

Omega-3 its Significance and Breeding Approaches in Linseed (*Linum usitatissimum* L.)

Satyapal Singh* and Parmeshwar Kr. Sahu

Department of Genetics and Plant Breeding,

Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh

*Corresponding Author E-mail: spsinghikv@gmail.com

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ABSTRACT

Flax (Linum usitatissimum L.) is currently focused on enhancing the oil content and nutritional value to meet the demand of nutraceutical market supply, as an alternate source of fish oil, a rich source of eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6). Flax seed is also rich in soluble and insoluble fibers and lignans, makes it useful as a dietary supplement. Intake of flaxseed in daily diet may reduce the risk of cardiovascular diseases such as coronary heart disease and stroke. Omega 3 fatty acids are an object of considerable medical and nutritional research for a wide range of human health conditions and disorders, including inflammation, joint and bone health, menopause, aging and obesity. With FDA-approved health claims that omega 3's may lower the risk of cancer and coronary heart disease, adults are increasingly seeking to incorporate these essential fatty acids into their daily diets. Breeding for fiber yield can be divided into two components, straw yield and fiber content. Linseed oil has more than 50% linolenic acid which is fit for its industrial application but where linseed oil is being used as edible oil, the linolenic acid needs to be reduced. Efforts in this direction have already been successful with the development of low linolenic acid varieties LINOLA in Australia in 1984 and SOLIN in Canada in 1990. In India too national linseed program in collaboration with BARC, Mumbai has developed some genotypes with less than 1% linolenic acid. Hence, the breeding efforts are needed further for development of low linolenic acid varieties, the oil of which can be widely used as cooking oil.

Key words: Flax, Fatty acid, Genetics, Omega-3, Oilseed.

INTRODUCTION

Omega-3 and omega-6 are types of essential fatty acids - meaning we cannot make them on our own and have to obtain them from our diet. Both are polyunsaturated fatty acids that differ from each other in their chemical structure. In modern diets, there are few

sources of omega-3 fatty acids, mainly the fat of cold water fish such as salmon, sardines, herring, mackerel, black cod, and bluefish. There are two critical omega-3 fatty acids, (eicosapentaenoic acid, called EPA and docosahexaenoic or DHA), that the body needs.

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Vegetarian sources, such as walnuts and flaxseeds contain a precursor omega-3 (alpha-linolenic acid called ALA) that the body must convert to EPA and DHA. EPA and DHA are the building blocks for hormones that control immune function, blood clotting, and cell growth as well as components of cell membranes.

Flax (*Linum usitatissimum* L.) is a valuable spinning and oil-yielding agricultural crop. Advanced technologies for processing natural fibers make it possible to produce an ecologically clean rich assortment of products. In the world production of textile fabrics, priority is given to flax fiber showing higher medicobiological and physicochemical properties than cotton fiber. Demand for flax and flax containing textile fabrics increases from year to year owing to such a unique set of flax properties as hygienic ability, high strength, low electric resistance, dust capacity, comfort ability, and natural bactericidal action (antiseptic and antiputrefactive action). The share of flax and flax containing fabrics in the total volume of textile fabric output in the world will increase to 70% by 2010 according to prognoses of leading French specialists. Whole flax seed (ground meal, powder or intact seed) contains 28% dietary fiber, (7 – 10% soluble fiber, 11 - 18% insoluble fiber), 40% fat (73% of it being polyunsaturated fatty acids), and 21% protein. Other flax seed nutrients include vitamins E and B, phytosterols, and mineral nutrients such as calcium, iron, and potassium. More than 50% of the fat in flax seed is an essential omega-3 fatty acid called alpha-linolenic acid (ALA), which makes flax seed the richest plant source of dietary omega-3 fatty acids. Flax seed is rich in antioxidants, such as lignans (also a phytosterogen) and other phenolic molecules. Unlike the ground meal and powder, flax seed oil contains no dietary fiber.

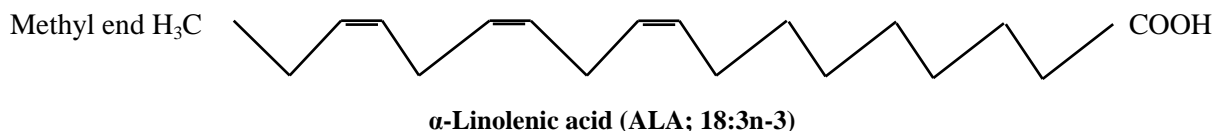
Omega-3 and omega-6 are types of essential fatty acids - meaning we can't make them on our own and have to obtain them from our diet. Both are polyunsaturated fatty acids that differ from each other in their chemical structure. In modern diets, there are few sources of omega-3 fatty acids, mainly the fat

of cold water fish such as salmon, sardines, herring, mackerel, black cod, and bluefish. There are two critical omega-3 fatty acids, (eicosapentaenoic acid, called EPA and docosahexaenoic or DHA), that the body needs. Vegetarian sources, such as walnuts and flaxseeds contain a precursor omega-3 (alpha-linolenic acid called ALA) that the body must convert to EPA and DHA. EPA and DHA are the building blocks for hormones that control immune function, blood clotting, and cell growth as well as components of cell membranes. Linseed oil has more than 50% linolenic acid which is fit for its industrial application but where linseed oil is being used as edible oil, the linolenic acid needs to be reduced. Efforts in this direction have already been successful with the development of low linolenic acid varieties LINOLA in Australia in 1984 and SOLIN in Canada in 1990. In India too national linseed programme in collaboration with BARC, Mumbai has developed some genotypes with less than 1% linolenic acid. Hence, the breeding efforts are needed further for development of low linolenic acid varieties, the oil of which can be widely used as cooking oil. As linseed is highly nutritious, efforts are needed to reduce its anti-nutrient components and also bio-convert its less acceptable omega-3 ALA into acceptable SDA. For achieving this objective, in addition to the conventional breeding, the biotechnological tools like marker-assisted breeding and genetic engineering may also be employed. Moisture stress being one of the major constraints, the varieties with inbuilt water stress tolerance may be given more emphasis to enhance and stabilize the productivity for making this crop more remunerative. More concerted efforts for development of varieties resistant to different diseases like wilt, rust, powdery mildew and *Alternaria* blight are also required by using the different resistant donors already available in this crop.

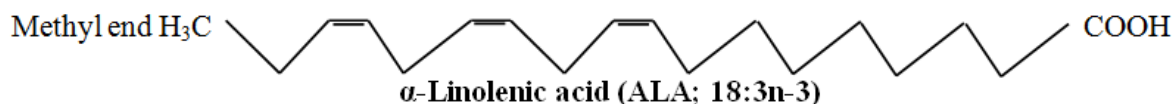
Types of Omega-3 Fatty Acids

1. Alpha-linolenic Acid (LNA) [18:3w3]
2. Eicosapentaenoic Acid (EPA) [20:5w3]
3. Docosahexaenoic Acid (DHA) [22:6w3]

Omega-3 Polyunsaturated Fatty Acids



Omega-3 Polyunsaturated Fatty Acids



Omega-3 fatty acids - More than half the fat in flaxseeds is alpha-linolenic fatty acid (ALA), the essential omega-3 fatty acid. Scientific studies reporting health benefits for omega-3 fatty acids show that these fatty acids are required for proper infant growth and development. Cholesterol can be reduced by adding flax to the diet. New research also suggests that ALA offers protective effects against both coronary heart disease and stroke. Omega-3s also have been shown to protect against hypertension, and inflammatory and autoimmune disorders. Long-term studies of flax effects on breast cancer now are underway.

Omega-6 fatty acids - An essential fatty acid, linoleic is the chief polyunsaturated fat in the North American diet. Most omega-6 fatty acids in the diet come from vegetable oils.

Ratio of omega-3s to omega-6s - Studies of hunter-gatherer populations show their diets contained roughly equal amounts of omega-6 and omega-3 fatty acids. Currently, researchers and nutrition experts recommend people replace some omega-6 fatty acids in their diet with omega-3 fatty acids like those found in flax.

Fatty Acid Composition of Flax Oil

Percent of total fatty acids	
Saturated fatty acids	9%
Monounsaturated	18%
Polyunsaturated fatty acids	
Omega-3 fatty acids	57%
Omega-6 fatty acids	16%

Genetics of Omega-3 in linseed

The size of the flax genome (686 Mbp) is around four times that of Arabidopsis and recently different research teams have developed reverse genetics and genomic approaches to learn more about fibre and seed

formation in this economically-important species.

For linoleic and linolenic acid content of the seed oil two QTLs were identified; this was expected since the E1747 parent was known to be a double mutant for two

desaturase genes that are involved in the proportion of linolenic and linoleic acid. Furthermore, this study showed that fiber content is a complex trait but accumulation of favourable alleles with additive and duplicate gene effect will result in high fiber content values. For the remaining traits co-localization of QTLs is discussed in terms of pleiotropic effects of single loci.

Fatty acid composition of seed oil - linoleic / linolenic acid content

As expected the trait values for linoleic and linolenic acid show a strong negative correlation ($r = -0.99$) in the RILs of VxE, because both components depend on the same substrate. Although linoleic and linolenic acid QTLs have been shown as separate traits on the VxE map, it is obvious that the minus allele for one trait should be allelic to the favourable allele for the other. The two QTL for both linoleic as linolenic acid (OLE-VxE_6.1/OLN-VxE_6.1 and OLE-VxE_13.1/OLN-VxE_13.1) are highly significant with LOD-values exceeding 30 and the sum of the explained phenotypic variance at both loci reached ~ 90%. This suggests that discrete classification of genotypes is nearly possible. The expected four genotypic classes are indicated and only the V1V1v2v2 and v1v1V2V2 classes showed overlap, where the symbol V1 and V2 represent the Viking derived dominant desaturase alleles at locus OLE-VxE_6.1/OLN-VxE_6.1 and OLE-VxE_13.1/OLN-VxE_13.1, respectively. Four groups are expected and indeed observed, because heterozygosity is highly improbable in F9 RILs.

Inheritance of polyunsaturated fatty acid composition

As expected two QTLs were identified that are involved in the proportion of linolenic and linoleic acid, because the E1747 parent was known to be a double mutant genotype for the two desaturase genes (Rowland 1991). The strong negative correlation between linoleic and linolenic acid is in close correspondence with the r-values reported by Green (1986) and Rowland (1991). This strong negative correlation suggests that the two QTLs

detected for linoleic acid and linolenic acid are allelic. Therefore, we assume that the two loci (OLE-/OLN-VxE_13.1 and OLE-/OLN-VxE_6.1) detected are the two mutated desaturase genes, which were present in the E1747 parent. It has reported a significant difference between the activities of both loci by comparing the two single mutant genotypic classes. This is in agreement with differences observed in sensitivity and activity of the desaturase genes in flax to high temperature during seed maturation (Green 1986).

Taxonomic status

The Linaceae family comprises of 22 genera of which genus *Linum* is the most wellknown. The more than 200 species present in the genus *Linum* are divided in five subsections (Tutin *et al.* 1968), of which subsection *Linum* contains the cultivated species *L. usitatissimum* L. and the ornamentals *L. grandiflorum* and *L. perenne*. However, the latter two species are of little economic importance. The number of chromosomes of the *Linum* species show a wide range varying from $2n = 16$ to $2n = 72$ (Fedorov 1974). *L. usitatissimum* and its wild relatives contain $2n = 30$ chromosomes (Muravenko *et al.* 2003). **The genome size (1C) of cultivated flax is 686 Mbp (Bennett and Leitch 2004).**

History of flax breeding

Centre of origin

The origin of flax (*Linum usitatissimum* L.) is uncertain. Some authors consider *L. bienne* as the progenitor of small seeded flax, originating from Kurdistan and Iran, whereas others consider *L. angustifolium* containing high oil content and seed weight, as progenitor, originating from the Mediterranean region (Murre 1955; Zeven and de Wet 1975). Other authors 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 (Tutin *et al.* 1968; Zohary and Hopf 1993). Recently, a study with molecular markers suggested that the three species originate from one common ancestor, *L. angustifolium* being most ancient (Muravenko *et al.* 2003). While *L.*

usitatissimum is an annual crop species, the wild forms can also be biannual or perennial. All species are predominantly self-pollinated (Zohary and Hopf 1993). Cross pollination may occur via honey bees (Williams 1988) or by artificial means.

Domestication

Flax was already grown 6000 – 8000 years ago in Egypt and Sumaria, and belongs (together with barley and wheat) to the oldest of cultivated plants. The distribution of flax from the Near East into Europe is well documented (Zohary and Hopf 1993). It is considered that flax cultivation in Western Europe (the Netherlands, Northern France, Belgium and Switzerland) started about 5000-3000 BC when semi-nomads, originating from the Middle East settled in Flanders and introduced flax cultivation (Dewilde 1983). Since the domestication of flax, there has been a preference for growing flax either for its fiber or oil. In the Western region of Eurasia, flax is mainly grown for its fiber, whereas in the Eastern region of Eurasia flax is grown for its oil (Gill 1987). Fiber flax has a long unbranched growth habit, whereas linseed (oil flax) is much shorter and highly branched. Throughout this thesis the distinction between fiber flax and linseed is made. *Modern plant breeding* Similar to most agricultural crops, commercial breeding of flax started at the end of the 19th century. However, already in 1816 Gelf Jensma developed the landrace Friesche Witbloei. First, he selected white flowering plants from blue flowering flax from Russia. Then after several rounds of selection in the white flowering plants, the landrace Friesche Witbloei originated with long stems (De Jonge 1942). Traditional flax breeding in the Netherlands was based on mass selection. Around 1900, pedigree selection of flax was introduced by prof. Broekema which later resulted in the high performing high yield or well performing cultivars Concurrent (Dorst), Wiera (Wiersema) en Resistentia (Hylkema) (De Haan 1952; Murre 1955). Nowadays, several breeding methods are available, but the pedigree method is the most common one used in flax breeding (Salas and Friedt 1995).

Utilization of flax as a crop species

The dual purpose of flax was already known in ancient times. In ancient Egypt, linen (derived from the fiber) was used for wrapping the royal mummies and additionally linseed oil was used to embalm the bodies of deceased Pharaohs (Dewilde 1983). For a long time flax has been cultivated as a dual-purpose crop, but nowadays fiber flax and linseed represent different gene pools. Fiber flax has been cultivated in the Netherlands and most likely in Belgium and Northern France since ancient times. The quality and fineness of the linen has been proven ever since (Bostock and Riley 1856; Stokkers *et al.* 2004). The application of flax is not restricted to the production of linen yarn. In fact almost the whole plant is used, justifying the name given by Linnaeus, *L. usitatissimum*, which means useful flax. The short fibers are used in paper, isolation material, matrix composites and linen painting textile. The wooden shives which are released during the scutching of flax can serve as an energy source, litter in cattle farming or as source material for pressurized wooden bricks (Stokkers *et al.* 2004). The seeds of fiber flax are mainly used as sowing seeds for the next year. Canada, China, the Russian federation and the United States in America are responsible for more than 65% of the world wide production of linseed. Besides the extraction of linseed oil, the seeds are also used in some food products, e.g. as an ingredient in bread. Linseed oil is high in linolenic fatty acid content (45–60%), making it a very effective drying agent. Although the oil is edible, it is used primarily for industrial purposes, such as the production of paints and oil-based coverings and the manufacture of linoleum flooring (Rowland 1998). Linseed oil also offers important nutritional benefits because of the high levels of omega-3 fatty acids. Animal experiments and clinical intervention studies indicate that omega-3 fatty acids have anti-inflammatory properties, and therefore, might be useful in the management of inflammatory and autoimmune diseases, including coronary heart disease, major depression, aging, rheumatoid arthritis,

Chrohn's disease and cancer (Simopoulos 2002). Recent breeding achievements are in the development of a new flax type called 'Solin'. This name is used for flax cultivars with low (<5%) linolenic acid content in the oil (Dribnenki and Green 1995). Solin, which is agronomically not different from regular linseed, is being developed for the edible oil market.

Agronomical aspects (Cultivation and processing of flax)

Fiber flax and linseed perform best in different regions. Fiber flax is mainly grown in climates with a relative low temperature and high air humidity, which is characteristic for northern temperate regions. The subtropical regions and highlands are ideal locations for linseed cultivation and therefore linseed should be more tolerant to prolonged periods of drought (Bunting 1951). Although the soil type is not the most important factor in flax cultivation, the sandy clay soils of the Netherlands, Belgium and Northern France are very suitable for fiber flax cultivation. Flax requires a wide crop rotation of about seven years. Also the preceding crop is important for growing flax to prevent the occurrence of diseases and lodging. Flax does not perform well after potatoes and sugar beets as the soil may be too loose and *Rhizoctonia* disease could be a problem. On the contrary, cereals and maize are good preceding crops. Flax also does well after legume crops, but *Rhizoctonia* might be a problem as well (Rowland 1998; Stokkers *et al.* 2004). As a rule of thumb, flax is sown at day 100 of the year and harvested at day 200, which is a growing period of 100 days. However, this depends somewhat on the cultivar and environmental conditions. The high sowing density of fiber flax of 110-130 kg/ha (Bonthuis *et al.* 2005) results in plant elongation due to the competition for light. This is important to obtain long high quality unbranched fibers. Linseed is sown with a lower density, 25-55 kg/ha (Rowland 1998), to stimulate branching in order to obtain higher numbers of flowers and an increased seed yield. Flax starts to flower approximately 11 to 14 weeks after sowing. The flowers are open

for only a couple of hours in the morning, after which the sepals fall off and petals close. Ten to 14 days after flowering the fruit reaches its final size, after which the weight remains stable until it decreases as a consequence of the ripening process. At the end of the development the flax plant hardens, turns yellow (senescence) and loses its leaves. At a certain point the plants are ready for the retting process, although the seeds might not be fully ripened. In the Netherlands the seeds are rippled while pulling, which implies that synchronization of fiber and grain maturation is important to reduce harvesting risks (Keijzer 1988). The retting process is the most crucial phase of flax cultivation, because it determines the yield and quality of the fiber. In the Netherlands dew retting is used, which is a natural rotting process where the fibers are released from the wood by bacterial enzymes. As dew retting depends on the weather conditions, this process is difficult to control (Stokkers *et al.* 2004). After the retting process, the straw is harvested and the fibers are released from the wood by scutching and smoothed by hackling. The fiber lint will be sent to spinning companies that produce the final linen yarn. Unlike fiber flax, linseed is harvested by straight combining or cutting with a swather and threshing later with a combine, depending on the maturity and dryness of the seeds. Traditionally, the straw of linseed is processed by companies that extract the flax fiber for the production of specialty papers (i.e. paper for cigarettes, currency, bibles and artwork etc.) (Rowland 1998).

Breeding for quantitative traits

Breeding for fiber yield can be divided into two components, straw yield and fiber content (Popescu *et al.* 1998). The low heritability found for fiber yield suggests a considerable environmental influence. Contrary, fiber content (ratio of fiber weight on the stem) is more heritable and easier to determine. However, in order to calculate fiber content, large numbers of stems are necessary and consequently large trial plots and big quantities of seeds (Fouilloux 1988).

Furthermore, it is known that both additive and dominant effects of genes are involved in the heredity of fiber content and both effects are influenced by environmental conditions (Popescu *et al.* 1998). However, it is still uncertain how many genes are involved in the heredity of fiber content. Likewise, the inheritance of straw yield is poorly understood. Straw yield is supposed to be controlled by several genes as well. A modest gain is to be expected from breeding for straw yield as heritabilities were shown to be low (Mourad and Abo-Kaied 2003). Considering

the genetic basis of both straw yield and fiber content, breeding for fiber yield should be mainly focused on the more heritable fiber content. Oil yield is the most important quantitative trait in linseed. Oil yield is dependent on the seed yield and linseed oil content. Low heritabilities were observed for seed yield in early generations. Contrary, selection on oil content, a character with comparatively high heritability, in an early stage, should be feasible and successful in linseed (Salas and Friedt 1995).



Resistance breeding

Three major diseases posed a threat to flax cultivation in the Netherlands over the past 100 years, namely rust, scorch and wilting. Most research focused on rust resistance, where the differential interaction between the pathogen (*Melampsora lini*) races and the resistant host genotypes, resulted in the widely known gene-for-gene theory (Flor 1956). Nowadays, the Dutch soils are no longer contaminated with rust and all cultivars in the Netherlands are resistant to flax rust (Bonthuis *et al.* 2005). Therefore, resistance breeding is now focused on scorch and wilting.

At this moment the soil-borne disease scorch is one of the major problems in Western European flax cultivation. In France 20 percent of the acreage is infected and depending on the cultivar and disease pressure, losses in harvest can vary from 10 up to 90

percent. To avoid these yield losses, either flax should be grown on soils free of scorch or resistant cultivars should be used (Cariou *et al.* 2003).

Breeding for scorch resistance is difficult, because little is known about the inheritance of scorch resistance and the causative pathogen is unclear. *Chalara Elegans*, *Pythium megalacanthum* and *Pythium buismaniae* have been suggested as the most likely pathogen of flax scorch (Cariou *et al.* 2003; Delon and Kiffer 1978; Wiersema 1955). Breeding for scorch resistance has a long history in the Netherlands! In 1893 Prof. L. Broekema selected the first resistant plants. Nevertheless, several of the current Dutch cultivars are still partly or fully susceptible to scorch (Bonthuis *et al.* 2005). Another important disease in flax is wilting caused by *Fusarium oxysporum* f.sp.

lini. Contrary to the scorch pathogen much more is known about the fungus *Fusarium*. Flax wilt occurs across all main flax and linseed growing countries and may cause severe losses. The main route of infection is through the roots. The symptoms may show up throughout the whole growing season. To select resistant cultivars and breeding lines field wilt nurseries are used. Recently, two in vitro screening methods were developed which proved to be useful in resistance screening, for evaluating race specificity of resistance and to study pathogenesis (Kroes *et al.* 1998). Little is known about the inheritance of resistance to *Fusarium*. The results concerning the

inheritance and durability reported so far are contradictory, but the majority of the reports mention the quantitative nature of the inheritance (Goray *et al.* 1987; Kamthan *et al.* 1981; Knowles and Houston 1955; Knowles *et al.* 1956; Kommendahl *et al.* 1970; Pavelek 1983). Recently Spielmeyer *et al.* (1999) identified two QTLs involved in the inheritance of *Fusarium* resistance explaining 38 percent and 26 percent of the phenotypic variance. Unfortunately, the AFLP marker loci and the linkage groups have been described in a way that does not allow identification of the same linkage groups and the same loci in other mapping populations.



Breeding for quality traits

Fiber quality is the most important trait related to quality. The flax fiber is an irregular strand of varying number of individual cells, the so-called elementary fiber cells. These cells also vary in diameter and in cell wall thickness. The cell wall consists mainly of cellulose. The quality of the fiber after retting and scotching can be defined by different physical and chemical parameters, i.e. strength, fineness and degree of polymerization of cellulose (D.P.-value) (Keijzer and Metz 1992). Recently near infrared (NIR) spectroscopy has been used for the prediction of fiber fineness and strength in flax NIR spectroscopy is a useful tool (Faughey and Sharma 2000), and is currently used in flax breeding. To identify genes involved in fiber quality a large number

of Expressed Sequence Tags (ESTs) have been generated from genes expressed during flax fiber cell formation (Day *et al.* 2005). This resulted in the identification of a number of highly expressed genes involved in the synthesis of cell wall polymers such as celluloses, hemicelluloses, pectins and lignins. Apparently, the inheritance of fiber quality could be a complex trait, but it should be feasible to test several candidate genes for the presence or absence of polymorphisms in expression level or DNA sequence. The quality of the linseed oil is determined by the fatty acid composition (especially the linoleic and linolenic acid content). Wild type linseed oil contains a high level (45-65%) of α -linolenic acid. This polyunsaturated fatty acid is highly susceptible to oxidation and

polymerization and therefore well suited for industrial purposes. Contrary, for edible purposes, linseed oil is not sufficiently stable, because autoxidation during storage will result in off flavors (Green 1986a). To improve linseed oil for edible purposes two different mutant genotypes flax were developed that contain low levels of linolenic acid in its seed oil (Green 1986b; Rowland 1991). These mutants resulted in the first low linolenic acid cultivar LINOLATM-947 of which the oil has great potential in food industry (Dribnenki and Green 1995).

Molecular Approach

Marker Assisted Selection (MAS)

Molecular markers are widely used in plant and animal research to understand the genetic basis of monogenic and polygenic complex traits (quantitative trait loci, QTL). Molecular markers are applied in breeding programs, where marker assisted selection can replace or complement the conventional phenotypic selection. Marker assisted selection is especially powerful for complex traits with low heritabilities (Knapp 1998; Lande and Thompson 1990). MAS is also a valuable tool in breeding for disease resistance, where reliable inoculation and scoring methods are often lacking (Young 2000). Another motivation for using MAS is to achieve a reduction in costs by replacing more expensive methods of phenotypic selection (Knapp 1998; Peleman and Van der Voort 2003; Stam 1994). The greatest benefits of MAS will be achieved in perennial crops because many traits are expressed only after several years of costly field maintenance (Liebhard *et al.* 2003). Furthermore, MAS showed to be very successful in the construction of Backcross Introgression Libraries (BILs) which serve as QTL detection and breeding material (Eshed *et al.* 1992; Howell *et al.* 1996; Jeuken and Lindhout 2004). To efficiently apply MAS, a few practical considerations concerning the limitations of its utility should be given proper attention. First, the marker loci and the trait loci should be tightly linked, which requires mapping studies with high density linkage maps (Lande and Thompson 1990). With the

introduction of multiplex PCR marker system such as AFLP (Vos *et al.* 1995) the construction of dense linkage maps have proven to be successful in almost all important agricultural crops such as potato, barley, rice, maize and soybeans etc. (Castiglioni *et al.* 1999; Eck *et al.* 1995; Lin *et al.* 1996; Maheswaran *et al.* 1997; Qi *et al.* 1998). Secondly, the association between marker alleles and trait genes should not only occur in a single experimental full sib population, but in a wider range of cultivated germplasm. This extrapolation will rely on linkage disequilibria, which are continuously.

Molecular markers as a tool in genetic studies in flax

The application of molecular marker techniques and genetic analysis of several studies in flax. By means of different diversity indices and cluster analysis the variation within and between species can be analyzed. Besides the investigation of the variation of flax we describe the application of molecular markers in genetic studies as well. Optimizing the AFLP fingerprinting technique is necessary for optimal genotyping of mapping populations. Furthermore, good quality QTL mapping relies on high quality linkage maps. The use of different methods and careful genotyping might be the key to success. At last, QTL analysis can be used to understand the genetic basis of traits by combining molecular marker data with genotypic trait values. One of the objectives of these studies is to obtain insight in the structure and size of the flax gene-pool. Furthermore, we want to provide recommendations to reveal new alleles via several new strategies. And the last objective is to make MAB ready to use in flax breeding. The latter will be mainly focused on several disease resistances and quality traits.

QTL mapping of resistance and quality traits in Flax

In QTLs were identified involved in resistance and quantitative traits by combining field observation on the offspring of the V x E and B x H mapping populations with the V x E and B x H genetic linkage maps described. Four traits important to flax breeding were

investigated in more detail: scorch and *Fusarium* resistance, fatty acid composition of the seed oil and fiber quality traits. The inheritance of flax scorch resistance is based on a major QTL that explained the largest part of the phenotypic variation. However year and location specific minor QTLs were observed as well, which contributed significantly to a more desirable resistance level. It would be interesting to investigate the effect of multiple environments on scorch resistance. In this way a better understanding of the genetic model of scorch resistance might be obtained. The heredity of linoleic versus linolenic acid content was in accordance with literature reports: two unlinked additive desaturase loci (mutated in the E1747 parent) are involved in the biosynthesis of linolenic acid out of linoleic acid. *Fusarium* resistance in the B x H population and fiber content in the V x E population appeared to be more complex traits. *Fusarium* is controlled by at least five QTLs of which two occurred at different time points. The genetic model for *Fusarium* resistance at time point two was investigated more carefully and consisted of two loci with additive gene effect and an epistatic interaction between the two other loci. The differences observed at the two points raise intriguing questions about the mechanism of *Fusarium* resistance. More detailed studies on plant pathogen interaction at the different time points might give some answers. Fiber content in the V x E population appeared to be controlled by at least four QTLs, of which two QTLs had additive effect and the two other loci showed duplicate gene epistatic interaction. Furthermore, the position of fiber QTLs on the V x E and B x H map coincided. In this case we assume that both QTLs represent the same locus. Many co-localizing QTLs involved in different traits could be explained by the presence of a single locus with a pleiotropic effect on those different traits. However, more detailed correlation studies at these loci might show the true relation between traits.

Is it possible to make progress in flax breeding with the current gene pool?

Apparently, it is difficult to develop new cultivars with improved fiber content, fiber

yield and disease resistance, as last ten years only few new cultivars appeared on the National List of varieties of field crops in the Netherlands. The results described for all traits one or more loci are present in the flax gene-pool which are polymorphic between cultivars. Combining all positive alleles at these QTLs might result in cultivars that outperform the current elite material. For fiber content we could not identify QTLs that were not present already in cultivar Hermes, and thus little perspective is offered for this trait due to the limited amount of genetic diversity in the fiber flax gene pool. This is confirmed by the modest achievements of breeders. The fiber content of the current new cultivars does not exceed the fiber content of Hermes, which was introduced approximately ten years ago. Only the straw yield could be improved slightly in the new cultivars. Combining all positive QTL alleles for loci involved in fiber content and straw yield might result in a high fiber yielding cultivar. With markers efficient pyramiding of positive alleles of the QTLs becomes feasible. A major drawback of QTL mapping, as a strategy to identify favourable alleles at loci involved in important traits, is the limited amount of germplasm involved. In our study only four parental genotypes have been studied for the presence of favourable alleles, which is only a fraction of the total number of cultivars. The parental genotypes were carefully chosen that they should carry favourable alleles with high probability. To guarantee the segregation of those favourable QTL the other parent should not carry those alleles. For example favourable alleles for scorch resistance, seed yield and lodging should be expected to segregate from Belinka; fiber content, fiber yield, plant length and *Fusarium* resistance should be expected to segregate from the Hermes; fiber content and *Fusarium* resistance should be expected to segregate from the Viking; oil content seed, linoleic acid content seed oil and lodging should be expected to segregate from E1747. This experimental design is such that crosses between a favourable and inferior parent are used, but these favourable alleles may already be fixed

in germplasm that is superior for the traits involved. An experimental design where a superior parent is crossed with another superior parent would either render unsuccessful, because QTLs are not segregating or transgressive segregation will expose favourable allele combinations that have not yet been achieved. Only in the case of oil content seed, seed yield, *Fusarium* resistance, plant length and lodging transgressive segregation has been observed. An experimental design that circumvents the disadvantages of QTL analysis is association

mapping. A large collection of unrelated genotypes is screened with a large number of markers to identify associations between the trait value and marker alleles. Although Markers that were positively associated with traits should be tested in the QTL approach by analyzing that specific AFLP primer combination on the mapping populations. And in reverse, the markers near QTLs that have been identified should be tested in the association mapping approach to verify if these marker QTL associations can be extrapolated to a wider range of germplasm.

Some Linseed Varieties rich in omega-3 (release from AICRP IGKV, Raipur)

Sr.	Varieties	Omega 3 (%)
1	SLS-61	62
2	JLS	58
3	LC-2279-4	55.6
4	R-552	52 (lignan-79%)
5	RLC-112	49

CONCLUSION

Linseed oil has more than 50% linolenic acid which is fit for its industrial application but where linseed oil is being used as edible oil, the linolenic acid needs to be reduced. Efforts in this direction have already been successful with the development of low linolenic acid varieties LINOLA in Australia in 1984 and SOLIN in Canada in 1990. In India too national linseed program in collaboration with BARC, Mumbai has developed some genotypes with less than 1% linolenic acid. Hence, the breeding efforts are needed further for development of low linolenic acid varieties, the oil of which can be widely used as cooking oil. As linseed is highly nutritious, efforts are needed to reduce its anti-nutrient components and also bio-convert its less acceptable omega-3 ALA into acceptable SDA. For achieving this objective, in addition to the conventional breeding, the biotechnological tools like marker-assisted breeding and genetic engineering may also be employed. Moisture stress being one of the

major constraints, the varieties with inbuilt water stress tolerance may be given more emphasis to enhance and stabilize the productivity for making this crop more remunerative. More concerted efforts for development of varieties resistant to different diseases like wilt, rust, powdery mildew and *Alternaria* blight are also required by using the different resistant donors already available in this crop.

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