected genetic advance (0.89) while per cent mean genetic advance (17.33 %) was moderate.

4.2.12 Stearic acid

Stearic acid per cent in seeds of these mutant lines varied with a narrow range of variation from 3.40 to 5.10 with an overall mean of 4.20 per cent (Table 5). The GCV and PCV were low with 4.42 and 6.81 per cent respectively. This trait exhibited a moderate heritability (42.04 %) with a low expected genetic advance (0.25) and lower per cent mean genetic advance (5.90 %).

4.2.13 Oleic acid

The mutant lines exhibited a variation for oleic acid varying from 16.3 to 22.10 per cent. The overall mean was 19.36. The genotypic and phenotypic coefficients of variations were 5.20 and 5.59 per cent respectively. This character displayed a high estimate of heritability (86.80 %) with a very low expected genetic advance (1.88) while per cent mean genetic advance (9.99 %) was also recorded low.

Table 5: Estimates of genetic variability parameters for productivity and fatty acid composition in linseed mutants

Characters	Parents mean		Mutants mean	Range		GCV%	PCV%	h^2(bs)	GA	GAM (%)
	Indira alsi	NL-115		Min	Max					
Days to 50% Flowering	56.00	59.00	60.59	48.00	70.00	5.99	6.14	95.20	7.31	12.03
Days to maturity	112.00	110.00	115.19	106.00	125.00	3.04	3.12	94.90	7.06	6.10
Plant height (cm)	45.90	42.30	42.22	31.80	52.40	10.64	11.52	85.83	9.92	23.50
Number of primary branches per plant	4.00	3.65	4.89	2.80	8.00	27.58	29.68	86.34	2.23	45.60
Number of secondary branches per plant	7.40	7.85	9.71	4.20	23.20	35.53	37.00	92.20	7.18	70.29
Number of capsules per plant	40.25	46.65	61.54	9.40	152.20	47.24	48.51	94.80	60.51	94.77
Number of seeds per capsule	7.30	7.60	6.82	4.60	10.00	14.81	15.98	85.89	2.00	29.33
Seed yield per plant (g)	1.82	2.36	2.78	0.19	8.83	32.31	35.82	85.62	1.68	60.43
1000 seed weight (g)	4.85	5.00	5.14	3.10	6.60	13.65	14.23	92.02	1.39	26.98
Oil (%)	40.00	41.98	34.64	21.74	49.40	13.81	14.08	96.24	9.38	27.90
Palmitic acid (%)	6.20	6.22	5.08	4.30	8.50	9.59	10.94	76.90	0.89	17.33
Stearic acid (%)	5.08	5.40	4.20	3.40	5.10	4.42	6.81	42.04	0.25	5.90
Oleic acid (%)	30.81	31.61	19.36	16.30	22.10	5.2	5.59	86.80	1.88	9.99
Linoleic acid (%)	26.03	18.83	15.23	14.00	17.10	3.43	4.19	67.24	0.87	5.80
Alpha Linolenic acid (%)	52.20	53.40	52.71	48.80	56.20	2.32	2.47	88.50	2.34	4.50

4.2.14 Linoleic acid

Seeds of these mutant lines had narrow variation for linoleic acid content ranging from 14 per cent to 17.10 per cent with a mean value of 15.23 per cent. Low genotypic and phenotypic coefficients of variations were observed with 3.43 and 4.19 per cent respectively. This character displayed a high estimate of heritability (67.24 %) with a very low expected genetic advance (0.87) while per cent mean genetic advance was 5.80 %.

4.2.15 Alpha linoleinic acid

Alpha linoleinic acid content in seeds showed a moderate variation from 48.8 per cent to 56.20 per cent with a mean value of 52.71 per cent. The genotypic and phenotypic coefficients of variations were 2.32 and 2.47 per cent respectively. This character displayed a high estimate of heritability (88.50 %) with expected genetic advance of 2.34 and low per cent mean genetic advance of 4.50 percent.

4.3 Association studies

Economically important trait seed yield, which is a complex trait and is affected by many other genetic and non-genetic factors. In order to know the magnitude of association between seed yield with other yield influencing traits, correlation analysis is an effective measure. This gives an idea about how the traits are associated with each other as well as with the seed yield. The results of the association studies are presented in Table 6.

4.3.1 Days to fifty per cent flowering

The days to fifty per cent flowering exhibited positive significant correlation with number of capsules per seed (0.301) at phenotypic level. This trait had negative significant association with stearic acid (0.337) and oleic acid (0.307). However, plant height (0.019), number of seeds per capsules (0.11), 1,000 seed weight (0.22), oil content (0.059) and linoleic acid (0.012) exhibited positive association with days to fifty per cent flowering. Days to maturity (-0.103), number of primary branches per plant (-0.09), number of secondary branches per plant (-0.214), seed yield per plant (-0.247),

palmitic acid (-0.256) and alpha linolenic acid (-0.097) showed negative and non-significant association with days to fifty per cent flowering.

4.3.2 Days to maturity

This trait exhibited positive non significant association with palmitic acid (0.043), oleic acid (0.074), linoleic acid (0.500) and alpha linolenic acid (0.350). This trait has negative significant association with plant height (-0.348). However, days to fifty per cent flowering (-0.103), number of primary branches per plant (-0.115), number of secondary branches per plant (-0.262), number of capsules per plant (-0.281), number of seeds per capsule (-0.163), seed yield per plant (-0.188), 1,000 seed weight (-0.280), oil content (-0.286) and stearic acid (-0.136) showed negative non-significant association with days to maturity.

4.3.3 Plant height

The plant height exhibited positive significant correlation with number of primary branches per plant (0.341), number of secondary branches per plant (0.441), number of capsules per plant (0.407), 1,000 seed weight (0.378) and oil content (0.321). Days to fifty per cent flowering (0.019), number of seeds per capsules (0.123), seed yield per plant (0.158), stearic acid (0.174), linoleic acid (0.049) and alpha linolenic acid (0.020) exhibited positive non-significant association with this trait. This trait had negative significant association with days to maturity (-0.348) and negative non significant with palmitic acid (-0.065) and oleic acid (-0.068).

4.3.4 Number of primary branches per plant

The number of primary branches per plant exhibited significant positive association with plant height (0.341), number of secondary branches per plant (0.811), number of capsules per plant (0.722) and seed yield per plant (0.501). It had positive but non-significant correlation with number of seeds per capsule (0.172), 1,000 seed weight (0.149), oil content (0.072), palmitic acid (0.085), stearic acid (0.059) and oleic acid (0.158) at phenotypic level. Number of primary branches per plant was negatively associated with days to fifty per cent flowering (-0.090), days to maturity (-0.115), linoleic acid (-0.105) and alpha linolenic acid (-0.069).

4.3.5 Number of secondary branches per plant

The number of secondary branches per plant showed positive significant correlation with plant height (0.441), primary branches per plant (0.811), number of capsules per plant (0.908), seed yield per plant (0.595) and oleic acid (0.367) at phenotypic level. This trait had positive non-significant correlation with number of seeds per capsule (0.205), 1,000 seed weight (0.263), oil content (0.047), palmitic acid (0.186) and stearic acid (0.192). This trait was negatively associated with days to fifty per cent flowering (-0.214), days to maturity (-0.262), linoleic acid (-0.101) and alpha linolenic acid (-0.069) at phenotypic level.

4.3.6 Number of capsules per plant

The number of capsules per plant exhibited positive significant correlation with days to fifty per cent flowering (-0.301), plant height (0.407), number of primary branches per plant (0.722), number of secondary branches per plant (0.908), seed yield per plant (0.548) and oleic acid (0.366). However, it showed a positive non-significant association with number of seeds per capsule (0.300), 1,000 seed weight (0.087), oil content (0.089), palmitic acid (0.100), stearic acid (0.136) and alpha linolenic acid (0.086) at phenotypic level. The number of capsules per plant showed a negative non-significant with days to maturity (-0.281), linoleic acid (-0.064) at phenotypic level.

4.3.7 Number of seeds per capsule

The number of seeds per capsule was positively associated with days to fifty per cent flowering (0.110), plant height (0.123), number of primary branches per plant (0.172), number of secondary branches per plant (0.205), number of capsules per plant (0.300), seed yield per plant (0.192), 1,000 seed weight (0.078) and oil content (0.039) at phenotypic level. The number of seeds per capsule was negatively associated with days to maturity (-0.163), palmitic acid (-0.105), stearic acid (-0.062), oleic acid (-0.080), linoleic acid (-0.133) and alpha linolenic acid (-0.009).

4.3.8 Seed yield per plant

The seed yield per plant showed significant positive correlation with number of primary branches per plant (0.501), number of secondary branches per plant (0.595)

number of capsules per plant (0.548) and alpha linolenic acid (0.350). While with remaining characters like plant height (0.158), number of seeds per capsule (0.192), stearic acid (0.164), oleic acid (0.306) and linoleic acid (0.025) showed positive but non-significant correlation with seed yield per plant. Further it was found to have negative non significant association with days to fifty per cent flowering (-0.247), days to maturity (-0.188), 1,000 seed weight (-0.007), oil content (-0.085), palmitic acid (-0.013).

4.3.9 Test weight (1000 seed weight in grams)

This trait exhibited significant positive association with only plant height (0.378). However, this trait had a positive association was observed with days to fifty per cent flowering (0.220), number of primary branches per plant (0.149), number of secondary branches per plant (0.263), number of capsules per plant (0.087), number of seeds per capsule (0.078) and palmitic acid (0.059) at phenotypic level. It showed negative non-significant association with days to maturity (-0.280), seed yield (-0.071), oil content (-0.020), stearic acid (-0.011), oleic acid (-0.007), linoleic acid (-0.163) and alpha linolenic acid (-0.089).

4.3.10 Oil content

The oil content of the seed exhibited a positive significant correlation with plant height (0.321). The oil content recorded positive but non-significant association with days to fifty per cent flowering (0.059), number of primary branches per plant (0.072), number of secondary branches per plant (0.047), number of capsules per plant (0.089), number of seeds per capsule (0.039), palmitic acid (0.027), stearic acid (0.145) and alpha linolenic acid (0.209). However, this trait showed negative significant association with linoleic acid (-0.331). Negative non-significant association was observed for oil content with days to maturity (-0.286), seed yield per plant (-0.085), 1,000 seed weight (-0.020) and oleic acid (-0.224).

4.3.11 Alpha-linolenic acid content

The alpha linolenic acid content of the seeds exhibited significant positive correlation with seed yield per plant (0.350), linoleic acid (0.324). However, days to

Table 6: Phenotypic correlation coefficients between seed yield and component characters in linseed mutants

	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}	X_{15}
X_1	1	-0.103	0.019	-0.09	-0.214	0.301*	0.110	-0.247	0.220	0.059	-0.256	-0.337*	-0.307*	0.012	-0.097
X_2		1	-0.348*	-0.115	-0.262	-0.281	-0.163	-0.188	-0.280	-0.286	0.043	-0.136	0.074	0.500	0.350
X_3			1	0.341*	0.441**	0.407**	0.123	0.158	0.378*	0.321*	-0.065	0.174	-0.068	0.049	0.020
X_4				1	0.811**	0.722**	0.172	0.501**	0.149	0.072	0.085	0.059	0.158	-0.105	-0.069
X_5					1	0.908**	0.205	0.595**	0.263	0.047	0.186	0.192	0.367*	-0.101	-0.016
X_6						1	0.300	0.548**	0.087	0.089	0.100	0.136	0.366*	-0.064	0.086
X_7							1	0.192	0.078	0.039	-0.105	-0.062	-0.080	-0.133	-0.009
X_8								1	-0.007	-0.085	-0.013	0.164	0.306	0.025	0.350**
X_9									1	-0.020	0.059	-0.011	-0.007	-0.163	-0.089
X_{10}										1	0.027	0.145	-0.224	-0.331*	0.209
X_{11}											1	0.133	0.057	-0.388*	0.089
X_{12}												1	0.125	0.179	0.026
X_{13}													1	0.002	0.015
X_{14}														1	0.324*
X_{15}															1

* and ** indicates Significant at 1 percent and Significant at 5 percent level of probability

X_1 = Days to 50% flowering
X_2 = Days to Maturity
X_3 = Plant height(cm)
X_4 = Number of Primary branches per plant
X_5 = Number of secondary branches per plant
X_6 = Number of capsules per plant
X_7 = Number of seeds per capsules
X_8 = Seed yield per plant (g)
X_9 = 1000 seed weight (g)
X_{10} = Oil content (%)
X_{11} = Palmitic acid
X_{12} = Stearic acid
X_{13} = Oleic acid
X_{14} = Linoleic acid
X_{15} = Alpha Linolenic acid

maturity (0.350), plant height (0.020), number of capsules per plant (0.086), oil content (0.209), palmitic acid (0.089), stearic acid (0.026) and oleic acid (0.015) showed a positive non-significant association with alpha linolenic acid at phenotypic level. The traits days to fifty per cent flowering (-0.097), number of primary branches per plant (-0.069), number of secondary branches per plant (-0.016), number of seeds per capsule (-0.009) and 1,000 seed weight (0.089) registered negative association with alpha linolenic acid content.

4.4 Mean performance of mutants and parents

Mean performance of mutants for all the characters are compared with parents NL-115 and Indira alsi (Table 5). Mean performance of mutants for the characters like primary branches per plant, secondary branches per plant, number of capsules per plant, seed yield per plant and alpha lilolenic acid content was higher than the parents NL-115 and Indira alsi. It showed that, there was increase in number of primary branches per plant, secondary branches per plant, number of capsules per in mutants which ultimately lead to increased yield compared to its parents.

Mean of alpha linolenic (ALA) content was also higher than parents, which shows that mutation has lead to alter the fatty acid composition which is useful to screen the desirable mutant with high ALA content in seed. Other characters like days to fifty per cent flowering, days to maturity, plant height, number of capsules per plant, oil content, palmitic acid, stearic acid, oleic acid and linoleic acid showed lesser mean performance than the parents (Table 5).

4.5 Selection of promising mutants for yield and alpha linolenic acid

Present study was mainly focused to select the promising mutants for higher seed yield and higher alpha linolenic acid content in seeds. Table 7 shows the mutants selected for higher seed yield and higher ALA content. The mutants 75-1 (208.00) and 57-2 (152.20) found significantly superior than NL-115 (60.21) with more number of capsules per plant followed by the mutant 85-1 with 121 capsules per plant, and significant higher seed yield was accounted for mutant 90-3 (7.08 g) followed by 85-1 (6.71 g) and 57-2(6.07 g) compared to check NL-115 (2.36 g).

Table 7: Superior mutants for yield and ALA content

Mutants	Number of secondary branches per plant	Number of capsules per plant	Seed yield per plant	Oil (%)	Alpha linolenic acid
46-1	7.60	104.40**	5.73**	27.79	50.30**
57-2	5.00	152.20**	6.07**	30.75	53.40**
85-1	17.20**	121.00**	6.71**	32.11	48.80**
92-2	19.00**	105.80**	5.26**	29.64	48.90**
90-3	16.80**	141.00**	7.08**	28.41	51.90**
103-2	14.60	90.60**	3.76**	28.78	52.90**
65-1	5.60	97.00**	4.73**	30.88	52.80**
43-5	4.40	109.20**	5.12**	36.80	54.30**
75-1	11.60**	208.00**	3.53**	26.06	50.90**
Indira alsi	7.40	40.25	1.82	41.00	52.2
NL-115	7.85	60.21	2.36	42.98	53.4
C. D. (5%)	1.69	11.27	0.79	1.47	0.69
C.V. (%)	2.98	12.93	1.40	2.59	1.23

a. 90-3

b. 46-1

c. 75-1

d. Indira Alsi (parent)

Plate 4: Variation for number of capsules of Indira Alsi mutants

a. 57-2 b. 103-2

c. Nl115 (parent)

Plate 5: Variation for number of capsules of NL 115 mutants

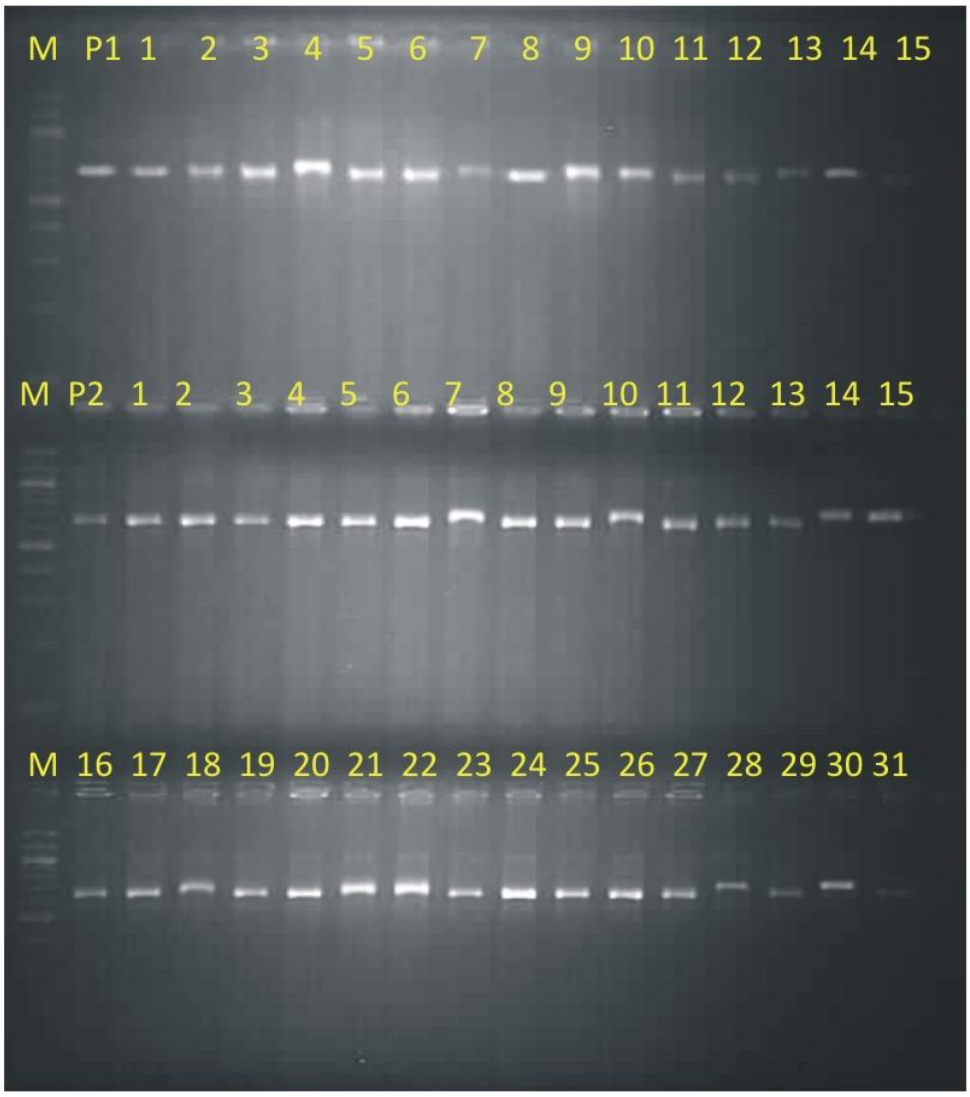
Oil content of mutants were non-significant with lower oil content than the check and higher ALA content was recorded in mutants. Mutant number 43-5 (54.30 %) accounted with significant higher ALA content followed by 57-2 (53.40 %) than Indira alsi (31.87 %) and NL-115 (37.94 %). It shows that, increase in number of capsules per plant will increase the seed yield and also with higher ALA content (Table 7).

4.6 Marker trait association

Marker-trait association identification will play an important role in plant MAS/QTL breeding programs, especially in orphan crops such as linseed with heterozygosity when no other genetic information such as linkage maps and Quantitative Trait Loci are available. However, such associations of markers identified in one population may be some times attributed to the presence of false positives and/or the use of structured populations (Roy *et al.,* 2006). So, there is a need to validate their association in different populations like F_2, backcross populations, RIL, DH population or mutant populations.

In this study, markers reported by Prasanna, 2014 were subjected for validation in M_5 generation of linseed mutants. A total of thirteen SSR markers (Lub11, Lua133, Lu236, Lu144a, Lu151, Lu143, Lua68, Lu138, Lu143, Lub14, Lua60, Lua125a and Lua49B) were reported to be associated with different traits like days to maturity, number of secondary branches per plant, number of capsules per plant, 1000 seed weight, seed yield per plant, oil content and ALA content in seed.

Total of thirteen SSR markers were used for genotyping mutants by following three major steps *viz*., isolation of genomic DNA from the 50 linseed mutants, and amplification of the DNA by SSR primers and analysis of amplified products. The SSR primers used in the present study produced scorable, unambiguous amplicons. In total of 13 markers, 5 markers (Lua60, Lua49B, Lub14, Lu138, Lu144a) showed polymorphism between mutant and non-mutant. Remaining eight markers exhibited a monomorphic banding pattern between parent and mutant. Analysis of amplified products was carried out by scoring the banding pattern. Scoring (A and B) given to amplified products of five markers was subjected for single marker analysis to find the marker trait association.

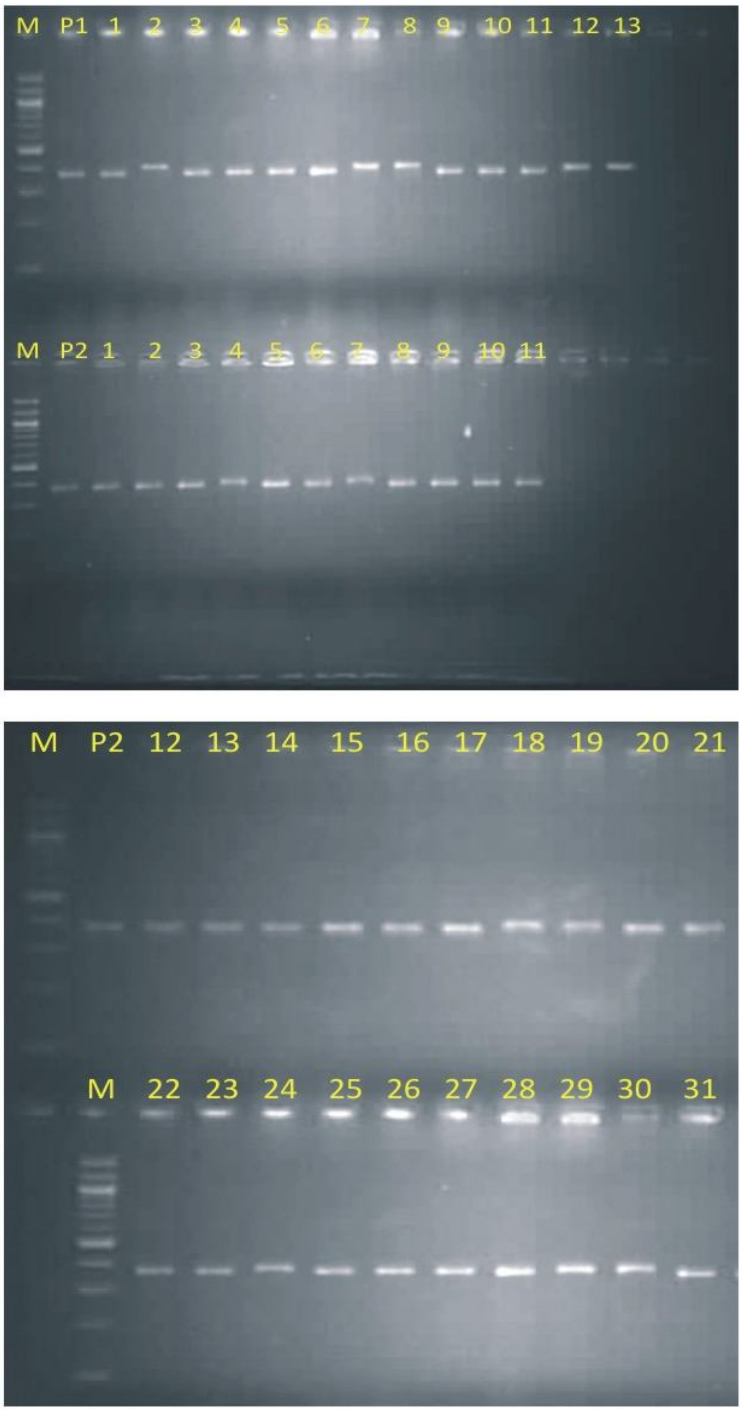


M – 100 bp ladder

P_1- NL-115, 1 to 14 – Mutants of P_1

P_2- Indira Alsi, 1 to 31 – Mutants of P_2

Plate 6: Amplified products of primer-Lu144a on gel indicating polymorphism between parent and mutants



M - 100bp ladder
P_1 – NL-115, 1 to 13 – Mutants of P_1
P_2 – Indira Alsi, 1 to 31 – Mutants of P_2

Plate 7: Amplified products of primer-Lua49B on gel indicating polymorphism between parent and mutants

4.6.1 Single marker analysis

Single marker analysis was performed using phenotypic and genotypic data of all 50 advanced mutant lines of linseed to determine the strength of association between five markers with yield and yield attributing traits. Strength of association between marker and trait was determined by calculating the *F* statistic value and phenotypic variance explained by markers which was measured as R^2 value (Table 8).

The markers Lua60, Lua49B, Lub14, Lu144a, and Lu138 showed significant association with different traits. Marker Lub14 was found to be associated with oil content with R^2 value 17.46. Number of capsules per plant established to be associated with two SSR markers Lu144a and Lu138 with R^2 value of 11.91 and 10.61 respectively. In case of fatty acid contents, two markers showed association with ALA content *viz.*, Lua49B and Lua60 with R^2 value of 6.04 and 7.93 respectively (Table 8). Besides the earlier reports these markers found to be associated with other traits also, Lu144a was found to be associated with plant height and secondary branches per plant and Lua49B was associated with plant height. Maximum of three traits were found to be associated with one marker (Lu144a).

Table 8: Marker trait association by Single Marker Analysis

Traits	Marker	P-Value	F-Value	R^2
ALA content	Lua49B	0.0008	12.28	6.04
	Lua60	0.0043	8.81	7.93
Number of capsules per plant	Lu138	0.0067	7.84	10.61
	Lu144a	0.0042	8.79	11.91
Oil percent	Lub14	0.0004	13.74	17.46
Height of the plant	Lu144a	0.0300	4.88	6.98
	Lua49B	0.0230	5.42	7.69
Secondary branches per plant	Lu144a	0.0004	13.96	17.67

5. DISCUSSION

There is a wide scope for increasing crop productivity through the use of high-yielding varieties. But the development of improved linseed cultivars is restricted by limited genetic variability. The creation of variation constitutes a primary step to get desirable plant types. Most genetic variability available today in plant collections is the result of past evolution involving spontaneous mutations, recombinations and exposure to the forces of natural selection. Induced mutation is one of the most widely used technique for creating additional variability in seed oil quality.

Mutation breeding has been perceived as an important tool to faster additional variability in qualitatively and quantitatively inherited traits in a number of crop plants. There are indications that a major part of domestication of crop plants occurred in the form of discrete steps mediated by single gene mutations (Gottlieb, 1984). The variability thus created enhances opportunities for selection of new genotypes with the desired characteristics. Induced mutation can play a vital role in the restructuring of the plant, leading to yield improvement. It could create additional genetic variability to supplement conventional crop breeding. Induced mutation has been employed successful in linseed by many workers (Badere and Choudhary, 2007; Baculis, 2001; Rai *et al.*, 2014; Tolba, 2000).

The extent of variability is detected by genotypic and phenotypic coefficients of variability (GCV and PCV), provides information about the relative amount of variation in different characters. Several researchers (Abideen *et al.*, 2013; Lodhi *et al.*, 2013; Singh *et al.*, 2015; Synram *et al.*, 2014; Hasan *et al.*, 2014) have discussed the importance of genetic components of variance in predicting responses in selection. Since yield and its component characters are controlled by polygenes, information on the extent of the heritable portion of variability induced by mutation is meaningful for developing improved varieties. Therefore, genetic variability in terms of coefficients of variability is not sufficient to determine the amount of heritable variability. The estimate of heritability acts as a predictive mechanism in expressing the consistency of phenotypic values. Hence, it helps plant breeders to make a selection for a particular character when heritability is high (Arulbalachandran *et al*., 2010).

Heritability estimates provide information on the extent to which a particular genetic character can be transmitted to successive generations, whereas genetic advance helps in formulating suitable selection indices. Such estimates smooth the progress of the evaluation of genetic and environmental effects, thereby aiding in selection (Begum and Dasgupta, 2014). Higher the genetic variability more will be the opportunities for improvement through suitable selection procedure. Thus, there is a need to generate information on phenotypic and genotypic variances as well as heritability, Genetic advance and interrelationships of yield and yield related quantitative traits in the crop like linseed, where very little information is available (Singh, 2015).

One of the most important objectives in oilseed breeding has been the genetic modification of seed oil quality by changing the proportion of fatty acids composition suitable for either nutritional or industrial purposes. Development of new lines with desirable alteration to the fatty acid composition of the seed oils can be achieved through conventional breeding, coupled in some cases with mutagenesis without adversely affecting agronomic characteristics of the plant. Such alterations in the fatty acid composition through the use of physical as well as chemical mutagens have been reported for many oil seed crops including soybean (Patil *et al.,* 2007). And also nutritional concerns, functionality in food manufacturing and the need for high stability and extended shelf life had a tremendous impact on developing and commercializing modified oilseeds (Kavera *et al*., 2013).

In recent years, there has been increasing awareness for nutritional quality improvement of every food crop. In oilseed crops, mainly fatty acid composition determines the nutritional qualities and oxidative stability of the oil. Linseed is one of the major oilseed crop grown in India. With the growing awareness of the health there is increasing demand for quality oil by the consumers. Also, there is tremendous loss of edible oil by auto oxidation due to tropical climate and poor storage conditions leading to the demand for improved storability of oil by the traders.

Modification of fatty acid composition of oilseeds to improve oxidative stability has been the focus of work in soybean, sunflower, safflower and rapeseed (Hammond *et al*., 1997). Similar work has not been successful in linseed due to the lack of ample genetic variability for low linoleic content (Robbelen and Nitsch, 1975). From

1970s onwards, the induction of mutations by treatment of seeds with mutagenizing agents was revealed as an effective system for modifying fatty acid profile. In fact, mutagenesis has proved to be one of the most successful approaches for creating novel oil types.

In the present study, when such an attempt was made by inducing mutation, a wide range of genetic variability was obtained for the yield traits and fatty acids especially for alpha linoleic acid. The results of the present investigation are discussed below under following headings:

5.1 Variability studies

5.2 Correlation studies

5.3 Selection of promising mutants

5.4 Validation of molecular markers

5.1 Variability studies

All the 50 mutants studied in this investigation displayed a considerable amount of differences in their mean performance with respect to characters studied. This has been revealed by significant values for entries (ignoring blocks) and for varieties, which precise that the accessions under study were genetically diverse.

The mutants showed a wide range of variation for all the characters studied except for number of seeds per capsule (Table 3) and stearic acid (Table 4). The wide range of variation provides generous scope for selection of superior and desired genotypes for the plant breeder to foster crop improvement programmes.

5.1.1 Phenotypic and genotypic coefficients of variability

In the present study, phenotypic coefficients of variation were higher than genotypic coefficients of variation for all the characters, and the difference between genotypic and phenotypic variance observed was low, which indicates that the traits considered under the investigation were less influenced by the environmental factors. High values of GCV and PCV were obtained for number of primary branches per plant,

number of secondary branches per plant, number of capsules per plant, seed yield per plant (Fig. 1) which indicates that the variation in these characters greatly contributes to the total variability. The wide range of variability observed in this investigation is in accordance with the reports made by previous researchers in linseed *viz.,* Mirza *et al*. (1996) for number of capsules per plant; Mahto and Mahto (1998) for number of primary branches per plant, number of secondary branches per plant, number of capsules per plant; Chandrashekhar *et al*. (1998) for number of secondary branches per plant, number of capsules per plant. Likewise, Mishra and Yadav (1999) reported wide range of variability for number of capsules per plant; Singh (2001) for number of primary branches per plant, number of secondary branches per plant; Akbar *et al.* (2003) for number of capsules per plant; Awasthi and Rao (2005) for number of primary branches per plant, number of secondary branches per plant, number of capsules per plant, seed yield per plant; Kailashram *et al.* (2008) for number of capsules per plant, number of secondary and primary branches per plant; Pujar (2012) for number of capsules per plant, number of secondary branches per plant, number of primary branches per plant; Lodhi *et al.* (2013) for number of secondary branches per plant, number of primary branches per plant and seed yield per plant; Prasanna (2014) for number of capsules per plant, number of secondary branches per plant, number of primary branches per plant. The existence of high genetic variability for seed yield per plant in linseed is in conformity with the findings of earlier workers (Sanjiv *et al.*, 2012; Tyagi *et al.*, 2014; Vardan and Rao 2012; Singh *et al.*, 2015, Reddy *et al.*, 2013, Kanwar *et al.*, 2014 and Dandigadasar *et al.*, 2011). This indicates good scope for the genetic improvement of these traits.

This study showed moderate values of PCV and GCV for plant height, number of seeds per capsule, 1,000 seed weight and oil content. The results were in accordance with Akbar *et al*. (2003); Burako *et al.* (2010) and Suresh *et al.* (2014) for plant height. In contrast to the present investigation, Mirza *et al.* (1996) and Singh (2001) reported high variability for plant height; Lodhi *et al.* (2013) for 1,000 seed weight. Further, Tadesse *et al*. (2010) reported low values for plant height; Pujar (2012) reported low variability for oil content and Prasanna (2014) reported low variability for 1,000 seed weight and oil content. Thus the presence of moderate genetic variability as indicated by the above results for the said characters provides greater scope for improvement of these

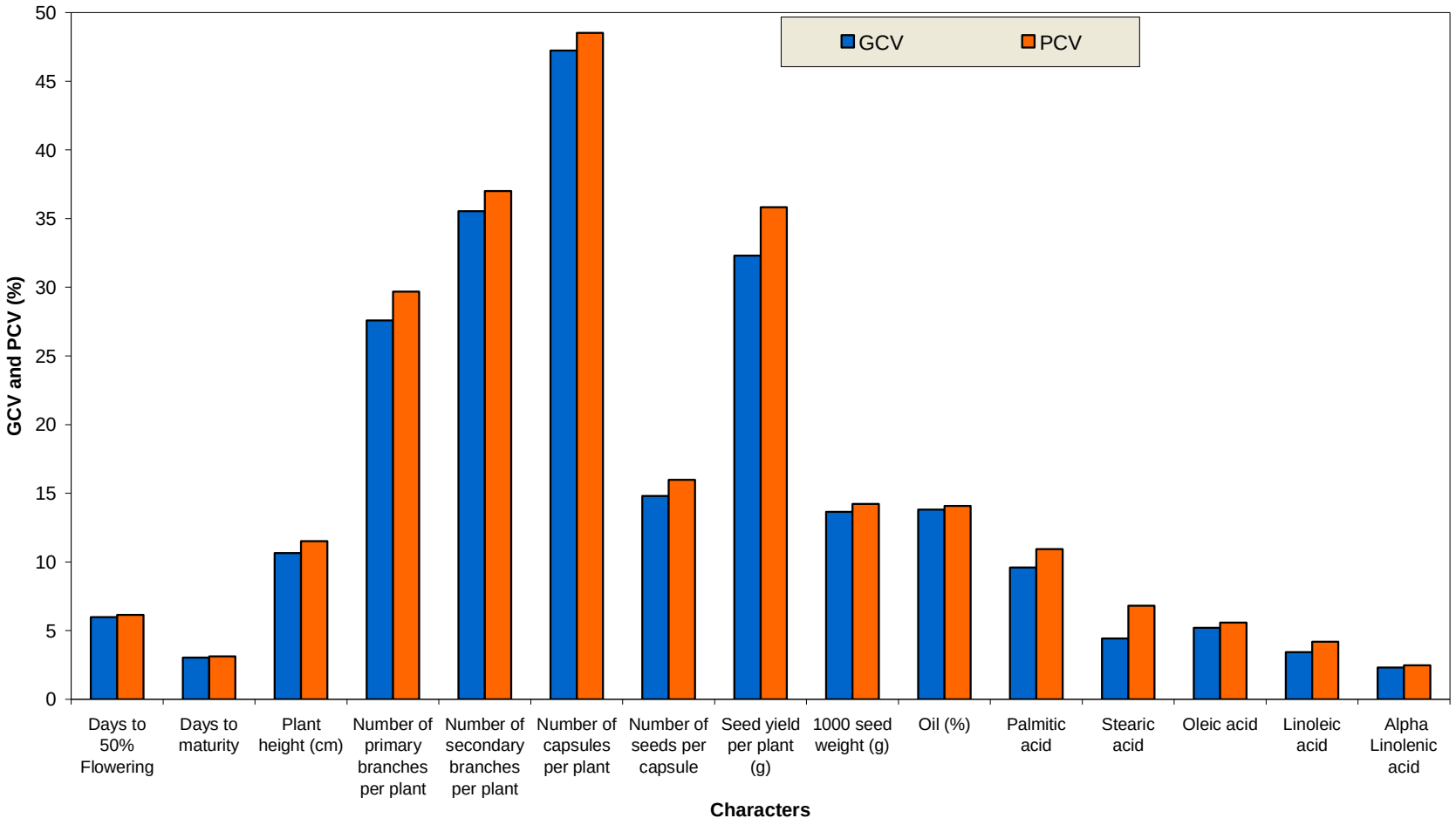


Fig. 1: GCV and PCV parameters for productivity and fatty acid composition in linseed mutants (M_5)

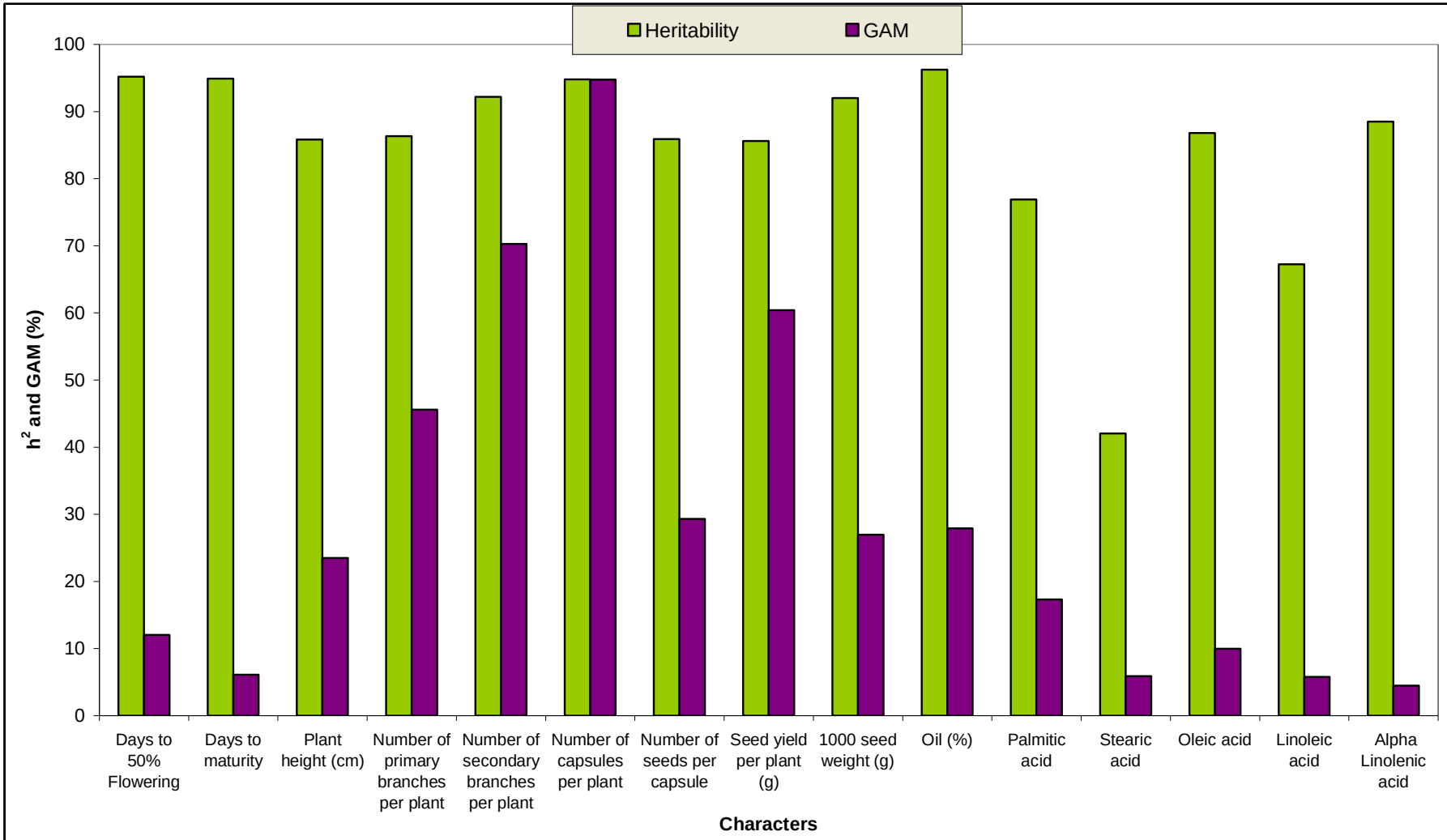


Fig. 2: Heritability and GAM for productivity and fatty acid composition in linseed mutants (M_5)

characters by direct selection. On the other hand days to fifty per cent flowering, days to maturity, palmitic acid, stearic acid, oleic acid, linoleic acid and alpha linolenic acid content exhibited low variability in the present investigation. However, Akbar *et al*. (2003) reported moderate values of PCV and GCV for days to maturity; Jeromela *et al*. (2011) reported similar findings with character days to fifty per cent flowering and days to maturity and Chandrashekhar *et al*. (1998) reported high PCV and GCV for 1,000 seed weight.

5.1.2 Heritability and genetic advance

The estimate of heritability acts as a predictive mechanism in expressing the consistency of phenotypic values. Hence, it helps plant breeders to make a selection for a particular character when heritability is high (Arulbalachandran *et al*., 2010). Ramanujan *et al.* (1967), while studying the genetic variability in red pepper, discussed the boundaries of assessing the heritability in broad sense as it includes both additive and non-additive gene effects. They concluded that heritability estimates in broad sense accompanied by genetic advance would be more reliable. As heritability is also influenced by the environment, the information on heritability alone may not be adequate in pinpointing characters enforcing selection. Therefore, an estimation of heritability coupled with genetic advance is needed to assess the heritable portion of total variation and the genetic gain expected for effective selection.

High value of heritability and predicted genetic advance indicates that selection among genotype would be effective (Ghandorah and EI-Shawaf, 1993). Heritability in broad sense estimates the ratio of total genetic variance, including additive, dominance and epistatic variance to the phenotypic variance (Falconer and Mackay., 1996). High broad sense heritability associated with high genetic advance reveals strong contribution of additive genetic variance for expression of the traits (Iqbal and Khan, 2003). To design an effective selection strategy for the utilization of the existing breeding materials, the knowledge on the extent of variation, heritability of the trait and genetic gain is important.

In the present study, most of characters showed very high estimates of broad sense heritability. The characters *viz*., days to 50 per cent flowering, days to maturity, plant height, number of primary branches per plant, number of secondary branches per

plant, number of capsules per plant, number of seeds per capsule, seed yield per plant, 1,000 seed weight and alpha linolenic acid content in seeds showed high estimates of broad sense heritability. Other characters like palmitic acid and linoleic acid showed a slightly high heritability and stearic acid recorded with low broad sense heritability.

These results are in accordance with the findings of Mirza *et al*. (1996) for plant height at maturity and number of capsules per plant; Mahto and Mahto (1998) for days to maturity; Chandrashekhar *et al.* (1998) for days to 50 per cent flowering and 1,000 seeds weight; Pradhan *et al*. (1999) for number of capsules per cent and seed yield per plant; Mishra and Yadav (1999) for days to maturity, number of seeds per capsule and number of capsule per plant; Akbar *et al.* (2003) for plant height at maturity, number of capsules per plant, 1,000 seed weight and seed yield per plant; Awasthi and Rao (2005) for seed yield per plant, number of primary branches per plant, number of secondary branches per plant, 1,000 seed weight; Nagaraja *et al.* (2009) for number of capsules per plant, plant height; Burako *et al.* (2010) for 1,000 seed weight, oil content and days to maturity; Pujar (2012) for all the traits; Yedlapalli (2014) for number of primary branches per plant, number of secondary branches per plant, 1,000 seed weight and days to fifty per cent flowering; Singh *et al*. (2015) for all the above traits.

In the present study, high heritability coupled with high per cent of mean genetic advance was obtained for the characters *viz*., plant height, number of primary branches per plant, number of secondary branches per plant, number of capsules per plant, number of seeds per capsule, seed yield, oil content and 1,000 seed weight (Fig. 2). These characters are more likely to be controlled by additive gene action and hence selection made through these characters would be effective. It provides very high response to selection due to their high heritability and genetic advance. Same results were reported by Mirza *et al*. (1996); Mishra and Yadav (1999) and Pradhan *et al*. (1999) except for seed yield per plant. Only number of capsules per plant was in accordance with Akbar *et al.* (2003). Similarly, the number of secondary branches per plant was in accordance with Tadesse *et al.* (2010). Similar results are in conformity with earlier findings of Singh *et al.* (2015) for plant height, number of secondary branches, number of capsules per plant and 1,000 seed weight.

High heritability coupled with moderate genetic advance over mean was observed for days to fifty per cent flowering and palmitic acid. Similar findings were reported by Lodhi *et al.* (2013) for days to fifty per cent flowering. However, traits like 1,000 seed weight, oil content and linoleic acid exhibited high heritability coupled with low genetic advance expressed over mean. This indicates possibility of obtaining reasonable response to selection in these traits owing to their high transmissibility. High estimates of broad sense heritability and low genetic advance may be due to presence of non-additive gene effects and high genotype and environment interactions (Panse and Sukhatme., 1957).

5.2 Correlation studies

Yield is the ultimate product in which breeder is interested. It is a highly complex quantitative character, which is governed by polygenes. Its expression depends largely on the environment, as polygenes are highly sensitive to the environment. Hence, selection of superior genotypes based on yield may not be effective. For a rational approach towards the improvement of yield, selection will be more rewarding when it is based on the components of yield. Association of yield components with yield thus, assumes special importance as the basis of indirect selection. The idea of correlation was elaborated by Fisher (1918) and Wright (1921). Breeder has to establish and understand the existing relationships between yield and yield attributing characters within a plant often arise because of either genetic linkage or pleiotropy (Heraland, 1939).

In the present study, yield traits were investigated for their relationship with yield as well as among themselves using phenotypic correlation analysis. The results obtained are discussed below.

5.2.1 Correlation between yield and its components

Number of primary branches per plant showed significant positive association with seed yield per plant. This is in consonance with reports made by Savita *et al*. (2011); Kailashram *et al.* (2008); Burako *et al*. (2010); Gauraha and Rao (2011); Pujar (2012); Prasanna (2014) and Leelavathi (2016). The number of secondary branches per plant was found to have significant positive association with seed yield per plant at

phenotypic level in the present study. This is in line with the findings of Yadav and Dalal (2001); Burako *et al.* (2010); Gauraha and Rao (2011); Pujar (2012); Prasanna (2014) and Leelavathi (2016).

Capsules per plant exhibited highly significant positive association with seed yield at phenotypic level which indicates that the number of capsules per plant may be the reliable yield indicator. Such significant association of number of capsules per plant with seed yield has also been observed by Kailashram *et al*. (2008); Ramakant *et al.*,(2008); Burako *et al*. (2010); Savita *et al.* (2011) and Rahimi *et al.* (2011); Gauraha and Rao (2011); and Pujar (2012), Pali and Mehta (2013); Reddy *et al.* (2013), Prasanna (2014) and Leelavathi (2016). This clearly indicates the increased capsule number per plant will increase seed yield hence while making selection for yield more emphasis should be given to this character.

Numbers of seeds per capsule and seed yield per plant are found to be correlated positively. This is in accordance with findings of Dandigadasar *et al.* (2011); Chaudhary *et al.* (2014); Tariq (2014); Prasanna (2014); Sanjiv *et al*. (2014) and Leelavathi (2016). Hence selection for plants having capsule with more number of seeds is likely to be effective in improving the seed yield per plant. Plant height found to be positively associated with seed yield and Alpha lilolenic acid also found positive significant association with seed yield per plant at phenotypic level which is similar to the studies Prasanna (2014). Hence, there is a scope for improving α-linolenic acid content and seed yield per plant simultaneously.

In the present investigation, negative association was observed between 1,000 seed weight and seed yield per plant which is in accordance with the previous studies Maduli and Patnaik (1994) and Kurt (1996). Days to 50 per cent flowering exhibited significant negative association with seed yield. But Joshi *et al.* (1961) observed negative but non-significant association of days to 50 per cent flowering with seed yield per plant. Similarly days to maturity exhibited negative association with seed yield per plant. This is in contrast to the results obtained by Mahto and Mahto (1998). Negative association was observed between oil content and seed yield.

5.2.2 Association among yield components

Days to 50 per cent flowering showed significant positive association with number of capsules per plant and positively associated with plant height, 1,000 seed weight, oil content and linoleic acid. There was a negative association of days to 50 per cent flowering with days to maturity, number of primary and secondary branches per plant, seed yield per plant and alpha linolenic acid which are in contrast to the results Leelavathi (2016). Days to maturity exhibited negative non significant association with the plant height.

The positive significant association of plant height at maturity with number of primary branches per plant, number of secondary braches per plant, number of capsules per plant and 1,000 seed weight and oil content was observed which indicate plant height also influences seed yield positively by associating with all these characters.

Number of secondary braches per plant exhibited positive and significant association with plant height, number of primary branches per plant, number of capsules per plant, seed yield per plant and oleic acid content in seed. This is in consonance with Jeswani and Murthy (1963); Pujar (2012) for positive association of number of secondary branches per plant with plant height. Number of capsules per plant exhibited positive and significant association with plant height, number of primary branches per plant, number of secondary braches per plant, seed yield per plant and oleic acid content. This is in consonance with the research findings of Pathak and Bajpayee (1964), Khorgade and pillai (1992), Kurt (1996) and Burako *et al.* (2010) and Pujar (2012). The result indicates that this trait is inter dependent on number of primary branches per plant will lead to increase in number of secondary branches, number of capsules and ultimately leads to increase in yield. So selection for this trait would be fruitful to improve yield. Number of capsules per plant exhibited negative significant association with days to fifty per cent flowering.

In the present investigation, oil content exhibited positive significant association with plant height and negatively associated with seed yield and linoleic acid. Seed yield was found to be positively associated with ALA content. Linoleic acid found to have positive significant association with alpha linolenic acid which indicates by increasing linoleic acid we can increase alpha linolenic acid in seed. And also linoleic acid was

positively associated with plant height, days to maturity, oil content, palmitic acid, stearic acid and oleic acid in seed.

5.3 Selection of promising mutants

Yield increment being the main objective in most of the plant breeding programmes, mutation breeding has played a key role. In the present investigation, mean performance of mutants were greater than parents for characters like number of primary branches per plant, number of secondary branches per plant, seed yield per plant and higher in ALA content (Table 7). This shows there was a positive type of mutation for these traits which are desirable to improve for yield and quality. Few of the mutants in the present experiment produced higher seed yield compared to parents (Table 7). Among the superior mutants selected for seed yield, mutant 90-3 has recorded highest mean yield of 7.08 g per plant compared to 1.821 g in check (Indira Alsi), this considerable increase in yield is owing to increase in number of capsules per plant (141) and enhanced secondary branches (19). Likewise in mutant 85-1 which recorded 6.71 g the increase in yield can be attributed to increase in number of secondary branches (17.2), number of capsules per plant (121). Similarly in other high yielding mutants *viz.*, mutant 57-2 (6.07g) and 46-1 (5.73 g) the amplified yield can be owed to improvement in yield attributing traits like number of secondary branches, number of capsules per plant and number of primary branches. Yield being a polygenic character, the yield advantage obtained might be due to the favorable gene mutation at more than one loci. Similar results were obtained by Badere and Choudhary (2007) where they isolated high yielding mutants with increased number of seeds per capsule. According to them the increase in number of seeds was due to enhancement in the yield contributing characters such as, capsules per plant and seeds per capsule. The improved mutants need to be evaluated for stability and if found consistently superior, it could be released as a variety.

Linseed oil is mainly industrial oil with high potential, due to its drying nature linseed oil is used for the production of paints, varnishes, inks and linoleum. The improvement in seed yield, oil yield and plant type of this crop would be helpful in increasing its quality (Badere and Choudhary, 2004). Therefore attempts have been

made to enhance the oil content by different workers Rath and Scharf (19680; Seetharam (1972); Green and Marshall (1981); Badere and Chaudhury (2004).

Linseed is being grown for its seed oil and therefore the improvement in seed oil percent is the major breeding objective in this crop (Salas and Friedt, 1995). In this study large variation was observed for oil content in linseed mutants. The oil content ranged from 21.74 per cent to 49.40 per cent with a mean value of 33.02. There was decrease in the mean performance of oil percentage compared to parents (Table 7) but increase in the yield can ultimately increase the oil productivity per unit area.

Fatty acid analysis of the flax seeds showed presence of five major fatty acids with predominance of 18 carbon species, α-linolenic acid (ALA 18:3), linoleic acid (18:2), oleic acid (18:1), stearic acid (18:0) and palmitic acid (16:0), all of which are members of the same pathway catalyzed by the elongase and desaturase enzymes. The amount of total poly unsaturated fatty acids in selected varieties was 63.1 per cent to 71 per cent and mono unsaturated fatty acids from 16.3 per cent to 21.9 per cent, while the amount of total saturated fatty acids ranged from 3.8 per cent to 12.8 per cent of the total oil. There are several reports of total unsaturated and saturated fatty acids in linseed seeds varying from 87 to 91 per cent and 9 to 12 per cent, respectively (Bhatty, 1995; El-Beltagi *et al.*, 2007; El-Beltagi *et al.*, 2011). The unique feature of linseed is the accumulation of large amounts of linolenic acid (Omega 3), the final product of three desaturation steps. Fatty acid analysis of the mutants generated in our study showed highest linolenic acid of 54.3 per cent in mutant 43-5 than its parent Indira alsi (31.87) followed by mutant 57-2 (53.4). These mutants with higher linolenic acid with higher seed yield can be further studied to use as high ALA source in pharmaceutical field and as high yielding varieties.

5.4 Validation of molecular markers

In recent years, molecular markers technology has greatly accelerated the breeding programme for the improvement of various crops. Among various markers, SSR markers are the choice for various genetic studies because of their random distribution throughout the genome, variable number of tandem repeats such as di, tri, tetra nucleotides, hyper variability, reproducibility, co-dominant nature and locus

specificity. Molecular markers linked with QTL/genes are being routinely identified in several crops using mapping populations such as F_2, RIL, DH populations, germplasms *etc.* Many molecular markers for traits of interest have been identified using germplasm collections (Virk *et al.,* 1996; Maccaferri *et al.,* 2005; Breseghello *et al.,* 2005; Skot *et al.,* 2005). At present study SSR markers associated with linseed yield and yield attributing traits were validated.

Recently many SSR markers have been developed using different approaches (Deng *et al.,* 2009), and also markers associated with different traits (Hasan *et al.,* 2008; Qin *et al.,* 2015) which can be utilized for number of further studies (Pali *et al.,* 2014; Cloutier *et al.,* 2011).

Once the markers are identified, they need to be validated to utilize them in further studies as it may lead to false positives, validation will remove those false positives and gives the accurate markers to use. Validation of several markers had been done in many crops by da Silva *et al.* (2007); Fondevilla *et al.* (2008); Sheeba *et al.* (2009); Kaur *et al.* (2012); Sukruth *et al.* (2015).

In the present study thirteen SSR markers (Table 2) reported earlier by Prasanna (2014) were used for genotyping linseed mutants. Scoring given to amplified products were subjected for single marker analysis to find the association of markers to different traits. Out of thirteen markers, five markers (Lua60, Lua49B, Lub14, Lu138, Lu144a) which were reported to be linked to ALA content (Lua60, Lua49B), oil content (Lub14) and number of capsules per plant (Lu138, Lu144a) were validated in the present study. Besides these traits, Lu144a was found to be associated with plant height and secondary branches per plant and Lua49B was associated with plant height which were not associated earlier. Similar results were observed in Gajjar *et al.* (2014); Anita *et al.* (20110; Tsilo *et al.* (2008).

Hence, above association studies could provide valuable information to screen the germplasm accessions for the specific traits of interest using the linked SSR marker. The genetic diversity analysis based on morphological data will facilitate the breeding efforts towards the development of nutraceutically improved, high yielding linseed varieties using markers linked to oil and ALA content.

Future line of work

1. Superior mutants identified with desirable fatty acid profile (increased alpha linoleic acid) need to be further tested for their agronomic performance in large scale trials over locations and in *rabi* seasons for confirming the stability and superiority.

2. The promising high linolenic acid containing mutants can be used as parents in future breeding programme to alter fatty acid composition for oil with high linolenic acid.

3. These mutants can be screened for their lignan (SDG) content.

4. Molecular markers (SSR) validated in the present study could be used for marker assisted selection in linseed.

6. SUMMARY AND CONCLUSIONS

Genetic variability is essential for the continued progress in breeding as well as for adaptation to future environmental challenges. The primary objective of induced mutation is to enhance the variability and to create desirable and heritable variations in them. With this view, the present investigation was undertaken to study induced genetic variability for desirable traits in two promising varieties of linseed *viz.,* Indira Alsi and NL-115 which are in M_5 population and to validate the markers which were previously reported for different traits. The salient findings of present study are summarized as below.

1. Data on each plants for days to fifty per cent flowering, days to maturity, plant height, primary branches per plant, secondary branches per plant, number of capsules per plant, number of seeds per capsule, seed yield per plant, test weight, oil content and fatty acid composition were recorded in the M_5 generation.

2. Analysis of variance revealed highly significant difference among the genotypes for all the characters studied except number of seeds per capsules and stearic acid content.

3. Genotypic and phenotypic coefficients of variation were high for number of primary branches per plant, number of secondary branches per plant, number of capsules per plant and seed yield per plant.

4. The characters *viz.,* plant height, number of primary branches per plant, number of secondary branches per plant, number of capsules per plant, number of seeds per capsule, seed yield, oil content and 1000 seed weight exhibited high heritability coupled with a high genetic advance as per cent of mean which indicated that simple selection scheme would be sufficient for these traits to bring about genetic improvement.

5. Number of primary branches per plant, number of secondary branches per plant, number of capsule per plant and number of seeds per capsules showed significant positive association with seed yield per plant. The result indicates that this trait is inter dependent on number of primary branches per plant which

will lead to increase in number of secondary branches, number of capsules and ultimately leads to increase in yield. So selection for this trait would be fruitful to improve yield. . Emphasis on these characters must be given in selection programme which ultimately improves the grain yield.

6. Alpha linolenic acid content in seeds exhibited significant positive correlation with seed yield per plant at phenotypic level. Hence, there is a scope for developing nutraceutically enriched high yielding varieties of linseed.

7. Screening of previously reported 13 SSR markers with parents and mutants resulted in identification of five polymorphic markers (Lua60, Lua49B, Lub14, Lu138, Lu144a).

8. Single marker analysis was performed to study marker–trait association. This showed significant association for five SSR markers with different traits under study.

9. The marker Lu144a was found to be associated with number of secondary branches per plant with R^2 value of 17.67. Two markers namely, Lua49B and Lua60 were associated with ALA content in seed with R^2 of 6.04 and 7.93 respectively. Marker Lub14 was found to be associated with oil content with R^2 value 17.46.

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VALIDATION OF MOLECULAR MARKERS LINKED TO QUALITY AND QUANTITATIVE TRAITS IN ADVANCED MUTANT POPULATION OF LINSEED (*Linum usitatissimum* L.)

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ABSTRACT

In the present investigation, fifty mutants (M_5) derived from two linseed cultivars *viz.,* Indira Alsi and NL-115 were sown in *rabi*, 2016-17 at AICRP on MULLaRP, MARS, Dharwad and evaluated for genetic variability and used to validate molecular markers previously reported to be associated with yield and quality components.

Higher GCV and PCV coupled with higher heritability and genetic advance were obtained for seed yield per plant, number of capsules per plant, number of secondary and primary branches per plant and also showed positive significant association between them. Hence, these characters are amenable for selection process and importance should be given for these traits to improve yield potential. Alpha linolenic acid (ALA) had positive significant association with seed yield which can be used as a source of high linolenic acid for pharmaceutical and health benefits.

Fatty acid analysis of the mutants generated in our study showed highest linolenic acid of 54.3 per cent in mutant no “43-5” with increased seed yield of 170 kg/ha followed by mutant no “57-2” (53.4 %) with seed yield 202 kg/ha. Higher seed yield was recorded in mutant no “90-3” with 236 kg/ha. These mutants with higher linolenic acid with higher seed yield can be used as genetic stock for breeding of high yielding and genotypes with rich omega-3 fatty acid.

In the present study thirteen SSR markers which are previously reported to be associated with yield and yield attributing traits were used for genotyping linseed mutants and to find the association of markers to different traits. Out of thirteen markers, five markers were validated which are reported to be associated with ALA content (Lua60, Lua49B) with R^2 value 6.04 and 7.93, oil content (Lub14) with R^2 value 17.46 and number of capsules per plant (Lu138, Lu144a) with R^2 value 10.61 and 11.91 respectively.