RESEARCH NOTE

Inheritance of deficient tocopherol accumulation in sunflower seeds

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Introduction

Tocopherols are the main antioxidants in oil seeds and seed oils (Padley *et al.* 1994). They constitute a family of four compounds, named alpha-, beta-, gamma-, and delta-tocopherol that exert antioxidant activity both *in vivo*, also known as vitamin E activity, as well as *in vitro*. Alpha-tocopherol is the form with maximum vitamin E effect (Traber and Sies 1996). Apart from its role as an antioxidant in the lipid phase of biomembranes, it has been suggested that alpha-tocopherol may play an important role in regulation of signalling cascades and gene expression (Azzi 2007). Unlike most oil seeds, which mainly accumulate gamma-tocopherol and delta-tocopherol (Padley *et al.* 1994), sunflower seeds mainly accumulate alpha-tocopherol, which account for more than 90% of the tocopherols in sunflower seeds and oils (Velasco *et al.* 2002).

The discovery of mutants with deficient accumulation of tocopherols has played an important role in the characterization of the tocopherol pathway (DellaPenna and Pogson 2006). The most relevant ones are the *Zea maize* mutant *sxd1* (Provencher *et al.* 2001; Sattler *et al.* 2003) and *Arabidopsis thaliana* mutant *vte 1* (Porfirova *et al.* 2002), which are devoid of tocopherols, and the *Arabidopsis thaliana* mutant *vte5-1* (Valentin *et al.* 2006), with seed tocopherol levels reduced to 20% of the wildtype.

A natural sunflower mutant named IAST-522 with seed tocopherol levels reduced to 29% of the wildtype (73.4 mg per kg seed compared to 250.9 mg per kg in the wildtype) has been identified (Velasco *et al.* 2010). This mutant is unique, as no other mutant with deficient tocopherol accumulation has been reported in sunflower. Therefore it is a valuable genetic source for studying biochemical and genetic aspects of tocopherol biosynthesis and accumulation in sunflower seeds. The objective of the present research was to study the inheritance of reduced seed tocopherol accumulation in seeds of the sunflower line IAST-522.

Materials and methods

Plant materials

IAST-522 is an $S_{4:5}$ sunflower line produced by a plant-to-row selection scheme for reduced total seed tocopherol content, mainly in the alpha-tocopherol form (>95% of total tocopherols), from an accession with reduced seed tocopherol levels identified in the evaluation of a collection of cultivated sunflower entries (Velasco *et al.* 2010). HA89 is an oilseed maintainer line with standard seed tocopherol content and composition (>95% of total tocopherols in the alpha-tocopherol form) released by the Texas Agricultural Experiment Station and the USDA-ARS in 1971.

Genetic study

Plants of IAST-522 grown in pots in the greenhouse were reciprocally crossed with plants of HA89 in the winter of 2004 and 2005. F_1 plants together with plants of both parents were grown in pots in a mesh cage enclosure under open air conditions in the spring of 2005 and 2006, where reciprocal crosses as well as backcrosses to both parents were made. The heads of all plants were bagged before flowering to produce seeds under selffertilization. At harvest, six-seed bulk samples from each plant were analysed for total tocopherol content as described by Velasco *et al.* (2002).

Plants of the parents, F_1, F_{1r}, F_2, BCP_1 and BCP_2 generations produced in 2005 and 2006 were grown in the field in 2006 and 2007, respectively, in a randomized complete block design with two replications. F_3 plants from an F_2 plant population of 76 individuals were grown in the 2007 field plot, with 12 F_3 plants per each F_2. In all cases, seeds were germinated in moistened filter paper, sown in small pots, and transplanted to the field after three weeks in the growth chamber.

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The field plots consisted of rows 5-m long with 1 m spacing between rows and 0.20 m spacing between plants in the row. Analyses of tocopherol content of F_1, F_2, F_3, and BC plants were conducted on six bulked F_2, F_3, F_4, and BC_1F_2 achenes, respectively, chosen at random.

Heritability estimates

Broad-sense heritability (H^2) and narrow-sense heritability (h^2) were estimated on a single-plant basis as (Allard 1960).

$$H^2 = \left(\sigma_{F_2}^2 - \left(\sigma_{P_1}^2 + \sigma_{P_2}^2 + \sigma_{F_1}^2 + \sigma_{F_{1r}}^2\right)/4\right)/\sigma_{F_2}^2$$

$$h^2 = \left(2\sigma_{F_2}^2 - \left(\sigma_{BCP_1}^2 + \sigma_{BCP_2}^2\right)\right)/\sigma_{F_2}^2 \,,$$

where $\sigma_{F_2}^2$, variance among F_2 plants; $\sigma_{P_1}^2$, variance among P_1 (HA89) plants; $\sigma_{P_2}^2$, variance among P_2 (IAST-522) plants; $\sigma_{F_1}^2$, variance among F_1 plants of the cross HA89 × IAST-522; $\sigma_{F_{1r}}^2$, variance among F_1 plants of the cross IAST-522 × HA89; $\sigma_{BCP_1}^2$, variance among plants from the backcross to HA89; $\sigma_{BCP_2}^2$, variance among plants from the backcross to IAST-522. The minimum number of genes (k) controlling the total tocopherol content was estimated following Wright (1968) as:

$$k = (P_1 - P_2)^2 /8 \left(\sigma_{F_2}^2 - \left(\sigma_{P_1}^2 + \sigma_{P_2}^2 + \sigma_{F_1}^2 + \sigma_{F_{1r}}^2\right)/4\,\sigma_{F_2}^2\right),$$

where P_1 and P_2 are the mean values of the two parents.

Estimate of heritability through parent–offspring correlation was computed as the correlation coefficient between tocopherol content in the F_2 and F_3 plant generations. The use of the correlation instead of the regression coefficient is recommended when the parent and offspring generations are evaluated under different environments, as this method of estimating heritability tends to reduce environmental effects (Frey and Horner 1957).

Results and discussion

Seeds of line IAST-522 showed a low average tocopherol content in 2006 (74 mg per kg) and 2007 (75 mg per kg) as compared to the wildtype line HA89 (240 and 207 mg per kg, respectively). F_1 plants from the cross IAST-522 × HA89 showed an average tocopherol content of 146 mg per kg in 2006 and 124 mg per kg in 2007, whereas F_1 plants from the reciprocal cross showed an average tocopherol content of 118 mg per kg in 2007 (table 1). The F_1 generation from the cross HA89 × IAST-522 could not be evaluated in 2006, as most F_1 plants produced no seeds or an insufficient number of seeds for tocopherol analysis. The results of 2007 indicated no significant differences ($t = 0.75$, $P > 0.05$) between reciprocal F_1s, suggesting absence of cytoplasmic effects.

Mean and standard deviation values for the F_2 and BC generations are presented in table 1. Only 19 plants from the BC to IAST-522 produced sufficient number of seeds for tocopherol analysis in 2006, which was considered insufficient

Table 1. Total tocopherol content (mean ± SD; mg per kg seed) of sunflower lines HA89, IAST-522, their F_1 and F_2 generations, and the backcross to both parents, grown in Córdoba, Spain in 2006 and 2007, and estimates of broad-sense heritability (H^2), narrow-sense heritability (h^2), and minimum number of genes controlling the trait (k).

Generation	Year 2006	Year 2007
HA89	240 ± 26	207 ± 31
IAST-522	74 ± 15	75 ± 18
F_1(HA89xIAST-522)	–	118 ± 24
F_1(IAST-522xHA89)	146 ± 26	124 ± 29
F_2(IAST-522xHA89)	154 ± 52	151 ± 46
BC to HA89	182 ± 41	170 ± 41
BC to IAST-522	–	128 ± 39
H^2	0.81	0.67
h^2	–	0.49
k	1.55	1.62

and accordingly h^2 could not be estimated in that year. Estimates of H^2 were 0.81 in 2006 and 0.67 in 2007. Estimate of h^2 in 2007 was 0.49 (table 1), indicating a major role of additive gene action. Estimate of heritability through parent–offspring correlation was 0.59 (figure 1). The estimate of the minimum number of genes underlying a deficient accumulation of tocopherols in seeds of IAST-522 was 1.55 in 2006 and 1.62 in 2007, suggesting that the trait is oligogenic.

There are no previous studies on the inheritance of deficient seed tocopherol accumulation in seeds of sunflower or other oilseeds. In rapeseed (*Brassica napus* L.), several inheritance studies have been conducted using breeding lines with different seed tocopherol levels. Goffman and Becker (2001a,b) concluded that total seed tocopherol content was mainly controlled by genes with additive effects, whereas Marwede *et al.* (2004) found low heritabilities (broad sense) for total seed tocopherol content, which ranged from 0.34 to
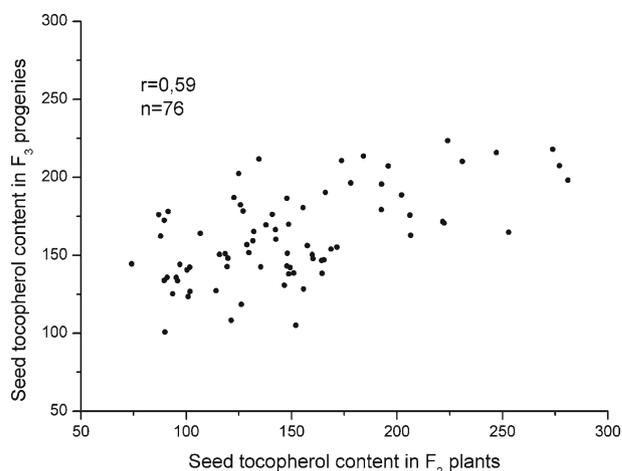

Figure 1. Scatter plot of total seed tocopherol content (mg per kg seed) of individual F_2 plants (bulked F_3 seeds) and their F_3 progenies (bulked F_4 seeds in 12 individual F_3 plants) from the cross between sunflower lines IAST-522, with reduced seed tocopherol content, and HA89, with wildtype seed tocopherol content.

0.48 in three environments. The present research identified H^2 of 0.67 and 0.81 for reduced tocopherol content in seeds of IAST-522, indicating that the trait is highly heritable. The h^2 was estimated as 0.49 in 2007 compared to H^2 of 0.67 under the same environment, suggesting the predominance of additive gene action for this trait. Our results also suggested that reduced tocopherol content in seeds of the sunflower line IAST-522 is controlled by a relatively small number of genes, which is in line with the results obtained in previous studies on the inheritance of total seed tocopherol content in rapeseed (Goffman and Becker 2001b; Marwede *et al.* 2005).

In conclusion, the present research revealed that deficient tocopherol accumulation in seeds of sunflower line IAST-522 is the result of genetic modifications in a relatively small number of genes, which results in a moderate-to-high heritability, with predominance of additive gene action. Although the breeding goal is to increase tocopherol content in sunflower seeds, the availability and characterization of a line with defective tocopherol accumulation is of great value for the identification of key genes involved in tocopherol accumulation in this crop.

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