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Tocopherol Content and Profile of Soybean: Genotypic Variability and Correlation Studies

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Abstract Plant seed oils, including soybean seed oil, represent the major source of naturally derived tocopherols, the antioxidant molecules that act as free radical quenchers preventing lipid peroxidation in biological systems and vegetable oil products. All four isomers of tocopherols, i.e. α, β, γ, δ tocopherols that exist in nature are found in soybean seeds. The biological activity and the contribution of these isomers in improving the oxidative stability of vegetable oil are in reverse order. Because of the nutritive value and the importance for oil stability, enhancement of tocopherol content, through breeding programs, in soybean seeds has become a new and an important objective. Genotypic variability, which is the basis of every breeding program, is scarcely reported for tocopherol content and profile in soybean seeds. In the present investigation, the tocopherol content and profile in seed samples of 66 genotypes of Indian soybean were determined. The ratios observed between the lowest and the highest values for α, β, γ, δ, total tocopherol content were 1:13.6, 1:10.4, 1:7.5, 1:9.1, 1:7.9, respectively. The mean contents for α, β, γ, δ and total tocopherols were 269, 40, 855, 241 and 1,405 μg/g of oil, respectively. Total tocopherol content was the highest in 'Co Soya2' followed by 'Ankur'. Concentration of α-tocopherol was the highest (27%) in 'Ankur' followed by 'MACS124' (26%) whereas gamma tocopherol concentration was the highest (69%) in 'VLS1' and 'PK327' followed by 'MACS13' (67%). In view of the fact that levels of unsaturated fatty acids, apart from tocopherols, also determine the oxidative stability of vegetable oils, the relationship of four isomers of tocopherols with each other as well as with different unsaturated fatty acids and oil content was also investigated in the present study. All the four isomers of tocopherols exhibited highly significant correlations with each other ($p < 0.001$) whereas γ-tocopherol and total tocopherol content showed a significant relationship with linoleic acid ($p < 0.05$).

Keywords Genotypic variability · Soybean seed oil · Tocopherol isomers · Fatty acids

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Tocopherols, natural antioxidant molecules that scavenge free radicals in biological systems, are abundantly present in plant seed oil. They exist in four isomers, i.e. α, β, γ and δ tocopherol, depending upon the number and position of methyl substituents on the chromanol ring, with α tocopherol having the maximum of three, β and γ tocopherol having two, and δ tocopherol having only one substituent. An increased tendency to release hydrogen atoms occurs with increasing numbers of methyl substituents on the chromanol ring. Thus, the antioxidative activities of tocopherols in biological systems against lipid peroxidation are $\alpha > \beta > \gamma > \delta$ (100, 50, 10 and 3% relative activity, respectively) [1]. Moreover, α tocopherol is preferentially retained and distributed in the body [2]. Medical evidence has indicated that an intake of 400 IU of tocopherols results in a decreased risk for atherosclerosis, cancer, degenerative diseases like Alzhemeir and Parkinson's disease and an improved immune system [3, 4]. With regard to the contribution of tocopherols in the protection of oxidation-sensitive fatty acids from the free radicals (under in vitro conditions), the relative antioxidant activities of tocopherols are $\alpha < \beta < \gamma < \delta$ which is the reverse of the in vivo activities.

Soybean oil constitutes about 30% of worldwide consumption of vegetable oils; and unlike other major vegetable oils available in the market, it contains all four isomers of tocopherols with the γ isomer possessing the maximum (60%) proportion of total tocopherols content [5]. All four naturally occurring tocopherol isomers in crude oil decrease in the refining process to varying degree [6, 7] mainly in the deodorization step, where γ tocopherol suffers the greatest loss [6]. This necessitates the need for a breeding program with the objective of enhancing the content of tocopherols in soybean seeds. As genotypic variability is the basis of any breeding program pertaining to the development of genotypes with an increased level of a particular quality trait, it becomes imperative to study the genetic variability for tocopherols in soybean genotypes to identify genotypes with high levels of tocopherols. However, very limited information concerning genotypic variation for tocopherols isomers in soybean seed oil is available in the literature [8, 9]. McCord et al. [8] investigated three isomers of tocopherol (α, γ and δ tocopherol) in three populations derived from three single crosses between normal linolenate and reduced linolenate lines. Panizzi and Erhan [9] investigated seeds of 161 Brazilian soybean cultivars for all four isomers of tocopherols. The tocopherol contents of Indian soybean genotypes have not yet been investigated.

Soybean seed oil contains three major unsaturated fatty acids viz. oleic acid (23%), linoleic acid (53%) and linolenic acid (8%). The rate of oxidation of linolenic acid, linoleic acid and oleic acid is in the ratio of 21.6:10.3:1 [10], respectively indicating that these fatty acids play an important role in the oxidation of soybean oil. Since the oxidative stability of vegetable oils is a function of tocopherols as well as the fatty acid composition, the relationship between tocopherol isomers and different unsaturated fatty acids needs to be investigated. In view of the limited reports focusing on the association of different tocopherols with fatty acids in soybean seed oil available in the literature [8, 11, 12], the present investigation was undertaken to survey the genotypic variability for tocopherols among soybean genotypes recommended for use in India and to study the relationship of tocopherols isomers with each other as well as with fatty acids and oil content.

Materials and Methods

Materials

Sixty-six cultivars of soybean were raised following standard agronomic practices in single row plots of 5 m long with 0.45 m spacing between rows in the experimental field of the National Research Centre for Soybean, Indore, (22°N), situated in the Malwa region of Madhya Pradesh, India, in the 2005 cropping season. Madhya Pradesh is the hub of soybean cultivation in India; and during the cropping season 2005, at Indore, the average daily mean temperature was 25.9 °C and the rainfall received was 620.4 mm. Of the selected genotypes, some of the genotypes have one parent from maturity group V–VII. Seeds of all the genotypes harvested at their respective maturity were evaluated for oil content, fatty acids and four tocopherols (i.e. α, β, γ, and δ tocopherol).

Methods

(1) *Extraction and estimation of oil content*. Oil from ground seeds (1 g) was extracted with 180 ml hexane in an automated Soxhlet unit (Pelican Equipments, Chennai, India) for 3 h. Percent oil content was determined by weight differences.

(2) *Determination of fatty acid composition*. Ground seeds (200 mg) were soaked in 2.5 ml petroleum ether (boiling point 40–60 °C) overnight at room temperature. The petroleum ether–oil mixture was decanted and kept in a water bath at 75 °C till the petroleum ether was completely removed. The oil extracted was transesterified using 1 N sodium methoxide in anhydrous methanol [13]. Fatty acid methyl esters (FAMEs) were prepared, separated and analyzed using Shimadzu GC 17A, using capillary column (SGE BPX70), with a length and diameter of 30 m and 0.32 mm, respectively. The oven temperature of GC was programmed at 140 °C for 3.6 min, then increased to 170 °C at the rate of 13.5°C/min and maintained for 3.8 min and finally increased to 182 °C at 5 °C/min. The flame ionization detector (FID) and injector were maintained at 240 °C. Nitrogen, the carrier gas used, was maintained at a flow rate of 15 ml/min with a column pressure of 90 kPa. Hydrogen and air were maintained at 50 kPa for proper detector operation. The peaks obtained for fatty acid methyl esters were identified by comparing the retention times with those of standard fatty acid methyl esters (Sigma-Aldrich, India).

(3) *Extraction and determination of tocopherols using HPLC*. Twenty randomly taken seeds of each soybean genotype, in triplicate, were ground using a

metallic pestle and mortar into a fine flour and sieved (500 μm size). Oil from the fine soy flour was extracted by soaking the flour in HPLC-grade hexane for 8 h. The hexane–oil mixture was transferred into vials and the solvent was evaporated using a freeze drier. The weight of the oil was determined gravimetrically in each vial and the samples were re-dissolved in HPLC-grade *n*-hexane to a fixed volume (1.0 ml). Tocopherol composition was determined using a Shimadzu HPLC system equipped with a UV detector and a silica-NH_2column (5 μm; Phenomenex; Spinco Biotech, India) with dimensions of 250 × 4.6 mm. Twenty microlitres of a syringe-filtered sample were injected into the column and eluted isocratically with HPLC-grade *n*-hexane and ethyl acetate (70:30 v/v) at a flow rate of 1.0 ml/min. The tocopherols were detected with a UV detector (SPD 10 AT *vp*) at a wavelength of 295 nm. The relative amounts of tocopherols were calculated by comparing their peak areas with a standard curve generated using different amounts of external standards of α, β, γ and δ tocopherol (Sigma-Aldrich, India). Total tocopherols content was computed by summing up the values of all the four isomers. The tocopherols data were expressed as μg/g oil basis. The contents of α, β, γ and δ tocopherol were also determined as a percentage of total tocopherol.

(4) *Computation of Vitamin E activity*. The vitamin E activity of soybean oil from different cultivars was taken as the sum of multiplication of α, β, γ and δ tocopherol contents by 1.0, 0.5, 0.1, and .03, respectively as previously reported [14].

Statistical Analyses

Genotypes were arranged in randomized block design and data were analyzed using the statistical programme MSTAT-C version 2.1 (Russell D. Freed, Michigan State University). Mean values were used for correlation studies.

Results and Discussion

Values for all the four isomers of tocopherols of the Indian genotypes investigated are presented in Table 1. Genotypic variation was observed for the four isomers of tocopherol, total tocopherol content and tocopherols' composition. The predominant tocopherol was γ tocopherol followed by δ tocopherol, α tocopherol and β tocopherol. α Tocopherol ranged from 58 μg/g for 'MACS13' to 794 μg/g for 'Co Soya2', with an overall mean value of 269 μg/g of oil. β Tocopherol ranged from 12 for 'Pusa16' to 121 for 'Co Soya2' with an overall mean value of 40 μg/g oil. γ Tocopherol ranged from 240 μg/g for 'SL96' to 1,794 μg/g for 'Co Soya2', with an overall mean value of 855 μg/g of oil while δ tocopherol ranged from 66 μg/g for 'KB79' to 602 μg/g for 'Co Soya2', with an overall mean value of 241 μg/g of oil. Total tocopherol content ranged from 422 μg/g for 'SL96' to 3,311 μg/g for 'Co Soya2', with an overall mean value of 1,405 μg/g of oil. α/γ Tocopherol ranged from 0.17 for 'MACS13' to 0.52 for 'JS2' with a overall mean value 0.308. With regard to percent tocopherol composition, α-tocopherol ranged from 11 for 'MACS13' to 26 for 'MACS124' with an overall mean value of 18%. β Tocopherol ranged from 2% for 'VLS1' to 6 for 'NRC2' with an overall mean value of 3%. γ Tocopherol ranged from 51 for 'JS2' to 69; 'VLS1' and 'PK327' with an overall mean value of 60% whereas δ tocopherol ranged from 15 for 'JS90-41' to 26 for 'NRC2' with an overall mean value of 18%. Total vitamin E activity (activity as α tocopherol equivalents) ranged from 102 for genotype 'MACS13' to 1,052 μg/g of oil for genotype 'Co Soya2'.

Genotypic variability was also observed for oil content and all the five major fatty acids viz. palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3) content (Table 2). The oil content ranged from 13 (genotypes 'JS79-81', 'NRC12', 'PK471') to 22 (variety 'VLS1') with an overall mean value of 17%. Of all the genotypes studied, three genotypes viz. ''VLS1', 'JS71-05', and 'Pusa24' exhibited an oil content of more than 20%. Among saturated fatty acids, palmitic acid (C16:0) ranged from 9% for genotype 'VLS1' to 15% for genotype 'KHSb2' while stearic acid ranged from 2% for genotype 'Ankur', 'Samrat', 'Shilajeet', and 'Shivalik' to 8% for genotype 'VLS1'. Overall average values for palmitic and stearic acid among all the genotypes studied were 11 and 4%, respectively. Monounsaturated fatty acid, i.e. oleic acid (C18:1) ranged from 19% for genotype 'PK327' to 36% for genotype 'Bhatt yellow', with an overall mean value of 25%. Linoleic acid (C18:2) and linolenic acid (C18:3) are two major polyunsaturated fatty acids found in soybean. Linoleic acid ranged from 42% for genotype 'Bhatt Black' to 59% for 'Pusa22' while linolenic acid ranged from 5% for 'NRC48' and 'Pusa24' to 11% for genotype 'SL96'. The overall mean linoleic and linolenic acid values, of all the soybean genotypes studied were 53 and 7% of the total fatty acids, respectively. Averaged overall the genotypes studied, mean value for M (monounsaturated fatty acid):P (polyunsaturated fatty acids) ratio, an indicator of oxidative stability of oil as linoleic acid and linolenic acid oxidize 10 and 21

Table 1 Tocopherols content (mean and standard deviation, n = 3) of some Indian soybean genotypes

Genotype	Tocopherol isomers[a] (μg/g oil)				Total	Genotype	Tocopherol isomers				Total
	α	β	γ	δ			α	β	γ	δ	
ADT-1	171 ± 28 (16)	32 ± 4 (3)	686 ± 44 (63)	196 ± 24 (18)	1,085	Monetta	350 ± 29 (24)	66 ± 7 (4)	809 ± 45 (54)	267 ± 21 (18)	1,492
Ankur	754 ± 69 (27)	108 ± 11 (4)	1,534 ± 85	403 ± 42 (14)	2,799	NRC2	87 ± 6 (16)	33 ± 4 (6)	290 ± 21 (52)	146 ± 7 (26)	556
Bhatt Black	126 ± 11 (14)	29 ± 3 (3)	576 ± 43 (63)	189 ± 8 (21)	920	NRC8	246 ± 13(16)	40 ± 3 (3)	998 ± 88 (65)	256 ± 22 (17)	1,540
Bhatt yellow	413 ± 29 (20)	75 ± 9 (4)	1,226 ± 77 (60)	322 ± 26 (16)	2,036	NRC12	361 ± 32 (17)	45 ± 5 (2)	1,298 ± 78 (62)	391 ± 29 (19)	2,095
CO Soya1	239 ± 18 (17)	49 ± 6 (4)	843 ± 63 (60)	258 ± 17 (19)	1,389	NRC37	310 ± 23 (20)	41 ± 2 (3)	889 ± 81 (57)	326 ± 21 (21)	1,566
CO Soya2	794 ± 73 (24)	121 ± 13 (4)	1,794 ± 112 (54)	602 ± 41 (18)	3,311	NRC48	110 ± 9 (13)	37 ± 4 (4)	501 ± 44 (60)	183 ± 16 (22)	831
Hardee	241 ± 19 (18)	34 ± 5 (3)	847 ± 73 (63)	216 ± 18 (16)	1,338	Palamsoya	355 ± 21 (23)	46 ± 6 (3)	870 ± 76 (57)	248 ± 23 (16)	1,519
GS1	164 ± 13 (12)	48 ± 4 (4)	845 ± 75 (62)	319 ± 13 (23)	1,376	PK262	428 ± 34 (20)	41 ± 4 (2)	1,373 ± 86 (65)	273 ± 16 (13)	2,115
GS2	447 ± 31 (25)	63 ± 8 (4)	908 ± 78 (54)	272 ± 16 (16)	1,690	PK308	111 ± 10 (12)	16 ± 2 (2)	620 ± 47 (66)	191 ± 14 (20)	938
Improved Pelican	126 ± 8 (25)	18 ± 2 (4)	289 ± 17 (57)	76 ± 3 (15)	509	PK327	207 ± 19 (13)	27 ± 3 (2)	1,127 ± 68 (69)	284 ± 20 (17)	1,645
IS9	211 ± 14 (16)	35 ± 4 (3)	850 ± 66 (64)	226 ± 11 (17)	1,322	PK416	318 ± 26 (23)	37 ± 3 (3)	834 ± 73 (59)	217 ± 16 (15)	1,406
JS2	376 ± 27 (26)	70 ± 6 (5)	727 ± 52 (51)	269 ± 21 (19)	1,442	PK471	263 ± 22 (16)	31 ± 3 (2)	1,042 ± 81 (63)	316 ± 21 (19)	1,652
JS71-05	250 ± 18 (17)	38 ± 5 (3)	957 ± 69 (64)	249 ± 17 (17)	1,494	PK1024	185 ± 14 (21)	25 ± 4 (3)	522 ± 42 (60)	143 ± 13 (16)	875
JS72-44	231 ± 21 (15)	39 ± 2 (3)	1,102 ± 99 (65)	271 ± 16 (18)	1,643	PK1029	320 ± 31 (20)	31 ± 2 (2)	1,024 ± 77 (65)	220 ± 17 (14)	1,595
JS72-280	240 ± 18 (22)	36 ± 4 (3)	644 ± 46 (59)	175 ± 13 (16)	1,095	PK1092	225 ± 12 (18)	50 ± 3 (4)	759 ± 49 (60)	239 ± 15 (19)	1,273
JS75-46	187 ± 16 (18)	29 ± 2 (3)	651 ± 51 (63)	171 ± 16 (17)	1,038	PB1	105 ± 7 (12)	34 ± 2 (4)	513 ± 44 (61)	192 ± 17 (23)	844
JS79-81	231 ± 14 (17)	49 ± 5 (4)	847 ± 72 (60)	276 ± 25 (20)	1,403	Pusa16	82 ± 6 (14)	12 ± 1 (2)	376 ± 39 (64)	110 ± 6 (20)	580
JS90-41	120 ± 9 (22)	20 ± 3 (4)	314 ± 26 (59)	82 ± 7 (15)	536	Pusa20	242 ± 16 (19)	53 ± 6 (4)	748 ± 65 (58)	250 ± 19 (19)	1,293
JS93-06	224 ± 19 (21)	34 ± 4 (3)	661 ± 53 (61)	173 ± 14 (16)	1,092	Pusa22	159 ± 14 (12)	21 ± 2 (2)	906 ± 81 (67)	268 ± 24 (20)	1354
JS335	326 ± 27 (21)	47 ± 6 (3)	933 ± 82 (59)	275 ± 19 (17)	1,581	Pusa24	210 ± 10 (16)	33 ± 4 (2)	853 ± 63 (65)	220 ± 13 (17)	1,316
Kalitur	410 ± 32 (24)	49 ± 4 (3)	1,024 ± 97 (60)	224 ± 16 (13)	1,707	Pusa37	234 ± 21 (16)	34 ± 4 (2)	948 ± 80 (64)	226 ± 15 (19)	1,442
KB79	107 ± 7 (25)	17 ± 2 (4)	244 ± 19 (56)	66 ± 7 (15)	434	Pusa40	267 ± 24 (18)	27 ± 3 (2)	978 ± 61 (67)	191 ± 18 (13)	1,463
KHSb2	216 ± 14 (17)	36 ± 5 (3)	787 ± 62 (61)	245 ± 17 (19)	1,284	RAUS5	394 ± 33 (25)	59 ± 6 (4)	817 ± 62 (53)	283 ± 21 (18)	1,553
Lee	304 ± 28 (21)	46 ± 5 (3)	874 ± 79 (59)	261 ± 29 (18)	1,485	Samrat	297 ± 18 (18)	30 ± 3 (2)	1,060 ± 73 (66)	232 ± 20 (14)	1,619
MACS13	58 ± 6 (11)	14 ± 1 (2)	348 ± 24 (67)	101 ± 9 (19)	519	Shilajeet	197 ± 12 (14)	33 ± 3 (2)	908 ± 60 (62)	328 ± 31 (22)	1,466
MACS124	474 ± 41 (26)	34 ± 2 (2)	1,101 ± 86 (61)	199 ± 21 (11)	1,808	Shivalik	239 ± 12 (18)	41 ± 4 (3)	817 ± 41 (59)	276 ± 24 (20)	1,373
MAUS1	310 ± 23 (16)	38 ± 2 (2)	1,298 ± 91 (65)	360 ± 27 (18)	2006	SL 96	87 ± 9 (21)	16 ± 1 (4)	240 ± 18 (57)	79 ± 6 (19)	422
MAUS2	245 ± 18 (22)	27 ± 3 (2)	686 ± 27 (62)	158 ± 18 (14)	1,116	SL295	159 ± 13 (18)	30 ± 3 (3)	527 ± 29 (59)	182 ± 13 (21)	898
MAUS32	310 ± 25 (21)	31 ± 4 (2)	872 ± 49 (59)	271 ± 29 (18)	1,484	SL459	262 ± 21 (20)	42 ± 4 (3)	809 ± 78 (61)	205 ± 15 (16)	1,318
MAUS47	564 ± 47 (24)	79 ± 8 (3)	1,359 ± 97 (57)	376 ± 24 (16)	2,375	T49	415 ± 33 (16)	53 ± 5 (2)	1,646 ± 97 (63)	484 ± 36 (19)	2,598
MAUS61	99 ± 8 (19)	15 ± 2 (3)	321 ± 21 (62)	83 ± 7 (16)	518	VLS1	247 ± 21 (16)	23 ± 2 (2)	1,077 ± 86 (69)	203 ± 18 (13)	1,550
MAUS61-2	224 ± 17 (20)	38 ± 4 (3)	677 ± 46 (60)	185 ± 12 (17)	1,124	VLS21	202 ± 18 (19)	32 ± 3 (3)	648 ± 42 (61)	176 ± 14 (17)	1,058
MAUS71	344 ± 23 (20)	57 ± 7 (3)	1,013 ± 78	320 ± 27 (19)	1,734	VLS47	534 ± 37 (20)	59 ± 5 (2)	1,738 ± 91 (64)	404 ± 35 (15)	2,735

LSD (p = 0.05) for α, β, γ, δ and total tocopherol was 36, 8, 77, 39, and 133, respectively

[a] Values given in parentheses indicate the percentage of the total tocopherol content

Table 2 Range and overall mean values for oil content and fatty acid composition of some of Indian soybean genotypes

Oil and fatty acids	Range	Mean
Oil %	13–22	17
Palmitic acid (C16:0) %	9–15	11
Stearic acid (C18:0) %	2–8	4
Oleic acid (C18:1) %	19–36	25
Linoleic acid (C18:2) %	42–59	53
Linolenic acid (C18:3) %	5–11	7

times faster than oleic acid [10], was 0.44. In general, soybean oil has an M:P ratio of 0.5 which is much lower than the value of 1.95, 1.65 and 4.0 for canola, peanut and olive oil, respectively, indicating that soybean oil is less oxidatively stable than canola, peanut and olive oils.

The ratios between the lowest and the highest values for α, β, γ, δ and total tocopherol in the present study were 1:13.6, 1:10.4, 1:7.5, 1:9.1, 1:7.9 as compared to 1:17.4, 1:10.7, 1:4.4, 1: 3.3, 1:3.5 reported in Brazilian cultivars, respectively [9]. Indian genotypes exhibited more variability for γ- and δ- and total tocopherol while lower variability for α tocopherol than observed in Brazilian genotypes. McCord et al. [8] investigated 20 normal and 20 reduced linolenate soybean lines for tocopherols and reported the ranges of 133–182, 713–740, 395–440 and 1,268–1,348 µg/g of oil for α, γ, δ and total tocopherol content, respectively with the ranges of 10.5–13.5 (% α), 53.85–67.2 (% γ) and 31.15–32.4 (% δ) tocopherol. In this study [8], β tocopherol was integrated into the γ tocopherol due to insufficient separation of β and γ tocopherol. Higher average percent α tocopherol content and lower average percent γ and δ tocopherol content in our study were observed compared to the values for the corresponding isomers in the results of McCord et al. [8]. These differences may be due to genotypic differences or may be attributed to the growing temperature as suggested by Britz and Kremer [15], who reported an increase in free α tocopherol content in seeds of soybean under warmer growing conditions during seed maturation. More specifically, they found an increase in ratio of α tocopherol/total tocopherol from 0.130 at 23 °C to 0.372 at 28 °C in the 'Essex' variety while this ratio increased from 0.128 to 0.280 in the 'Forrest' variety. Interestingly, in the current study, the average value for α-/total tocopherol was 0.192 and the prevailing average daily temperature during seed development was 26.27 °C. The value obtained for α tocopherol/total tocopherol in our investigation lies in between the values obtained for the ratio at 23 and 28 °C in the earlier study [15]. Genotypic variability for tocopherol isomers has been reported in other oilseed crops as well [11, 16, 17]. Dolde et al. [11] reported ranges of 504 to 687 and 534 to 1,858 µg/g of oil in canola and sunflower, respectively. In sunflower, Velasco et al. [16] also reported genotypic variation for tocopherol content ranging from 562 to 1,872 µg/g of oil and the α tocopherol, being the predominant fraction of total tocopherol content in sunflower seeds, ranged from 88.4 to 96.3% of the total tocopherol. In corn, Rocheford et al. [17] reported ranges of 11.8–66 and 43–229 µg/g of oil for α and γ tocopherol, respectively in 45 hybrids of corn. In this study, α and γ tocopherol accounted approximately 20 and 80% of the total tocopherol content, respectively.

Table 3 shows the correlation of different tocopherols with oil content and fatty acids. Our results indicated a highly significant ($p < 0.001$) positive correlation of all the four tocopherols isomers and total tocopherol content with each other. Similar results were also shown in an earlier investigation [8], but with lower level of significance ($p < 0.01$). Furthermore, in the present study, soybean genotypes did not exhibit any significant association of α, β, γ, δ tocopherol and total tocopherol with linolenic acid, which is in contrast to the results of Dolde et al. [11]. These authors [11]

Table 3 Correlation studied between four tocopherol isomers, total tocopherols, total vitamin E activity, unsaturated fatty acids and monounsaturated (M):polyunsaturated fatty acids (P)

	Tocopherol isomers				Total Tocopherol	Total Vitamin E	Oleic acid	Linoleic acid	Linolenic acid	M:P
	α	β	γ	δ						
Oil	NS	NS	NS	NS	NS	NS	–0.314**	NS	NS	–0.292*
Tocopherol isomers										
α		861***	0.831***	0.773***	0.909***	0.827***	NS	NS	NS	NS
β			0.664***	0.778***	0.779***	0.804***	NS	NS	NS	NS
γ				0.892***	0.982***	0.663***	NS	0.282*	NS	NS
δ					0.924***	0.695***	NS	NS	NS	NS
Total tocopherols						750***	NS	0.258*	NS	NS
Total vitamin E							NS	NS	NS	NS

*, ** and *** indicate $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively

found a positive association of linolenic acid with γ tocopherol but a negative association with α tocopherol. However, as the investigation was conducted only in four genotypes, the correlations obtained in their study may be misleading. A couple of reports demonstrating positive association between linolenic acid and tocopherols isomers are also available in the literature [8, 12]. Almonor et al. [12], in an investigation of 14 soybean genotypes, reported a significant positive association between tocopherols and linolenic acid [12]. McCord et al. [8] also observed positive association of α, δ tocopherol and total tocopherol with linolenic acid in an analysis of tocopherols in 20 normal and 20 low linolenic acid soybean lines. The authors suggested that it was possible to breed low linolenate soybean lines with reasonable amount of total tocopherols as some lines with low linolenic acid showed appreciable amount of tocopherols. Significant positive correlations ($p < 0.05$) of total tocopherols and γ tocopherol content with linoleic acid as well as PUFA content observed in the present study indicated that the alleles that govern the expression of ω-6 desaturase gene, responsible for conversion of oleic acid (C18:1) to linoleic acid (C18:2), influence the tocopherols levels as well. A similar observation was reported in cv 'N85-2176' carrying homozygous recessive allele for ω-6 desaturase when compared with cv 'Dare' carrying homozygous dominant allele for ω-6 desaturase [12]. Significant positive correlation between α tocopherol content and PUFA content has been reported in corn hybrids also [18]. In the current study, no correlation between tocopherols fractions and oil content was observed. Reports focusing on the study of the relationship between tocopherols and oil content in soybean seeds are unavailable, however, significant association of tocopherols with oil content has been reported in other oilseed crops [19, 20]. Negative association of α and γ tocopherol with oil content in canola seeds [19] whereas a positive association of β, and γ tocopherol with oil content has been reported in *Cannabis sativa* seeds [20]. In the present study, oil content exhibited a significant ($p < 0.01$) negative correlation with oleic acid and M:P.

α Tocopherol is biosynthesized from γ tocopherol by γ tocopherol methyltransferase (γ TMT). Shintani and DellaPenna [21] achieved 95% of the total tocopherol pool as α-tocopherol by overexpression of γ tocopherol methyltransferase (γ TMT) gene in *Arabidopsis* seeds. Apart from this transgenic approach, enhanced levels of a specific tocopherol can be achieved through traditional or molecular breeding. A highly significant correlation ($p < 0.001$) of tocopherol isomers with each other and total tocopherol contents in our study suggests that any breeding program focusing on enhancement of one type of tocopherols would result in enhancement of other three tocopherols. Therefore, enhancement of total tocopherols content remains the only way to enhance specific type of tocopherol isomer. Furthermore, molecular markers for high levels of tocopherols need to be identified in soybean, as accomplished in corn [18], to facilitate the selection of genotypes with high levels of tocopherols in large populations in breeding programs. Genotypes, 'CoSoya2' and 'Ankur', exhibited comparatively higher values for total tocopherols content, seem to be good donors for breeding programs focusing on the enhancement of total tocopherol content. Notwithstanding, the significant ($p < 0.05$) positive relationship observed for γ-tocopherol and total tocopherol content with linoleic acid in the present study, there are some cultivars, which showed a comparatively high total tocopherol with low linoleic acid suggesting that it may be possible to improve the oxidative stability of soybean oil by decreasing the linoleic acid content without compromising the tocopherol content.

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