



Genotype and environment effects on tocopherol and plastochromanol-8 contents of winter oilseed rape doubled haploid lines derived from F1 plants of the cross between yellow and black seeds



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ABSTRACT

To determine the effect of genotype and the environment on tocopherol (T) and plastochromanol-8 (PC-8) contents in winter oilseed rape (*Brassica napus* L.), a three-year study was performed. The plant material consisted of 25 doubled haploid (DH) lines derived from the F₁ hybrid obtained from the cross between two DH lines (Z × H), (Z-114- yellow seeds and H₂-26- black seeds). Quantitative contents of α-T, β-T, γ-T, δ-T, and PC-8 were determined by HPLC. Total tocopherol content ranged between 40.62 and 51.20 mg/100 g and PC-8 5.43 – 8.92 mg/100 g. The α-T/γ-T ratio ranged from 0.77 to 1.23. The heritability in the broad sense was examined for the following components: PC-8, α-T, β-T, γ-T, δ-T, α/γ-T, and γ-T contents in oil. Heritability levels were 0.60, 0.58, 0.53, 0.50, and 0.43, respectively. This suggest the superiority of the effect of genotype on tocopherol contents. The *h*² values for δ-T and β-T were 0.36 and 0.32, which demonstrates a great influence of environmental conditions. Among all analyzed genotypes, eleven were selected as satisfactory and stable regarding tocopherol or PC-8 contents.

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1. Introduction

Vitamin E consists of α-, β-, γ-, and δ-tocopherol and the corresponding α-, β-, γ-, and δ-tocotrienol. The tocopherols are characterized by the 6-chromanol ring structure methylated to varying degrees at the 5, 7, and 8 positions. At position 2 there is a C₁₆ saturated side chain. The specific tocopherols therefore differ in the number and positions of the methyl groups on the 6-chromanol ring (Eitenmiller and Lee, 2004). Rapeseed is one of the most important oil crops, and is grown mostly in temperate climates worldwide. Rapeseed oil contains high amounts of vitamin E, an essential component in human nutrition and health. The

content of tocopherols in rapeseed ranges from 210 to 1200 mg/kg of seeds (Marwede et al., 2005). It is mainly α-T and γ-T, accompanied by small quantities of β-T and δ-T (Goffman and Becker 2001; Marwede et al., 2004). The ratio of α-T to γ-T ranges between 0.54 and 1.70 (Marwede et al., 2004). Plastochromanol-8 is a derivative of γ-tocotrienol, which has a longer side chain (Przybylski and Mag, 2002). This compound was detected in canola and flax oils. It was found that PC-8 activity is 1.5 fold greater than that of α-T (Olejnik et al., 1997).

The tocopherol biosynthetic pathway has been elucidated several years ago (Soll et al., 1980). Most of the genes (VTE, loci 1–5; PDS1) encoding the key enzymes of the core biosynthetic pathway have been identified and characterized in *Arabidopsis thaliana* and *Synechocystis* sp. PCC3903 (Norris et al., 1998; Collakova and DellaPenna, 2003). Tocopherols are present in all anatomical parts of plants, i.e., roots, tubers, leaves, stems, and flowers. However, there is a great variation both in the total contents of tocopherols and their homologues in particular plant parts. Two tocopherol

Abbreviations: DH, doubled haploid; PC-8, plastochromanol-8; T, tocopherol; α-T, alpha-tocopherol; β-T, beta-tocopherol; γ-T, gamma-tocopherol; γ-T3, gamma-tocotrienol; δ-T, delta-tocopherol; γ-TMT, gamma-methyl transferase.

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homologues, which are the most common in plants, are α -T and γ -T. The predominant form is the α -T homologue, which protects the photosynthesizing organ from reactive oxygen species and free radicals. In seeds (non-photosynthesizing tissues) γ -T predominates, which serves the role of an antioxidant of polyenoic fatty acids (Bramley et al., 2000; Eitenmiller and Lee, 2004; Szymańska and Kruk, 2007).

The use of genotypes with a very low glucosinolate content in breeding in conjunction with the earlier elimination of erucic acid resulted in double improved (double zero -"00") varieties being introduced to cultivation in the late 1980's (Booth and Gunstone, 2004; Eskin, 2013). Obtaining varieties that are raw materials for various sectors of the industry is made possible by a combination of modern biotechnological methods and conventional breeding techniques. Qualitative changes must be accompanied by an increase in fertility of the oilseed rape varieties as well as their adaptation to the cultivation and agricultural technology (Friedt and Snowdon, 2009). Qualitative traits of rapeseeds are improved by increasing their contents of fatty acids, protein and biological active compounds (tocopherols, sterols, phenolic compounds). Improved varieties contain different proportions and compositions of fatty acids, while levels of anti-nutritional compounds such as glucosinolates, fiber, and flavonoids are reduced (Friedt and Snowdon, 2009). Studies on breeding yellow-seed, triple-zero varieties (low in erucic acid, glucosinolates, and dietary fiber) have been conducted since the 1980's. One of the main objectives in the development of double improved yellow-seed canola varieties has been to increase oil content in seeds and the content and availability of protein (Meng et al., 1998; Rashid et al., 1994; Relf-Eckstein et al., 2003; Rahman and Mc Vetty, 2011).

The use of double haploids (DH) in plant breeding increases selection of both qualitative and quantitative traits. Androgenesis in vitro of *Brassica napus* F1 hybrids is a routine method for obtaining homozygous lines of genetically diverse parental gene recombination. DH lines are completely homozygous and traits controlled not only by dominant genes but also by recessive genes can be fully expressed. Variation that also occurred in the dominant and recessive genes could easily be identified (Cegielska-Taras et al., 2002). A large doubled haploid population derived from a single hybrid results in significantly different genotypes. DH lines derived from F1 plants represent a large scale of possible genetic recombination of traits of parental lines and they are an ideal material for selection (Szała et al., 2003; Cegielska-Taras et al., 2002). Many authors present the results of genotype \times environment interactions for contents of tocopherols, fatty acids, phytosterols, or sinapate ester in *B. napus* (Marwedee et al. 2003; 2004; Zhang et al., 2004; Zhao et al., 2008; Amar et al., 2008). However, there are no reports concerning the population of doubled haploid seeds obtained by crossing yellow and black seeds oilseed rape. Similarly, no data are available concerning PC-8 content in rapeseed. Therefore, it was decided to undertake such studies to analyze the impact of genotype \times environment effects in a population of DH lines derived from a cross between DH lines with black- and yellow-seeds *B. napus*.

2. Material and methods

2.1. Material

The object of this study was the population of doubled haploid lines (DH) of winter rapeseed (*B. napus* L.) derived by in vitro culture of isolated microspores (Cegielska-Taras et al., 2002). Population was obtained from F1 hybrids by crossing yellow-seeded (Z-114) and black-seeded (H2-26) doubled haploids of winter rapeseed with 00-quality. The biological material consisted of 25 different DH lines (population Z \times H) and two parental lines.

Yellow-seeded DH Z-114 line has derived from breeding materials with yellow seeds obtained in plant breeding and Acclimatization Institute–National Research Institute in Poznan, Poland. The origin this materials is from a natural bright-seeded mutant and Canadian spring line selected from segregating lines from cross between *B. napus* \times *Brassica rapa* (Bartkowiak-Broda et al., 2009). Field trials were carried out in Łagiewniki, Poland in the growing seasons of 2008/2009, 2009/2010, and 2010/2011 on plots of 4.5 m \times 1.2 m (length \times width). In one experimental plot 400 seeds were sown. All field experiments were carried out in 3 replicates. Seeds were sown on August 28, August 31, and August 30 in 2008–2010, respectively. Each year fertilizers were applied at a concentration of 205 kg N ha⁻¹, 60 kg P₂O₅ ha⁻¹, and 120 kg K₂O ha⁻¹. Weeds were controlled by application of Butisan 400 SC just after sowing and then mechanically. Plots were harvested on July 23, 2009. On July 21, 2010 and on July 22, 2011 by combine in full maturity. Seeds for sowing in the next season were collected separately before mechanical harvest from individual plants which flowers were covered with bags.

Seed color was determined using spectrophotometer ColorFlex on a scale from 0 (black) to 5 (yellow) (Michalski, 2009).

2.2. Tocochromanol contents

Samples of seeds (2 g) were saponified using 60% KOH (2 ml), ethanol (20 ml), and pyrogallol (0.5 g). Saponification was carried out at the ethanol boiling point (78 °C) for 30 min. After saponification unsaponifiable substances were extracted using 50 ml *n*-hexane/ethyl acetate (90:10 v/v). Tocopherols and PC-8 were qualitatively and quantitatively identified using HPLC (Waters 600 Asc. Milford, MA, USA). A LiChrosorb Si60 column (250 \times 4.6 mm; 5 μ m) was used. The mobile phase consisted of *n*-hexane and 1,4-dioxane (97:3 v/v). Flow rate was 1.5 ml/min. The fluorometric detector (Waters 474 Asc. Milford, MA, USA) worked at an excitation of $\lambda = 295$ nm and an emission of $\lambda = 330$ nm for tocochromanols and PC-8 (Ryynänen et al., 2004; Gawrysiak-Witulska et al., 2011). Standards of α -, β -, γ -, and δ -tocopherol (α -T, γ -T, β -T, δ -T) (95%, purity HPLC) were purchased from Merck (Darmstadt, Germany) and used to determine retention times and quantify tocopherol.

2.3. Statistical analysis

The effect of genotype, environment and genotype by environment interaction (G \times E) was analyzed using the following mixed linear model:

$$Y_{ijk} = \mu + g_i + e_j + ge_{ij} + \epsilon_{ijk}$$

where Y_{ijk} is the observation of genotype i in environment j in replication k , μ is the general mean, g_i is the effect of genotype i ($i = 1, \dots, G$), e_j is the effect of environment j ($j = 1, \dots, E$), ge_{ij} is the genotype \times environment interaction of genotype i with environment j and ϵ_{ijk} is the residual error of genotype i in environment j in replication k . The genotypes were considered as fixed, whereas environment was considered as a random variable (Mądry and Kang 2005).

Main effects for individual genotypes $g_i = \mu_i - \mu$ were tested to find satisfactory and unsatisfactory genotypes. Positive and significant estimations of main effects are considered to be satisfactory. Positive, but non-significant or negative effects are considered as unsatisfactory effects. The significance of main effects is tested by the F statistics:

$$F_{gi} = \frac{E(E-1)g_i^2}{S_{ge,ii}}$$

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