

Linseed improvement in oil and fibre quality

Vikender Kaur¹ and Rashmi Yadav²

National Bureau of Plant Genetic Resources, New Delhi 110 012

Linum usitatissimum (Linseed or flax) is important for the seed oil, stem fibre, paper, wax, nutraceutical and food processing industries. Flax seeds are being used for fortification of food products as a health benefit ingredient because they are a rich source of micro-nutrients, dietary fiber, vitamin B₆ and the essential fatty acid α -linolenic acid, also known as ALA or omega-3. Recently, there has been a growing interest in linseed as a functional food due to its probiotic and anticancer properties. However, linseed oil is not much used for cooking in spite of its health benefits due to occurrence of rancidity in the oil after storage. Hence, efforts have been made to lower the linolenic acid content in the oil to make linseed oil 'premium' cooking oil. The present article is intended to appraise the readers about the major developments in linseed improvement in oil and fibre quality. Moving ahead from the conventional approaches like selection and hybridization for improving grain and fibre yield, biotic and abiotic stresses; induced mutagenesis was resorted to for conferring novel properties like low linolenic acid content to the crop. Later, with the progress in biotechnology and molecular biology tools genetic manipulation was also attempted in linseed to improve its oil and fibre quality, resistance to pathogens etc.

Key words: Fibre, Linseed, Oil

LINSEED (*Linum usitatissimum*) is an annual, self-pollinated species believed to have originated in either the Middle-East or Indian regions and spread throughout Asia and Europe, prior to its introduction into the New World. Great genetic diversity of this crop present in Indian sub-continent. It is an important crop world-wide, the total world production of linseed reached approximately 2.5 million tonne in 2014, with Canada (34%), Russian Federation (15%) and China (13.6%) being the main producers. India is an important linseed growing country in the world ranking fourth in area (13%) and sixth in production (5.5%), respectively (Refer EAOSTAT, 2016). Currently the fibre type is the third largest textile fibre crop, and the oil-type is fifth oil crop in the world although with a magnitude lower production than the major fibres (cotton and jute) or



other oil crops (maize, soybean, palm and *Brassica*).

The terms 'flaxseed' and 'linseed' are often used interchangeably, although North Americans use 'flaxseed' to describe flax when it is eaten by human beings and 'linseed'

to describe flax when it is used for industrial purposes. In Europe, the term 'flaxseed' describes the varieties grown for making linen. Linseed oil has edible as well as industrial uses. It contains high i.e. more than 50% of α -linolenic acid (ALA); a polyunsaturated fatty acid with high degree of unsaturation which provides quick drying properties to the oil. Thus, it was once popular as a drying agent in paints and varnishes. Besides being popular as a drying agent in paints and varnishes, diverse uses of linseed oil include the manufacturing of hardboards, brake linings, printers' ink, caulking compounds, manufacturing of linoleum, oil-cloth, and soaps. Although, the petroleum-based products have replaced linseed oil in such application but they pose threat to the environment. Hence, the interest in linseed oil is rejuvenating in present era. Its stems produce

good quality fibers that are flexible, lustrous and soft so are used in high value linen products and have many end uses including paper, technical fiber, thread/rope and packaging materials, biofuels, and fiber is being used as the interior panelling of some cars. The straw and short fibre for pulp to produce special papers for cigarettes, currency notes and artwork; and the wooden part serves as biomass energy or litter in cattle farming.

Linseed oil is also used as cooking medium, to some extent as lower grade cooking oil or *salad* oil, mainly in India and former USSR. As such the linseed oil is considered to be good for health; however, at the same time high degree of unsaturation makes it prone to oxidation, which is responsible for rancidity in the oil. This rancidity makes the oil lesser desirable as a cooking medium; thus, impeding its popularity. Hence, the major breeding objective in linseed is to lower the α -linolenic acid content in its oil and make the oil suitable for cooking without compromising the health benefits.

QUALITY IMPROVEMENT

Hybridization and mutations methods are used widely, through initially, introduction and pure line selection were the employed in improvement of linseed. Later, brief description and salient features of linseed varieties released in recent years are discussed in Table 1. Recently Biotechnology and Genetic Engineering have also emerged as effective way towards improvement of linseed.

DEVELOPMENTS IN LINSEED

Conventional breeding

Hybridization in linseed was first started in India in 1924 at IARI, New Delhi with a view to combine high yield, high oil and bold seededness in new varieties. Pedigree and modified pedigree methods have been used mainly along with backcrossing in some cases. Later on, modification of fatty acid profile was also attempted to reduce the α -linolenic acid levels through inter-specific hybridization. However,

these attempts were not successful due to pollen sterility, chromosomal aberration and incompatibility. Even, inter-varietal crosses would have not been successful in this regards due to insufficient variation in fatty acid composition of the varieties. To improve the yield potential, direct selection based on capsules per plant and seed index and reciprocal recurrent selection to concentrate maximum favourable genes for yield and its components were proposed. Alongwith this, simultaneous consideration of plant height and number of branches, while making selections, was also suggested to improve yield. Capsules per plant and 1,000 seed weight were major yield components; thus, suggesting due weightage for both these components, in formulating selection programme were aimed at the improvement of yield.

Linseed varieties for culinary uses

'Solin' (Linola™ 947), a mutant strain of linseed having fatty acid profile suitable for culinary uses was developed in Australia in early 1990's by the Commonwealth Scientific and Industrial Research Organization (CSIRO) and first commercially grown in 1994. Solin is the generic name for low-ALA flax; the trade-marked name is Linola™. Here 'sol' refers to sun and light and 'lin' is for linseed. Solin oil has less than 5% ALA and is being used in premium margarines, especially in Europe. Linola™ 989 refers to the second solin cultivar released; developed by backcross breeding method followed by pedigree selection. Yield of Linola™ 989 varies from 1,960 to 2,190 kg/ha, depending upon the soil. It contains 46.2% oil with 2.6% linolenic acid. Linola™, 1,084 is another yellow seeded, low linolenic acid flax cultivar. Its yield and oil content is higher than both Linola™ 989 and Linola™ 947. At the same time total saturated fats are also lower than the other two. It is immune to North American races of rust and is moderately resistant to *Fusarium* wilt.

Induced mutagenesis

Both, chemical and physical

mutagens were used for mutating linseed which includes ethyl methanesulphonate, nitrosomethylurea, sodium azide, X-rays, gamma (γ) rays etc. More than last three decades have witnessed radical changes in linseed only due to induced mutations. Mutagenizing linseed by EMS was successful in modifying the fatty acid composition. Later several viable mutants affecting height, branching, leaf size, flower colour, seed colour and size, high seed number, high test weight, high oil content, high meal protein content, and reduced linolenic acid were isolated.

Biotechnology

In the improvement of linseed, several biotechnological approaches have been attempted. Regeneration of whole plant from the hypocotyl, cotyledons and about induction of somatic embryogenesis in linseed were successful. For the time plants were regenerated from the protoplasts of *Linum usitatissimum* as well as from wild species of *Linum*, such as *L. lewisii* and *L. strictum*. Protoplast fusion of cv. Linda with those of wild species of *Linum* was also attempted and was successful. Inter-simple sequence repeat and randomly amplified polymorphic DNA (RAPD) primers has been utilized in linseed. Plants derived from the same callus had identical Polymerase Chain Reaction (PCR) patterns at five loci. Thus, they were likely to be derived from the same microspore.

Genetic engineering is indispensable when some new trait like production of epoxy fatty acid in linseed is to be conferred to plant. Stable transformation, in progenies of transgenic flax plants using an *A. tumefaciens* strain, carrying a disarmed Ti-plasmid vector containing a chimeric npt-II gene and a glyphosate resistance gene has been reported. Transformed plantlets with curled leaves, short internodes and a root system characterized by plagiotropic behavior were produced and no detrimental effect of the T-DNA on important agronomic traits was found. Herbicide resistant genes have also been successfully used in transformation experiments.

Table 1. Brief description and salient features of linseed varieties released.

Variety	Year of release	Oil content (%)	Average yield (kg/ha)	Recommended states/ Regions	Specific features
LC-2063	2008	41	1,200 Irrigated	Irrigated areas of Punjab	Tall plant, medium maturity, and blue brown seed,
Azad Alsi-1 (LMS 9-2K)	2008	39.9	1,610 Irrigated	Bundelkhand of Madhya Pradesh and Rajasthan	Disk shape violet blue flower, dark brown seed, resistant to rust, wilt, powdery mildew and bud fly
Himani (KL-214)	2008	36.4	583 Utera	Himachal Pradesh, Jammu and Kashmir, Haryana and Punjab	Blue flowered, small brown, seeded moderately resistant to rust and powdery mildew
PKV NL 260	2009	37.7	963 Rainfed	Maharashtra	Medium height, early, light violet funnel-shaped flower, moderately resistant to <i>Alternaria</i> blight, powdery mildew, and bud fly
Shival (SLS 67)	2010	40.2	1,252 Rainfed	Bundelkhand of Uttar Pradesh, Madhya Pradesh and Rajasthan	Dwarf, star-shaped, early maturity, light brown seed, moderately resistant to powdery mildew and rust
Jawahar Linseed-66 (SLS 66)	2010	42.8	1,200 Rainfed	Madhya Pradesh	Resistant to rust, moderately resistant to wilt, powdery mildew, and matures in 109 to 120 days
Bhagsu (KL-215)	2010	36.4	428 Utera	Punjab, Haryana, Himachal Pradesh, Jammu and Kashmir, and Uttarakhand	Matures in 175-201 days, resistant to rust and moderately resistant to <i>Alternaria</i> blight, powdery mildew and wilt
Ruchi (LCK-5021)	2011	39.9	1,366 Irrigated 1,055 Rainfed	Uttar Pradesh (Excl. Bundelkhand), Bihar, Jharkhand, West Bengal, Asom and NEH Region	Matures in 129-135 days, resistant to rust, wilt and powdery mildew
MAU Azad Alsi-2 (LMS-149-4)	2011	40	1,000 Rainfed	Chhattisgarh, Maharashtra, Karnataka, and Odisha	Early to medium duration maturity, resistant to rust, wilt, powdery mildew and bud fly
Jawahar Linseed-41 (PKDL-41)	2012	40	1,600 Irrigated	Madhya Pradesh	Resistant to wilt, powdery mildew, rust and tolerant to <i>Alternaria</i> blight and bud fly, and matures in 115-120 days
Pratap Alsi 2	2013	41.8	1,957 Irrigated	Rajasthan	Matures in 129-135 days, moderately resistant to <i>Alternaria</i> blight, powdery mildew and wilt

Source: *Status Paper on Oilseeds (2014)*, Oilseed Division, Department of Agriculture and Co-operation, Union Ministry of Agriculture, Government of India.

Resistance to stress has also been conferred to linseed through genetic manipulations. Transgenic linseed with increased pathogen resistance was produced by introduction of chitinase gene and α -1,3 glucanase DNA from potato.

Transgenic linseed over-expressing key enzymes of flavonoid biosynthetic pathway was produced to impart enhanced stability to the oil. This transgenic linseed expressed genes encoding chalcone synthase, chalcone isomerase and dihydroflavonol reductase which resulted in significant antioxidant property. The fiber quality of linseed has been greatly improved through genetic manipulations. The lowering of lignin content of fiber by silencing CAD gene; substantial improvement in elastic properties of linseed fiber by transforming linseed with genes encoding β -ketothiolase (Phb A), acetoacetyl-CoA reductase (Phb B)

and PHB synthase (Phb C) has been achieved. Besides, increased retting properties of linseed fibers by over expression of polygalacturonase and rhamnogalacturonase has been reported also.

SUMMARY

Flax oil is widely recognized for its probiotic properties having beneficial effects on coronary heart disease, neurological and hormonal disorders, colon tumor, breast cancer and atherosclerosis. Because of various health benefits, the demand for whole and milled-flax seed, and cold pressed flaxseed oil is increasing; where seeds and oil are being used for fortification of food products as a health benefit ingredient. Hence, the interest in linseed is rejuvenating in present era. The narrow genetic base used for the development of linseed cultivars, scarce availability of related species to incorporate new variation,

lack of hybrid production systems and the limited molecular breeding have hampered yield and quality improvements, limiting linseed competitiveness. To improve economic value of linseed, varieties with fatty acid profile tailored to specific need, good meal and fiber quality, high yield and stress tolerance are needed. The whole genome sequence achieved in 2012 is enabling more detailed study of the genes and diversity in commercially important accessions of linseed. Further research is important for adapting linseed cultivation to changing environmental conditions or to changing market needs as well as its continued improvement into a truly multi-purpose crop.

¹Scientist (GED), ²Scientist, Division Genomic Resources. Corresponding author's (e mail: drvikender@yahoo.in)