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RESEARCH

Genotype by environment interaction influence on functional molecules (tocopherols and sterols) accumulation in sunflower oil

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Abstract Tocopherol and sterol are non-dietary functional molecules in sunflower oil, which act as antioxidants, reduce cholesterol and improve immunity against diseases. The present study was designed to determine tocopherol and sterol contents in 13 high and two low oleic acid sunflower hybrids across two seasons (spring and autumn) and four locations under subtropical conditions of Pakistan with contrasting reproductive phase temperatures. The results showed that tocopherol and sterol contents varied across the seasons and locations. Autumn planting produced high tocopherol content. Moreover, locations under high temperature during reproductive phase negatively affected the sterol and tocopherol contents. High oleic acid hybrids yielded 38% higher tocopherol content than low oleic acid hybrids. High oleic acid hybrids produced higher sterol contents at all locations and seasons. Hybrids such as H4 and H5 are considered stable due to comparatively close values of tocopherol and sterol contents across the four locations when compared with standard checks and other hybrids during spring season as indicated from various stability parameters. Hybrids H8, H4 and H5 also manifested higher magnitude of heterosis for tocopherol and sterol contents that may be due to overdominance gene action. Breeding lines such as B.116.P, B.112.P and RH.365 were positive combiners for the investigated traits, thus likely carrying positive alleles for both tocopherol and sterol traits.

Keywords Hybrid vigor · Functional molecules · Oil contents · Overdominance

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Introduction

Sunflower is an important source of edible oil, which is consumed for cooking and salad dressing. The oil is rich source of polyunsaturated and monounsaturated fatty acids, which are important forreducing the low density lipid profile of blood stream. Sunflower is also a rich source of non dietary functional

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molecules such as tocopherols and sterols. They act as antioxidants, lower cholesterol and improve human immunity against pathogens. Hence, there is a need to improve the availability of these functional molecules within sunflower oil and seed. Tocopherol contents range between 314 to 1024 mg kg^{-1} in seed and from 563 to 1873 mg kg^{-1} in oil (Velasco et al. 2002). The α-tocopherol is the main tocopherol comprising about 88 to 96% of the total. Concentration of other tocopherol species can be enhanced through mutagenesis (Velasco et al. 2004). The tocopherol content is affected by genotype × environment and genotype × location interactions in sunflower (Velasco et al. 2002; Rauf et al. 2017). Despite tocopherol and sterol contents have been well investigated in various sunflower accessions, there is need to continuously improve both functional molecules in elite germplasm and hybrids and to determine interaction of genotypes with various environmental conditions (Rauf et al. 2020). High intercepted radiation have negative influence on the tocopherol accumulation in sunflower oil (Nolasco et al. 2004). Selection for high tocopherol contents lead to the increase of tocopherol contents (467 mg kg^{-1}) in newly constituted lines as compared to standard check (251 mg kg^{-1}) (Velasco et al. 2010).

Moreover, it was observed that sterol contents may be improved through genetic modification and agronomic practices (Roche et al. 2010a). Genetic variation among the inbred lines and hybrids was observed in sunflower (Roche et al. 2010a). Generally, more than 70% of the sterols were stored in the embryos while the remaining were found in hulls (Roche et al. 2010b). Multigenic inheritance was determined through quantitative trait loci mapping that showed that 13 genomic regions on 9 linkage groups, contribute to the variability of sterol content in sunflower, of which LG1, LG4 and LG7 were particularly important genomic regions (Merah et al. 2012). High temperature and water stress conditions also enhanced the accumulation of sterol content (Roche et al. 2006). Sowing date and genotypes were the most important factors affecting the accumulation of sterol content in sunflower (Roche et al. 2006).

The aim of this research was on determining tocopehrol and sterol contents in mid and high oleic acid sunflower hybrids. Our preliminary results revealed apositive correlation between these three traits in 65 sunflower accessions that were screened for oleic acid, α-tocopehrol and sterol contents. This study also investigated the effect of seasons and locations on α-tocopehrol and phytosterol contents in different sunflower genotypes including hybrids having higher oleic acid content than two standard checks.

Materials and methods

A part of this study was done at the Department of Plant Breeding & Genetics, College of Agriculture, University of Sargodha, Pakistan during autumn and spring seasons in 2019 and 2020. All the plant materials and cross combinations were maintained and developed at College of Agriculture, University of Sargodha, Pakitsna. College of Agriculture (Latitude: 32.13367° or 32° 8′ 1″ north and Longitude 72.68705° or 72° 41′ 13″ east), Sargodha is situated in the mixed cropping zone of the central Punjab, Pakistan,

Selection of parental material and development of crosses

Our initial screening of diversified sunflower germplasm showed a positive relationship between oleic acid, tocopherol and sterol. Therefore, parental lines were selected on the basis of oleic acid content, with the aim to determine the concentration of tocopherol and sterol in high oleic acid sunflower hybrids.

Cytoplasmic male sterile (CMS) lines were crossed with fertility restorer (FR) lines by planting at 2:1 in field trials. Each plant of CMS and FR lines was covered with net bag before the onset of anthesis to avoid insect pollinators. CMS lines were hand pollinated on daily basis early in the morning at 8:00 a.m. until all the stigma of disc floret withered (7 days after start of the first pollination). CMS lines were also maintained by pollinating with maintainer "B" line, while "B" and FR lines were maintained by bagging two floral heads. Floral heads were manually harvested along with their tags after reaching physiological maturity (turned golden yellow). Heads were dried under the shade and threshed manually. Threshed seeds were cleaned and unfilled achenes were removed. Seeds were stored at room temperature. Seeds of the commercial checks namely "Hysun-33" (Imported: LCI, Pakistan) and "FH-331" (Local early maturing hybrid developed by Oilseed Reseach Institute, Faisalabad,

Pakistan) were purchased from the market for comparison.

Plantation of trials

During spring season, 13 single cross hybrids along with two commercial checks ("Hysun-33" and "FH-331") were planted at four locations (Sargodha, Faisalabad, Multan, Bahawalpur) on 15 February, 2020. Autumn trials were sown at two locations on 20 August, 2019 (Table 1). Salient features of each location are shown in Table 1). All hybrids (Table 2) were sown on ridges in a randomized complete block design with 3 replications. Each hybrid was sown in 3 rows of 4 m length within each block. Distance between rows was 72 cm; whereas, plant to plant distance was 22 cm. Field trials were fertilized with 120 kg ha^{-1} of urea and 60 kg ha^{-1} of diammonium phosphate (DAP). DAP was added at the time of field preparation while half of the urea was added at field preparation and half during initiation of reproductive phase (R1 with miniature star like bud as narrated by Schneiter and Miller scale (1981). Plots were sprayed with 1 L ha^{-1} pre-emergence herbicide (Dual Gold, 960 g L^{-1} S-metolachlor) to control weeds. Spring trials were sprayed with recommended dose (60 mL per 100 L) of Lufenuron to inhibit the infestation of armyworm (*Spodoptera frugiperda*). Single irrigation was done soon after sowing in autumn and spring crops for uniform emergence of seedling. Trials at Sargodha and Faisalabad was grown under a rainfed condition, while fields in Multan and Bahawalpur were irrigated 6 and 4 times, respectively, to avoid any water stress. At maturity, 5 plants were manually harvested from each row and the seeds were threshed, placed in kraft paper bags and stored at 25 °C for further analysis (Table 2).

Oil extraction

Oil was extracted through manual hand extractor. Samples of 50 g achene were dehulled, kernel oil was squeezed using hand extractor and oil samples were placed in Eppendorf tubes. To eliminate sample contamination, extractor was cleaned with hexane to remove residues of oil and cleaned with paper towel before extraction of the next sample. Oil samples

Table 2 Sunflower hybrids and check codes, hybrid parentage and oleic acid contents

Code	Hybrid parentage	Oleic acid (%)
FH.331	N/A [commercial check]	54
Hysun-33	N/A [commercial check]	42
H1	C.112.P×RH.344	71
H2	C.112.P×RSIN.82	60
H3	C.112.P×RH.365	67
H4	C.250×RH.345	72
H5	C.112.P×RH.347	79
H6	C116.P×RH.365	59
H7	C.249×RH.345	73
H8	C.116.P×RH.344	72
H9	C.250×RH344	70
H10	C.249×RH.447	67
H11	C.249×RH.347	70
H12	C.259×RH.447	68
H13	C.259×RH.344	67

Table 1 Salient characterization features of each location during autumn and spring seasons

Feature	Spring				Autumn	
	Sargodha	Faisalabad	Bahawalpur	Multan	Sargodha	Faisalabad
Elevation	190 m	186 m	214 m	122 m	190 m	186 m
Soil texture	Sandy loam	Sandy loam	Sandy	Loam	Sandy loam	Sandy loam
Soil pH	7.62 ± 0.13	7.51 ± 0.17	7.60 ± 0.10	8.80 ± 0.20	7.43 ± 0.12	7.53 ± 0.18
Soil EC	1.70 ± 0.12	2.06 ± 0.11	2.20 ± 0.20	1.82 ± 0.15	1.64 ± 0.11	1.71 ± 0.10
Organic matter (%)	0.63	0.74	0.52	0.42	0.66	0.75
Rainfall (mm)	180	170	70	120	70	70
Total ° days	1560	1850	2745	2810	1700	1600
Supplemental irrigation (75 mm)	1	1	4	6	1	1

were refrigerated at 4 °C until use for the chromatographic analysis of tocopherol and sterols.

Chromatography

Oil samples of 40 µL were mixed with 960 µL of isoproponal and shaken to dissolve oil within solvent. 10 µL was injected in a high performance liquid chromatograph (HPLC). Mobile phase was 50% methanol and 50% acetonitrile with a flow rate of 1 mL min^{-1}. Column was C18 reverse phase with initial temperature of 40 °C. Detector had excitation wave length of 290 mm and emission wave length of 312 mm.

Sterol contents was determined through gas chromatography by mixing 50 mg oil, 200 µL internal standards and 5 mL 2 M KOH prepared in ethanol and the mixture was heated at 75 °C for 30 min for saponification. Unsponfiable matter was removed by adding 2 mL deionized water and 5 mL hexane. The same procedure was repeated three times. Hexane fraction was removed through steam of nitrogen. 500 µL of Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was added to the residual sterols. The vial were heated at 75 °C for 30 min and then cooled to room temperature for further analysis. Analysis was done with gas chromatography, set with a capillary column with helium as carrier gas at flow a rate of 1.2 mL min^{-1}. Temperature was increased up to 290 °C with increment of 50 °C min^{-1} and was held for 15 min.

Statistical and biometrical analysis

The experiment was conducted in spring and autumn seasons as a randomized complete block design with three blocks and two factors; i.e., hybrids and locations. The data were subjected to analysis of variance and the means were compared by the least significant difference ($P \leq 0.05$). Stability parameters were determined through online "STABILITYSOFT" developed by Pour-Aboughadareh et al. (2019), which analyzed several biometrical stability parameters including: Wricke (1962) ecovalence (Wi^2) (As per ecovalance, stability of the ith genotype was its interaction with environments which was summed and sequared across environment), Shukla (1972) ($\sigma^2 i$), Kang (1988) (KR), "bi' (Eberhart and Russell 1966) and S^2_{di} (Finlay and Wilkinson 1963). Combining ability analysis was performed as outlined by Kempthorne (1957).

Results

Analysis of variance showed significant influence of hybrids and locations (Table 3). Hybrids × locations effects were also significant ($P \leq 0.05$), which showed that hybrids ranking was not similar across locations for both tocopherol and sterol (Table 3). There was seasonal effect on the accumulation of sterol and α-tocopherol. α-tocopherol contents were higher in the autumn season; whereas sterol contents were higher in the spring. Among locations, the highest contents of both α-tocopherol and sterol were at Sargodha, followed by Faisalabad and Multan during spring season; whereas Bahawalpur had the lowest contents (Fig. 1). Hybrid H6 had the highest α-tocopherol content followed by the check at Sargodha (Fig. 1A). The highest α-tocopherol content at Faisalabad produced by hybrids H1 and H7; whereas hybrids H6 and H1 had the highest content at Multan during spring season.

Table 3 Mean sum of square for α-tocopherol and sterol contents resulting from the analysis of variance

Factor	Autumn			Spring		
	DF	Tocopherol	Sterols	DF	Tocopherol	Sterol
Blocks	2	3661.28^{NS}	1978.57^{NS}	2	7143.66^{NS}	3661.28^{NS}
Hybrids	11	82,863.61**	67,023.05**	11	1,250,242.66**	82,863.61**
Locations	1	266,949.93**	506,522.49**	3	9,961,810.48**	266,949.93**
Hybrids × Locations	11	11,102.97*	139,774.90*	33	848,011.40**	11,102.97*
Residual	46	5185.21	8328.70	94	52,252.03	5185.21
Total	71	21,780.63	53,540.19	143	535,303.88	21,780.63

NS, ** and *indicate non-significant, highly significant ($P \leq 0.01$) and significant ($P \leq 0.05$) factors, respectively

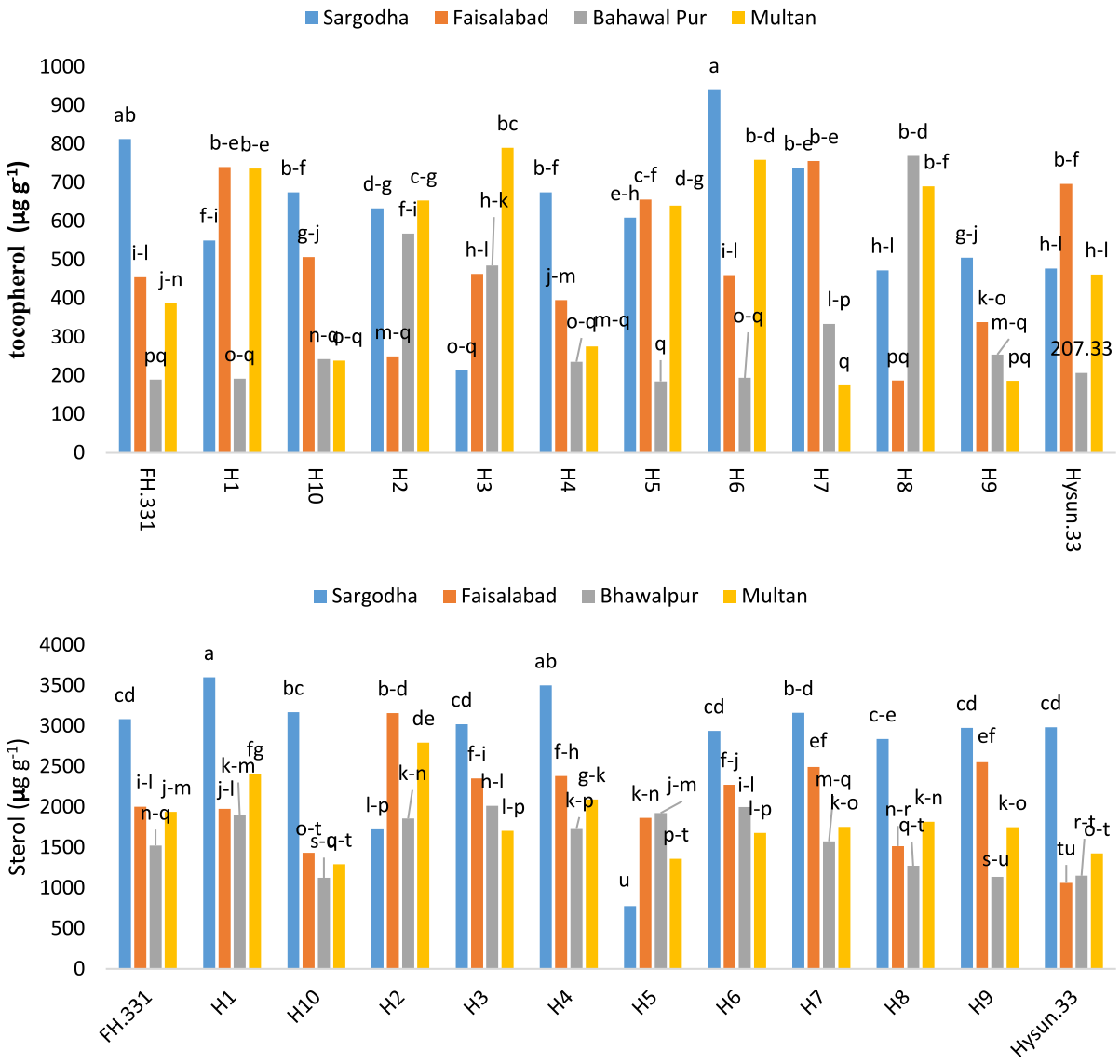

Fig. 1 Response of newly developed hybrids for tocopherol and sterol contents at Sargodha, Faisalabad, Bahawalpur and Multan during the spring season

Sterol accumulation was the highest at Sargodha followed by Faisalabad and Multan. H1 and H4 had the highest sterol contents at Sargodha; whereas H2 was the highest at Faisalabad and Multan during spring season (Fig. 1B). H13 had the highest α-tocopherol content at Sargodha location during autumn season, while H3 had the highest sterol contents during autumn season (Fig. 2). Stability parameter of α-tocopherol is shown in Table 4. H6 followed by H1 had the highest α-tocopherol across all locations (Table 4). Wricke (1962) proposed ecovalence (Wi^2) as contribution of genotype to environment interaction. H4 had the lowest Wi^2 value and thus considered stable for α-tocopherol contents. Similarly, low value for Shukla's stability variance ($\sigma^2 i$) also indicate stable hybrids. H1 had the lowest $\sigma^2 i$ value. Coefficient of regression (bi) is another stability parameter and value close to unity is considered stable along with insignificant deviation from regression. Hybrid H8 had high "bi", thus showing

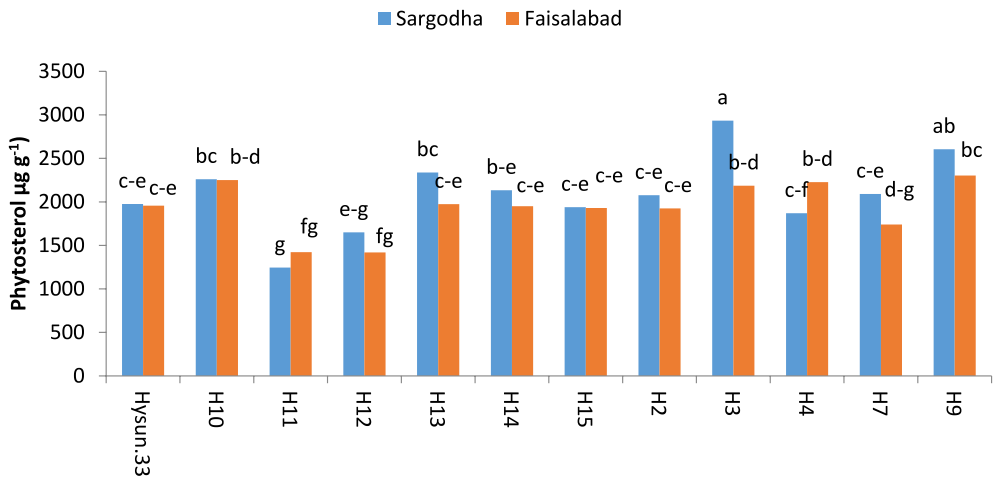

Fig. 2 Response of newly developed hybrids for tocopherol and sterol contents at Sargodha and Faisalabad during the autumn season

Table 4 Estimation of various stability parameters for sterol contents (µg g^{-1}) across four locations during the spring season

Hybrids	Y	W_i^2	σ^2_i	s^2d_i	b_i	CVi	$\theta_{(i)}$	θ_i	R
FH.331	2138.213	84,675.83	5603.331	4757.665	1.25	31.11	307,857.9	170,864.1	8
H1	2471.515	453,302.4	153,053.9	50,790.29	1.34	31.80	294,453.3	237,887.1	8
H10	1756.195	639,328.4	227,464.3	24,845.81	1.75	54.15	287,688.7	271,710	19
H2	2384.155	2,969,713	1,159,618	193,828.9	−0.39	29.52	202,947.5	695,416.3	14
H3	2273.75	159,369.7	35,480.87	22,726.93	0.98	24.87	305,141.8	184,444.8	7
H4	2426.675	174,176.6	41,403.63	546.221	1.45	31.53	304,603.3	187,137	6
H5	1481.233	3,087,052	1,206,554	38,474.44	−0.84	36.11	198,680.6	716,750.6	24
H6	2223.328	156,066	34,159.4	21,641.55	0.93	24.12	305,261.9	183,844.1	8
H7	2246.98	186,322.6	46,262.04	12,364.75	1.35	32.42	304,161.6	189,345.3	10
H8	1861.953	237,266.5	66,639.6	28,332.5	1.21	37.04	302,309.1	198,607.9	15
H9	2103.455	462,316.2	156,659.5	42,862.8	1.44	39.07	294,125.5	239,526	16
Hysun.33	1656.218	718,521.8	259,141.7	69,358.87	1.53	54.32	284,808.9	286,108.8	21

Y: mean tocopherol contents (µg g^{-1}) across all locations; Wi^2: Wricke (1962) ecovalence, σ^2i: Shukla (1972), KR: Kang (1988), bi; Eberhart and Russell (1966) regression coefficient, S^2_{di}: Finlay and Wilkinson (1963), deviations from regression, $\theta_{(i)}$: Plaisted and Peterson (1959) mean and GE variance component

adaptability to environment that yielded higher α-tocopherol contents; while lesser than "1" indicated adaptability to low α-tocopherol-yielding environment such as Bahawalpur (Table 4). Kang ranking (KR) considers both high mean value and stability parameter to rank hybrids stability. According to KR value, H5 had the lowest ranking and thus considered more stable with comparatively better α-tocopherol content (Table 4).

H4 had the highest sterol contents across all four locations during spring season. FH.331 had the lowest Wi^2 and σ^2i followed by H3 and H4 and thus considered stable. H4 had insignificant deviation from regression with bi value higher than unity showing adaptability to environment yielding high sterol contents such as "Sargodha". H5 and H6 had the bi value close to "1" showing stable hybrids with insignificant deviation from regression (S^2_{di}). H4 also had the lowest KR ranking. Following Kang (1988), hybrids with the lowest ranking such as H4 (6) had the highest sterol contents and were stable

(closeness to the performance of sunflower hybrids) across locations (Table 5).

Combining ability and mean values for parental lines for α-tocopherol contents are shown in Fig. 3. Breeding line B.116 followed by RH.347 and RH.34 had the highest α-tocopherol at Sargodha, while RSIN.82 and RH.347 were positive combiner at Faisalabad. B.116.P and RH.345 had the highest combining ability and mean value for α-tocopherol during the spring season at Bahawalpur. RH.365, B.116.P and B.112.P were positive combiner along with the highest α-tocopherol value at Multan (Fig. 3).

B.112.P and R.SIN.82 were the best combiners for sterol contents; whereas B.249 had the highest sterol content at Sargodha during the spring season (Fig. 4). B.250, RH.365 and RH.344 were the best combiners for sterol content at Faisalabad. RH.365 and R.SIN.82 and B.112.P were the best combiners at Bahawalpur, and R.SIN.82 and B.112.P were better combiners at Multan (Fig. 4).

The highest heterotic effect was estimated at Bahawalpur by H3 and H8 for tocopherol content during spring season. H8 showed the highest magnitude of heterosis at Bahawalpur and Multan for tocopherol content during the spring season (Table 6). H4 followed by H5 and H6 showed the highest heterosis for sterol content at Bahawalpur followed by Multan (Table 7). H4 also had the highest heterosis at Multan followed by H2 and H6 (Table 7).

Discussion

Sunflower oil and seed are considered healthy food due to rich source of unsaturated fatty acids and functional molecules to improve the immunity against various pathogens under the current pandemic conditions (Rauf et al. 2020). Tocopherol and sterol are important functional molecules that act as antioxidants to improve immunity against pathogens (Velasco et al. 2002; Belo et al. 2017, 2018). Sterol contents control plasma cholesterol levels and thus preventing various cardio vascular diseases (Belo et al. 2018). Tocopherols can neutralize the peroxy radical and thus prevent oil and lipids from peroxidation, thereby improving their shelf life (Sadiq et al. 2019).

Tocopherol and sterol contents were found to be affected by the seasons and locations. Generally, tocopherol contents (440–815 μg g^{-1}) were higher in the autumn season than in the spring season (330–747 μg g^{-1}) across Sargodha and Faisalabad; whereas sterol contents ranged between 1334 and 2560 μg g^{-1} in the autumn and from 1320 to 2942 μg g^{-1} in the spring seasons. Bahawalpur and Multan had significant lower tocopherol and sterol contents when compared with Sargodha and Faisalabad (Fig. 1).This may be due to higher available reproductive degree days during spring season than autumn season, which may had negative impact on the accumulation of tocopherol content specifically. Moreover, Multan and Bahawalpur locations had higher reproductive degree days accumulation

Table 5 Estimation of various stability parameters for tocopherol contents (μg g^{-1}) across four locations during the spring season

Genotype	Y	W_i^2	σ^2_i	s^2d_i	b_i	CVi	$\theta_{(i)}$	θ_i	R
FH.331	461.22	71,504.63	23,942.66	3494.63	2.06	56.38	48,650.91	38,626.38	13.00
H1	555.05	115,750.56	41,641.04	15,114.44	1.49	46.40	47,041.97	46,671.10	9.00
H10	416.06	62,014.11	20,146.46	7966.28	1.38	51.31	48,996.02	36,900.83	14.00
H2	526.40	135,317.88	49,467.96	14,986.65	0.15	35.69	46,330.43	50,228.79	12.00
H3	488.36	267,967.23	102,527.70	20,967.22	− 0.69	48.33	41,506.81	74,346.85	18.00
H4	395.63	42,379.07	12,292.44	5107.50	1.40	50.14	49,710.02	33,330.82	13.00
H5	522.89	58,145.50	18,599.01	5961.72	1.62	43.19	49,136.70	36,197.45	8.00
H6	588.39	147,661.02	54,405.22	5772.75	2.59	55.89	45,881.59	52,473.00	10.00
H7	501.04	190,619.83	71,588.74	26,782.90	1.27	58.29	44,319.45	60,283.69	16.00
H8	530.08	338,815.34	130,866.95	21,869.32	− 1.10	49.19	38,930.52	87,228.33	15.00
H9	321.46	35,351.35	9481.35	4690.20	0.76	42.85	49,965.57	32,053.06	13.00

Y: mean tocopherol contents (μg g^{-1}) across all locations; Wi^2: Wricke (1962) ecovalence, σ^2i: Shukla (1972), KR: Kang (1988), bi; Eberhart and Russell (1966) regression coefficient, S^2_{di}: Finlay and Wilkinson (1963), deviations from regression, $\theta_{(i)}$: Plaisted and Peterson (1959) mean and GE variance component

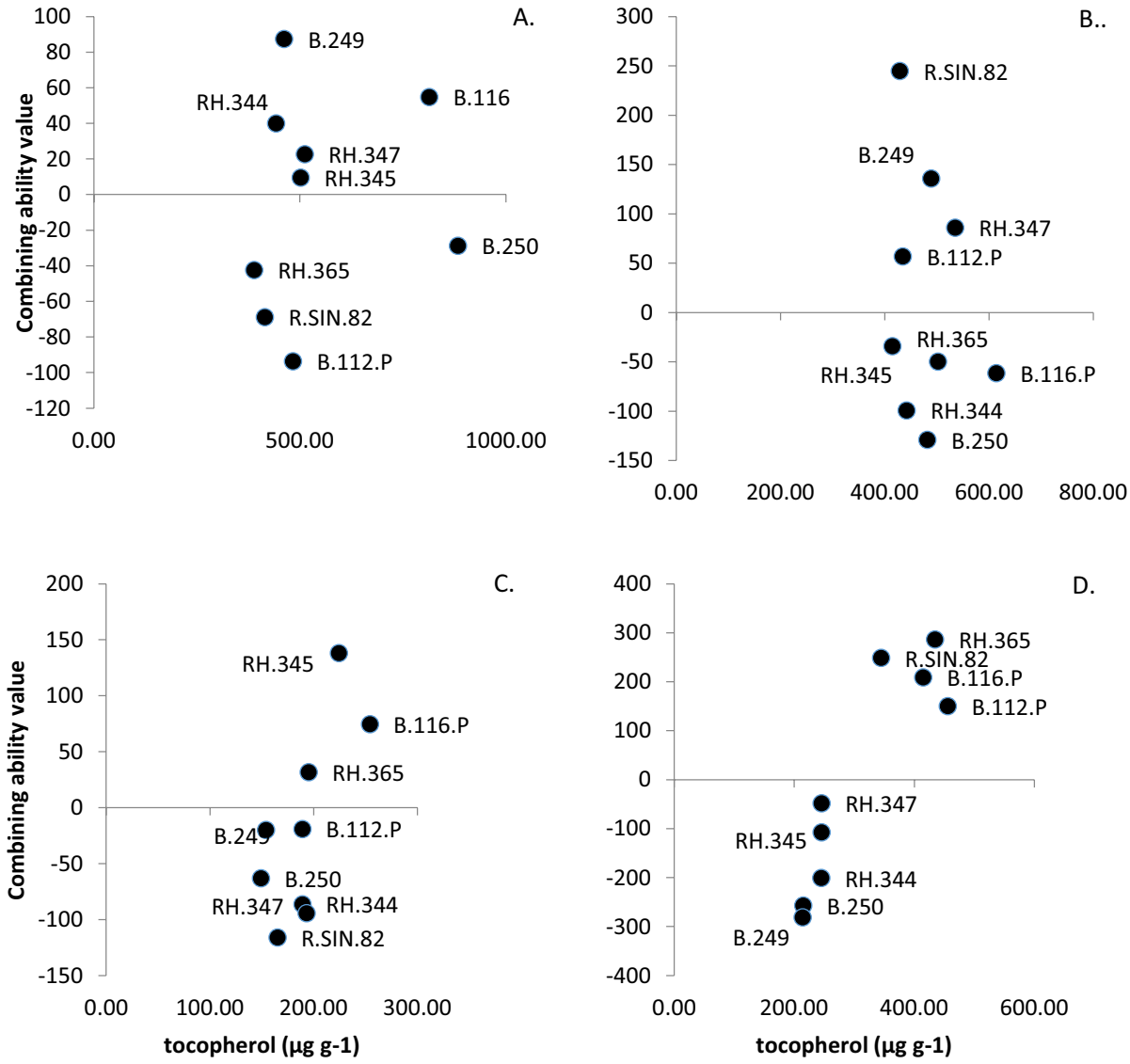

Fig. 3 Combining ability and mean values for tocopherol contents in sunflower parental lines across four locations **A** Sargodha, **B** Faisalabad, **C** Bahawalpur and **D** Multan during the spring season

than Sargodha and Faisalabad, which had a negative impact on the accumulation of both tocopherol and sterol contents.

Tocopherol and sterol contents accumulation has been affected by various environmental factors including increased intercepted radiations, which also increased the tocopherol accumulation period at specific range in sunflower and other species such as almond (Nolasco et al. 2004; Velasco et al. 2010; Belo et al. 2017; Kodad and Alonso 2018). Heat and drought stresses were detrimental for tocopherol accumulation in almond. It was also identified that α and βtocopherol contents in the species was affected by the genotypes and environment (Fritsche et al. 2017; Kodad and Alonso 2018). Results showed significant genotype × location effects affect the tocopherol or sterol production in sunflower (Fritsche et al. 2017; Belo et al. 2018; Kodad and Alonso 2018). In this context, the stability parameters indicated that some of the new hybrids such as H4 and H5 had

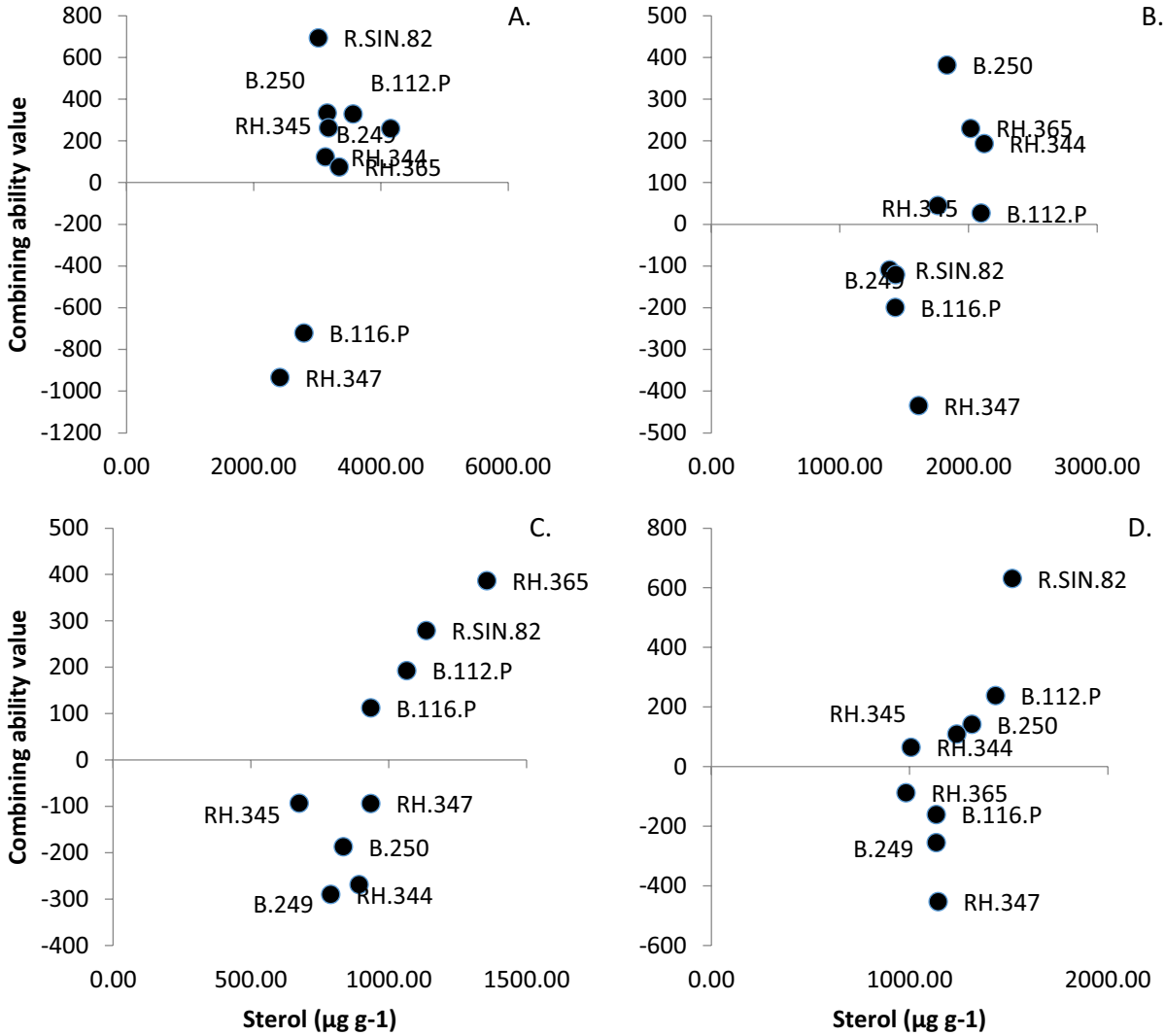

Fig. 4 Combining ability and mean values for sterol contents in sunflower parental lines across four locations **A** Sargodha, **B** Faisalabad, **C** Bahawalpur and **D** Multan during the spring season

relatively stable tocopherol and sterol contents across locations. Hence, these hybrids may be recommended for cultivation to produce oil with high oleic acid.

Overall, the tocopherol content of high oleic acid in the hybrids was not higher than standard low oleic acid hybrids in the autumn season or in the spring season at locations with relatively low temperature. However, high oleic acid hybrids comparatively had 38% higher tocopherol content at warmer locations such as Multan and Bahawalpur. On the other hand, high oleic acid hybrids produced higher sterol content in bot seasons and all locations. This result suggests that high oleic acid oil hybrids favors the accumulation of tocopherol content under warm climate. High oleic acid content in sunflower is also known to be triggered under warm temperature, which may have induced oxidative stability to sunflower oil and provided advantage for tocopherol accumulation as compared to low oleic acid hybrids (Angeloni et al. 2017; Akkaya et al. 2019). A previous finding also showed that oleic acid, sterol and tocopherol contents were positively correlated (Hussain 2021).

The high magnitude of heterosis for various hybrids such as H8, H4 and H5 may be an evidence

Table 6 Mid-parental heterosis in various hybrids for tocopherol contents in sunflower oil per location during spring season

Hybrid	Sargodha	Faisalabad	Bahawalpur	Multan
H1	75.46*	3.64^{NS}	0.15^{NS}	10.47^{NS}
H2	22.45^{NS}	71.36*	8.52^{NS}	83.75*
H3	− 51.00*	8.97^{NS}	152.56*	77.29*
H4	− 2.61^{NS}	− 19.65^{NS}	26.30^{NS}	19.55^{NS}
H5	− 8.16^{NS}	14.18^{NS}	− 17.15^{NS}	93.78*
H6	56.18*	− 10.58^{NS}	− 13.58^{NS}	78.45*
H7	53.18*	52.52*	76.62^{NS}	− 24.05^{NS}
H8	− 28.17^{NS}	− 66.47*	221.54*	108.89*
H9	− 23.74^{NS}	− 26.80^{NS}	50.37*	− 18.94^{NS}
H10	38.50*	− 0.93^{NS}	39.61*	3.99^{NS}

* and NS indicate significant at $P \leq 0.05$) and non-significant ($P \geq 0.05$), respectively

Table 7 Mid-parental heterosis in various hybrids for sterol contents in sunflower oil at each location during the spring season

Hybrid	Sargodha	Faisalabad	Bahawalpur	Multan
H1	− 7.83^{NS}	− 5.06^{NS}	64.35*	58.81*
H2	9.47^{NS}	13.23^{NS}	72.47*	63.28*
H3	− 12.48^{NS}	14.29^{NS}	66.35*	40.99*
H4	10.65^{NS}	32.30^{NS}	129.01*	63.92*
H5	− 70.22*	22.48^{NS}	105.83*	19.30^{NS}
H6	− 4.15^{NS}	31.86^{NS}	74.34*	58.59*
H7	− 13.65^{NS}	55.81*	100.98*	47.80*
H8	− 4.75^{NS}	− 5.11^{NS}	58.01*	53.12*
H9	− 5.26^{NS}	28.92^{NS}	39.77*	50.54*
H10	− 3.42^{NS}	− 5.06^{NS}	23.10^{NS}	13.40^{NS}

* and NS indicate significant at $P \leq 0.05$), and non-significant ($P \geq 0.05$), respectively

of overdominance type of gene action for tocopherol and sterol contents. Heterosis has been shown to result by interallelic interactions causing an overdominance type of gene action (Khotyleva et al. 2017). Combining ability analyses was done to select positive combiners for tocopherol and sterol contents. Breeding lines such as B.116.P, B.112.P and RH.365 were best combiners for tocopherol. Breeding with positive general combining ability may carried preponderance of positive alleles for these traits (Khalil et al. 2016). Hence, these breeding lines may be further use in breeding programs for developing hybrids with high tocopherol contents.

Conclusion

The present investigation showed that tocopherol and sterol contents in sunflower are influenced by season and location. The autumn season is associated with higher tocopherol content than spring season; in contrast, sterol content was higher under spring season. The warmer the location the higher the had tocopherol content in oleic acid hybrids; whereas, it was observed their higher sterol content across locations as than in low oleic checks. Newly developed hybrids showed significant heterosis for tocopherol and sterol contents. Breeding lines B.116.P, B.112.P and RH.365 were best combiners for the investigated traits.

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Declarations

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