Research Communication

Identification of Soybean Varieties with High α -Tocopherol Content

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α -Tocopherol (Toc) is the tocopherol isoform with the highest vitamin E activity. Since soybean seeds contain a low percentage of α -Toc, increasing the α -Toc content to improve vitamin E activity is a breeding objective. Little is known about the genetic variability of the tocopherol content and composition in soybeans. In the present study, we analyzed the tocopherol composition of 1,109 germplasm accessions of cultivated and wild soybean by high performance liquid chromatography (HPLC) and identified three varieties, Dobrogeance, Dobruzoa 14 Pancevo and Keszthelyi Aprozemu Sarga with a high α -Toc content. Dobrogeance showed a similar Toc composition to that of Dobruzoa 14 Pancevo with the α -Toc content being four to seven times higher than that of standard varieties and almost the same as that of total Toc. Keszthelyi Aprozemu Sarga showed a markedly increased content of α -Toc, but a significantly lower total Toc content.

Key Words: soybean, wild soybean, tocopherol (vitamin E), genetic variability.

Introduction

Tocopherols are lipophilic antioxidants that protect lipids from peroxidation. They are known as vitamin E and play many important roles in human nutrition, for example, they prevent cardiovascular diseases and cancer, and enhance the immune function. They consist of a polar chromanol head group and a hydrophobic phytyl tail. Four different tocopherols, α -, β -, γ - and δ -tocopherols occur in nature and they differ from one another based on the number and position of methyl groups on the chromanol head group. Among the four tocopherols, α -Toc displays the highest biological activity since it harbors the most potent form of vitamin E, while in the case of β -, γ - and δ -Toc, the vitamin E activity is 40, 10 and 1–2%, respectively, of that of α -Toc. It is considered that the α -tocopherol transfer protein (α -TTP) specifically binds to the fully methylated form of tocopherol, α -Toc, in the human body and enhances its transfer into the liver (Mino and Fukuzawa 1996, Arai 1997, Bramley *et al.* 2000).

Tocopherol composition is different among plant organs. Among the tocopherols, α -Toc is the most predominant form in photosynthetic tissues such as leaves and stems and among the tocopherols present in these tissues, α -Toc accounts for about 90%. On the other hand, in seeds, oil

crops such as soybean and rape generally contain a low proportion of α -Toc, although some crops such as sunflower and safflower contain a high proportion of α -Toc (70–80%) in seeds (Kajimoto and Hasebe 1982, Munne-Bosch and Alegre 2002). Since the vitamin E activity of corn and soybean oil is low from a nutritional point of view, increasing the α -Toc content and enhancing the vitamin E activity in oil crops are breeding objectives. In maize, quantitative trait loci that determine the content of individual tocopherols and total tocopherol have been identified (Rocheford *et al.* 2002, Wong *et al.* 2003). Recently, using transgenic technologies, coexpression of a gene identified in *Arabidopsis* that encodes the tocopherol biosynthetic enzyme 2-methyl-6-phytylbenzoquinol methyltransferase with a γ -tocopherol methyltransferase gene has resulted in an increase of the α -Toc content in soybean seeds from 10% to >95% (Van Eenennaam *et al.* 2003). However, γ -Toc plays other important nutritional and functional roles. It shows a moderate antioxidant activity and appears to be a better food additive as antioxidants than α -Toc. Recent investigations have revealed that its derivatives promote the excretion of sodium in urine, preventing renal failure and swelling, and this function is more effective when α - and γ -Toc are taken at the same time (Christein *et al.* 1997). Therefore, manipulation of the α - and γ -Toc contents may be a more suitable breeding strategy for improving the nutrition and function of the tocopherols than increasing the content of only one type of tocopherol.

Although soybean is one of the most important oil crops, the genetic variability of the tocopherol content and

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composition is poorly documented. In the present study, we analyzed the tocopherol composition of more than one thousand cultivated and wild soybean accessions from the germplasm collection and we identified novel soybean varieties with high α -Toc contents.

Materials and Methods

Seed materials

Nine hundred and nine soybean (*Glycine max* (L.) Merr.) and 200 wild soybean (*Glycine soja* Sieb. and Zucc.) accessions were evaluated. The germplasm was obtained from the gene bank of the National Institute of Agrobiological Sciences in Tsukuba, Japan.

Methods for screening the tocopherol composition

Based on standard vitamin E analytical methods (Okabe and Nakamura 1985), a simple and reliable analytical procedure was used for screening the tocopherol composition in seeds. Fifty mg of ground seeds were weighed in 10 ml screw-capped test tubes and supersonicated in 1 ml of 80% aqueous ethanol for 15 min at room temperature. Two ml of hexane saturated with pyrogallol was added and allowed to stand for 30 min at room temperature (25°C). After centrifugation, 20 μ l of the hexane layer (upper phase) was subjected to HPLC (Hitachi LaChrom Elite, Hitachi High-Technologies Corp., Japan) on Inertsil ODS-3 reverse phase column (3.0 $\times$ 250 mm, GL Sciences, Japan) at 40°C with a 0.5 ml/min flow rate using CH₃CN/CH₃OH (75:25 v/v) for 25 min. Tocopherols were detected by UV at 295 nm.

Quantification of individual tocopherols and total tocopherol

As an internal standard, tocol (Tama Biochemical Co., Ltd., Japan) was mixed with an extraction buffer (80% aqueous ethanol) for the quantification of individual tocopherols and total tocopherol. Fifty mg of ground seeds were weighed in 10 ml screw-capped test tubes and supersonicated in 1 ml of 80% aqueous ethanol containing 3–5 μ g tocol for 15 min at room temperature (25°C). Then after, the same procedure was used in the screening method. Individual tocopherols were quantified based on the ratio of the area of each isoform peak compared to the internal standard. Total tocopherol content was calculated by summing up the content of the individual tocopherols.

Results and Discussion

Identification of soybean varieties with increased contents of α -tocopherol

We analyzed the tocopherol composition of cultivated and wild soybean accessions by HPLC. Three major peaks which corresponded to δ -, γ - and α -Toc were obtained for all the accessions. In the separation procedure used in the present investigation, β - and γ -Toc could not be separated, and since the β -Toc content was very low in soybean seeds,

the β -Toc peak was not considered. Figure 1-A and -B show that the HPLC chromatograms for cv. Ichihime and cv. Toyokomachi, respectively, gave large peaks of δ - and γ -Toc and a small peak for α -Toc. Standard tocopherol concentrations of δ -, γ - and α -Toc in mature seeds of common soybean varieties are about 30%, 65% and 5%, respectively (Kajimoto and Hasabe 1982). Most of the accessions analyzed showed similar chromatograms to those observed in Figure 1-A and -B. However, three varieties, Dobrogeance, Dobruzoa 14 Pancevo and Keszthelyi Aprozemu Sarga gave different chromatograms and displayed larger peak areas for α -Toc. All the varieties with increased α -Toc contents identified so far had consisted of cultivated soybean accessions.

The varieties with high α -Toc contents (referred to as "high α -Toc varieties") can be classified into two types based on the elution profile of the individual tocopherols in the HPLC chromatograms: (1) that of vars. Dobrogeance and Dobruzoa 14 Pancevo with a smaller peak of δ -Toc, similar peak area of γ -Toc and larger peak area of α -Toc compared to the typical accessions analyzed (Fig. 1-C); and (2) that of

Fig. 1. HPLC chromatograms of tocopherols in soybean seeds for (A) cv. Ichihime, (B) cv. Toyokomachi, (C) var. Dobrogeance and (D) var. Keszthelyi Aprozemu Sarga. Peaks 1, 2 and 3 correspond to δ -, γ - and α -tocopherol, respectively.

Table 1. Tocopherol composition of soybean varieties

Accessions	Tocopherols (mg/100 g meal) ¹⁾				Genebank stock number	Origin
	δ	γ	α	Total		
Dobrogeance	1.59±0.26	5.72±0.55	3.71±0.29	11.02±1.05	00034330	Romania
Dobrudza 14 P.	1.53±0.24	6.60±0.16	3.26±0.12	11.39±0.46	00034283	Yugoslavia
Keszthelyi A.S.	1.09±0.10	2.65±0.08	4.26±0.32	8.00±0.30	00034187	Hungary
Toyokomachi	5.35±0.16	5.11±0.17	0.49±0.42	10.95±0.73	00058196	Japan
Ichihime	4.90±0.08	6.93±0.11	0.96±0.15	12.79±0.25	—	Japan

¹⁾ Values are mean of three replicates±SD.

var. Keszthelyi Aprozemu Sarga with smaller peak areas of δ - and γ -Toc and a significantly larger peak of α -Toc compared to the typical accessions analyzed (Fig. 1-D).

Quantitative analysis of tocopherols of the high α -Toc varieties

Quantitative analysis of the tocopherol concentrations in seeds from the three high α -Toc varieties using tocol as the internal standard is summarized in Table 1. Seeds of var. Dobrogeance and var. Dobruoza 14 Pancevo were grown at the National Agricultural Research Center for Tohoku Region, Akita in 2001. Seeds of var. Keszthelyi Aprozemu Sarga were grown at the National Shikoku Agricultural Experiment Station, Kagawa in 1998. Seeds of cv. Toyokomachi and cv. Ichihime used to represent standard cultivars grown in Japan were grown at the Hokkaido University Agricultural Farm in 2003.

Three high α -Toc varieties, var. Dobrogeance, var. Dobruoza 14 Pancevo and var. Keszthelyi Aprozemu Sarga, respectively, showed higher α -Toc contents than those of cv. Toyokomachi and cv. Ichihime (Table 1). In the present study, the high α -Toc trait of these varieties was considered to be stable among different years. However, although the total tocopherol content of var. Dobrogeance and var. Dobruoza 14 Pancevo was similar to that of cv. Toyokomachi and cv. Ichihime, the content of var. Keszthelyi Aprozemu Sarga was significantly lower.

Further studies are under way to analyze the genetic control of the high α -Toc trait in the three high α -Toc varieties identified. In addition, it will be necessary to determine the extent to which the tocopherol composition is influenced by environmental conditions such as growth temperature, because it was reported that the tocopherol composition and/or contents significantly influenced by the temperature during the seed ripening time (Almonor *et al.* 1998, Dolde *et al.* 1999, Britz and Kremer 2002).

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