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Varietal selection of oilseeds: the prospective nutritional and technological benefits
Perspectives offertes par la sélection variétale sur la qualité nutritionnelle et technologique des oléagineux

New sunflower seeds with high contents of phytosterols

Leonardo Velasco*, Álvaro Fernández-Cuesta and José M. Fernández-Martínez

Institute for Sustainable Agriculture (IAS-CSIC), Alameda del Obispo s/n., 14004 Córdoba, Spain

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Abstract – Dietary phytosterols have a positive nutritional impact because they contribute to reduce cholesterol levels in blood. Accordingly, foods rich in phytosterols are required in a healthy diet. Vegetable oils are the richest source of phytosterols in the diet, though sunflower oil has lower phytosterol content than other seed oils such as rapeseed and corn. Increasing phytosterol content in sunflower oil requires optimizing first selection procedures. In this way, the development of accurate methods for analyzing phytosterol content in seeds instead of oils has opened up recently the way for large-scale screening for this trait. Large variability for seed phytosterol content has been identified in sunflower germplasm, from which we have developed a line, IASP-18, with about twofold seed phytosterol content than conventional sunflower. The trait is expressed across environments. Genetic studies are underway to characterize its inheritance and assess the feasibility of introgressing genes for high phytosterol content into elite sunflower germplasm.

Keywords: Sunflower / phytosterols / genetic variation / analytical methods / inheritance / genetics / molecular markers

Résumé – Nouvelles graines de tournesols à hauts taux de phytostérols. Les phytostérols alimentaires ont un impact bénéfique sur la santé, car ils contribuent à réduire les niveaux de cholestérol sanguin. Aussi, les aliments enrichis en phytostérols sont-ils recommandés pour une alimentation saine. Les huiles végétales représentent la source la plus riche en phytostérols alimentaires ; l'huile de tournesol affiche le plus faible taux en phytosterols comparée aux autres huiles de graines comme l'huile de colza ou de maïs. L'augmentation de la concentration en phytostérols de l'huile de tournesol nécessite en premier lieu l'optimisation des procédures de sélection. Ainsi, le développement de méthodes précises pour analyser le contenu en phytostérols dans les graines au lieu des huiles a récemment ouvert la voie à une sélection à grande échelle concernant ce caractère. Une grande variabilité des concentrations en phytostérols des graines a été identifiée dans le germplasme du tournesol, dont nous avons développé une lignée, IASP-18, contenant de l'ordre du double de la concentration habituelle en phytostérols du tournesol. Ce caractère est exprimé quel que soit l'environnement. Des études génétiques sont en cours pour caractériser son héritabilité et évaluer la faisabilité de l'introduction de gènes de haut taux de phytostérols dans le germplasme de tournesols de qualité supérieure.

Mots clés : Tournesol / phytostérol / variation génétique / méthodes d'analyse / génétique / marqueurs moléculaires

1 Introduction

Phytosterols or plant sterols are steroid compounds with similar chemical structure to mammalian cholesterol, consisting of a tetracyclic cyclopenta[a]phenanthrene structure and an alkyl side chain with 8–10 carbons (Hartmann, 1998). Figure 1 shows the close chemical structure of cholesterol and β-sitosterol, the predominant sterol in vegetable oils (Gunstone, *et al.*, 2007). Because of their structural similarity with cholesterol, phytosterols reduce absorption of cholesterol and contribute to reduce blood cholesterol levels (Plat and Mensink, 2005).

Vegetable oils are the richest natural sources of phytosterols in the diet, followed by cereal grains and nuts (Piironen, *et al.*, 2000). Vegetable oils differ for phytosterol content. Amongst commercially important oils, corn oil possesses the

* Correspondence: lvelasco@ias.csic.es

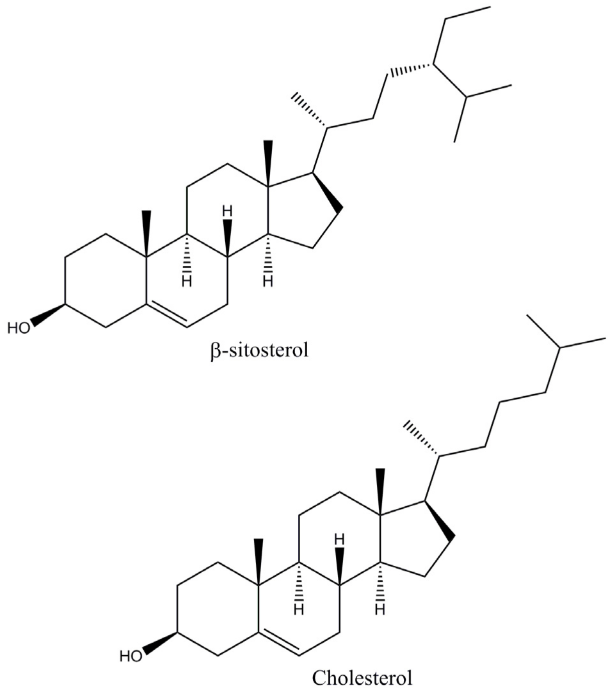

Fig. 1. Chemical structure of *β*-sitosterol and cholesterol.

maximum phytosterol content (ca. 11 120 mg kg^{-1}) followed by rapeseed/canola (ca. 7099 mg kg^{-1}). Sunflower oil has an estimated average phytosterol content of 4436 mg kg^{-1} (Velasco and Ruiz-Méndez, 2014). Increasing total phytosterol content is therefore an important breeding objective for this oil crop.

2 Factors influencing selection for increased seed tocopherols in sunflower

In the evaluation of variability for phytosterols in sunflower, these compounds have been commonly measured in crude extracted oils. In these, Ayerdi-Gotor, *et al.* (2007) and Piironen, *et al.* (2000) reported ranges of variation between 1250 to 7650 mg kg^{-1} and 3740 and 7250 mg kg^{-1}, respectively. From a breeding perspective, phytosterol content in the oil is the result of several variables: phytosterol content in the seeds, oil content of the seeds, and efficiency of the oil extraction method. Additionally, oil is commonly extracted from bulks of seeds from several plants, which does not allow evaluating plant to plant variation. For selection purposes, all these variables need to be considered separately. A first consequence of the above discussion in that selection for total phytosterol content must be conducted by analyzing the phytosterol content in the seeds of individual plants (Fernández-Cuesta, *et al.*, 2014).

Methods for the analysis of phytosterol content in small samples of seeds, valid for the analysis at the single plant level, were proposed by Amar, *et al.* (2008) in rapeseed and Roche, *et al.* (2010a) in sunflower. The methods basically consist in the extraction of the unsaponifiable lipid fraction directly from seeds, derivatization of sterols to trimethyl-silyl ethers, and GLC analysis (Amar, *et al.*, 2008). Fernández-Cuesta, *et al.* (2012a) analyzed the phytosterol content of the seeds of 87 sunflower inbred lines and their extracted crude oils, in the latter case using conventional procedures. After transforming seed phytosterol content into oil phytosterol content using the known oil content of the seeds, they found a correlation coefficient of 0.85 between the conventional analysis in extracted oils and the method of analysis in seeds. The results clearly supported the validity of analyzing phytosterols in seeds instead of oils for breeding research.

3 Variability for seed phytosterol content in sunflower germplasm

The availability of methods for analyzing seed phytosterol content has enabled evaluations of germplasm and selection programs in recent years. Merah, *et al.* (2012) reported a variation from 1523 to 2490 mg kg^{-1} seed (whole achene) in a population of recombinant inbred lines. Other studies based on the analysis of seed kernels have reported ranges of variation from 1426 to 4710 mg kg^{-1} kernel in 87 sunflower inbred lines (Fernández-Cuesta, *et al.*, 2012a), from 2,179 to 3555 mg kg^{-1} kernel in 12 confectionery landraces (Fernández-Cuesta, *et al.*, 2012b), from 2954 to 3810 mg kg^{-1} kernel in 22 commercial hybrids (Velasco, *et al.*, 2013), from 1319 to 5119 mg kg^{-1} kernel in 985 accessions of a world collection (Fernández-Cuesta, *et al.*, 2014), and from 1344.0 to 2942.5 mg kg^{-1} kernel in a collection of 137 Spanish landraces of confectionery sunflower (Velasco, *et al.*, 2014).

Several studies have shown high heritability of total seed phytosterol content, but also a marked effect of environmental factors. Broad sense heritability was estimated in 0.72 by Merah, *et al.* (2012) and 0.85 by Velasco, *et al.* (2013). Amongst the main factors influencing seed phytosterol content, Roche, *et al.* (2006; 2010b) found that higher temperatures enhanced phytosterol accumulation, whereas water stress has been also found to favour phytosterol accumulation in sunflower seeds (Roche, *et al.*, 2006).

The above mentioned studies have emphasized the genetic potential for selecting for increased phytosterol content in sunflower, but also the necessity of evaluating selected germplasm across a range of environments to minimize environmental effects.

4 Selection for increased seed tocopherols in sunflower

From the initial variation between 1319 to 5119 mg kg^{-1} kernel identified by Fernández-Cuesta, *et al.* (2014) in 985 accessions of a sunflower world collection, the authors conducted selection during two years and evaluated the best accessions during two additional years in replicated field trials. The accession with the highest phytosterol content had an average phytosterol content of 3682 mg kg^{-1} kernel compared to 2686 mg kg^{-1} kernel in the control, far from the highest values identified in the original germplasm collection that probably reflected strong environmental effects.

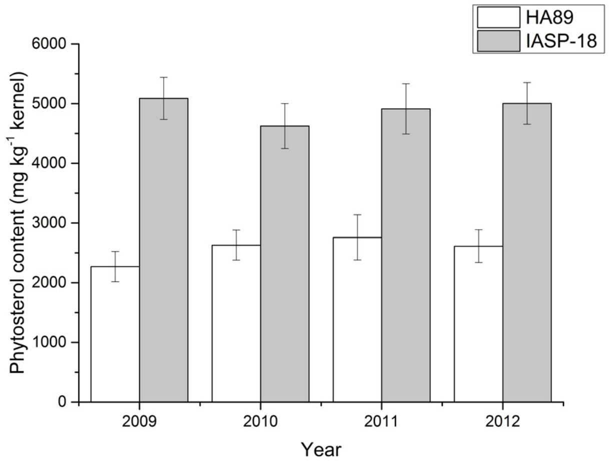

Fig. 2. Kernel phytosterol content in line IASP-18, selected for high phytosterol content and control line HA89 grown in the field during four years from 2009 to 2012.

In the evaluation of 87 sunflower inbred lines, Fernández-Cuesta, *et al.* (2012a) identified a large range of variation from 1426 to 4710 mg kg^{-1} kernel. Selection at the single-plant level under several environments (unpublished results) led the isolation of the line IASP-18 with around twofold phytosterol content than the control line HA89. Figure 2 shows phytosterol content of both lines in four environments, in which IASP-18 averaged 4889 mg kg^{-1} kernel compared to 2529 mg kg^{-1} kernel in HA89. Line IASP-18 derived from germplasm with a low seed oil content around 28% (Aguirre, *et al.*, 2012), compared to values around 50% in current sunflower cultivars (Fernández-Martínez, *et al.*, 2009). Studies on the feasibility of combining high seed phytosterol content and high seed oil content are currently ongoing.

5 Genetics of seed phytosterol content

A segregating F_2 population was developed from the cross between lines IASP-18 and HA89. The analysis of seed (kernel) phytosterol content in this F_2 population showed a continuous variation that suggested that the trait is under polygenic control (Fig. 3). Further genetic studies on the high phytosterol content of line IASP-18 are under way. Previous genetic studies on seed phytosterol content in sunflower confirm the genetic complexity of the trait. Haddadi, *et al.* (2012) identified four quantitative trait loci (QTL) associated with seed phytosterol content that explained only 27% of the observed phenotypic variance. Merah, *et al.* (2012) reported 13 regions on 9 linkage groups that were involved in different phytosterol traits.

6 Conclusions

Sunflower germplasm contains large variability for seed phytosterol content. Since the trait shows high heritability, there is room for selection for high seed phytosterol content and to develop cultivars with higher levels of these compounds. Because of the beneficial nutritional effects of dietary phytosterols, oils with enhanced phytosterols levels may have commercial advantages, at least for specific market niches. We have selected a line, IASP-18, that exhibits around twofold phytosterol content in seeds than conventional sunflower. Even though the line has low oil content and poor agronomic performance, we are currently studying whether the trait can be introgressed into elite sunflower material with high oil content and good combining ability for production of hybrids with good agronomic performance.

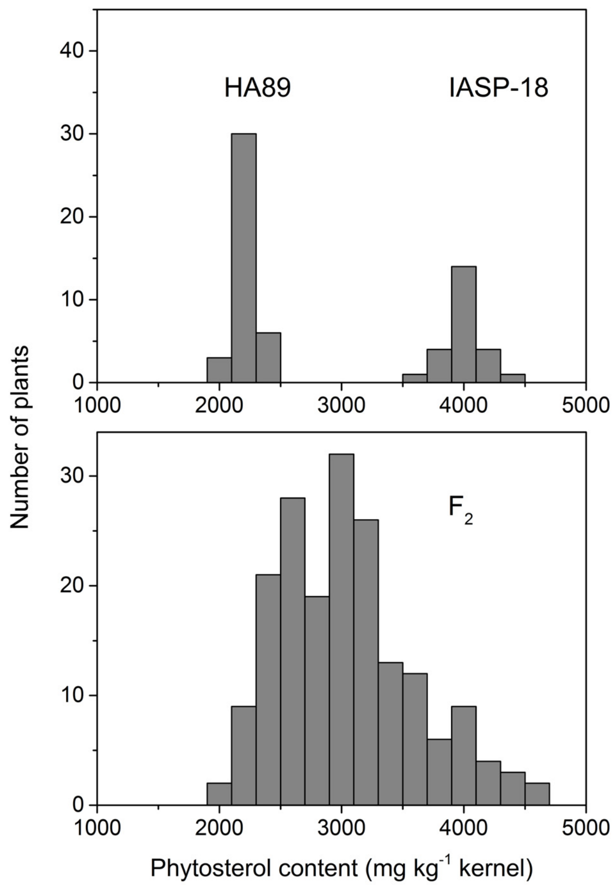

Fig. 3. Kernel phytosterol content in line IASP-18, selected for high phytosterol content, control line HA89, and an F_2 population developed from them.

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Conflict of interest: none.

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