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Response of Carotenoids and Tocols of Durum Wheat in Relation to Water Stress and Sulfur Fertilization

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ABSTRACT: Lipophilic antioxidants are essential components of plant defense against stressful conditions. The response of carotenoids and tocols to water deficit and sulfur fertilization was investigated in durum wheat cultivars. The amounts of tocols and carotenoids were evaluated in both whole meal and semolina samples. Differences among cultivars were observed. Simeto cultivar showed a significant effect of water regime on whole meal and semolina carotenoids, with about 20% and 15% increase, respectively. Also tocols and tocotrienols of Simeto were positively affected by water stress (about 10% increase and 15% increase in whole meals and semolinas). Sulfur fertilization positively impacted mainly Ofanto whole-grain and semolina carotenoids, semolina tocols, and tocotrienols. In conclusion, water deficit occurring under a Mediterranean environment was responsible for an improvement of lipophilic antioxidant content in durum wheat; in contrast sulfur supplementation did not improve the response of the antioxidant pool under water deficit.

KEYWORDS: *tocols, carotenoids, antioxidants, water stress, agronomic conditions, durum wheat*

INTRODUCTION

Whole-grain foods have been the focus of scientific and commercial research during the past 10 years since epidemiological studies have shown their protective role against the risk of many chronic diseases.[1] Growing evidence suggests that cereal and cereal-based products contain notable levels of bioactive compounds of health benefit including natural antioxidants such as tocols and carotenoids.[2−4]

Carotenoids are a diverse group of yellow-orange pigments found in many biological systems, which can be divided into two general classes: carotenes and xanthophylls. The nutritional importance of carotenoids comes mainly from the provitamin A activity of β-carotene, β-cryptoxanthin, and others with at least one intact nonoxygenated β-ionone ring. In addition to their role as precursors of vitamin A, carotenoids are antioxidants,[5] and besides β-carotene, other carotenoids without provitamin A activity have recently been involved in the prevention of or protection against serious human disorders, such as cancer and cardiovascular diseases. The xanthophyll cycle pool, the carotenoids violaxanthin, anteraxanthin, and zeaxanthin, are involved in light energy dissipation of vegetable green tissues. Increasing evidence shows that carotenoids may act as radical scavengers both as singlet oxygen quenchers and as quenchers of triplet excited states of molecules,[6] thus limiting membrane damage during environmental stress. Fruits and vegetables are the main sources of carotenoids, which are minor constituents in cereal grains. Besides their nutritional and health benefits, they are responsible for the attractive bright yellow color of food products such as durum wheat pasta.[7] The role of carotenoids in seeds is less clear than in other tissues, but it is emerging. Carotenoid production in the seed is important for abscissic acid production and seed dormancy. Furthermore carotenoids contribute to the antioxidant system in seeds, with the function of limiting free radical-induced membrane deterioration and seed aging.[6]

Tocopherols and tocotrienols (tocols) are a group of fat-soluble lipid compounds recognized as a generic term for vitamin E, which differ only in the degree of saturation of their hydrophobic prenyl side chains and are essential in maintaining membrane integrity. α-Tocopherol is the major form found in the green parts of plants, while tocotrienols are mostly found in seeds. Vegetable oils are the main source of tocols; however, substantial amounts of these compounds are also reported in most cereal grains (10.2 to 74.7 mg/kg), including barley, oat, wheat, rye, and rice.[3,8−11] Biological activities of tocols are generally believed to be due to their antioxidant action by inhibiting lipid peroxidation in biological membranes. α-Tocopherol has been labeled as the most efficient antioxidant for breaking free radical-driven chain reactions. However, results indicate that α-tocotrienol is at least 3-fold more efficient as a scavenger of peroxyl radicals than α-tocopherol.[12]

The capacity of the plant cellular antioxidative and photoprotective defense toward harmful reactive oxygen species (ROS), produced by natural and man-made stress situations, is determined by the pool size of the antioxidants and protective pigments. Changes in these parameters reflect the impact of environmental stresses on plant metabolism.[13] In the last years several environmental conditions have been identified as able to modify the amount of antioxidant compounds. In particular, water stress was found to increase the protective carotenoids and de-epoxidation state of the xantophyll cycle, as well as total glutathione and tocopherol concentrations.[14] An increase in α-tocopherol and β-carotene by 2.5-fold was observed in wheat leaves under drought.[15] Also sulfur fertilization may have an important role in determining drought tolerance, due to the

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antioxidative properties of glutathione (GSH), directly involved in the protection of cells from ROS damage and in regeneration of oxidized substrates. Furthermore, also thioredoxins, small protein thiols, are essential for the adjustment of metabolism to environmental conditions.[16] However, most of these studies dealt with the photosynthetic tissue during vegetative growth stages of crops, because these are critical phases in plant survival and yield formation.[17,18] In contrast, fewer studies reported data on the antioxidant concentrations in the harvested fractions of crops as influenced by environmental stresses. A range of antioxidant components was investigated, including the lipophilic classes of carotenoids and tocopherols. The effect of water stress on tocopherol and carotenoid levels was investigated by several authors, in different plant systems, with different results.[14,19,20]

A high level of consensus was seen on the stimulating effect of various types of environmental stresses on tocopherol (vitamin E) concentration. A tendency toward increasing concentration was observed, although with more frequent exceptions.[21] Such data were mainly reported for fruits and vegetables, in which the antioxidant capacity is a major factor determining the quality and health effects in human diets.

The level of accumulation of tocols within the cereal kernel has been found to be influenced by genotype, environmental effects of different growing locations, agronomic inputs, and the interaction of these factors.[4,11,22] In corn seed oil a considerable increase in tocopherols was observed due to water stress.[23] Genotype and environment were found to influence carotenoid levels in durum wheat,[24,25] with an increase observed under salt stress.[25] These increments have been partly explained by a concentration effect.[21]

Despite several works dealing with the antioxidant response to environmental conditions, only very few studies report the effect of water stress and fertilization on the antioxidant profile of cereals and especially of durum wheat, a species well adapted to Mediterranean environments, where water limiting conditions often occur. Water stress and nitrogen fertilization were proved to affect grain quality and protein composition in durum wheat.[26−28] Also sulfur fertilization was proved to affect grain quality, both by influencing the relative amounts of storage proteins and in the formation of sulfhydryls and disulfide bonds, which are important for the stabilization of protein structures, being also responsible for the viscoelastic properties of wheat gluten.[16,29] While many studies were carried out on the effect of water stress on technological quality, very few studies are relative to the effect on antioxidants and are mainly limited to hydrophilic compounds such as phenols,[30] which were found to increase under stress. Furthermore to our best knowledge no information is available on the effect of sulfur fertilization on lipophilic antioxidants in durum wheat.

In order to gain insight into the changes in the grain lipophilic antioxidant profile under abiotic stress, the primary goal of this study was the investigation of the effect of drought stress on the content of carotenoids and tocols in different durum wheat cultivars. Moreover the influence of sulfur fertilization was investigated, due to the global sulfur deficiency occurring in arable lands, mainly due to phasing out of sulfur-containing fertilizer and decrease in atmospheric SO_2 deposition, and also in light of its effects on plant response to abiotic stresses. The exact amounts of carotenoids and tocols in wheat samples, as whole meals and semolinas, were determined by a HPLC technique after an extraction procedure involving saponification of the food matrix.[9,31]

MATERIALS AND METHODS

Chemicals. α-Carotene, β-carotene, and lutein were from Sigma (St. Louis, MO). β-Cryptoxanthin and zeaxanthin were from Extrasynthese (Z.I. Lyon-Nord, Genay, France). α-, β-, γ-, and δ-tocopherol standards were from Fluka (Sigma-Aldrich, St. Louis, MO); α-, γ-, and δ-tocotrienol standards were obtained from a saponified barley fraction enriched by pearling, and β-tocotrienol was obtained from a saponified sample of durum wheat.[9] All other reagents were of analytical or HPLC grade and were purchased from Carlo Erba (Milano, Italy).

Samples and Agronomic Conditions. Field experiments were carried out in Foggia (southern Italy, 41°, 46′ N; 15°, 54′ E), during two consecutive growing seasons, on a clay soil with a mean value of 1360 mg/kg total nitrogen, 21.2 mg/kg assimilable phosphorus, 475 mg/kg exchangeable potassium, 1.5% organic matter. In both years a nitrogen fertilizer level of 60 kg/ha was applied. A split plot experimental design with two replicates was carried out, with water regime (irrigated and rain-fed) in the main plots and during the second season two sulfur fertilizer levels (S0 and S33, corresponding to 0 and 33 kg sulfur/ha, respectively) in plots. Four durum wheat cultivars in the first season, Ofanto (Adamello × Appulo, Cereal Crop Centre, Roma), Simeto (Capeiti × Valnova, Stazione Sperimentale Granicoltura, Caltagirone, CT), Appio (Cappelli × Gaviota × Yuma, Federconsorzi, Roma), and Creso (Cymmit × Cp B144, ENEA, Roma), and two cultivars (Ofanto and Simeto) in the second season were cultivated in subplots. Each subplot was 20.4 m^2. In both seasons two different water regimes were applied: irrigated (I) and rain-fed (NI). In the irrigated treatment, water was applied in order to establish field capacity of the 0.4 m soil profile whenever the threshold of 50% available water was reached. Despite a similar total rainfall of 312 and 322 mm in the two growing seasons, differences in the rainfall trend were observed. In particular, in the second year a marked water deficit was observed in grain filling, with 67 and 24 mm of total rainfall in this stage being observed in the first and second year, respectively. This caused differences in irrigation between the two years with one application of 473 m^3/ha at the booting stage in the first year and two applications of 475 m^3/ha in the second year at the booting and grain filling stage. Once harvested, cleaned, and mixed, grains from the two replicates of the different experimental conditions of the field trials were stored at 4 °C and were used within 15 days of storage to produce whole meals and, only for the second season, semolina samples. An aliquot of samples was stored at −20 °C and analyzed within 3 months, in case more than three analytical repetitions were necessary. Immediately before analysis, the samples were ground in a Cyclotec 1093 laboratory mill with a 0.8 mm sieve (FOSS Italia, Padova, Italy) and carefully mixed. Semolinas were produced by a Buhler MLU 202 (Uzwill, Switzerland), equipped with three break and three reduction rolls and six steel screens. Semolina yield was 65−68%.

Equipment. Chromatography was performed using a HPLC analytical system, comprising a Waters model 510 solvent delivery system (Milford, MA), equipped with an injector with a 50 μL loop (Rheodyne, Cotati, CA), a 2996 diode array detector, and a programmable model 470 spectrofluorimeter. Results were evaluated by a Waters Millennium chromatography system.

Sample Preparation. *Saponification.* Tocols and carotenoids were extracted according to the methods given in refs 9 and 31, respectively: 2 g of cereal sample was saponified under nitrogen in a screw-capped tube by adding 5 mL of ethanolic pyrogallol (60 g/L) as antioxidant, 2 mL of ethanol (95%), 2 mL of sodium chloride (10 g/L), and 2 mL of potassium hydroxide (600 g/L). The tubes were placed in a 70 °C water bath and mixed every 5−10 min during saponification. After alkaline digestion at 70 °C for 45 min, the tubes were cooled in an ice bath, and 15 mL of sodium chloride (10 g/L) was added. The suspension was then extracted twice with a 15 mL portion of *n*-hexane/ethyl acetate (9:1 v/v). The organic layer was collected and evaporated to dryness; the dry residue was dissolved in 2 mL of isopropyl alcohol (10%) in *n*-hexane. For tocol determination the sample was evaporated to dryness and suspended in 2 mL of isopropyl alcohol (1%) in *n*-hexane.

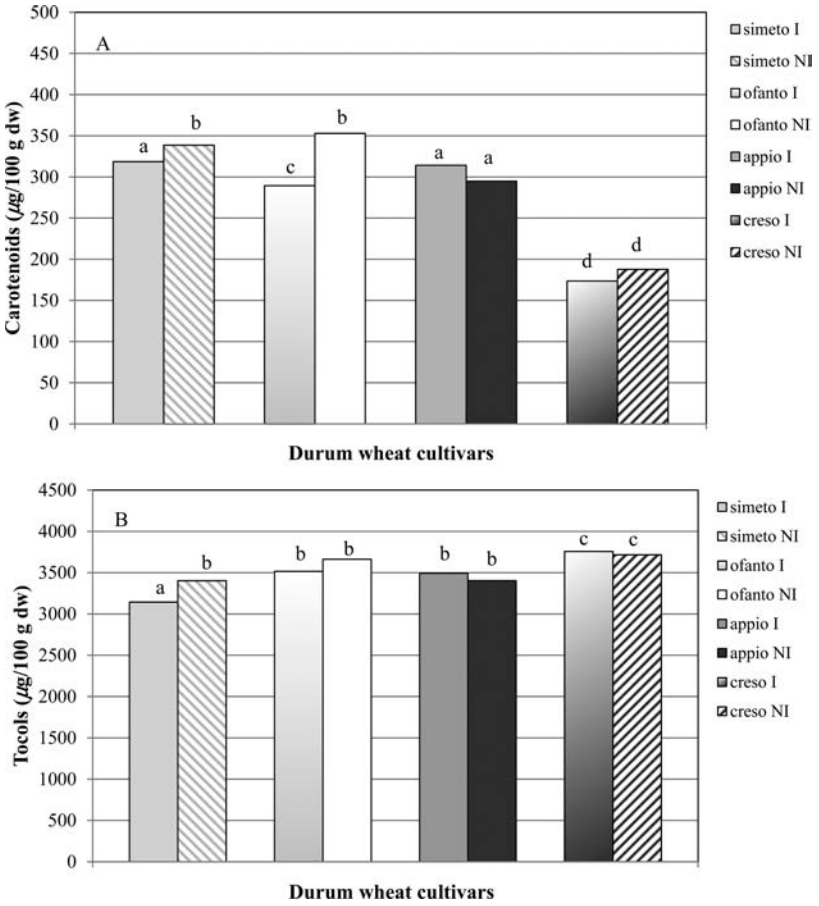

Figure 1. Total carotenoid (A) and tocol (B) content in whole meals under water stress in the first growing season. I: irrigated; NI: rain-fed. Same letters indicate no significant differences at $p \geq 0.05$ according to Tukey's test.

Allowing the opening up of the cell wall matrix and releasing compounds that might be strongly linked to cellular components, the saponification procedure used gives more precise and reliable results than those obtained without saponification. In fact, carotenoids and tocols have been proved to be partially bound to matrix components and therefore are hardly extracted by using single solvents;[9,31] moreover the ratio between the bound and the free forms could be influenced by environmental factors and technological processes. The method, previously published, resulted to be sensitive, selective, and reliable for the determination of the qualitative and quantitative distribution of these compounds in cereals.[9,31,32]

HPLC Analysis. The chromatographic separation of the compounds was achieved by means of a 250 mm × 4.6 mm i.d., 5 μm particle size, Kromasil Phenomenex Si column (Torrance, CA). For tocol analysis,[9] the mobile phase was *n*-hexane/ethyl acetate/acetic acid (97.3/1.8/0.9 v/v/v), at a flow rate of 1.6 mL/min. For carotenoid analysis,[31] the mobile phase was *n*-hexane/isopropyl alcohol (5%) at a flow rate of 1.5 mL/min. Tocols were spectrofluorimetrically detected (excitation wavelength 290 nm, emission wavelength 325 nm). Spectrophotometric detection of carotenoids was achieved by means of a diode array detector set in the range 350−500 nm. Peaks were detected at 450 nm. Carotenoids and tocols were identified, as reported in the original papers,[9,31] through comparison of their retention times with known standard solutions and were quantified on the basis of their respective standard solution, with the exception of unidentified carotenoids that were quantified as lutein. After every 10 injections the column was reactivated with a solution of 10% isopropyl alcohol in *n*-hexane (v/v). The performances of the used analytical methods are reported in the original papers.

Statistical Analysis. Data from this study are reported as mean for at least three replications for each sample. For all the investigated parameters, the analysis of variance (ANOVA) was performed using a split−split plot design by the MSTAT-C computer package (version 2.1, Crop and Soil Sciences Department, Michigan State University, 1991). Significant differences among the mean values were calculated following Tukey's test.[33]

RESULTS AND DISCUSSION

First Crop Season: Effect of Water Stress. In the first crop season the whole meals of four cultivars were investigated regarding the effect of water stress, as described in Materials and Methods.

Analysis of variance did not show a significant effect on yield of different genotypes (about 4.7 t/ha, as average value) and water regime (4.8 t/ha under water regime versus 4.3 t/ha under water stress).

Among the different cultivars, a significant difference was observed for carotenoid and tocol levels of whole meals ($p \leq 0.05$). Simeto had the highest carotenoid amounts, about 300 μg/100 g of dry weight (dw), while Creso had the lowest carotenoid (about 180 μg/100 g dw) and the highest tocol levels (about 3700 μg/100 g dw). A positive effect ($p \leq 0.05$) of water regime on the amounts of the analyzed compounds was observed; in particular, for carotenoids, in rain-fed (NI) Simeto and Ofanto cultivars, while, for tocols, only in rain-fed Simeto cultivar. No significant effect was found for Appio and Creso cultivars (Figure 1).

Second Crop Season: Carotenoid and Tocol Amounts. In light of the results obtained in the first crop season, in the second year of experimentation the investigation was carried

Table 1. Average Amounts (Micrograms/100 g of Dry Weight) of Tocols and Carotenoids in Simeto and Ofanto Whole Meals and Semolinas

	Simeto		Ofanto	
compound	whole meals	semolinas	whole meals	semolinas
β-carotene	11.1	7.9	10.3	8.2
lutein	300.7	329.9	247.4	302.2
zeaxanthin	23.2	8.7	19.3	10.6
total carotenoids[a]	335.0	346.5	277.0	321.0
α-tocopherol	698.6	158.5	648.9	181.5
β-tocopherol	170.8	34.1	211.0	55.0
total tocopherols	869.4	192.6	859.9	236.5
α-tocotrienol	423.6	206.8	349.8	205.2
β-tocotrienol	2319.3	1322.8	2706.0	1999.2
total tocotrienols	2742.9	1529.6	3055.8	2204.4
total tocols[b]	3612.3	1722.2	3915.7	2440.9

[a]Mean values are expressed as sum of β-carotene, lutein, and zeaxanthin. [b]Mean values are expressed as sum of total tocopherols and total tocotrienols.

out only on Ofanto and Simeto cultivars. Both cultivars under study are high-yielding varieties adapted to water-limited environments, with Simeto showing an earlier heading time, a higher test weight, and commercial value being still largely cultivated in South Italy, due to its better grain technological quality.[26] The two cultivars were analyzed under different water conditions (see Materials and Methods). Moreover, due to its controversial effects on the antioxidant content in different vegetables, also sulfur fertilization was investigated.

Several authors[10,34] showed that, across the wheat grain, from the central endosperm to the outer layers, a different distribution pattern for carotenoids and tocols exists, depending on genotype. Therefore, for a more in-depth evaluation of the effect of agronomical treatments, not only whole meals but also the respective semolinas should be analyzed.

In order to show differences between whole meals and semolinas, in Table 1 the amounts of the main representative carotenoids and tocols of the two different genotypes are summarized. Total carotenoids, total tocols, tocotrienols, and tocopherols, expressed as the sum of the main compounds, are also reported. Values are expressed as mean contents of those found under all the different tested conditions.

In the two tested wheat genotypes the main representative carotenoid was lutein (about 90% and 95% of total carotenoids in whole meals and semolinas, respectively), followed by zeaxanthin (7% and 3%, respectively) and β-carotene (3% and 2%, respectively). Low amounts of β-cryptoxanthin and other minor compounds that have been tentatively identified as cis isomers (9, 9′, 13, 13′) of lutein[31] have been detected. Data from Table 1 report a higher carotenoid content in Simeto whole meals than that found in the analogous Ofanto samples ($p \leq 0.05$). Whereas in Simeto cultivar the same total carotenoid contents were found between whole meals and

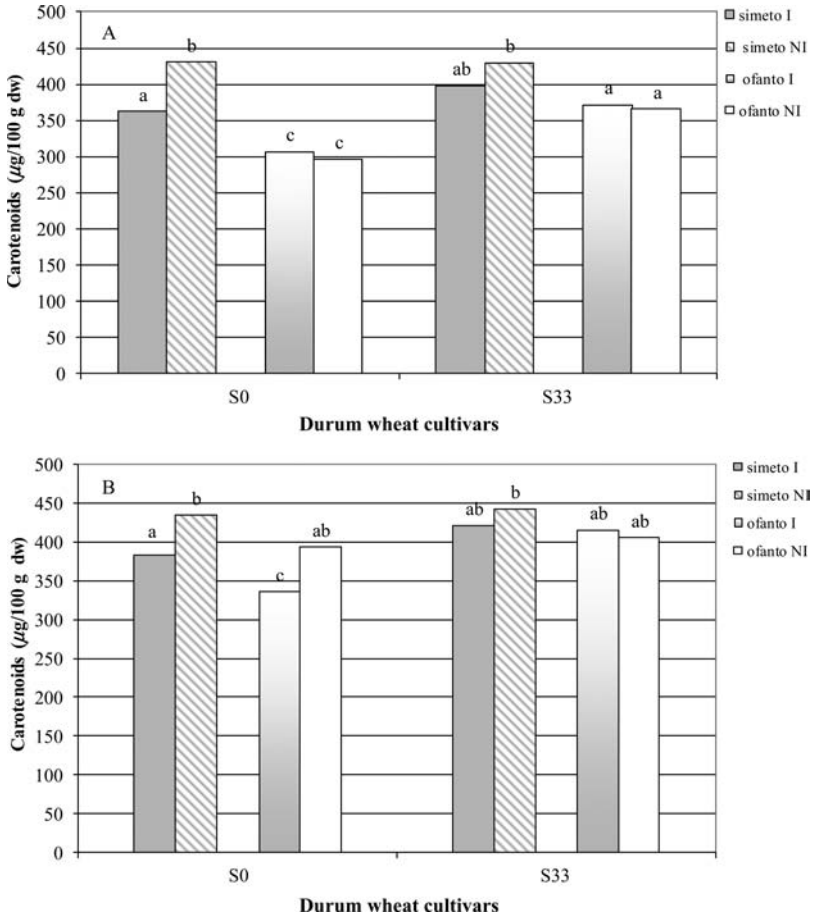

Figure 2. Total carotenoid content in Simeto and Ofanto whole meals (A) and semolinas (B) under water stress and sulfur fertilization in the second growing season. I: irrigated; NI: rain-fed. Same letters indicate no significant differences at $p \geq 0.05$ according to Tukey's test.

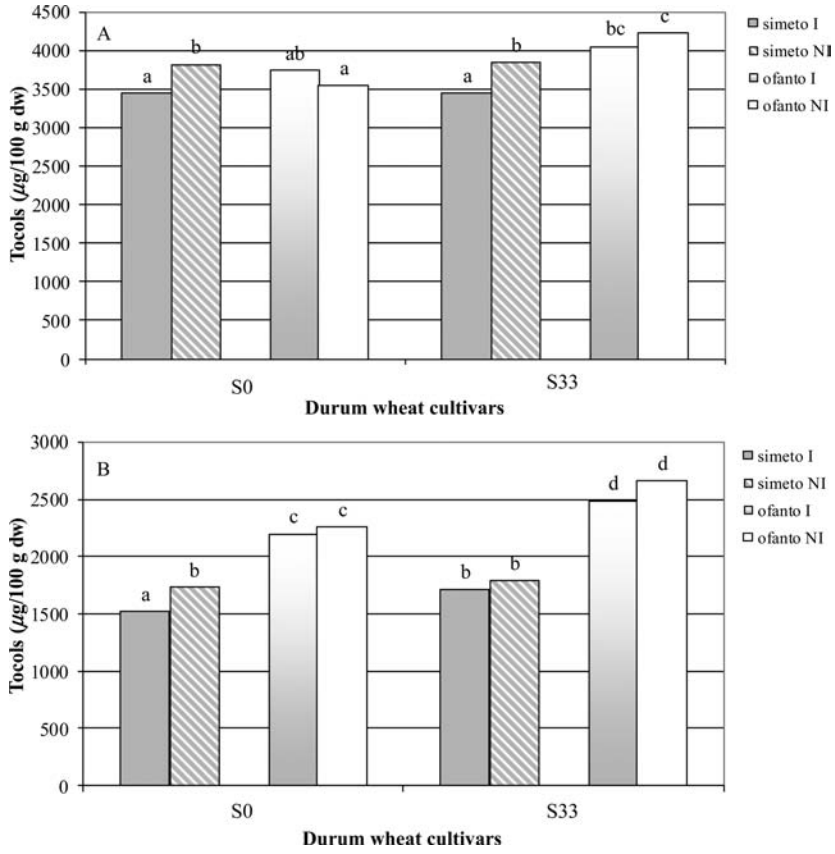

Figure 3. Total tocol content in Simeto and Ofanto whole meals (A) and semolinas (B) under water stress and sulfur fertilization in the second growing season. I: irrigated; NI: rain-fed. Same letters indicate no significant differences at $p \geq 0.05$ according to Tukey's test.

semolinas, in Ofanto semolinas, a 20% average increment of lutein with respect to the raw samples was measured. Due to this increase, similar amounts of carotenoids were found in Ofanto and Simeto semolinas (by an average of 321.0 μg/100 g dw and 346.5 μg/100 g dw, respectively) (Table 1). The increase in lutein content in semolinas with respect to whole meals could be due to the fact that in milled samples the bran and the germ of the wheat kernel are removed during the refining process. Environmental conditions that could have affected the development of Ofanto kernels could have determined an increase in lutein concentration in the endosperm, which became more evident after the removal of the peripheral parts, as already observed in a previous work.[7]

The main vitamin E vitamers found in Ofanto and Simeto cultivars, under all the different tested conditions, are α and β isomers of tocopherols and tocotrienols. β-Tocotrienol was the most representative isomer, ranging, with respect to total tocols, from 65% to 70% in whole samples and about 80% in semolinas. These results are in accordance with previous works.[9,35] From the data in Table 1, the Ofanto cultivar showed higher amounts of tocols and tocotrienols in whole meals and tocols, tocopherols, and tocotrienols in semolinas than Simeto. In the Simeto genotype, from whole meals to semolinas, a reduction of about 50% of total tocols, in particular of about 45% of tocotrienols and of 90% of tocopherols, was observed. In the Ofanto genotype about a 40% reduction of total tocols (about 30% of tocotrienols and 70% of tocopherols) was measured.

Second Crop Season: Effect of Water Stress and Sulfur Fertilization. Data from the different experimental conditions were analyzed in order to verify the effect of water stress and sulfur fertilization.

From the analysis of variance Simeto showed a higher yield value (3.2 t/ha versus 3.1 t/ha, $p \leq 0.01$), consistent with a higher 1000 kernel weight (52 g versus 48 g, $p \leq 0.01$) than Ofanto. Under water stress a significant decrease of yield (3.1 t/ha versus 3.3 t/ha, $p \leq 0.01$) and 1000 kernel weight (47 g versus 52 g, $p \leq 0.01$) were observed with respect to the irrigated treatment. Furthermore, under water stress, Simeto showed a higher decrease in 1000 kernel weight (48.9 g versus 54.6 g) than Ofanto (46.2 g versus 48.9 g).

Regarding tocol and carotenoid levels, the cultivar effect was always significant, with the exception of semolina carotenoids. A significant effect of water regime on whole meal carotenoids ($p \leq 0.05$), tocols ($p \leq 0.05$), and tocotrienols ($p \leq 0.01$) and semolina carotenoids ($p \leq 0.01$) was observed. Sulfur fertilization showed a significant effect on whole meal carotenoids ($p \leq 0.05$) and semolina carotenoids ($p \leq 0.01$), tocols ($p \leq 0.01$), and tocotrienols ($p \leq 0.01$). Moreover, the analysis of variance did not show any additional effect of sulfur supplementation on the response of compounds to water stress ($p \leq 0.01$).

The combined effect of water regime, sulfur fertilization, and genotype was studied. The total carotenoid content in whole meals and semolinas, in the two analyzed cultivars, under the two water regimes and sulfur fertilization, is reported in Figure 2A and Figure 2B. Total carotenoids were expressed as the sum of every detected compound.

An increase in whole meal and semolina carotenoids was observed, under water deficit. In particular a 20% increase in

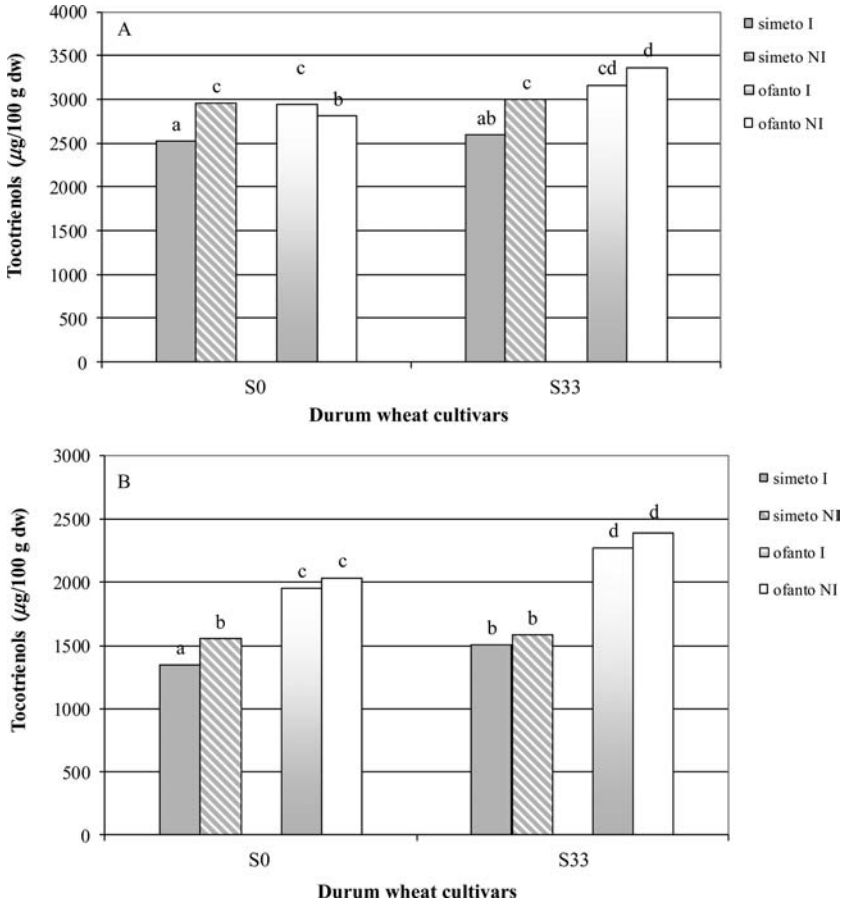

Figure 4. Total tocotrienol content in Simeto and Ofanto whole meals (A) and semolinas (B) under water stress and sulfur fertilization in the second growing season. I: irrigated; NI: rain-fed. Same letters indicate no significant differences at $p \geq 0.05$ according to Tukey's test.

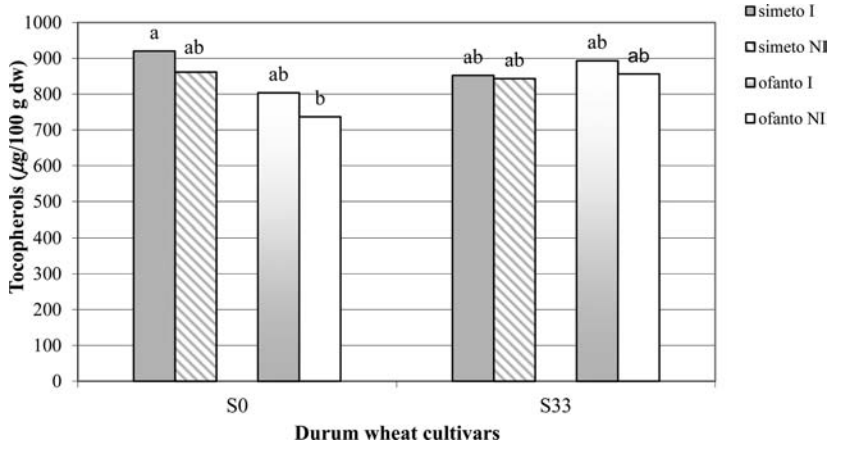

Figure 5. Total tocopherol content in Simeto and Ofanto whole meals under water stress and sulfur fertilization in the second growing season. I: irrigated; NI: rain-fed. Same letters indicate no significant differences at $p \geq 0.05$ according to Tukey's test.

total carotenoid content was found in Simeto whole meals and in the absence of sulfur fertilization (S0), from about 360 μg/100 g dw (I) to about 430 μg/100 g dw (NI) (Figure 2A). Considering single carotenoids, lutein and zeaxanthin increased about 20%, while β-carotene was not affected by water deficit (data not shown). No significant effect was observed in Ofanto whole meals. Regarding carotenoids in semolinas (Figure 2B), a positive effect of water deficit (about 15% increase) was observed in both cultivars in S0 samples.

Sulfur fertilization led to a significant carotenoid increase in Ofanto whole meals under both water conditions, while in semolinas only in irrigated samples. No effect was observed in Simeto samples.

The amounts of total tocols in whole meals and semolinas of the two analyzed cultivars, under the two water regimes and sulfur fertilization, are reported in Figure 3A and Figure 3B, respectively. Tocotrienols are reported in Figure 4A and Figure 4B. In Figure 5 only tocopherols in whole meals are shown. Their content in semolinas was not considered, since most

tocopherols were removed going from whole meals to semolinas.

Exposure to water stress caused an increment of tocols and the corresponding tocotrienols only in Simeto, which appeared more responsive to drought. In particular, water stress caused about a 10% increment in tocols of whole meals of S0 and S33 (Figure 3A) and about 15% in respective semolinas of S0 (1.73 mg/100 g dw versus 1.52 mg/100 g dw) (Figure 3B) ($p \leq 0.05$). Similar increases were observed for tocotrienols (Figure 4). Among tocotrienols, β-tocotrienol went from 2.09 mg/100 g dw to 2.51 mg/100 g dw in whole samples of S0 and from 1.18 mg/100 g dw to 1.38 mg/100 g dw in corresponding semolinas. Finally, relative to tocopherols of whole meals, changes due to water regime were not observed (Figure 5).

Sulfur fertilization led to an increase in tocols and tocotrienols, mainly in the Ofanto cultivar (Figure 3 and Figure 4, respectively). In particular, their contents were positively affected by sulfur treatment only in NI whole meals, while in semolinas, under both water regimes. The tocotrienol increase was about 15% (Figure 4) and was mainly due to β-tocotrienol (from about 1.80 mg/100 g dw to about 2.10 mg/100 g dw). In the Simeto cultivar, a significant total tocol and tocotrienol increment, about 13%, was observed only in semolinas from irrigated samples (I) (Figure 3B and Figure 4B). Regarding tocopherols, no significant effect was observed (Figure 5).

On the whole, the above-reported results showed a significant cultivar effect on whole meal carotenoid and tocol levels, as already reported by several authors.[2−4,11,22,36]

Some of the genotypes were more sensitive than others to the impact of water stress. In fact, while in some cultivars no effect on lipophilic antioxidants was observed, in others a general positive trend was found toward an increase in their concentration, under water deficit, with a more marked increase of carotenoids in semolinas. The different response toward water stress is consistent with literature data, indicating constitutive differences in water use efficiency among durum wheat cultivars.[37] The increase in lipophilic antioxidant content due to environmental conditions is in accordance with results previously reported by other authors in different systems,[21] and the cooperative interaction among carotenoids, tocopherols, and tocotrienols under stress conditions has been the focus of several studies.[38,39] The observed increases could depend either on biosynthesis or on the storage capacity of the plant tissue.[21] Moreover, they could be also partly explained by a simple concentration effect due to quantitative yield losses caused by abiotic environmental stresses on crops.

Regarding the effect of sulfur application, several authors reported its influence on the antioxidant contents in different plant systems, and controversial results have been obtained.[40−42] Under our conditions, sulfur fertilization positively affected the content of carotenoids and tocols, mainly in the Ofanto cultivar, causing no further increments in response to water stress. This behavior needs further investigation, also in relationship to other antioxidant systems linked to sulfur utilization.

AUTHOR INFORMATION

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Notes

The authors declare no competing financial interest.

I, irrigated; NI, rain-fed; S0, without sulfur; S33, 33 kg ha^{-1} of sulfur level

REFERENCES

(1) Ward, J.; Poutanen, K.; Gebruers, K.; Piironen, V.; Lampi, A. M.; Nyström, L. The HEALTHGRAIN cereal diversity screen: concept, results and prospects. *J. Agric. Food Chem.* **2008**, *56*, 9699−9709.

(2) Abdel-Aal, E. M.; Young, J. C.; Rabalski, I.; Hucl, P.; Fregeau-Reid, J. A. Identification and quantification of seed carotenoids in selected wheat species. *J. Agric. Food Chem.* **2007**, *55*, 787−794.

(3) Adom, K. K.; Sorrelis, M. E.; Liu, R. H. Phytochemical profiles and antioxidant activity of wheat varieties. *J. Agric. Food Chem.* **2003**, *51*, 7825−7834.

(4) Andersson, A. A. M.; Lampi, A. M.; Nyström, L.; Piironen, V.; Li, L.; Ward, J. L.; Gebruers, K.; Courtin, C. M.; Delcour, J. A.; Boros, D.; Fras, A.; Dynkowska, W.; Rakszegi, M.; Bedo, Z.; Shewry, P. R.; Åman, P. Phytochemical and fibre components in barley varieties in the HEALTHGRAIN diversity screen. *J. Agric. Food Chem.* **2008**, *5*, 9767−9776.

(5) Palozza, P.; Krinsky, N. I. Antioxidant effects of carotenoids in vivo and in vitro: an overview. *Methods Enzymol.* **1992**, *213*, 403−420.

(6) Pinzino, C.; Capocchi, A.; Galleschi, L.; Saviozzi, F.; Nanni, B.; Zandomeneghi, M. Aging, free radicals, and antioxidants in wheat seeds. *J. Agric. Food Chem.* **1999**, *47*, 1333−1339.

(7) Fratianni, A.; Panfili, G.; Irano, M.; Acquistucci, E. Estimation of color of durum wheat. Comparison of WSB, HPLC and reflectance colorimeter measurements. *J. Agric. Food Chem.* **2005**, *53*, 2373−2378.

(8) Finocchiaro, F.; Ferrari, B.; Gianinetti, A.; Dall'Asta, C.; Galaverna, G.; Scazzina, F. Characterization of antioxidant compounds of red and white rice and changes in total antioxidant capacity during processing. *Mol. Nutr. Food Res.* **2007**, *51*, 1006−1019.

(9) Panfili, G.; Fratianni, A.; Irano, M. Normal phase high-performance liquid chromatography method for the determination of tocopherols and tocotrienols in cereals. *J. Agric. Food Chem.* **2003**, *51*, 3940−3944.

(10) Panfili, G.; Fratianni, A.; Di Criscio, T.; Marconi, E. Tocol and β-glucan levels in barley varieties and in pearling by-products. *Food Chem.* **2008**, *107*, 84−91.

(11) Lampi, A. M.; Nurmi, T.; Piironen, V. Effects of the environment and genotype on tocopherols and tocotrienols in wheat in the HEALTHGRAIN diversity screen. *J. Agric. Food Chem.* **2010**, *58*, 9306−9313.

(12) Packer, L. Nutrition and biochemistry of the lipophilic antioxidants; vitamin E and carotenoids. In *Nutrition, Lipids, Health, and Disease*; Ong, A. S. H., Niki, E., Packer, L., Eds.; American Oil Chemists' Society: Champaign, IL, 1995; pp 8−35.

(13) Polle, A.; Rennenberg, H. Photoprotective stress in trees. In *Causes of Photooxidative Stress and Amelioration of Defence Systems in Plants*; Foyer, C. H.; Mullineaux, P. M., Eds.; CRC Press: Boca Raton, FL, USA, 1994; pp 199−218.

(14) Herbinger, K.; Tausz, M.; Wonisch, A.; Soja, G.; Sorger, A.; Grill, D. Complex interactive effects of drought and ozone stress on the antioxidant defence systems of two wheat cultivars. *Plant Physiol. Biochem.* **2002**, *40*, 691−696.

(15) Bartoli, C. G.; Simontacchi, M.; Tambussi, E.; Belatrano, J.; Montaldi, E.; Puntuarulo, S. Drought and watering-dependent oxidative stress: effect on antioxidant content in *Triticum aestivum* L. leaves. *J. Exp. Bot.* **1999**, *50*, 375−383.

(16) Tausz, M.; Gullner, G.; Komives, T.; Grill, D. The role of thiols in plant adaptation to environmental stress. In *Sulphur in Plants*; Abrol, P. Y.; Ahmad, A., Eds.; Kluwer Academic Publishers: The Netherlands, 2003; pp 221−244.

(17) Flagella, Z.; Pastore, D.; Campanile, R. G.; Di Fonzo, N. Photochemical quenching of chlorophyll fluorescence and drought tolerance in different durum wheat (*Triticum durum*) cultivars. *J Agric. Sci.* **1994**, *122*, 183−192.

(18) Flagella, Z.; Campanile, R. G.; Stoppelli, M. C.; De Caro, A.; Di Fonzo, N. Drought tolerance of photosynthetic electron transport

under CO_2-enriched and normal air in cereal species. *Physiol. Plant.* **1998**, *104*, 753−759.
(19) Galleschi, L.; Capocchi, A.; Ghiringhelli, S.; Saviozzi, F.; Calucci, L.; Pinzino, C.; Zandomeneghi, M. Antioxidants, free radicals, storage proteins, and proteolytic activities in wheat (*Triticum durum*) seeds during accelerated aging. *J. Agric. Food Chem.* **2002**, *50*, 5450−5457.
(20) Keleş, Y.; Öncel, I. Response of antioxidative system defence system to temperature and water stress combinations in wheat seedlings. *Plant Sci.* **2002**, *163*, 783−790.
(21) Wang, Y.; Frei, M. Stressed food - The impact of abiotic environmental stresses on crop quality. *Agric. Ecosys. Environ.* **2011**, *141*, 271−286.
(22) Cavallero, A.; Gianinetti, A.; Finocchiaro, F.; Delogu, G.; Stanca, A. M. Tocols in hull-less and hulled barley genotypes grown in contrasting environments. *J. Cereal Sci.* **2004**, *39*, 175−180.
(23) Quasim, A.; Ashraf, M.; Anwar, F. Seed composition and seed oil antioxidant activity of maize under water stress. *J. Am. Oil. Chem. Soc.* **2010**, *87*, 1179−1187.
(24) Van Hung, P.; Hatcher, D. W. Ultra-performance liquid chromatography (UPLC) quantification of carotenoids in durum wheat: Influence of genotype and environment in relation to the colour of yellow alkaline noodles (YAN). *Food Chem.* **2011**, *125*, 1510−1516.
(25) Borrelli, G. M.; Ficco, D. B. M.; Giuzio, L.; Pompa, M.; Cattivelli, L.; Flagella, Z. Durum wheat salt tolerance in relation to physiological, yield and quality characters. *Cereal Res. Comm.* **2011**, *39*, 525−534.
(26) Flagella, Z. Qualità nutrizionale e tecnologica del frumento duro. *Ital. J. Agron.* **2006**, *1*, 123−159.
(27) Flagella, Z. M.; Giuliani, M. M.; Giuzio, L.; Volpi, C.; Masci, S. Influence of water deficit on durum wheat storage protein composition and technological quality. *Eur. J. Agron.* **2010**, *33*, 197−207.
(28) Giuliani, M. M.; Giuzio, L.; De Caro, A.; Flagella, Z. Relationships between nitrogen utilization and grain technological quality in durum wheat: II. Yield parameters and technological quality. *Agron. J.* **2011**, *103*, 1668−1675.
(29) Pompa, M.; Giuliani, M. M.; Giuzio, L.; Gagliardi, A.; Di Fonzo, N.; Flagella, Z. Effect of sulphur fertilization on grain quality and protein composition of durum wheat (*Triticum durum* Desf.). *Ital. J. Agron.* **2009**, *4*, 159−170.
(30) Shewry, P. R.; Piironen, V.; Lampi, A. M.; Edelmann, M.; Kariluoto, S.; Nurmi, T.; Fernandez-Orozco, R.; Ravel, C.; Charmet, G.; Andersson, A. A. M.; Aman, P.; Boros, D.; Gebruers, K.; Dornez, E.; Courtin, M. C.; Delcour, J. A.; Rakszegi, M.; Bedo, Z.; Ward, L. J. The HEALTHGRAIN wheat diversity screen: effects of genotype and environment on phytochemicals and dietary fiber components. *J. Agric. Chem.* **2010**, *58*, 9291−9298.
(31) Panfili, G.; Fratianni, A.; Irano, M. Improved normal-phase high-performance liquid chromatography procedure for the determination of carotenoids in cereals. *J. Agric. Food Chem.* **2004**, *52*, 6373−6377.
(32) Fratianni, A.; Caboni, M. F.; Irano, M.; Panfili, G. A critical comparison between traditional methods and supercritical carbon dioxide extraction for the determination of tocochromanols in cereals. *Eur. Food Res. Technol.* **2002**, *215*, 353−358.
(33) Steel, R. G. D.; Torrie, J. H.; Dickey, D. A. *Principles and Procedures of Statistics, a Biometrical Approach*, 3rd ed.; McGraw-Hill Co. Inc.: New York, NY, 1997.
(34) Borrelli, G. M.; De Leonardis, A. M.; Platani, C.; Troccoli, A. Distribution along durum wheat kernel of the components involved in semolina colour. *J Cereal Sci.* **2008**, *48*, 494−502.
(35) Fratianni, A.; Di Criscio, T.; Mignogna, R.; Panfili, G. Carotenoids, tocols and retinols evolution during egg pasta−making processes. *Food Chem.* **2012**, *131*, 590−595.
(36) Blanco, A.; Colasuonno, P.; Gadaleta, A.; Mangini, G.; Schiavulli, A.; Simeone, R.; Digesu, A. M.; De Vita, P.; Mastrangelo, A. M.; Cattivelli, L. Quantitative trait loci for yellow pigment concentration and individual carotenoid compounds in durum wheat. *J. Cereal Sci.* **2011**, *54*, 255−264.
(37) Rizza, F.; Ghashghaie, J.; Meyer, S.; Matteu, L.; Mastrangelo, A. M.; Badeck, F. W. Constitutive differences in water use efficiency between two durum wheat cultivars. *Field Crop Res.* **2011**, *125*, 49−60.
(38) Skibsted, L. H. Carotenoids in antioxidant networks. Colorants or radical scavengers. *Food Chem.* **2012**, *60*, 2409−2417.
(39) Leif, H.; Skibsted, L. H.; Tesoriere, L.; Bongiorno, A.; Pintaudi, A. M.; D'Anna, R.; D'Arpa, D.; Livrea, M. A. Synergistic interactions between vitamin A and vitamin E against lipid peroxidation in phosphatidylcholine liposomes. *Archiv. Biochem. Biophys.* **1996**, *396*, 57−63.
(40) Kopsel, D. E.; Kopsel, D. A. Kale carotenoids remain stable while flavor compounds respond to changes in sulfur fertility. *J. Agric. Food Chem.* **2003**, *51*, 5319−5325.
(41) Reif, C.; Arrigoni, E.; Neuweiler, R.; Baumgartner, D.; Nyström, L.; Hurrell, F. R. Effect of sulfur and nitrogen fertilization on the content of nutritionally relevant carotenoids in spinach (*Spinacia oleacea*). *J. Agric. Food Chem.* **2012**, *60*, 5819−5824.
(42) Hell, R.; Dahl, C.; Knaff, D. B; Leustek, T. *Sulfur Metabolism in Phototrophic Organisms*; Springer-Verlag New York Inc.: New York, NY, 2008.