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Genotype and Environment Effects on Tocols of Barley and Oats[1]

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ABSTRACT

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Grain of 12 oat and 30 barley genotypes, each from three locations, was analyzed for tocols (tocopherols and tocotrienols) by high-performance liquid chromatography with fluorescence detection. The objective was to assess the variation in levels of tocols among genotypes and locations. Significant genotype differences existed for most tocols in both species. Total tocol concentrations for genotypes ranged from 19 to 30 mg kg^{-1} for oats and from 42 to 80 mg kg^{-1} for barley. Location differences were significant for oats but not for barley. Only a small percentage of the variance was associated with the interaction of genotype and location. α-Tocotrienol and α-tocopherol were the predominant tocol isomers in both species; β- and γ-tocotrienol were also present in significant amounts in barley. The major isomers of barley, but not of oats, were generally positively correlated with each other. The data indicate the feasibility of attempting to increase tocol concentration in these crops by hybridization and selection. That could lead to food products that might lower serum low-density-lipoprotein cholesterol, a risk factor for cardiovascular disease.

Vitamin E activity results from the complex of tocols found in various foodstuffs. Tocols include tocopherols and tocotrienols, the difference being a saturated side-chain in tocopherols and a triunsaturated side-chain in tocotrienols. Each class of tocols consists of at least four isomers, differing in the number and position of methyl substituents on the benzene ring (Pennock et al 1964).

Many biological activities of vitamin E are believed to result from its antioxidant action, specifically the inhibition of lipid peroxidation in biological membranes (Burton and Traber 1990). Although α-tocopherol is considered to have the greatest biological activity (Taylor and Barnes 1981), recent evidence suggests that α-tocotrienol may have 40–60 times higher antioxidant activity (Serbinova et al 1991).

Recently, a cholesterol biosynthesis inhibitor identified as α-tocotrienol was found in barley (Qureshi et al 1986). Further research has shown that tocols from barley, oats, palm oil, and other sources lower the cholesterol levels in chickens, swine, and humans (Qureshi et al 1991a–c). γ-Tocotrienol and δ-tocotrienol appeared to be more effective than the more abundant α-tocotrienol (Weber et al 1990, Qureshi et al 1991c). Also, α-tocopherol and α-tocotrienol had opposite effects on cholesterol metabolism in chicks, indicating that a higher ratio of tocotrienols to tocopherols in the diet may be important in metabolic regulation (Qureshi et al 1989).

Cereal grains are rich sources of tocols. The quantities of tocols in various cereal grains (Barnes 1983, Cort et al 1983) and cereal products (Piironen et al 1986) have been reported. The lack of a standardized method for extraction and analysis until recently (AOCS 1989) has led to a wide variation in quantities of the various tocols reported, especially in the earlier literature. In barley, all eight tocol isomers have been detected; α-tocotrienol is highest in concentration, followed by γ-tocotrienol, α-tocopherol, and β-tocotrienol (Barnes 1983). In oats, α-tocotrienol is also highest, followed by α-tocopherol (Barnes 1983). Most reports on oats have identified only the α and β isomers, but Lásztity et al (1980) found quantities of all eight.

We have found no reports indicating that variation in tocol concentrations may exist among barley cultivars and only a single report for oats (Lásztity et al 1980). Nor have there been any reports indicating that variation may exist due to different growing conditions. One study reported effects of air and moisture on tocols of stored barley grain (Hakkarainen et al 1983).

As more research results on the benefits of tocols in the human diet become available, there will be an interest among plant breeders in developing new cultivars of cereals having higher levels of total tocols and more favorable ratios of the isomers, i.e., more γ- and δ-tocotrienols. For that reason, we have measured the concentration of tocol isomers in a number of barley cultivars and experimental lines and oat cultivars grown in diverse environments. Our objective was to determine the variation in concentrations of tocols among diverse genotypes, the effects of location, the presence or absence of a location-genotype interaction, and the relationships among the isomers.

MATERIALS AND METHODS

Samples

Barley samples grown in 1990 were obtained from the USDA Western Regional Spring Barley Nursery. The nursery contained 30 entries, consisting of advanced breeding selections of feed and malting barleys and check cultivars. Samples from three replications at each of three locations (Ontario, OR; Aberdeen, ID; and Fargo, ND) were chosen for analysis. Oat samples were 12 popular cultivars that were grown in replicated trials at a number of university experiment stations in 1989. As with barley, oat seed supplied to each location for planting was from a common lot. Seed lots from each of three replications from Carrington, ND; W. Lafayette, IN; and Ithaca, NY, were chosen for analysis.

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Sample Preparation and Extraction

Whole-grain barley samples were ground, but oat samples were dehulled before grinding. Grain samples were dried overnight in a vacuum oven at 70°C and then ground in a Retsch ZM-1 ultracentrifugal mill (Brinkman Instruments Co., Westbury, NY) to pass a 0.5-mm screen. Prior drying improved the grinding characteristics of oats and was essential for accurate results (S. Paisley, *personal communication*). Ground samples were stored desiccated at room temperature until extraction; samples were extracted within a few days of grinding, usually the next day, as some deterioration in tocol profiles was noted upon prolonged storage.

Duplicate 0.5-g portions of ground grain were weighed into screw-capped culture tubes and extracted with 7 ml of methanol on a horizontal shaker (Eberbach Corp., Ann Arbor, MI) for 15 min at 220 oscillations per minute. After centrifugation (15 min at 1,000 × *g*), the supernatants were decanted into conical centrifuge tubes and solvent was evaporated under vacuum at 38°C (HBI vortex evaporator, Haake Buchler Instruments, Saddle Brook, NJ). For oats, the residues were extracted a second time with 7 ml of methanol, and these extracts were added to the conical tubes after most of the solvent from the first extraction had evaporated. Tocols in the dried residues were extracted with 2 ml of hexane by vortexing (SP multi-tube vortexer, American Scientific Products, McGaw Park, IL) for 3 min, followed by centrifuging for 5 min at 1,000 × *g*.

Analysis

The tocols were separated by high-performance liquid chromatography (HPLC) on a Waters μPorosil 10-μm particle size, 3.9 × 300-mm column (Millipore, Bedford, MA). Twenty microliters of each sample was injected with an autoinjector. The mobile phase was 0.5% isopropanol in hexane. Flow rate was 1.3 ml/min, and the peaks were detected with a Shimadzu RF-535 fluorescence monitor (Shimadzu Scientific Instruments, Columbia, MD) using an excitation wavelength of 295 nm and emission wavelength of 330 nm. Data were recorded and peak heights measured with a Shimadzu C-R3A Chromatopac. Standards (α-tocopherol and α-, β-, γ-, and δ-tocotrienol) were purified from palm oil by Kim Wright, Bristol-Myers Squibb. A mixture of these five, at 1 μg/ml each, in hexane was routinely analyzed. β-, γ-, and δ-Tocopherol peaks were quantitated against their corresponding tocotrienol standards.

Statistical Analysis

The data for each location were analyzed by the GLM procedure of SAS (SAS 1989). After checking for homogeneity of error variances by Bartlett's test (Steele and Torrie 1980), a combined analysis of variance was performed. The cultivar means at each location were calculated, and both Pearson product-moment and Spearman rank correlation coefficients were computed between locations. Variance components were calculated by the SAS VARCOMP procedure to determine what proportion of the variance could be attributed to cultivar, location, and their interaction. Phenotypic and genotypic correlation coefficients were computed among the tocol isomers (Falconer 1989) using sum of squares and cross products matrices generated by the SAS MANOVA procedure, based on genotype means. The standard error for evaluation of genotypic coefficients was computed as described by Mode and Robinson (1959).

RESULTS AND DISCUSSION

The total tocol concentration of the 12 oats averaged over locations ranged from 19 to 30 mg kg^{-1} (Table I). The mean value for total tocols (26 mg kg^{-1}) compared favorably with previous reports: 26.5 mg kg^{-1} (Lásztity et al 1980), 18.8 mg kg^{-1} (Barnes 1983), and 32 mg kg^{-1} (Piironen et al 1986). The range was less than reported by Lásztity et al (1980) for 13 (mostly European) varieties (13–43 mg kg^{-1}). The oat samples contained predominantly α-tocotrienol and α-tocopherol (86–91% of the total), with lesser amounts of the β-isomers, γ-tocopherol, and δ-tocotrienol. Only trace amounts of γ-tocotrienol and δ-tocopherol were detected in some samples. Lásztity et al (1980) found measurable quantities of all eight isomers, including substantial quantities of γ-tocotrienol; those findings have not been confirmed in other laboratories. Barnes (1983) found only α- and β-isomers. There is complete agreement that α-tocotrienol is the predominant isomer, followed by α-tocopherol.

The barley samples were substantially higher in total tocols, averaging 58 mg kg^{-1}, with a range of 42–80 mg kg^{-1} (Table II). Previous reports have indicated lesser amounts (about 30–32 mg kg^{-1}) (Barnes 1983, Piironen et al 1986). As for oats, α-tocotrienol was the major fraction, followed by α-tocopherol. However, barley also had substantial quantities of β- and γ-tocotrienols. All eight isomers were detected in the barley samples. Previous reports showed that α-tocotrienol was the major fraction, but γ-tocotrienol was higher than α-tocopherol in those earlier studies (Barnes 1983, Piironen et al 1986), whereas we found the opposite. The barley samples had a higher proportion of tocotrienols (74–83% of total tocols) as compared to oats (53–74%). That was due to a higher ratio of α-tocotrienol to α-tocopherol and to the greater amounts of the β- and γ-tocotrienols in barley.

The analysis of variance showed significant differences among oat genotypes for all isomers (except β-tocopherol) and for total tocols (Table III). Cultivar Webster (Appendix I) was highest in total tocols. Cultivars Steele and Porter were lowest. There were significant differences among locations for all tocols except β-tocopherol and δ-tocotrienol (Table III, Appendix II), and the genotype × location (G × L) interactions were also significant for all but β-tocopherol (Table III).

For barley, the error variances from each location were not homogeneous by Bartlett's test. Therefore, a nonparametric combined analysis of variance was performed, using the rankings

TABLE I
Genotype and Location Ranges and Overall Means for Oat Tocol Concentrations

Tocol[a]	Tocol Concentration, mg kg^{-1}		
	Genotype[b]	Location[c]	Mean
α-T	5.53 — 9.56[d]	7.18 — 9.58[d]	8.13
α-T3	9.10 — 18.56[d]	11.65 — 18.33[d]	14.91
β-T	0.72 — 1.26	0.70 — 1.13	0.90
β-T3	0.50 — 1.50[d]	0.53 — 1.39[d]	0.87
λ-T	0.52 — 1.25[d]	0.77 — 1.05[e]	0.91
δ-T3	0.00 — 0.57[d]	0.09 — 0.28	0.21
Total T+T3	19.00 — 30.32[d]	20.92 — 31.49[d]	25.94

[a] Tocols include tocopherols (T) and tocotrienols (T3).
[b] $n = 12$.
[c] $n = 3$.
[d] Differences between genotypes or locations significant at $P = 0.01$.
[e] Differences between genotypes or locations significant at $P = 0.05$.

TABLE II
Genotype and Location Ranges and Overall Means for Barley Tocol Concentrations

Tocol[a]	Tocol Concentration, mg kg^{-1}		
	Genotype[b]	Location[c]	Mean
α-T	7.15 — 11.60[d]	8.81 — 11.05[e]	9.75
α-T3	23.80 — 43.02[d]	30.46 — 36.26	33.23
β-T	0.50 — 1.26	0.02 — 1.93[d]	0.72
β-T3	3.10 — 12.05[d]	6.68 — 7.49	7.08
λ-T	0.37 — 1.22[d]	0.68 — 0.74	0.72
λ-T3	2.40 — 9.61[d]	4.97 — 5.61	5.36
δ-T	0.39 — 1.40[d]	0.35 — 1.31	0.71
δ-T3	0.20 — 2.00[d]	0.74 — 1.09	0.90
Total T+T3	42.16 — 80.03[d]	56.71 — 60.75	58.47

[a] Tocols include tocopherols (T) and tocotrienols (T3).
[b] $n = 30$.
[c] $n = 3$.
[d] Differences between genotypes or locations significant at $P = 0.01$.
[e] Differences between genotypes or locations significant at $P = 0.05$.

of the cultivars at each location. The F ratios and conclusions about the significance of the results were unchanged from those resulting from analysis of the actual data (Table III). The barley analysis showed that, like oats, most genotype effects were significant (except β-tocopherol). Unlike oats, location differences were only significant for α-tocopherol and β-tocopherol. Significant G × L interactions were found only for α-tocotrienol and total tocols. These results could be explained by a greater stability of barley across locations or by similar environments at the chosen locations. The highest concentration of total tocols was 80 mg kg^{-1} in genotype WA903584; that was almost double the lowest concentration of 42 mg kg^{-1} in OR1209 (Appendix III).

In a further attempt to assess the role of genotype, location, and their interaction on tocol concentrations, variance components were calculated and expressed as a percentage of the total variance (Table IV). For oats, most of the variance was associated with genotype and location and very little with their interaction. The minor components, β-tocopherol and δ-tocotrienol, had a large proportion of the variance associated with error. For barley, almost all the variance was associated with genotype for isomers present in substantial amounts, the exception being α-tocopherol.

TABLE III
Mean Squares for Combined Analysis of Variance for Oat and Barley Tocols

Species and Source	DF	Tocol[a]								
		α-T	α-T3	β-T	β-T3	γ-T	γ-T3	δ-T	δ-T3	Total T+T3
Oat										
Location (L)	2	55.76[b]	379.26[b]	1.719	7.209[b]	0.610[c]	...	...	0.391	962.1[b]
Error a										
(Reps/L)	6	1.55	4.21	1.302	0.064	0.064	...	...	0.257	17.9
Genotype (G)	11	11.22[b]	70.87[b]	0.157	0.710[b]	0.481[b]	...	...	0.235[b]	89.5[b]
G × L	22	1.0[c]	5.56[b]	0.062	0.074[b]	0.050[b]	...	...	0.071[c]	12.3[b]
Error b										
(G × R/L)	64[d]	0.49	1.38	0.054	0.009	0.013	...	...	0.039	3.6
Barley										
L	2	121.5[c]	761	99.1[b]	14.7	0.083	10.7	24.9	2.77	386
Error a										
(Reps/L)	6	11.6	351	2.65	15.4	0.217	13.8	8.9	1.34	912
G	29	11.6[b]	263[b]	0.21	54.5[b]	0.430[b]	28.7[b]	0.55[b]	1.79[b]	918[b]
G × L	58	1.03	31[b]	0.17	2.2	0.039	1.7	0.12	0.17	62[c]
Error b										
(G × R/L)	174	0.81	18	0.19	1.8	0.036	1.5	0.10	0.15	43

[a] Tocols include tocopherols (T) and tocotrienols (T3).
[b,c] Significant at $P = 0.05, 0.01$, respectively.
[d] There were two missing plots.

TABLE IV
Percentage of Total Variance for Tocols Associated with Each Source of Variation

Species and Source	Tocol[a]								
	α-T	α-T3	β-T	β-T3	γ-T	γ-T3	δ-T	δ-T3	Total T+T3
Oat									
Location[b] (L)	44	50	6	65	17	...	...	3	61
Genotype[c] (G)	35	36	6	24	52	...	...	22	21
G × L	4	6	2	7	13	...	...	10	6
Barley									
L[b]	33	7	80	0	0	0	28	4	0
G[d]	32	41	0	71	50	61	8	46	54
G × L	2	7	0	2	1	2	1	2	4

[a] Tocols include tocopherols (T) and tocotrienols (T3).
[b] $n = 3$.
[c] $n = 12$.
[d] $n = 30$.

TABLE V
Average Correlation Coefficients Between Locations for Tocol Concentrations and Ranks of 12 Oat and 30 Barley Genotypes

Species and Coefficient[b]	Tocol[a]								
	α-T	α-T3	β-T	β-T3	γ-T	γ-T3	δ-T	δ-T3	Total T+T3
Oat[c]									
r_p	0.76 ± 0.03[d]	0.79 ± 0.11	0.44 ± 0.26	0.79 ± 0.06	0.76 ± 0.06	...	...	0.38 ± 0.42	0.67 ± 0.12
r_s	0.63 ± 0.10	0.57 ± 0.12	0.39 ± 0.32	0.83 ± 0.08	0.78 ± 0.05	...	...	0.39 ± 0.34	0.28 ± 0.27
Barley[e]									
r_p	0.79 ± 0.04	0.72 ± 0.00	−0.01 ± 0.25	0.90 ± 0.03	0.77 ± 0.06	0.85 ± 0.02	0.57 ± 0.07	0.79 ± 0.06	0.83 ± 0.01
r_s	0.78 ± 0.05	0.73 ± 0.05	−0.05 ± 0.07	0.88 ± 0.03	0.82 ± 0.05	0.84 ± 0.02	0.50 ± 0.07	0.75 ± 0.08	0.85 ± 0.04

[a] Tocols include tocopherols (T) and tocotrienols (T3).
[b] r_p = Pearson product-moment correlation coefficient; r_s = Spearman rank correlation coefficient.
[c] $r > 0.58, 0.71$ are significant at $P = 0.05, 0.01$, respectively, for individual correlations.
[d] Mean of 3 individual correlations ± standard deviation.
[e] $r > 0.36, 0.46$ are significant at $P = 0.05, 0.01$, respectively, for individual correlations.

The δ isomers had a high error variance, and variance associated with the G × L interaction was very low.

Another way to estimate G × L interactions is to determine correlations between locations. We determined both the Spearman rank correlation coefficients and the Pearson product-moment correlation coefficients; the averages of three comparisons between locations are shown in Table V. For oats, the correlation coefficients were generally high and significant for the α-isomers, β-tocotrienol, and γ-tocopherol. For the other isomers and total tocols, the results were varied. Results of the Pearson and Spearman analyses were similar except for total tocols; the Pearson coefficients were significant, but the Spearman coefficients were not. These differences resulted from two cultivars with tocol concentrations that were considerably lower than the others, which strongly influenced the regressions based on concentration. The coefficients for barley were uniformly high and significant, except for that of β-tocopherol.

Phenotypic and genotypic correlations among the tocol isomers were calculated. For oats, only three of 21 phenotypic correlations were significant (Table VI). Significant positive correlations were noted for α-tocopherol with γ-tocopherol ($r = 0.62$) and for α-tocotrienol with β-tocotrienol ($r = 0.68$). There was a high positive correlation of α-tocotrienol with total tocols ($r = 0.93$) as expected because α-tocotrienol is the main constituent. Genotypic correlations were generally similar to phenotypic ones. For barley (Table VII), most isomers were significantly positively correlated, except β-tocopherol. Genotypic coefficients corresponded with the phenotypic ones.

CONCLUSIONS

There is significant genetic diversity in tocol concentrations among both oats and barley, amounting to a 1.5-fold (oats) and almost twofold (barley) difference from low to high, averaged over locations for these genotypes. Concentrations in none of the oats were as high as those in three Scottish cultivars reported by Lásztity et al (1980). A survey encompassing more genotypes, including unadapted and wild species, would probably reveal even greater diversity. Locations also significantly affected tocol levels in oats but not barley. This may have been a result of sampling only three locations, and it is possible that barley grown in other locations would differ. There was general stability across locations for both species, indicated by nonsignificant interaction mean squares in the analysis of variance (barley), a small component of variance associated with the interactions, and generally significant correlation coefficients between locations. Another test of stability, regression analysis of genotype means on location means, was not meaningful in this study because of only one degree of freedom for three locations. These results indicate that plant breeders could attempt to improve grain quality by selecting for high tocols and that testing in only a few environments is sufficient.

Barley, with a higher concentration of total tocols and a greater proportion of tocotrienol to tocopherol has a particularly good potential for food use. The tocols survive the malting and brewing process and are present in the brewers' spent grains in high concentration (90–120 mg kg^{-1}), much of the carbohydrate and

TABLE VI
Phenotypic and Genotypic Correlation Coefficients Among Oat Tocols Measured for 12 Genotypes at Three Locations

Tocol[a]	Coefficient[b]	α-T	α-T3	β-T	β-T3	γ-T	δ-T3
α-T3	r_p	−0.06					
	r_g	−0.08					
β-T	r_p	−0.26	−0.57				
	r_g	−0.35	−0.72				
β-T3	r_p	−0.39	0.68[c]	−0.19			
	r_g	−0.41	0.69	−0.24			
λ-T	r_p	0.62[c]	0.23	−0.55	−0.28		
	r_g	0.63	0.23	−0.71	−0.29		
δ-T3	r_p	0.21	0.05	0.36	−0.25	−0.05	
	r_g	0.23	0.05	0.30	−0.27	−0.05	
Total T+T3	r_p	0.32	0.93[d]	−0.60	0.52	0.45	0.16
	r_g	0.29	0.93	−0.80	0.52	0.45	0.16

[a] Tocols include tocopherols (T) and tocotrienols (T3).
[b] r_p = phenotypic correlation coefficient; r_g = genotypic correlation coefficient. Standard errors of genotypic correlations averaged 0.24 ± 0.08.
[c,d] Probability of $> r = 0.05, 0.01$, respectively.

TABLE VII
Phenotypic and Genotypic Correlation Coefficients Among Barley Tocols Measured for 30 Genotypes at Three Locations

Tocol[a]	Coefficient[b]	α-T	α-T3	β-T	β-T3	γ-T	γ-T3	δ-T	δ-T3
α-T3	r_p	0.50[c]							
	r_g	0.49							
β-T	r_p	−0.32	−0.23						
	r_g	−0.98	−0.72						
β-T3	r_p	0.56[c]	0.71[c]	−0.09					
	r_g	0.58	0.72	−0.27					
λ-T	r_p	0.41[c]	0.33	0.03	0.57[c]				
	r_g	0.44	0.34	0.08	0.58				
λ-T3	r_p	0.45[d]	0.73[c]	−0.03	0.82[c]	0.34			
	r_g	0.48	0.75	−0.15	0.83	0.34			
δ-T	r_p	0.39[d]	0.45[d]	−0.05	0.74[c]	0.86[c]	0.50[c]		
	r_g	0.43	0.50	−0.50	0.80	0.92	0.54		
δ-T3	r_p	0.30	0.64[c]	0.11	0.76[c]	0.37[d]	0.91[c]	0.52[c]	
	r_g	0.33	0.67	0.32	0.77	0.37	0.92	0.55	
Total T+T3	r_p	0.62[c]	0.93[c]	−0.17	0.89[c]	0.48[c]	0.88[c]	0.62[c]	0.79[c]
	r_g	0.63	0.94	−0.58	0.90	0.49	0.89	0.67	0.81

[a] Tocols include tocopherols (T) and tocotrienols (T3).
[b] r_p = phenotypic correlation coefficient; r_g = genotypic correlation coefficient. Standard errors of genotypic correlations, except those involving β-T, averaged 0.11 ± 0.05. Those for correlations involving β-T averaged 0.49 ± 0.11.
[c,d] Probability of $> r = 0.05, 0.01$, respectively.

protein having been removed (Qureshi et al 1991a). Oats are also favorable, with a high concentration of α-tocotrienol. Oats have more than double the oil percentage of barley (Price and Parsons 1975), and the tocols extract with the oil fraction. If oats are further developed to produce food oils, as has been suggested (Frey and Hammond 1975, Branson and Frey 1989), the oil would contain the cholesterol-lowering α-tocotrienol. In human feeding experiments, 30 g of barley bran or 3 g of barley oil daily significantly lowered total and low-density lipoprotein cholesterol in hypercholesterolemic subjects (Weber et al 1990, 1991). That indicates that dietary intervention with moderate levels of the barley products can have a positive therapeutic effect.

The separation and analysis of tocols by HPLC and fluorescence detection is relatively easy (Taylor and Barnes 1981, Cort et al 1983, Tan and Brzuskiewicz 1989) and could be performed on selected lines from breeding nurseries. Because the analysis method provides results for each isomer, selection for individual isomers or total tocols could be performed. The high correlations between γ- and δ-tocotrienols and total tocols in barley indicate that one could select for either to make progress in increasing both.

We have discovered that some oat and barley samples, including a group of high-oil oat genotypes, have an additional peak or peaks eluting more slowly than δ-tocotrienol. The identity and activity of these compounds are being investigated.

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APPENDIX I
Tocol Concentrations of 12 Oat Genotypes Averaged over Three Growing Locations

Genotype	Tocol[a] concentration, mg kg^{-1}						
	α-T	α-T3	β-T	β-T3	γ-T	δ-T3	Total T+T3
Steele	7.19 ± 0.40[b]	9.1 ± 0.8	1.26 ± 0.21	0.62 ± 0.12	0.55 ± 0.03	0.29 ± 0.10	19.0 ± 1.3
Porter	7.75 ± 0.51	10.0 ± 1.1	0.87 ± 0.09	0.52 ± 0.12	0.95 ± 0.12	0.06 ± 0.04	20.1 ± 1.9
Lodi	7.50 ± 0.61	14.8 ± 1.3	0.93 ± 0.13	0.95 ± 0.15	0.78 ± 0.05	0.03 ± 0.03	25.0 ± 2.1
Valley	8.29 ± 0.39	14.8 ± 1.4	0.82 ± 0.11	0.96 ± 0.20	0.81 ± 0.07	0.27 ± 0.10	25.9 ± 2.1
Otee	9.41 ± 0.51	14.5 ± 1.2	0.72 ± 0.13	0.50 ± 0.08	1.09 ± 0.08	0.10 ± 0.06	26.3 ± 1.9
Hazel	5.53 ± 0.40	18.5 ± 1.2	0.83 ± 0.12	1.50 ± 0.21	0.52 ± 0.04	0.00 ± 0.00	26.9 ± 1.8
Ogle	8.68 ± 0.54	15.6 ± 0.9	0.79 ± 0.12	0.81 ± 0.13	1.14 ± 0.08	0.11 ± 0.07	27.1 ± 1.7
Garry	9.19 ± 0.55	15.0 ± 0.8	0.94 ± 0.14	0.99 ± 0.12	0.85 ± 0.04	0.31 ± 0.10	27.3 ± 1.5
Dal	8.33 ± 0.31	16.4 ± 1.3	0.95 ± 0.15	0.75 ± 0.15	0.75 ± 0.04	0.57 ± 0.13	27.8 ± 1.8
Horicon	7.45 ± 0.51	17.2 ± 1.4	0.90 ± 0.14	0.76 ± 0.16	1.15 ± 0.07	0.33 ± 0.10	27.8 ± 2.2
Proat	9.56 ± 0.34	15.4 ± 0.7	0.91 ± 0.15	1.03 ± 0.12	1.04 ± 0.04	0.15 ± 0.10	28.1 ± 1.1
Webster	8.71 ± 0.31	18.0 ± 0.5	0.89 ± 0.14	1.14 ± 0.09	1.24 ± 0.07	0.29 ± 0.12	30.3 ± 1.0
LSD (0.05)	0.66	1.1	NS[c]	0.09	0.11	0.19	1.8

[a] Tocols include tocopherols (T) and tocotrienols (T3).
[b] Mean ± standard error.
[c] No significant difference.

APPENDIX II
Tocol Concentrations at Each of Three Locations, Averaged over 12 Oat Genotypes and 30 Barley Genotypes

Species and Location	Tocol[a]								
	α-T	α-T3	β-T	β-T3	γ-T	γ-T3	δ-T	δ-T3	Total T+T3
Oat									
Carrington, ND	7.18 ± 0.24[b]	11.7 ± 0.5	0.70 ± 0.09	0.53 ± 0.04	0.77 ± 0.04	...	...	0.09 ± 0.04	20.9 ± 0.7
W. Lafayette, IN	7.66 ± 0.21	14.8 ± 0.6	1.13 ± 0.06	0.70 ± 0.05	0.91 ± 0.05	...	...	0.26 ± 0.06	25.4 ± 0.7
Ithaca, NY	9.58 ± 0.23	18.3 ± 0.5	0.87 ± 0.02	1.39 ± 0.07	1.05 ± 0.05	...	...	0.28 ± 0.05	31.5 ± 0.6
Barley									
Ontario, OR	11.05 ± 0.19	36.3 ± 0.9	0.02 ± 0.01	6.68 ± 0.30	0.68 ± 0.03	4.97 ± 0.25	0.35 ± 0.04	0.74 ± 0.07	60.8 ± 1.5
Aberdeen, ID	9.38 ± 0.15	33.0 ± 0.8	0.21 ± 0.04	7.49 ± 0.28	0.73 ± 0.03	5.61 ± 0.23	0.45 ± 0.05	1.09 ± 0.07	57.9 ± 1.3
Fargo, ND	8.82 ± 0.13	30.5 ± 0.7	1.93 ± 0.08	7.07 ± 0.31	0.74 ± 0.03	5.51 ± 0.20	1.31 ± 0.09	0.88 ± 0.05	56.7 ± 1.2

[a] Tocols include tocopherols (T) and tocotrienols (T3).
[b] Mean ± standard error.

APPENDIX III
Tocol Concentrations of 30 Barley Genotypes Averaged over Three Growing Locations

Genotype	Tocol[a] concentration, mg kg^{-1}								
	α-T	α-T3	β-T	β-T3	γ-T	γ-T3	δ-T	δ-T3	Total T+T3
OR1209	8.42 ± 0.49[b]	23.8 ± 1.6	0.51 ± 0.25	3.89 ± 0.20	0.62 ± 0.04	3.79 ± 0.33	0.54 ± 0.23	0.59 ± 0.13	42.2 ± 2.2
ID8540	8.93 ± 0.48	26.8 ± 1.2	0.75 ± 0.39	3.10 ± 0.18	0.45 ± 0.06	2.40 ± 0.20	0.39 ± 0.24	0.20 ± 0.07	43.0 ± 1.5
Trebi	8.11 ± 0.48	26.3 ± 1.8	0.62 ± 0.37	4.87 ± 0.26	0.37 ± 0.10	4.04 ± 0.19	0.42 ± 0.22	0.54 ± 0.05	45.3 ± 2.1
UT502358	8.82 ± 0.53	27.6 ± 1.9	0.82 ± 0.39	5.70 ± 0.44	0.90 ± 0.09	2.47 ± 0.21	0.79 ± 0.26	0.23 ± 0.08	47.3 ± 2.5
UT1378	9.48 ± 0.57	26.0 ± 2.1	0.73 ± 0.33	5.27 ± 0.35	1.14 ± 0.13	3.66 ± 0.30	0.93 ± 0.21	0.47 ± 0.10	47.7 ± 2.8
WP584118	7.15 ± 0.33	24.9 ± 1.9	1.27 ± 0.54	5.99 ± 0.27	0.51 ± 0.06	6.39 ± 0.65	0.41 ± 0.18	1.49 ± 0.23	48.1 ± 2.8
UT1705	9.87 ± 0.45	27.7 ± 1.8	0.71 ± 0.35	4.73 ± 0.62	0.61 ± 0.04	4.30 ± 0.63	0.54 ± 0.14	0.69 ± 0.21	49.1 ± 2.7
ND10278	11.36 ± 0.53	26.6 ± 1.3	0.64 ± 0.29	5.71 ± 0.23	0.74 ± 0.03	3.85 ± 0.28	0.48 ± 0.20	0.82 ± 0.07	50.0 ± 1.8
ND9866	10.08 ± 0.63	29.5 ± 1.4	0.68 ± 0.34	4.79 ± 0.16	0.39 ± 0.06	4.56 ± 0.27	0.41 ± 0.24	0.32 ± 0.08	50.7 ± 2.2
Steptoe	8.60 ± 0.40	31.3 ± 2.0	0.69 ± 0.31	6.06 ± 0.40	0.88 ± 0.11	3.89 ± 0.21	0.76 ± 0.20	0.58 ± 0.08	52.7 ± 2.2
OR2	8.31 ± 0.50	32.3 ± 2.9	0.98 ± 0.46	4.25 ± 0.46	0.61 ± 0.06	5.52 ± 0.59	0.57 ± 0.40	0.93 ± 0.16	53.4 ± 4.2
Morex	8.92 ± 0.31	34.3 ± 1.3	0.65 ± 0.35	5.63 ± 0.39	0.51 ± 0.04	3.51 ± 0.19	0.39 ± 0.19	0.53 ± 0.09	54.5 ± 1.9
ND10277	11.60 ± 0.77	29.4 ± 1.6	0.86 ± 0.40	7.00 ± 0.56	0.78 ± 0.02	4.49 ± 0.54	0.61 ± 0.18	0.80 ± 0.14	55.5 ± 2.7
MN52	9.08 ± 0.40	34.3 ± 1.9	0.83 ± 0.42	5.67 ± 0.36	0.48 ± 0.04	3.70 ± 0.30	0.56 ± 0.38	0.97 ± 0.18	55.6 ± 2.8
PB107	9.22 ± 0.55	30.6 ± 2.4	0.67 ± 0.28	9.35 ± 0.72	0.59 ± 0.05	5.40 ± 0.37	0.90 ± 0.21	0.69 ± 0.05	57.4 ± 3.6
BA2601	9.49 ± 0.40	36.1 ± 2.2	0.50 ± 0.23	5.51 ± 0.50	0.48 ± 0.05	4.60 ± 0.48	0.54 ± 0.29	0.77 ± 0.18	58.0 ± 3.4
ID71966	9.15 ± 0.35	35.0 ± 1.4	0.71 ± 0.35	7.07 ± 0.58	0.73 ± 0.04	3.99 ± 0.31	0.73 ± 0.22	0.73 ± 0.07	58.1 ± 2.0
OR006	9.28 ± 0.47	36.4 ± 2.1	0.81 ± 0.34	5.86 ± 0.47	0.64 ± 0.04	4.98 ± 0.30	0.73 ± 0.26	0.92 ± 0.10	59.6 ± 2.5
MT860756	11.23 ± 0.42	34.7 ± 1.1	0.56 ± 0.27	7.53 ± 0.27	0.41 ± 0.02	6.69 ± 0.41	0.42 ± 0.20	0.81 ± 0.10	62.4 ± 1.8
OR3	9.64 ± 0.45	37.2 ± 2.9	0.59 ± 0.24	5.69 ± 0.85	0.73 ± 0.10	6.68 ± 0.92	0.76 ± 0.33	1.25 ± 0.23	62.6 ± 4.5
BA854026	9.91 ± 0.36	35.5 ± 1.0	0.71 ± 0.35	8.22 ± 0.41	0.73 ± 0.05	5.81 ± 0.24	0.81 ± 0.20	1.00 ± 0.10	62.7 ± 1.3
WA9448-83	10.36 ± 0.58	35.1 ± 2.1	0.79 ± 0.43	9.95 ± 0.70	0.93 ± 0.09	5.82 ± 0.30	0.99 ± 0.24	0.87 ± 0.09	64.8 ± 2.9
MT851012	11.54 ± 0.61	38.0 ± 2.4	0.89 ± 0.40	8.57 ± 0.51	0.93 ± 0.08	6.29 ± 0.41	0.85 ± 0.25	0.94 ± 0.11	68.0 ± 3.5
Klages	10.52 ± 0.54	40.2 ± 1.5	0.68 ± 0.27	7.74 ± 0.64	0.86 ± 0.08	6.58 ± 0.46	0.71 ± 0.27	1.05 ± 0.14	68.4 ± 2.5
MT851195	9.59 ± 0.36	41.5 ± 1.3	0.67 ± 0.29	8.40 ± 0.27	0.77 ± 0.03	6.51 ± 0.40	0.55 ± 0.16	0.93 ± 0.11	68.9 ± 1.7
BA865169	9.72 ± 0.34	35.9 ± 1.8	0.81 ± 0.34	11.41 ± 0.58	1.02 ± 0.06	7.88 ± 0.30	1.15 ± 0.22	1.59 ± 0.17	69.4 ± 2.1
OR1	11.11 ± 0.43	38.3 ± 1.5	0.66 ± 0.29	9.52 ± 0.60	0.88 ± 0.05	7.25 ± 0.41	1.00 ± 0.24	1.14 ± 0.17	69.9 ± 2.1
ID842974	11.29 ± 0.57	38.7 ± 2.2	0.64 ± 0.27	11.37 ± 0.73	1.22 ± 0.12	7.46 ± 0.78	1.40 ± 0.26	1.66 ± 0.31	73.7 ± 3.7
WA9029	10.70 ± 0.54	40.3 ± 3.5	0.55 ± 0.27	11.50 ± 0.76	0.80 ± 0.05	8.71 ± 0.74	0.86 ± 0.29	1.79 ± 0.26	75.2 ± 4.7
WA903584	10.98 ± 0.49	43.0 ± 3.0	0.67 ± 0.32	12.05 ± 0.66	0.81 ± 0.05	9.64 ± 0.62	0.85 ± 0.19	2.01 ± 0.21	80.0 ± 4.1
LSD	0.83	3.9	NS[c]	1.24	0.18	1.13	0.29	0.36	6.1

[a] Tocols include tocopherols (T) and tocotrienols (T3).
[b] Mean ± standard error.
[c] No significant difference.

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