Breeding of oilseed rape (*Brassica napus* L.) for modified tocopherol composition – synergy of conventional and modern approaches

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ABSTRACT

Modification of seed composition has been an important objective in the genetic improvement of major oilseed crops, including soybean (*Glycine max*), oilseed rape (*Brassica napus*) and sunflower (*Helianthus annuus*). Due to successful breeding double-low rapeseed (canola) is highly estimated as food raw material, but further enhancement of its nutritional value and health effects has become more important in the last few years. Tocopherols (TOC), although present in small amounts (500-700 mg tocopherol · kg^{-1} oil), are important phytonutrients in edible oils showing bioactivity as vitamin E and reducing the autoxidation of unsaturated fatty acids, the production of off-flavours and rancidity. To improve rapeseed oil quality, it is considered necessary to increase total TOC content and to breed for higher individual content, either for more vitamin E (α-TOC) or higher antioxidant effect during storage (γ-TOC). To accomplish these objectives conventional breeding and metabolic engineering is applied.

Key words: Tocopherol - oilseed rape - *Brassica napus* – breeding – genetic engineering

INTRODUCTION

It has been about 80 years since vitamin E was discovered as dietary factor and essential element for maintaining reproduction in vertebrates, and yet we are just beginning to understand its physiologic functions and potential benefits in human health. Vitamin E occurs in nature in at least 8 structurally related forms, i.e., 4 tocopherols (α-, β-, γ- and δ-TOC) and 4 tocotrienols (α-, β-, γ- and δ-T3), differing in both molecular structure and biological effectiveness, but all of which are potent lipid-soluble antioxidants (Kamal-Eldin and Appelqvist 1996, Bramley et al. 2000, Brigelius-Flohé and Traber 1999, Ricciarelli et al. 2002).

Because humans and animals do not synthesize their own vitamin E, they primarily acquire tocopherols mainly from plants, which are the major organisms capable of making vitamin E. α-TOC is the predominant form of vitamin E in human and animal tissues and is the primary form in most supplements (Food and Nutrition Board - Institute of Medicine 2000, Brigelius-Flohé et al. 2002). γ-TOC is the major form of vitamin E in many vegetable oils and in diets, but has drawn little attention in medicine and nutrition compared with α-TOC. However, recent studies indicate that γ-TOC possesses unique features that distinguish it from α-TOC, and it may contribute significantly to human health in ways not recognized previously (Jiang et al. 2001).

There is an increasing commercial interest to develop breeding lines of important oil crops as potential higher-yielding sources of these value-adding micro constituents. In the course of the German joined project NAPUS 2000 dealing with the improvement of the nutritional value of rapeseed (*B. napus)* products both conventional breeding procedures and genetic engineering are applied to create novel genetic variation for TOC composition (cf. Friedt and Lühs 1999, Leckband et al. 2002).

MATERIALS and METHODS

Using relevant tocopherol gene constructs hypocotyl segments of spring rapeseed were transformed and regenerated to intact plants using a protocol as described in detail by Raclaru et al. (2003). Selected T2 plants were grown under controlled temperature (day/night: 15/10 °C) and 16 hours day-light conditions in the greenhouse in order to increase seed material and confirm the transgenic phenotype in T3 seeds. Following conventional breeding procedures selection for TOC content in winter oilseed rape was done in field grown material at Göttingen and Hohenlieth, Germany, from 1999-2002. Samples were originating from diverse breeding lines including material derived from mutagenesis (Marwede et al. 2003). Oils were obtained either by extraction from seed samples with petroleum ether or were authentic oils obtained from commercial sources. TOC were analysed by HPLC with fluorescence detection, total content was calculated as the sum of α-, γ- and δ-TOC as well as plastochromanol P-8, with β-TOC as internal standard and iso-octane as sample solvent (Thies 1997, Olejnik et al. 1997; cf. Fig. 1).

RESULTS and DISCUSSION

Reported total content and composition of TOC in rapeseed oil have been variable, probably in part due to differences in methods of seed analysis. Compared to other seed oils, such as linseed (400-600 ppm), sunflower (700-1,000 ppm) or soybean (1,000-1,500 ppm), rapeseed oil has a moderate average TOC content of 500-700 ppm with about 25-40% α-TOC and 55-70% γ-TOC as predominant tocopherols, 1-2% of δ-TOC and small amounts of plastochromanol P-8 (Marquard 1990, Kamal-Eldin and Andersson 1997, Cole et al. 1998, Dolde et al. 1999, Abidi et al. 1999). Preliminary results from our analyses of breeding material revealed that actual winter rapeseed germplasm shows a considerable variation in total TOC content ranging from 214 to 1,175 ppm in the oil, whereas the major components range from 86 to 866 ppm (α-TOC) and 61 to 784 ppm (γ-TOC) in the oil, respectively. Goffman et al. (1999a) have used tocopherols as chemotaxonomic parameter in order to study their relationship with oil content and fatty acid profile in a collection of 91 Brassicaceae species. The collection showed a wide variability for total content (70-2,500 mg kg^{-1} oil) and composition of tocopherols. Individual tocopherols were found to have great taxonomic value in the crucifer family.

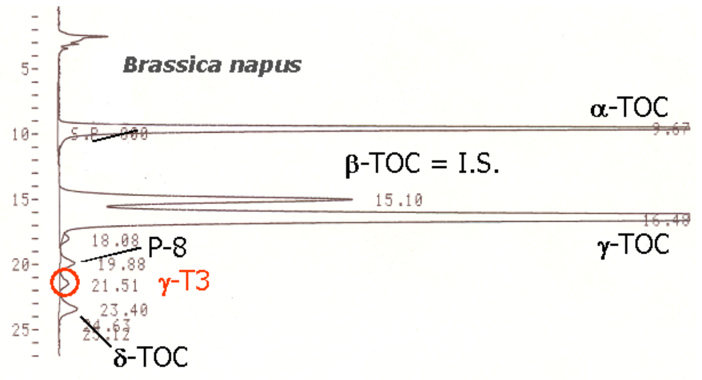

Fig. 1: Asides the main tocopherol forms (α-TOC, γ-TOC) rapeseed oil contains small amounts of δ-TOC and plastochromanol-8. In this sample traces of γ-tocotrienol (γ-T3) were detected. β-TOC is used as internal standard (I.S.).

The inheritance of tocopherol composition has been studied in sunflower seeds (cf. Demurin et al. 1996) and in winter rapeseed (Goffman et al. 2001a, 2001b, Marwede et al. 2003). Furthermore, studies on canola and sunflower oils have indicated that altered content and composition of tocopherols could be accomplished independently from selection for modified fatty acid composition (Kamal-Eldin and Andersson 1997, Dolde et al. 1999, Abidi et al. 1999). However, changes in fatty acid composition have not always resulted in a desirable enhancement of stability expected in new oil types. That may be particularly important for canola oil possessing a beneficial content of α-linolenic acid and narrow ratio of n-6 to n-3 fatty acids (cf. Kamal-Eldin and Yanishlieva 2002). Therefore, tocopherols functioning as natural antioxidants will gain much more importance in breeding programs for new rapeseed varieties.

At present, it is generally accepted that TOC biosynthesis in oily seeds is related to plastid development, rather than to storage oil accumulation. However, little is known about the sub-cellular compartmentation and storage of tocopherols as well as the histochemical distribution within the seed (Newton and Pennock 1971, Janiszowska and Pennock 1976, Soll et al. 1985, Soll 1987, Schultz 1990, Hess 1993, Keller et al. 1998, Yoshida et al. 1998, Goffman et al. 1999b). Apart from *G. max*, it was found that TOC accumulation in major oil crops is inversely related to seed oil content, where lower temperatures during seed development lead to higher oil content and a decrease in total TOC content in the oil due to a dilution effect (Marquard 1990).

Tocopherol biosynthesis in plants has not yet been fully characterized, but a probable pathway has been formulated. Most of the work on genetic modification has been performed on *Arabidopsis thaliana* (cf. Tsegaye et al. 2002, Shintani et al. 2002, Collakova and DellaPenna 2003, Koch et al. 2003, Hofius and Sonnewald 2003). Shintani and DellaPenna (1998) demonstrated that a single transgenic event can alter ratios of α- to γ-TOC to an extent most likely not possible with traditional selection. The strategy of our transgenic approach is to elevate TOC levels in *B. napus* by metabolic engineering of enzymes that catalyse relevant biosynthetic steps, such as 4-hydroxyphenylpyruvate dioxygenase and homogentisate phytyltransferase. Selected T2 plants bearing different transgene constructs were grown under controlled greenhouse conditions to examine the altered phenotype in T3 seeds (cf. Raclaru et al. 2003).

SUMMARY AND OUTLOOK

Although the major source of dietary vitamin E is derived from plant foods, the regulation of TOC biosynthesis in plants is largely unknown. In order to achieve an entire metabolic engineering of the biosynthetic pathway the deregulation of quite a number of enzymatic steps is required and the focus must be shifted to enzymes that occur earlier in the pathway. Future breeding selection programs aimed at developing rapeseed oils with modified fatty acid patterns will have to include evaluation of tocopherols and possibly other components in order to obtain the maximum stability of the new product. Tocopherol-enriched rapeseed oil is considered as a nutraceutical, which can offer several benefits to the consumer. In the case that there are problems with the acceptance of genetically modified plant-based products hampering commercialization, the strategy is to develop material without transgenic plants, using the variation found by conventional breeding including mutagenesis.

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