High Oleic and Low Linolenic *Brassica napus*

CHUNGU Chibwe[1], MURRAY Bruce [1], THOMPSON Steve.[2], KUBIK, Tom[1],FREEMAN Sara.[1], GORE Sherry.[1]

[1] Dow AgroSciences Canada Inc., 104-111 Research Drive, Saskatoon, SK S7N 3R2, Canada
[2] Dow AgroSciences LLC., Trait Development, 9330 Zionsville Road, Indianapolis IN 46268 U.S.A

Abstract:

The development of canola oilseed, *Brassica napus* L., with a high oleic, low linolenic (HOLL) acid content is of interest for nutritional and functional purposes. Relatively high levels of linoleic acid (C18:2) and linolenic acid (C18:3) fatty acids reduce the oxidative stability and limit the utility of canola oil for cooking unless it is hydrogenated. The development of canola cultivars with high oleic (C18:1) and low C18:3 has produced a nutritionally desirable oil and opened new niche markets. The inheritance of the HOLL trait is polygenic and strongly influenced by the environment. Several studies have shown that C18:1 content increases with increasing ambient temperature, while C18:3 is reduced at elevated temperatures. Molecular markers linked to these traits hold potential as an environmentally insensitive tool for use in breeding HOLL canola.

Introduction

Canola *(Brassica napus* L). is a major edible oilseed crop in many parts of the world. The quality of canola oil and its suitability for different end uses are largely determined by its fatty acid composition. Canola oil is considered superior to other oils due to its low levels of saturated fatty acids, however, the presence of a moderately level of C18:3, although nutritionally desirable, can contribute to cooking odors and reduced shelf life of the oil and its products. Standard canola oil contains about 60% oleic acid (C18:1), 20% linoleic acid (C18:2) and 10% linolenic acid (C18:3) which are the major unsaturated fatty acids in canola. Linolenic acid is recognized as a desirable fatty acid because of the role it plays in reducing plasma cholesterol levels (Eskin et al. 1996). However, natural oxidation of linolenic acid produces compounds that cause poor oil flavor. Reducing C18:3 to an acceptable level requires hydrogenation to stabilize the oil. The process of hydrogenation can result in the formation of trans fatty acids, which tend to raise the serum low-density lipoprotein cholesterol (LDL-C) levels and reduce serum high density lipoprotein cholesterol (HDL-C). Lowered levels of HDL-C are associated with enhanced risk of cardio-vascular disease.

To reduce the need for hydrogenation and increase the stability of canola oil, low C18:3 oils have been developed through plant breeding. Varieties with reduced C18:3 form lower levels of free fatty acids during frying, resulting in better flavor quality of fried products (Przybylski, R. unpublished data). The development of low C18:3 canola types indirectly raises the C18:1 levels and produces a canola oil with greater heat stability (high C18:1) and reduced potential for rancidity (low C18:3). This type of canola is of interest nutritionally as well as for industrial

purposes. In recent years, the functional and nutritional aspects of canola oil have had a significant influence on the development of different canola niche markets, i.e, high C18:1 and/or low C18:3. Various breeding institutions including Dow AgroSciences Canada Inc. are involved in developing varieties with HOLL profiles to meet the changing consumer demands. The main focus of Dow AgroScience's breeding program is the development of HOLL varieties which are trademarked as Natreon* varieties. A challenge being faced by the industry is to determine the most desirable oil profile for emerging market segments such as HOLL.

Breeding for High Oleic and Low Linolenic Fatty Acids

Today, there is a substantial effort by both private and public breeding programs to develop canola varieties with high C18:1 and low C18:3. These traits have been produced by applying mutagenesis to seed and/or microspore derived embryos of *B. napus* (Rakow, 1973; Robbelen and Nitsch, 1975; Wong *et al*. 1991, Auld et al. 1992), and through transgenic modification (Debonte and Hitz, 1996). Seed mutagenesis followed by crossing and selection has resulted in varieties with >85% C18:1 and low levels of C18:3 (<3.0%). Canola oil with >86% C18:1, < 7% C18:2 and <2.5% C18:3 content has been produced in plants containing seed-specific inhibition of microsomal oleate desaturase and microsomal linoleate desaturate gene expression (Debonte and Hitz, 1996). This inhibition has been created through co-suppression or antisense technology.

Breeding for high C18:1 and low C18:3 levels is challenging since the genes are inherited in a recessive manner (Auld et al. 1992; Rakow 1973; Röbbelen and Nitsch 1975). In *B. napus* these traits are controlled by 4 to 5 recessive genes (Dow AgroSciences data not reported) making it difficult to identify heterozygotes in segregating populations. In addition, the low C18:3 trait is significantly influenced by the environment, making selection of plants by half seed analysis from greenhouse grown material unreliable. Genetic analysis of progenies derived from crosses of the first low C18:3 variety «Stellar» × Drakkar, showed that the low C18:3 trait is controlled by two major genes, L1 and L2, with additive effects (Jourdren *et al.* 1996b). Jourdren *et al.* (1996a) found that a *fad3* desaturase gene was associated with L1 and a second *B. napus fad3* gene was found to be linked to L2 (Barret *et al*., 1999).

Rakow (1973) demonstrated that maternal influence on linolenic acid was low, amounting to only 20% of the influence of the embryo. Similar findings were reported by Thomas and Kondra (1973). In contrast, Bartkowiak-Broda and Krztmanski (1983) found that maternal genotype exclusively influenced C18:3 concentration. Kondra and Wilson (1976) found that the quantities of C18:1 and C18:3 were affected by both the maternal and embryo genotypes, and no cytoplasmic effects were evident in F1 reciprocal crosses. In another study, (Kondra and Thomas, 1975), it was demonstrated that C18:1 and C18:3 levels are controlled by simple additive gene effects.

Heritability for low C18:3 content in canola seed oil was estimated to be 26 - 59% by Kondra and Thomas (1975) whereas heritability was estimated to be 82% by Rajcan *et al*. (1997). Somers *et al*. (1988) suggested that at least three major genes may be involved in the desaturation of C18:2 to C18:3, whereas Rücker and Röbbelen (1996) indicated that probably several minor genes are involved in the desaturation step. In contrast heritability esimates for

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C18:1 have been found to be 53% to 78% (Kondra and Thomas 1975), and 94% (Schierholt and Becker, 1999). The higher heritability of C18:1 content suggests that this trait may be more environmentally stable than 18:3. Inheritance of C18:3 may differ depending on genotypes. Relatively high stability of the low C18:3 trait over locations and years has been observed in the DAS breeding program

Effect of Environment of Oleic Acid and Linolenic Acid

Post-flowering temperatures are considered to have a major effect on the fatty acid composition in a number of oilseed crop species. The degree of unsaturation of oils generally increases as post-flowering temperatures decrease (Canvin, 1965). The relative content of C18:1 and C18:3 are strongly influenced by environmental conditions (Deng and Scarth, 1998). Oleic acid content is generally elevated in areas with warm and wet conditions, and lower in cool and moist environments. These results are consistent with the findings of several workers (Tremolieres *et al*., 1982; Pritchard *et al*., 1999) who showed that low temperature increased desaturation of C18:1 and C18:2 resulting in higher C18:3 levels in mature canola seed.

Tribol-Blondel and Renard (1999) also found that high temperature resulted in a significant increase in C18:1 content and a decrease of C18:3, consistent with the results of Trémolières et al. (1982) and Pleines et al. (1987). In addition, the C18:1 content decreased and the C18:3 content increased at low temperature and under conditions of water stress. A study of the influence of the environment on the high oleic trait showed a high heritability, with significant environmental and G × E components (Schierholt and Becker 1999).

Methods: Half-seed and Microspore Culture

The utilization of the half-seed technique developed by Downey and Harvey (1963) has played a significant role in the identification of plants with the desired fatty acid profile. Microspore culture techniques (Fletcher *et al*., 1998), which are routinely used in breeding programs to produce pure breeding double haploid plants, have also contributed to the production of lines with more stable fatty acid profiles. The efficiency with which DH lines with the low C18:3 trait is produced can be improved by using a combination of breeding methods e.g., use pedigree methods to obtain agronomic data prior to producing pure breeding low C18:3 lines.

Molecular Markers and Their Application in Breeding Programs

Identification of *B. napus* lines with fatty acid profiles low in C18:3 early in the breeding program is essential. The application of the co-dominant markers in breeding programs can help improve efficiencies towards developing low C18:3 canola varieties. Brown and Scarth (1999) have reported a RG-PCR test that can specifically detect two mutations conferring the 'low C18:3' trait in 'Stellar'. These markers are co-dominant, and therefore can be used to select individual plants homozygous for L1 and L2 mutations in segregating populations. Since C18:3 content is greatly influenced by the environment and often not reproducible between field and greenhouse conditions, such markers are ideal for evaluating the trait as the environment has no effect on molecular markers, permitting efficient selection for low C18:3 from greenhouse grown plants.

Several research groups have identified and developed markers for low C18:3 content, but robustness of these markers has not been established. Clearly, markers that are effective in lines

derived from different crosses would be ideal. A robust marker would provide breeders with greater flexibility in a marker assisted selection breeding program. Several papers have been published where markers are identified that are associated with QTLs that control the phenotypic variation in C18:3. The present challenge is to develop markers that have cross applicability in breeding.

Agronomic performance of Natreon varieties

It is a myth that HOLL varieties are associated with a yield drag. It is true that the first low C18:3 variety «Stellar«, released in 1988 and Apollo registered in 1994 yielded less than the controls by 20% and 8%, respectively. However, Rucker and Robbellen (1996) evaluated the impact of low C18:3 content on seed yield of winter oilseed rape. They demonstrated that by crossing and backcrossing low C18:3 selections to high yielding genotypes, C18:3 selections in the BC_4 generations were not associated with a decreased seed yield. We have also demonstrated with Natreon varieties that the HOLL traits do not limit productivity (Fig. 1).

Conclusion

Development of mutants with a fatty acid composition of high 18:1 and low C18:3 have resulted in greater oxidative stability of canola oil and opened new niche markets. The agronomic performance (seed yield and Blackleg resistance) of HOLL varieties has shown great improvement and further improvements are in the pipeline. It is envisioned that marker assisted selection will play a larger role in the early identification of breeding lines carrying these traits, especially in greenhouse grown lines. Development of co-dorminant markers that have cross application will result in more user friendly breeding systems.

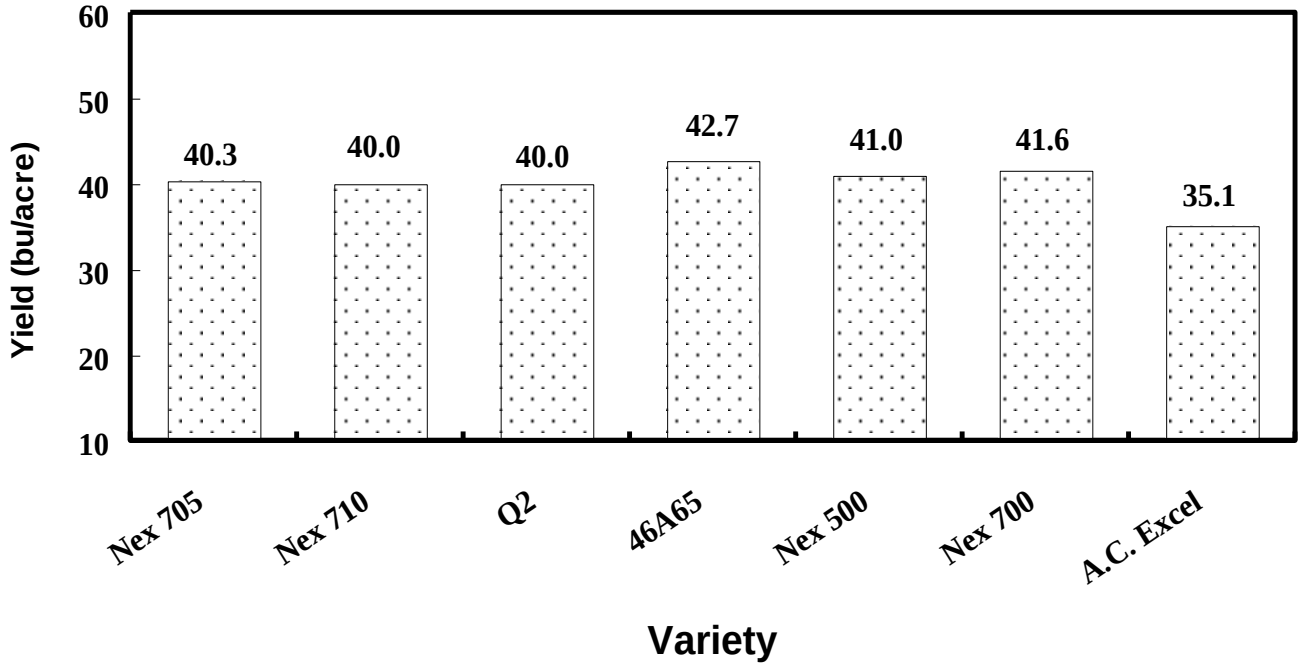

Fig.1. Mean seed yield of Natreon varieties (Nex 705, Nex 710, Nex 700 compared to the conventional check varieties Q2, 46A65 and A.C. Excel grown in 17 megaplots sites in 1999 and 2000

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