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Modifications of Strawberry Fruit Antioxidant Pools and Fruit Quality under NaCl Stress

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The responses of fruit antioxidants in two strawberry cultivars differing in their sensitivity to NaCl stress were studied. The sensitive cv. Elsanta and the less sensitive cv. Korona were treated with NaCl solutions of 40 and 80 mmol/L in the nutrient solution. In general, moderate salinity resulted in increases of antioxidant capacity. In cv. Korona, salt stress increased the activity of superoxide dismutase and the contents of glutathione, phenols, and anthocyanins, while that of ascorbic acid decreased. In cv. Elsanta, changes of superoxid dismutase activity and of ascorbic acid concentration were comparable to those of cv. Korona, but the decrease of ascorbic acid was more distinct. The contents of anthocyanins decreased, and those of phenols remained similar. The glutathione content was reduced at the highest NaCl level. The results indicate that less salt-sensitive strawberry cultivars may be grown under moderate salinity stress to optimize fruit quality.

KEYWORDS: Glutathione; ascorbic acid; SOD; FRAP value; phenolics; anthocyanins; *Fragaria* × *ananassa*

INTRODUCTION

Strawberry is one of the most consumed and healthiest fruits with a rapidly increasing area under cultivation. However, strawberry production in the field and also under glass is limited, among others, by a low quality of irrigation water, because water resources of drinking quality are limited. Therefore, water with a moderate salinity level has been applied, for example, in hydroponics, although negative influences of salinity on strawberry plant growth and yield are well-documented (*1*−*6*). Nevertheless, information on the impact of salinity on fruit quality is limited. Environmental stress such as salt stress may increase the formation of reactive oxygen species (ROS), which mediates the degradation of membrane components, the oxidation of protein SH groups, and the loss of membrane functions (*7*). Superoxide and hydrogen peroxide lead to cascade reactions resulting in the production of hydroxyl radicals or lipid peroxides and the degradation of components (*8*). These potentially toxic species are scavenged by a number of the plants' own antioxidants such as enzymes and nonenzymatic substances (*9*). Superoxide dismutase (SOD) catalyzes the dismutation of superoxide to hydrogen peroxide and oxygen. H_2O_2 is still toxic and is further detoxified by catalase or different peroxidases to water and oxygen. H_2O_2 is also decomposed by the ascorbate−glutahione cycle. Ascorbic acid and glutathione can directly interact with ROS and detoxify them (*10*). Plants with high constitutive or induced levels of antioxidants have been reported to be more resistant to oxidative damage (*7, 11*).

Strawberry is considered sensitive to salinity (*2*), but variations in sensitivity have been observed among cultivars (*5*, *6*). Negative influences of salinity on strawberry plant growth and yield are well-documented (*3*−*6*, *12*). Few investigations reported improvements of fruit quality under salinity, for instance, increases in contents of sugars and acids due to a lower fruit water content (*3*, *4*). However, other experiments (*5*) described a decrease in soluble solids and a lower acceptance of strawberry fruits by consumers. Only few researchers have focused on the influence of salt stress on antioxidants in fruits, and most of the studies were on tomatoes. A higher antioxidant capacity of tomato fruits, which was related to carotinoids, lycopene, and ascorbic acid, was reported at moderate levels of salinity (*13*, *14*). Salt stress may result in an elevated production of antioxidative compounds, as indicated in a preliminary 1 year study (*6*), reporting higher contents of glutathione, proteins, and phenols in strawberry fruits.

Although strawberry fruit represents an important source of bioactive phytochemicals in the human diet (*15*), information on their overall antioxidative activity and their contents of important antioxidants such as ascorbic acid, anthocyanins, and tocopherols under NaCl salinity is limited. However, these antioxidant pools are considered important for human health due to their anticarcinogenic and dietary properties as well as their involvement in the protection against cardio- and cerebrovascular diseases and cancer mortality through scavenging of free radicals (*16*). Therefore, it was the aim of the present study to investigate the differences in the influence of moderate and elevated salinity stress onto fruit quality in the salt-sensitive cv. Elsanta and the less-sensitive cv. Korona (*5*, *6*) with special attention to these bioactive compounds.

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Table 1. Composition of Modified Hoagland Solution (pH Value 6.5)

cation	mequiv L^{-1}	anion	mequiv L^{-1}
Ca^{2+}	5.0	NO_3^-	6.0
Mg^{2+}	4.0	PO_4^{3-}	3.0
K^+	4.0	SO_4^{2-}	7.0
NH_4^+	1.0		
H^+	2.0		
sum	16.0		16.0

MATERIALS AND METHODS

Material. Experiments were replicated twice during two successive vegetative periods in 2002 and 2003 in Göttingen, Germany, with strawberry (*Fragaria* × *ananassa* Duch., *Rosaceae*) cvs. Elsanta (NaCl-sensitive) and Korona (less sensitive). Salt levels applied for 4 months were moderate (40 mmol/L NaCl, E_c = 3.9 dS/m) and excessive (80 mmol/L NaCl, E_c = 7.5 dS/m) in addition to a control treatment (0 mmol/L NaCl, E_c = 0.0013 dS/m). Commercial strawberry plantlets (Frigo, class A+) were purchased from Kraege Beerenobst Spezialkulturen (Telgte, Germany). Frigo is a designation for runner plants that are taken out in mid-November and stored frozen without leaves during winter to initiate fruit setting the following year. For experiments, plantlets were selected for similar size and cultivated in 6 L metallic Mitscherlich containers filled with quartz sand (0.7−1.2 mm ∅). The experiments were conducted from the end of April to the middle of September. Plants were located randomly (12 plants per m^2) with ten replications per combination to ensure a statistical design and to exclude position effects. They were grown in a greenhouse to avoid a dilution of salt applied to the plants by rainfall. When not raining, pots were placed outside of the greenhouse. The recorded mean temperature and humidity from beginning of cultivation until end of harvest were 17 °C (max temp = 22.7 °C; min temp = 11.3 °C) and 77% relative humidity. Mineral requirements of strawberry plants were covered by application of 200 mL of modified Hoagland solution (pH 6.5) per plant twice a week (**Table 1**). Three weeks after planting, salt treatment started and 100 mL of solutions containing 0, 40, or 80 mmol/L NaCl was supplied four times a week to each plant. Once a week, 200 mL of demineralized water was supplied to all treatments. Surpluses of solutions were allowed to pass the containers to ensure salt stress in the root medium at a given concentration but to avoid anoxia by water logging. All of the plants received extra water when needed to avoid an additional water stress. To improve fruit quality, runners were removed immediately. Fruits were harvested at the optimum of fruit maturity, when about 90% of the fruit surface had reached a fully red color. The fruits were divided into sepals and fruit flesh. Only fruit flesh was used for further investigations. Fruits were frozen in liquid nitrogen immediately after harvest and stored at −30 °C until further analyses of entire or freeze-dried (Epsilon 2-40, Christ, Germany) fruits. Dry matter contents were recorded, and dried fruits were milled to a fine powder.

Analyses. Ascorbic acid, glutathione, and protein contents in addition to antioxidant enzyme activities were quantified from entire fruits. Tocopherol, phenol, anthocyanin, contents, and FRAP (ferric reducing antioxidant power) values were obtained from freeze-dried material. All of the results were expressed per fruit fresh mass (FM).

For the determination of ascorbic acid and glutathione, fruits were treated with 15% *meta*-phosphoric acid (5 mL g^{-1} fruit mass) and centrifuged (20 min, 15000*g* at 4 °C), and the supernatants were used for analyses. Reduced ascorbic acid (AA) and dehydroascorbic acid (DHAA) contents were measured titrimetrically (*17*, *18*), using a standard curve of purified AA (96% p.a., Merck, Germany).

Contents of total glutathione, measured as reduced glutathione (GSH) plus glutathione disulfide (GSSG), were determined based on the reaction of GSH with 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) in the presence of glutathione-reductase and the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH). Concentrations of GSSG were measured after derivatization of GSSG with 2-vinyl pyridine to GSH (*19*).

For the determination of total anthocyanins, total phenolic compounds, and antioxidative activity (FRAP), freeze-dried strawberry fruits (0.2 g) were extracted with methanol for 10 min at room temperature three times to the final volume of 10 mL and then were centrifuged for 10 min at 10000 rpm min^{-1} and 4 °C. The total anthocyanin content was measured using the pH differential method (*20*, *21*). Results were expressed in mg pelargonidin-3-glucoside equivalents. The total phenolic content was measured using the Folin−Ciocalteu method (*22*), and the results were expressed in mg gallic acid equivalents (GAE). The antioxidative activity was measured using the FRAP assay (*23*). $FeSO_4$ in the range of 0−1 mmol/L was used as a standard.

To distinguish between free and cell wall-associated enzymes, two phases were extracted (*24*). For the free compounds, 0.1 mol/L potassium phosphate buffer at pH 6.0 (2.5 mL g^{-1} FM) was used, and for cell wall-associated constituents, double extractions with 1 mol/L NaCl solution (5 mL g^{-1} sample) were performed. Concentrations of soluble proteins were measured using a Bio-Rad Protein Assay with bovine serum albumin (*25*). For both fractions, independent standards, solved in either potassium phosphate buffer (0.1 mol/L) or NaCl solution (1 mol/L), were prepared. The SOD activity (EC1.15.1.1) was determined using nitroblue tetrazolium (NBT) salt (*26*). One unit of SOD was defined as the amount of enzyme that yielded a 50% inhibition of the reduction of NBT. The activity of SOD was determined for each fraction separately.

α-Tocopherol was by far the main tocopherol in strawberry, which were extracted with isooctane (4 mL 100 mg^{-1} dry mass) from freeze-dried fruits (*27*). The HPLC system used for their identification and quantification contained the HPLC-Pump 2248 (Pharmazia-LKB), a Waters Autosampler 717+, a separation column (25 cm × 3 mm i. ∅; LiChrospher 100 Diol 5 μm) with precolumn (5 mm × 4 mm ∅ LiChrosper Si 60, 5 μm), and a Dionex Fluorescence detector (Ex, 295 nm; Em, 320 nm) with Varian-Integrator 4400. Isooctane/*tert*-butylated methyl ether (96:4 v/v) with a flow rate of 0.7 mL/min was used as the eluent. To ensure the detection of individual tocopherols (α, β, δ, and γ) and to calculate tocopherol recovery, strawberry samples were pretested by adding defined tocopherol concentrations. During the measurements, δ-tocopherol was added as an internal standard, because this form does not occur in strawberry fruit.

The obtained data were analyzed with the SPSS 12.0 statistical program (SPSS Inc., 1989−1999). All data sets were tested for normal distribution and variance homogeneity (P = 0.05). An influence of year was not statistically significant; hence, data of both years of experiment were lumped. In the case of homogenous sample variance, calculated means were compared by Duncan; in the case of nonhomogenous variance, means were compared by Tamhane tests (P = 0.05). Correlation and multiple regression procedures between normally distributed quality parameters were performed using Pearson correlation coefficients.

RESULTS AND DISCUSSION

Antioxidants are involved in scavenging of stress-induced reactive oxygen species such as superoxide, hydrogen peroxide, and hydroxyl radicals. Previous research has established a relationship between salt stress and antioxidant levels (*6*). Elevated levels of AA and GSH were found, in addition to higher activities of enzymes such as SOD, ascorbate peroxidase, and catalase (*7*, *11*).

In strawberry cvs. Elsanta and Korona, the increasing salt stress caused a significant increase of antioxidant capacity, determined as the FRAP value, in fruits of both cultivars (**Table 2**). The FRAP value, which is considered to express general antioxidant activity of fruit, responded similarly in cvs. Korona and Elsanta (**Table 2**). Fruits of both cultivars were characterized by a high antioxidant capacity, and the measured FRAP values were well within the range reported for different strawberry genotypes (*28*, *29*).

The AA content of strawberry fruit has been reported to vary between 10 and 100 mg 100 g^{-1} FM, with an average of 60 mg 100 g^{-1} FM (*28*−*32*) for different strawberry genotypes. The results of control plants are within these ranges and similar

Table 2. Response of Fruit Antioxidant Parameter of Two Strawberry Cultivars to NaCl Salinity Stress during Growth[a]

	FRAP value (mmol Fe)[x]	AA + DHAA (mg)[x]	AA/TAA ratio (relative units)[x]	GSH + GSSG (mg)[y]	GSH/GSSG (relative units)[y]	total SOD (mg)[x]	protein (mg)[x]	α-tocopherol (mg)[x]
				Korona (less salt sensitive)				
C	3.6 ± 0.8 c	46.9 ± 4.8 b	0.72 ± 0.03 a	0.21 ± 0.07 b	1.9 ± 1.4 bc	38.0 ± 10.1 bc	57.3 ± 9.2 d	0.60 ± 0.28 a
S1	4.2 ± 0.5 bc	40.8 ± 4.5 c	0.63 ± 0.02 bc	0.28 ± 0.04 b	2.2 ± 0.9 bc	48.6 ± 13.1 b	63.3 ± 9.1 cd	0.95 ± 0.40 a
S2	4.9 ± 0.6 a	32.8 ± 4.5 d	0.60 ± 0.05 cd	0.40 ± 0.09 a	4.4 ± 2.2 a	68.5 ± 24.5 a	66.9 ± 5.9 c	0.94 ± 0.31 a
				Elsanta (salt sensitive)				
C	3.7 ± 0.6 c	55.6 ± 4.4 a	0.64 ± 0.02 b	0.18 ± 0.03 b	2.3 ± 0.8 bc	28.6 ± 10.4 c	62.8 ± 11.7 cd	0.49 ± 0.31 a
S1	4.4 ± 0.8 ab	33.2 ± 3.8 d	0.60 ± 0.05 cd	0.27 ± 0.01 b	3.6 ± 1.8 ab	47.6 ± 11.9 bc	78.2 ± 7.1 b	0.45 ± 0.22 a
S2	4.9 ± 0.8 a	28.4 ± 4.3 e	0.59 ± 0.04 d	0.10 ± 0.03 c	1.6 ± 0.3 c	52.8 ± 18.1 b	100.5 ± 20.4 a	0.53 ± 0.38 a

[a] Data are expressed per 100 g FM. Different letters indicate significant differences by Duncan (*x*) or Tamhane (*y*) tests at $p \leq 0.05$ (C, control; S1, 40 mmol NaCl/L; S2, 80 mmol NaCl/L; FRAP, antioxidant capacity; and GSSG, oxidized glutathione).

to those reported for cvs. Elsanta and Korona in the literature (*5*, *6*, *33*, *34*). In controls of cv. Elsanta, levels of total ascorbic acid (TAA) (56 mg 100 g^{-1} FM) and DHAA (20 mg 100 g^{-1} FM) were significantly higher than in cv. Korona (TAA, 47 mg 100 g^{-1} FM; DHAA, 13 mg 100 g^{-1} FM). The contents of TAA decreased progressively with rising salinity (**Table 2**). In fruits of cv. Korona, the decrease of TAA was 13 and 30%, respectively, as compared to control fruits, while in cv. Elsanta, it accounted for 40 and 49%, respectively. This reduction was mainly caused by the suppression of AA accumulation, accounting for 24.4 and 42.4% in cv. Korona and 44.1 and 52.7% in cv. Elsanta for moderate and elevated salt stress, respectively. The rate of DHAA decreased significantly only in fruits of the more sensitive cv. Elsanta, while in cv. Korona, it remained fairly constant. The changes in ascorbic acid fractions due to salt stress are reflected by the decrease of the redox state, which is the ratio of AA to TAA (**Table 2**). Smaller values indicate a relatively lower portion of AA. The results suggest that the rate of ascorbic acid oxidation exceeded the capacity of the regenerative system. A lower decrease of AA under moderate and elevated salt stress as observed in cv. Korona may be indicative of a smaller salt sensitivity. Similar results were observed for tomato fruits (*13*).

Glutathione is a further member of the ascorbate−glutathione cycle and involved in the response to oxidative stress and scavenging of reactive oxygen species. Lower susceptibility to salt stress appeared to be associated with higher glutathione content in fruits. The total glutathione content increased sharply in fruits of cv. Korona from 210 μg 100 g^{-1} FM in control plants to 398 μg 100 g^{-1} FM, when subjected to the elevated salt stress (**Table 2**). In contrast, in fruits of cv. Elsanta, 80 mmol NaCl/L caused the significant decrease as compared to the control. Higher total glutathione levels could be referred to as an accumulation of GSH. The level of GSSG remained in both cultivars fairly constant. The GSH/GSSG ratio (**Table 2**) reflects differences in glutathione turnover rates. Salt tolerance is combined with a higher GSH/GSSG ratio. A higher ratio could be a result of higher glutathione reductase activity, which was observed in other plants under salinity (*11*), drought (*35*), or chilling stress (*36*). An elevated GSH/GSSG ratio was observed in fruits of cv. Korona subjected to the elevated salt stress, while in Elsanta, the value remained fairly constant. The synthesis of GSH by an oxidative stimulus plays a crucial role in determining the susceptibility of cells to oxidative stress (*11*), and experimental data indicate the importance of maintaining sufficient GSH pools for adaptive processes (*37*). In the present experiment, elevated levels of GSH under salinity can be explained either by an enhanced de novo synthesis, a more effective regeneration, or a reduced consumption of GSH. Under salt stress, cv. Korona showed in tendency higher concentrations of total glutathione, GSH, and GSSG than cv. Elsanta, but significant differences were observed only at 80 mmol NaCl/L (**Table 2**). This indicates a more active ascorbate−glutathione cycle in fruits of the less sensitive cv. Korona and suggests an important role of GSH in maintaining strawberry fruit quality. GSH may even serve as a link between an environmental stress factor and an adaptive process (*37*).

NaCl stress was also accompanied by increasing protein contents in strawberry fruit, with cv. Elsanta showing a distinctly larger response (**Table 2**). Higher amounts of soluble proteins were due to an increase in the free protein fraction, related to the symplast, whereas the contents of cell wall-associated, i.e., mainly apoplastic proteins, remained stable (**Figure 1**). The increase in soluble proteins is best explained by a de novo synthesis of stress-related proteins (*38*), which includes the production of antioxidative enzymes.

The contents of one of these enzymes, total SOD, rose due to NaCl in the root medium (**Table 2**), and higher concentrations were available as free SOD (**Figure 2**). This is interpreted as an increase of symplastic SOD. Fruits of cv. Korona were characterized by higher free SOD concentrations than those of cv. Elsanta (**Figure 2**). The content of cell wall-associated SOD rose significantly only in cv. Elsanta. The present results correspond to previous reports (*5*), indicating that an increase in the activity of antioxidative enzymes, e.g., of SOD, can be regarded as an adaptive response to salinity stress.

In addition to antioxidative enzymes, anthocyanins and phenolic compounds are involved in protecting plants against reactive oxygen species and increases in their concentrations are known to contribute to plant tolerance (*38*, *39*). In strawberry fruit, they contribute to the stability of fruit color and, together with phenols, are involved in unwelcome browning reactions (*40*). Thus, anthocyanin and phenol contents are not only crucial with respect to their physiological importance, but they are also decisive factor of consumers' acceptance.

Concentrations of phenolics compounds of both cultivars varied between 212 and 264 mg 100 g^{-1} FM irrespective of salt stress (**Figure 3**), which correspond well to earlier reports (*31*, *34*). In the less sensitive genotype Korona, moderate salinity increased the total soluble phenol content, but a further increase from 40 to 80 mmol NaCl/L in the root medium did not result in an additional rise. It is concluded that the phenylpropanoid and flavonoid pathways are still intact and functioning in this cultivar, enabling fruit tissues to respond to endogenous and external signals for defense requirements (*21*). By contrast, in fruits of the NaCl-sensitive cv. Elsanta, contents of phenolic compounds did not change significantly (**Figure 3**). In general, the composition and amount of phenolic compounds are influenced by several factors. The most significant influence is attributed to the genotype (*28*, *41*, *42*), followed by the stage

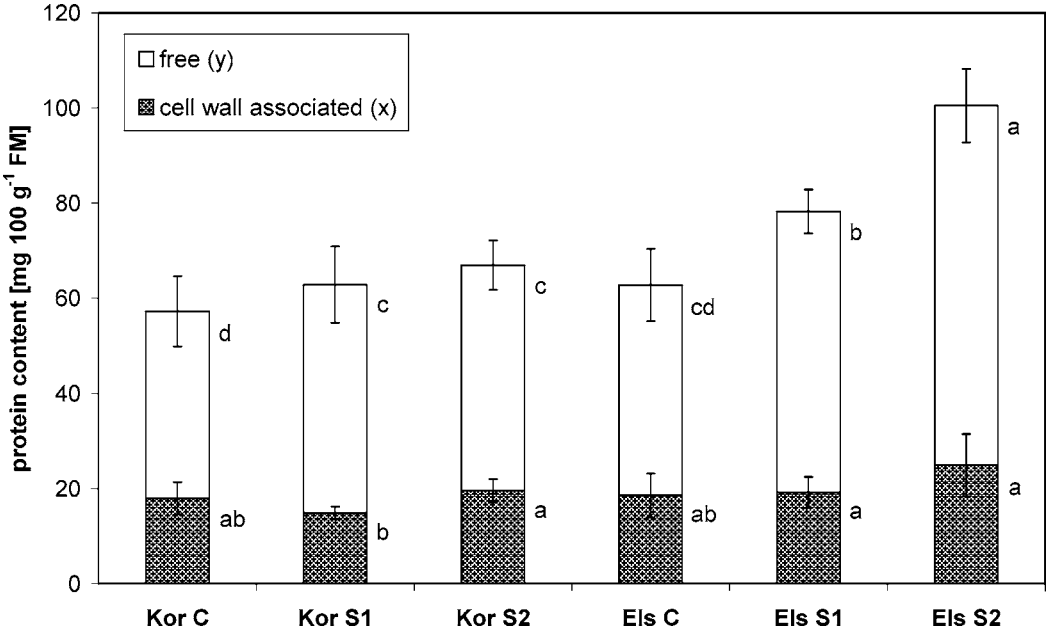

Figure 1. Changes in free and cell wall-associated fruit proteins of two different strawberry cultivars in response to moderate and elevated salt stress (Kor, less sensitive cv. Korona; Els, sensitive cv. Elsanta; C, control; S1, 40 mmol NaCl/L; and S2, 80 mmol NaCl/L). Different letters indicate significant differences by Duncan (x) or Tamhane (y) tests at $p \leq 0.05$ ($n = 12$); vertical bars indicate ± SD.

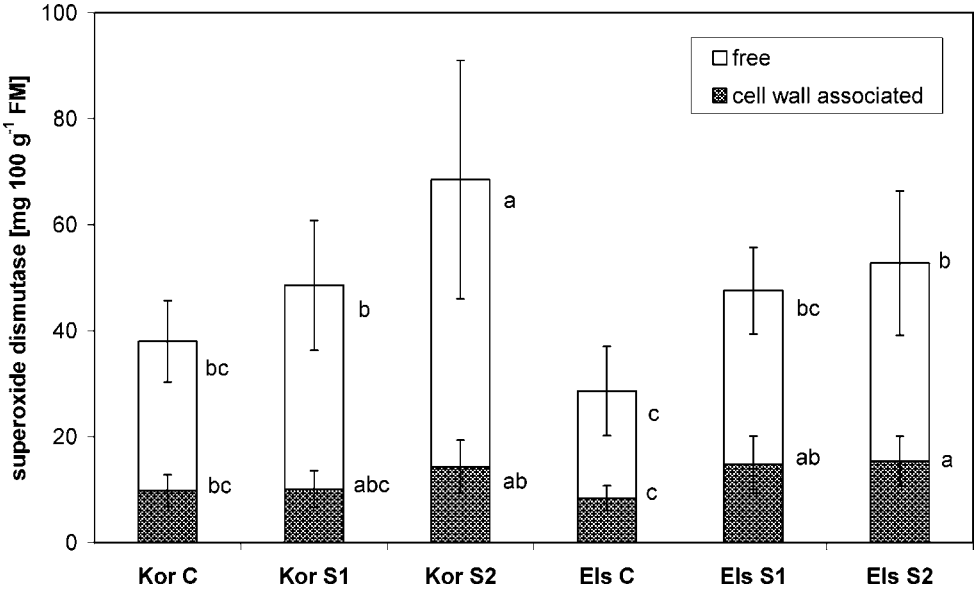

Figure 2. Levels of free and cell wall-associated SOD in strawberry fruits grown under moderate and elevated salt stress (Kor, less sensitive cv. Korona; Els, sensitive cv. Elsanta; C, control; S1, 40 mmol NaCl/L; and S2, 80 mmol NaCl/L). Different letters indicate significant differences by Duncan's test at $p \leq 0.05$ ($n = 12$); vertical bars indicate ± SD.

of development (*21*, *31*), and last by environmental conditions (*32*, *43*). In consequence, larger changes in fruit phenol content due to NaCl salinity might not be expected.

Anthocyanin pelargonidin-3-glucoside is responsible for the red color of strawberry fruits. It is the most abundant anthocyanin in strawberry. Its concentration changed significantly in both strawberry cultivars under salt stress. In cv. Korona, NaCl salinity led to the accumulation of anthocyanin, whereas in cv. Elsanta, a considerable reduction was observed (**Figure 3**). Similar to total phenols, cv. Korona was characterized by higher amounts of anthocyanin. In this cultivar, salt stress did not influence the portion of anthocyanin per total phenolic compounds, which remained fairly constant at ca. 17%. In fruits of cv. Elsanta, not only a decrease of anthocyanin contents but also a reduction of their portion per total phenolic compounds from 18 to 14% was observed. In contrast, in the strawberry cvs. Senga Sengana and Polka, the relative content of anthocyanin per total phenols accounted for 44% (*15*), which is higher than in the studied cultivars. These discrepancies are within the range of cultivar differences but may also be referred to differences in cultivation conditions. In the present experiment, the anthocyanin content was lower than in Senga Sengana and Polka (40−60 mg 100 g^{-1} FM) with 34 and 38 mg 100 g^{-1} FM for control fruits of cvs. Korona and Elsanta, respectively, which is well in line with the results of refs *31* and *34*.

An influence of cultivar and salt stress on the α-tocopherol content of strawberry fruit was not detected, but a tendency toward slightly higher amounts seemed to exist in cv. Korona (**Table 2**). The lipophilic free radical scavenger α-tocopherol is present within biological membranes, where it interrupts free

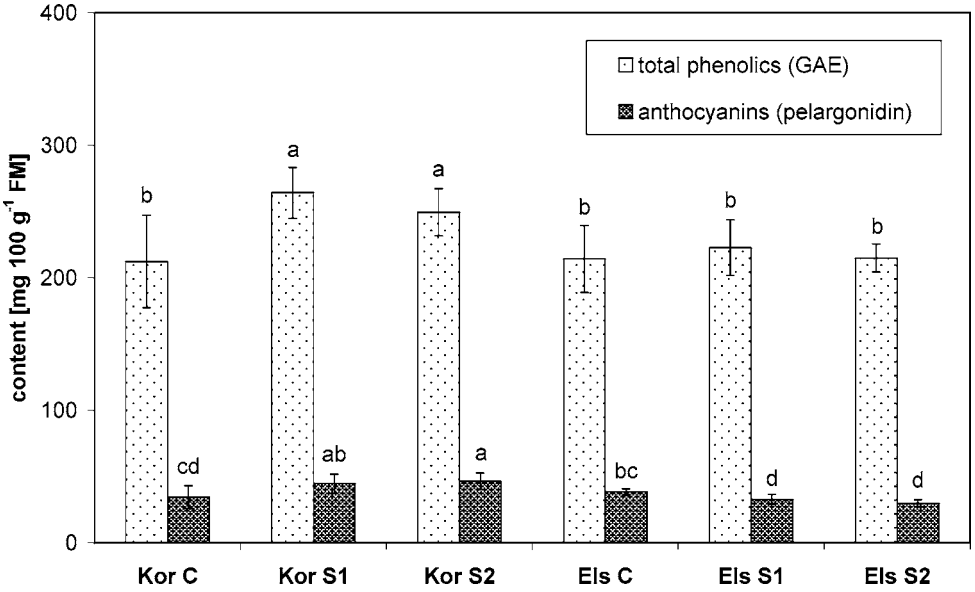

Figure 3. Development of total phenolic compounds and anthocyanins in fruits of two strawberry genotypes differing in their sensitivity to salt stress (Kor, less sensitive cv. Korona; Els, sensitive cv. Elsanta; C, control; S1, 40 mmol NaCl/L; and S2, 80 mmol NaCl/L). Different letters indicate significant differences by Tamhane's test at $p \leq 0.05$ ($n = 12$); vertical bars indicate ± SD.

Table 3. Relationship between Selected Antioxidant Properties of Strawberry Fruit Grown under Salt Stress Expressed as Pearson Correlation Coefficients Irrespective of Salt Stress Level and Cultivar[a]

	FRAP	Anth	Tocoph	Phen	AA	DHAA	TAA	GSH	GSSG	Glutath	F SOD	CW SOD	SOD	F prot.	CW prot
Anth	NS														
Tocoph	NS	0.742^{xx}													
Phen	NS	0.580^{x}	0.768^{xx}												
AA	–0.772^{xx}	NS	NS	NS											
DHAA	NS	NS	NS	NS	0.677^{x}										
TAA	–0.752^{xx}	NS	NS	NS	0.976^{xx}	0.822^{xx}									
GSH	NS	0.715^{xx}	0.684^{x}	NS	NS	NS	NS								
GSSG	NS	NS	0.607^{x}	NS	NS	NS	NS	0.587^{x}							
Glutath	NS	0.736^{xx}	0.715^{xx}	NS	NS	NS	NS	0.988^{xx}	0.705^{x}						
F SOD	0.721^{xx}	NS	0.742^{xx}	NS	–0.726^{xx}	NS	–0.733^{xx}	0.712^{xx}	NS	0.650^{x}					
CW SOD	0.695^{x}	NS	NS	NS	–0.931^{xx}	–0.707^{xx}	–0.931^{xx}	NS	NS	NS	0.625^{x}				
SOD	0.764^{xx}	NS	0.632^{x}	NS	–0.821^{xx}	–0.643^{x}	–0.827^{xx}	0.650^{x}	NS	0.595^{x}	0.985^{xx}	0.750^{xx}			
F prot.	NS	NS	NS	NS	–0.685^{x}	NS	–0.645^{x}	NS	–0.680^{x}	NS	NS	0.704^{x}	NS		
CW prot.	NS	NS	NS	NS	NS	NS	NS	NS	–0.668^{x}	NS	NS	NS	NS	0.611^{x}	
protein	NS	NS	NS	NS	–0.637^{x}	NS	–0.598^{x}	NS	–0.726^{xx}	NS	NS	0.705^{xx}	NS	0.984^{xx}	0.743^{xx}

[a] Abbreviations: NS, not statistically significant; ^{xx}, significance at $P = 0.05$; ^{x}, significance at $P = 0.01$; FRAP, antioxidant capacity (mmol Fe); Anth, anthocyanin; Tocoph, α-tocopherol; Phen, total phenolics; TAA, total ascorbic acid; GSSG, oxidized glutathione; Glutath, total glutathione; F SOD, free superoxide dismutase; CW SOD, cell wall-associated superoxide dismutase; SOD, total superoxide dismutase; F prot., free proteins; CW prot., cell wall-associated proteins; and protein, total proteins.

radical chain reactions (*37*). On the basis of the available data, it is concluded that the metabolism of α-tocopherol in strawberry fruit remained fairly unaffected by salt stress.

To relate antioxidant capacity of strawberry fruit, measured as FRAP value, with antioxidative compounds, a linear correlation analysis was performed. The FRAP value was positively correlated with free and cell wall-associated SOD (**Table 3**), but the relationship was not very strong ($r < 0.800$). In contrast to SOD, the relationship between FRAP value and AA was negative (**Table 3**). Similar results are reported in the literature for berry fruits such as strawberry, raspberry, and blueberry (*44*). Some authors mentioned only a comparatively small contribution of AA to total antioxidant capacity (*45*, *46*). Contrary to the present results, a high contribution of ascorbic acid to the antioxidant capacity of strawberry fruit was observed when using ORAC (*32*). However, this strong correlation between ascorbic acid and antioxidative capacity was only observed in case where the ascorbic acid content exceeded 70 mg 100 g^{-1} FM. For lower levels, the contribution of ascorbic acid to the total antioxidant capacity remained low (*32*).

The results of the present study could not confirm a significant correlation between phenolic compounds, glutathione, or protein fractions and strawberry antioxidant capacity. However, there are several records in the literature (*40*, *41*, *45*−*48*) that favor a strong contribution of phenolic compounds to total antioxidant capacity in several fruit and vegetable cultivars. The differences between these and the present results are at least partly explained by the fact that the mentioned authors used different test assays. Because in these assays different generators of free radicals, quantification systems, and target components are involved, the selection of the test system may be crucial. Also, differences in the plant commodities have to be considered (*29*). A further difficulty may represent the limited degree of standardization of assays. In this context, it is worthy of note that an antioxidative activity may be detectable even in the absence of phenolic compounds (*48*), indicating that further compounds, for instance, soluble fiber, may exhibit antioxidative activity.

The present study revealed that differences in the composition of antioxidants exist in strawberry fruit, which depend on genotype. Nevertheless, the expression of the genetic potential

was strongly influenced by environmental conditions such as salt stress. As compared to cv. Elsanta, fruits of cv. Korona responded more positively to salinity. In the latter cultivar, the antioxidant capacity (FRAP value) rose significantly and concentrations of total glutathione, SOD activity, total phenolic compounds, and anthocyanin were significantly increased. Nevertheless, the reduction of ascorbic acid content and of its redox state is regarded as a serious disadvantage for human nutrition and health. The present results revealed that moderate salt stress may provide fruits with significantly higher contents of antioxidative compounds, if a less salinity-sensitive genotype is cultivated. This is of practical relevance for strawberry cultivation in hydroponical systems, where the EC value of the nutrient solution could be elevated during part of the day, or for production systems, where seawater is added to the nutrient solution in case of water shortage. In areas where a slight salinization of the soil represents a limitation for the cultivation of rather salt-sensitive crops, salt-tolerant strawberry genotypes represent an interesting alternative in order to produce fruits of higher antioxidant capacity and a more attractive color. However, the observed decrease in ascorbic acid content in strawberry fruit grown under moderate NaCl salinity indicates that in any case a rich diet of different fruit and vegetable is recommended to ensure the consumption of a wide range of antioxidants for the human diet.

ABBREVIATIONS USED

AA, reduced ascorbic acid; DHAA, dehydroascorbic acid; TAA, total ascorbic acid; DTNB, 5,5′-dithio-bis(2-nitrobenzoic acid); FM, fruit fresh mass; FRAP, ferric reducing antioxidant power; GAE, gallic acid equivalent; GSH, reduced glutathione; GSSG, glutathione disulfide; L-AA, L-ascorbic acid; NADPH, reduced form of nicotinamide adenine dinucleotide phosphate; NBT, nitroblue tetrazolium salt; SOD, superoxide dismutase.

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LITERATURE CITED

(1) Kaya, C.; Kirnak, H.; Higgs, D. An experiment to investigate the ameliorative effects of foliar potassium phosphate sprays on salt stresses strawberry plants. *Aust. J. Agric. Res.* **2001**, *52*, 995−1000.

(2) Martinez-Barosso, M. C.; Alvarez, C. E. Toxicity symptoms and tolerance of strawberry to salinity in the irrigation water. *Sci. Hortic.* **1997**, *71*, 177−188.

(3) Awang, Y. B.; Atherton, J. G. Effect of plant size and salinity on the growth and fruiting of glasshouse strawberry. *J. Hortic. Sci.* **1995**, *70*, 257−262.

(4) Awang, Y. B.; Atherton, J. G.; Taylor, A. J. Salinity effects on strawberry plants grown in rockwool. II. Fruit quality. *J. Hortic. Sci.* **1993**, *68*, 791−795.

(5) Saied, A. S.; Keutgen, A. J.; Noga, G. The influence of NaCl salinity on growth, yield and fruit quality of strawberry cvs. 'Elsanta' and 'Korona'. *Sci. Hortic.* **2005**, *103*, 289−303.

(6) Keutgen, A. J.; Keutgen, N. Influence of NaCl salinity stress on fruit quality in strawberry. *Acta Hortic. (ISHS)* **2003**, *609*, 155−157.

(7) Gossett, D. R.; Millhollon, E. P.; Lucas, M. C. Antioxidant response to NaCl stress in salt-tolerant and salt-sensitive cultivars of cotton. *Crop Sci.* **1994**, *34*, 706−714.

(8) Noctor, G.; Foyer, C. Ascorbate and glutathione: keeping active oxygen under control. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1998**, *49*, 249−279.

(9) Vaidyanathan, H.; Sivakumar, P.; Chakrabarty, R.; Thomas, G. Scavenging of reactive oxygen species in NaCl-stresses rice (*Oryza sativa* L.)—Differential response in salt tolerant and sensitive varieties. *Plant Sci.* **2003**, *165*, 1411−1418.

(10) Polle, A. Dissecting the superoxide dismutase-ascorbate-glutathione-pathway in chloroplasts by metabolic modeling. Computer simulation as a step towards flux analysis. *Plant Physiol.* **2001**, *126*, 445−462.

(11) Gossett, D. R.; Banks, S. W.; Millhollon, E. P.; Lucas, M. C. Antioxidant response to NaCl stress in a control and an NaCl-tolerant cotton cell line grown in the presence of paraquat, buthionine sulfoximine, and exogenous glutathione. *Plant Physiol.* **1996**, *112*, 803−809.

(12) D'Anna, F. D.; Incalcaterra, G.; Moncada, A.; Miceli, A. Effects of different electrical conductivity levelson strawberry grown in soilless culture. *Acta Hortic. (ISHS)* **2003**, *609*, 355−360.

(13) D'Amico, M. L.; Izzo, R.; Navari-Izzo, F.; Tognoni, F.; Pardossi, A. See water irrigation; antioxidants and quality of tomato berries (*Lycopersicon esculentum* Mill.). *Acta Hortic. (ISHS)* **2003**, *609*, 59−65.

(14) De Pascale, S.; Maggio, A.; Fogliano, V.; Ambrosino, P.; Ritieni, A. Irrigation with saline water improves carotinoids content and antioxidant activity of tomato. *J. Hortic. Sci. Biotechnol.* **2001**, *76*, 447−453.

(15) Törrönen, R.; Määttä, K. Bioactive substances and health benefits of strawberries. *Acta Hortic. (ISHS)* **2002**, *567*, 797−803.

(16) Hertog, M. G. L.; van Poppel, G.; Verhoeven, D. Potentially anticarcinogenic secondary metabolites from fruits and vegetables. In *Phytochemistry of Fruits and Vegetables*; Tomás-Barberán, F. A., Robins, R. J., Eds.; Clarendon Press: Oxford, 1997; pp 313−329.

(17) Roe, F. H. Ascorbic, dehydroascorbic and diketogluconic acids. In *Methods of Biochemical Analysis*; Glick, D., Ed.; Interscience Publishers Inc.: New York, 1961; Vol. I, pp 115−139.

(18) Nakamura, M.; Kurata, T. Effect of L-ascorbic acid on the rheological properties of wheat flour-water dough. *Cereal Chem.* **1997**, *74*, 647−650.

(19) Anderson, M. E.; Tissue glutathione. In *Handbook of Methods for Oxygen Radical Research*; Greenwald, R. A., Ed.; CRC Press; Boca Raton, FL, 1985; pp 317−323.

(20) Giusti, M. M.; Wrolstad, R. E. Characterization and measurement of anthocyanins by UV-visible spectroscopy. *Current Protocols in Food Analytical Chemistry*; John Wiley and Sons Inc.: New York, 2000; Unit F1.2.

(21) Cheng, G. W.; Breen, P. J. Activity of phenylalanine ammonia-lyase (PAL) and concentrations of anthocyanins and phenolics in developing strawberry fruit. *J. Am. Soc. Hortic. Sci.* **1991**, *116*, 865−869.

(22) Singleton, V. L.; Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagent. *Am. J. Enol. Vitic.* **1965**, *16*, 144−158.

(23) Benzie, I. E. F.; Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Anal. Biochem.* **1996**, *239*, 70−76.

(24) Civello, P. M.; Martinez, G. A.; Chave, A. R.; Añón, M. C. Peroxidase from strawberry fruit (*Fragaria* × *ananassa* Duch.): Partial purification and determination of some properties. *J. Agric. Food Chem.* **1995**, *43*, 2596−2601.

(25) Anonymous. *Bio-Rad Protein Assay*; Bio-Rad Laboratories: Munich, 1994.

(26) Oberley, L. W.; Spitz, D. R. Assay of superoxide dismutase using nitroblue tetrazolium. In *Handbook of Methods for Oxygen Radical Research*; Greenwald, R. A., Ed.; CRC Press: Boca Raton, FL, 1985; pp 217−221.

(27) Thies, W. Development of starting material with enhanced α- or γ-tocopherol contents in the seed oil for the breeding of new rapeseed cultivars. *J. Appl. Bot.* **1997**, *71*, 62−67.

(28) Wicklund, T.; Rosenfeld, H. J.; Martinsen, B. K.; Sundfør, W. M.; Lea, P.; Bruun, T.; Blomhoff, R.; Haffner, K. Antioxidant capacity and colour of strawberry jam as influenced by cultivar and storage conditions. *LWT* **2005**, *38*, 387−391.

(29) Pellegrini, N.; Serafini, M.; Colombi, B.; del Rio, D.; Salvatore, S.; Bianchi, M.; Brighenti, F. Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. *J. Nutr.* **2003**, *133*, 2812−2819.

(30) Kafkas, E.; Kosar, M.; Paydaş, S.; Kafkas, S.; Başer, K. H. C. Quality characteristic of strawberry genotypes at different maturation stages. *Food Chem.* **2007**, *100*, 1229−1236.

(31) Cordenunsi, B. R.; do Nascimento, J. R. O.; Genovese, M. I.; Lajolo, F. M. Influence of cultivar on quality parameters and chemical composition of strawberry fruits grown in Brazil. *J. Agric. Food Chem.* **2002**, *50*, 2581−2586.

(32) Wang, S. Y.; Zheng, W.; Galletta, G. J. Cultural system affects fruit quality and antioxidant capacity in strawberries. *J. Agric. Food Chem*. **2002**, *50*, 6534−6542.

(33) Hakala, M.; Lapveteläinen, A.; Huopalahti, R.; Kallio, H.; Tahvonen, R. Effects of varieties and cultivation conditions on the composition of strawberries. *J. Food Compos. Anal.* **2003**, *16*, 67−80.

(34) Schöpplein, E.; Krüger, E.; Rechner, A.; Hoberg, E. Analytical and sensory qualities of strawberry cultivars. *Acta Hortic. (ISHS*) **2002**, *567*, 805−808.

(35) Boo, Y. C.; Jung, J. Water deficit-induced oxidative stress and antioxidative defenses in rice plants. *J. Plant Physiol.* **1999**, *155*, 255−261.

(36) Kocsy, G.; Kobrehel, K.; Szalai, G.; Duviau, M.-P.; Buzás Galiba, G. Abiotic stress-induced changes in glutathione and thioredoxin *h* levels in maize. *Environ. Exp. Bot.* **2004**, *52*, 101−112.

(37) Wang, S. Y.; Jiao, H. Scavenging capacity of berry crops on superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen. *J. Agric. Food Chem*. **2000**, *48*, 5677−5684.

(38) Stintzing, F. C.; Carle, R. Functional properties of anthocyanins and betalains in plants, food, and in human nutrition. *Trends Food Sci. Technol.* **2004**, *15*, 19−38.

(39) Gould, K. S.; McKelvie, J.; Markham, K. R. Do anthocyanins function as antioxidants in leaves? Imaging of H_2O_2 in red and green leaves after mechanical injury. *Plant Cell Environ.* **2002**, *25*, 1261−1269.

(40) Gil, M. I.; Holcroft, D. M.; Kader, A. A. Changes in strawberry anthocyanins and other polyphenols in response to carbon dioxide treatments. *J. Agric. Food Chem.* **1997**, *45*, 1662−1667.

(41) Connor, A. M.; Luby, J. J.; Hancock, J. F.; Berkheimer, S.; Hanson, E. J. Changes in antioxidant activity among blueberry cultivars during cold temperature storage. *J. Agric. Food Chem.* **2002**, *50*, 839−898.

(42) Häkkinen, S. H.; Törrönen, A. R. Content of flavonols and selected phenolic acids in strawberries and *Vaccinum* species: Influence of cultivar, cultivation site and technique. *Food Res. Int.* **2000**, *33*, 517−524.

(43) Wang, S. Y.; Zheng, W. Effect of plant growth temperature on antioxidant capacity in strawberry. *J. Agric. Food Chem*. **2001**, *49*, 4977−4982.

(44) Kalt, W.; Forney C.; Martin, A.; Prior, R. L. Antioxidant capacity, vitamin C, phenolics, and anthocyanins after storage of small fruits. *J. Agric. Food Chem*. **1999**, *47*, 4638−4644.

(45) Gorinstein, S.; Zachwieja, Z.; Katrich, E.; Pawelzik, E.; Haruenkit, R.; Trakhtenberg, S.; Martin-Belloso, O. Comparison of the contents of the main antioxidant compounds and the antioxidant activity of white grapefruit and his new hybrid. *LWT* **2004**, *37*, 1−7.

(46) Vinson, J. A.; Su, X.; Zubik, L.; Bose, P. Phenol antioxidant quantity and quality in foods. *J. Agric Food Chem.* **2001**, *49*, 5315−5321.

(47) Rababah, T. M.; Ereifej, K.; Howard, L. Effect of ascorbic acid and dehydration on concentration of total phenolics, antioxidant capacity, anthocyanins, and color in fruits. *J. Agric. Food Chem.* **2005**, *53*, 4444−4447.

(48) Llorach, R.; Tomás-Barberán, F. A.; Ferreres, F. Lettuce and chicory byproducts as a source of antioxidant phenolic extracts. *J. Agric. Food Chem*. **2004**, *52*, 5109−5116.

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