Sodium Chloride in Nutrient Solutions Can Affect Onion Growth and Flavor Development

Pai-Tsg Chang and William M. Randle
Department of Horticulture, 1111 Plant Sciences Building, University of Georgia, Athens, GA 30602-7273

Abstract. Onion is classified as a salt-sensitive crop, though it is found in production on saline soils around the world. While onion flavor intensity has been studied in response to various growing conditions, little is known about its response to salt stress. To understand if NaCl affects growth, flavor development, and mineral content in onion, ‘Granex 33’ plants were grown to maturity with six different concentrations of NaCl ranging from 0 (control) to 125 mM in nutrient solutions. NaCl affected onion fresh weight and altered onion flavor intensity and quality. Plants did not survive the 125 mM NaCl treatments and are not included in the results. As bulb Na⁺ and Cl⁻ content increased in response to increasing NaCl concentrations, leaf and bulb fresh weight of mature plants decreased. Total bulb S content also decreased with increasing NaCl solution concentrations, while bulb SO₄²⁻ content increased linearly, indicating that less S was entering the S metabolic stream. Though bulb soluble solids content was not influenced by NaCl concentrations, pungency increased, but only at the highest NaCl concentration. Total flavor precursors and methyl cysteine sulfoxide content increased in response to NaCl, but only at the 100 mM treatment. 1-Propenyl cysteine sulfoxide was generally unresponsive to the salt treatment. Propyl cysteine sulfoxide content decreased then increased in responses to increasing NaCl levels, but was found as a minor flavor precursor. Peptide intermediates measured in the pathway leading to 1-propenyl cysteine sulfoxide and propyl cysteine sulfoxide decreased linearly with increasing NaCl exposure. While NaCl affected onion flavor in this study, severe reductions in growth would prevent onion production under similar saline conditions. For practical purposes, the effects of NaCl on flavor are, therefore, minimal.

Soil salinity is a major agricultural problem that directly affects crop productivity and quality. Using FAO world salinity maps, the total area of saline soils is estimated at 397 million ha with 45 million ha under irrigation (Oldeman et al., 1991). Furthermore, productive land can become salinized through continued irrigation with saline water, (Epstein et al., 1980).

Onion (Allium cepa L.), valued for its characteristic flavors, is classified as a salt sensitive crop that has a 1.2 dS·m⁻¹ electrical conductivity (EC) threshold (Palif, 1960; Mass and Hoffman, 1977). Previous studies showed that onion flavor was influenced by environmental factors such as sulfate availability (Freeman and Mossadeghi, 1970), sulfur fertility (Randle, 1992; Randle et al., 1995), nitrogen availability (Randle, 2000), temperature (Platenius and Knott, 1941), and water supply (Freeman and Mossadeghi, 1973). Changes in onion flavor intensity when grown under saline conditions are not known.

Higher plants are thought to have evolved chemical defenses as protective mechanisms when exposed to environmental stress (Rennenberg, 1982; Bergmann and Rennenberg, 1993). Rennenberg (1984) indicated that glutathione could serve as temporary storage for reduced S which could be used to maintain cellular cysteine levels (Schmidt and Jager, 1992). Noctor et al., (1998) confirmed that glutathione not only functioned as a storage and transport form of reduced S, but also as a signal for the regulation of sulfur assimilation. Salt stress increased glutathione biosynthesis in Brassica napus to ameliorate oxidative stress (Ruz and Blumwald, 2002). Glutathione is metabolically connected to the flavor compounds in Allium. Glutathione is the proposed starting point in the biosynthetic pathway leading to the synthesis of the S-alk(en)yl cysteine sulfoxides (ACSOs) flavor precursors(Lancaster and Shaw, 1989). As such, flavor development may be affected by salt stress.

Flavor development in onion begins with sulfate (SO₄²⁻) uptake followed by its reduction to sulfide, and assimilation into cysteine (Leustek et al., 2000). Cysteine is then metabolized to the tripeptide glutathione which is the proposed start of the flavor pathway (Block, 1992). Sulfur terminates in what are collectively called the S-alk(en)yl cysteine sulfoxides (ACSOs) and through enzymatic decompression by allinase (EC 4.4.1.4) form the primary flavor attributes which are characteristic of this family. There are three principal flavor precursors found in onion, each imparting different flavor characteristics to onion (Block, 1992). Generally, 1-propenyl cysteine sulfoxide (1-PRENCOS) is the main precursor found in onion, and responsible for the mouth burn and lachrymatory sensations. Methyl cysteine sulfoxide (MCSO), is found in lesser abundance but under certain condition, can accumulate in large amounts (Randle et al., 1995; Kopsell and Randle, 1999; Randle, 2000). MCSO imparts fresh onion and cabbage-like flavors. Propyl cysteine sulfoxide (PCSO) is found in the lowest concentration in onion and produces onion and chive-like notes when decomposed (Randle et al., 1994).

The primary products from the hydrolysis of ACSOs are volatile and unstable thiosulfinates, pyruvate, and ammonia. Pyruvate, because it is stable, has been used as a measure of overall flavor intensity for onion (Wall and Corgan, 1992).

Because onions are grown on saline soils around the world, and S metabolism in plants has been influenced by salt-stress, we investigated whether or not increasing levels of NaCl in nutrient solutions would influence onion flavor.

Materials and Methods

Plant culture. On 10 Jan. 2002, seeds of ‘Granex 33’ onion (Asgrow Seeds, Kalamazoo, Mich.) were planted in growing cubes (Grodan; Hedenhusene, Denmark) covered with vermiculite, and watered as needed. After the cotyledons emerged, seedlings were fertilized with 400 mL of Peters 20N–20P–20K nutrient solution (Scotts-Sierra Co., Marysville, Ohio) at a rate of 200 mg·L⁻¹ weekly. After five true leaves developed, the seedlings were transplanted to 30-L tubs (Rubbermaid, Inc., Wooster, OH) in a greenhouse under natural photoperiods (=24° N latitude) with day and night temperature set points of 20 and 12 °C, respectively. Onion seedlings were grown hydroponically with half-strength modified of Hoagland solution (Hoagland and Arnon, 1950) for 2 weeks before NaCl treatments were applied.

Each tub was filled with 28 L of deionized water and all plants received a nutrients at levels of 0.47 g·L⁻¹ Ca (NO₃)₂·4H₂O, 0.30 g·L⁻¹ KN0₃, 0.057 g·L⁻¹ NH₄·H₂PO₄, 0.246 g·L⁻¹ MgSO₄·7H₂O, 0.08 mg·L⁻¹ CuSO₄·5H₂O, 0.02 mg·L⁻¹ H₂MoO₄·H₂O, 0.02 mg·L⁻¹ ZnSO₄·7H₂O, 1.81 mg·L⁻¹ MnCl₂·4H₂O, 2.86 mg·L⁻¹ H₂BO₃, and 10.0 mg·L⁻¹ Fe chelate. The experiment was set as a completely randomized design with six NaCl treatments (0, 25, 50, 75, 100, and 125 mM) arranged in individual tubs with ten plants per treatment, and four replications. Tubs were refilled daily with deionized water to volume. The nutrient solutions were replaced and renewed to initial treatment levels every two weeks. Plants were supported with wire mesh suspended above the tubs. Water potential was determined in the initial treatment cycle using a thermocouple psychrometer (model SC-10;Decagon Devices Inc., Pullman, Wash.) equipped with a nanovoltmeter (model NT-3, Decagon Devices Inc.). Water potential was calculated against a sodium chloride standard curve. Values ranged from −0.18 Mpa to −0.48 Mpa over the NaCl treatments. To determine if the NaCl treatments were purely an osmotic effect, polyethylene glycol (PEG) was added separately to the standard nutrient solutions at rates equivalent to the osmotic potential of the NaCl treatments. However, long-term exposure to PEG proved to be phytotoxic to the plants, severely restricting growth and performance. The PEG would polymerize on the roots, forming a gelatinous mass soon after addition to the solutions. Consequently, our interpretation of
the results obtained in this study could not be wholly separated from the fact that the effect may have been the result of osmotic potential versus NaCl concentration.

Plants were harvested when mature as defined when 80% of plants in a treatment had soft pseudostems. Bulb and leaf FW were measured at harvest. Bulbs were then dried at ambient greenhouse temperature for one week before analyses.

Plant analysis. The eight most uniform bulbs from each replication and treatment were used. From each bulb, three 5-mm-thick wedges were cut longitudinally and the tissue from the eight bulbs combined. One wedge group was used to measure total pyruvic acid (TPY) and soluble solids content (SSC), a second wedge group was used for ACSOs and γ-glutamyl peptides ((GPs) analysis, and a third wedge group was used to determine bulb Na⁺, Cl⁻, S and SO₄²⁻ content.

Soluble solids content and total pyruvic acid. Bulb tissue was crushed in a pneumatic press (Univ. Georgia design) and the juice collected. A few drops of the fresh juice were placed on a refractometer (Kernco, Tokyo, Japan) to measure SSC. Pungency of the onion was determined by the method of Randle and Bussard (1993) where total pyruvic acid (TPY) was determined with 1 mL of juice diluted 40 fold, and the juice collected. A few drops of the fresh juice were placed on a refractometer (Kernco, Tokyo, Japan). A 0.5-g sample of ground tissue was ashed at 500 °C for 4 h. Ten milliliters of HPLC-grade water was added to the ash and shaken at 75 rpm for 1 h. The extracts were filtered through a 0.22-µm nylon syringe filter (Fisher Scientific) into 1-mL plastic vials (National Scientific Company, Lawrenceville, Ga). Samples were run on the Waters 2690 Separations Module with a Waters 432 conductivity detector (Waters Corp.). Forty microfilters of extract solution were injected into an IC-PAK Cation HR column, linked to an IC-PAK Cation Guard Pak (Waters corp.). Column temperature was set at 30°C, and 0.1 mM EDTA mixed 189 µL nitric acid eluent was used at a flow rate of 1 mL·min⁻¹. Peaks were quantified and integrated on Millennium Chromatography Software (Version 3.05, Waters corp.). Chloride amount was measured according to Rieger and Litvin (1998) using a solid-state electrode (Fisher Scientific), a silver-silver chloride double junction reference electrode (Fisher Scientific), and a corning 350 ion analyzer (Corning Inc., N.Y.). A 0.5-g sample of dried-ground tissue was dissolved in 20 mL of 0.5 N HNO₃ for 15 min. Concentrations were measured 1 min after the electrode was inserted into the solution.

ACSOs and peptide intermediates. The content of ACSOs and (GPs from onion bulbs was measured according to Randle (2000). Fresh wedges were weighed and were extracted twice in 12 methanol : 3 water (5 mL·g⁻¹ FW) and then once in 12 ethanol : 3 water solution (5 mL·g⁻¹ FW). One milliliter of γ-glutamyl glutamic acid (γG; 0.2 mg·g⁻¹ FW), and (z)-S-butyly-cysteine sulfoxide (BCSO; 1.0 mg·g⁻¹ FW) were used as internal standards and added into 15 mL of the combined extracts (1.0 g FW equivalent).

The solutions were dried by forced air and redissolved in 1 mL of HPLC-grade water. A 0.5 mL aliquot of rehydrated solution was placed on a 10 × 40 ion exchanged column (Bio-Rad, Hercules, CA.) with 3 mL Dowex 1 × 8 resin (200 to 400 mesh; Bio-Rad). Fractionation of the samples was done using 0.1, 0.2, 2.0, and 5.0 mM acetic acid. The 0.1 and 2.0 mM fractions contained the ACSO and γGP compounds, respectively, and were dried by forced air. The fractions were rehydrated with 1 mL HPLC grade water, and 100 µL solution was placed in a 1.5-mL microcentrifuge vial and vacuumed dry using a Labconco Centrivap Concentrator (Kansas City, Mo.). A 250-µL aliquot of a ethanol: triethylamine (TEA): HPLC grade water (1:1:1) solution was added and then dried again. Samples were then derivatized by adding 100 µL 7:1:1:1 ethanol: TEA: phenylisothiocyanate (PTC): HPLC-grade water. Vials were immediately flushed with nitrogen, capped and stored at room temperature for 19 min. The derivatizing samples were dried under vacuum. Dried samples were redissolved in 1 mL of 7 HPLC grade water : 2 acetoniitrile and transferred to 1.5-mL glass vials before HPLC analysis.

Samples were analyzed by a Waters 2690 separator module (Waters corp.) with a 996 photodiode array detector (Waters corp.). A 40-µL sample was injected into a 5 µm, 250 × 4.6-mm column (Spheri-5 RP-18; Applied biosystems, Foster City, Calif.) fitted with a 15 × 3.2-mm, 7-µm guard column (RP-18 Newgard; Applied biosystems) for separation. Column temperature was maintained at 30 °C. Eluents were A) aqueous acetonitrile (60%), B) 0.14 M sodium acetate with 0.05% TEA buffered to pH 6.35 using glacial acetic acid. All eluents were filtered through 0.45-µm nylon filters (Millipore, Molsheim, France). Flow rate was 1 mL·min⁻¹. The eluent gradient was 15 % A for 1.1 min, 15% to 45% A for next 21.1 min, 45% to 100% A over 1 min, and 100% A for 14 min. The gradient was returned to the initial 15% A and 85% B over 1 min. and the column was conditioned and equilibrated for 12.9 min before the next sample was injected.

Statistical analysis. Data were subjected to analysis of variance (ANOVA) and linear and
polynomial regression procedures using SAS statistical software (Version 8.2, SAS, Cary, N.C.). When regression equations for the variables were not significant, data was presented as means with standard error bars.

**Results and Discussion**

*Plant growth observation.* Generally plants react to salinity by reducing growth. In our study, reduced plant growth was visible within one week in all but the lowest NaCl solution concentrations. Onions grown at the highest NaCl were greatly affected and did not survive. Therefore, data from this treatment were unavailable. As NaCl concentration increased, plant growth reductions were more pronounced.

Leaf tips were chlorotic and curly at 100 mNaCl/L. Onion roots and shoots were equally affected. Plant growth reductions were more pronounced. Unavailable. As NaCl concentration increased, plant growth was observed with plants grown in 25 mM NaCl/L. The relationships were linear (bulb Na = 0.93) from 350 g per bulb to 44.8 g per bulb as NaCl levels increased. Leaf FW also decreased linearly with increasing NaCl concentrations (Leaf FW = 171.505 – 1.514 NaCl, r² = 0.91) and ranged from 156 g per plant to 21.6 g per plant. High levels of NaCl have been shown to decrease leaf FW (Malik et al., 1978).

**Bulb and leaf fresh weight.** Increasing NaCl concentrations reduced bulb (P ≤ 0.001) and leaf (P ≤ 0.001) FW (Fig. 1). Bulb FW decreased linearly (bulb FW = 356.124 - 3.129 NaCl, r² = 0.93) from 350 g per bulb to 44.8 g per bulb as NaCl levels increased. Leaf FW also decreased linearly with increasing NaCl concentrations (Leaf FW = 171.505 - 1.514 NaCl, r² = 0.91) and ranged from 156 g per plant to 21.6 g per plant. As NaCl concentration increased, older leaves had a yellowish coloration, but root length was more reduced at 75 mM compared to 50 mM. Little difference in growth was observed with plants grown in 25 mM NaCl compared to the control.

**Sodium and chloride content.** Onion is classified as salt-sensitive with a low tolerance threshold (EC = 1.2 Ds m⁻¹) (Mass and Hoffman, 1977). For comparative purposes to our study, this level is equivalent to ∼12 mM NaCl/L concentrations (Marschner, 1995). Sodium (P ≤ 0.001) and Cl⁻ (P ≤ 0.001) content increased in bulbs as salinity increased in the solutions (Fig. 2). However, NaCl was undetectable at the zero NaCl solution concentration. On average, the Na⁺ content in plants is <1 mg g⁻¹ on a dry weight basis (Marschner, 1995). However, high Na⁺ levels inducing stress can suppress plant growth through a variety of mechanisms. In citrus high Na⁺ concentrations caused greater negative osmotic potential which, in turn, inhibited water uptake (Lea-Cox and Syvertsen, 1993). In soybean, high Na⁺ concentrations disrupted general plant metabolic activity (Nonami et al., 1995). Furthermore, the absorption of other cations were affected by high Na⁺ levels in tomato (Al-Karaki, 2000).

Total bulb sulfur and sulfate. Sulfur is the major element to affect onion flavor intensity (Randle and Lancaster, 2002). Hence, factors that affect S uptake or influence the metabolic activities of S are important to onion flavor development. In this study, total bulb S accumulation responded significantly to increasing NaCl concentrations (P ≤ 0.03). A significant linear decrease was found for total bulb S with increasing NaCl levels (bulb S = 5.0847 - 0.0311 NaCl, r² = 0.46) (Fig. 3). Total bulb S ranged from 5.04 mg g⁻¹ DW with no NaCl in the nutrient solution, to 3.69 mg g⁻¹ DW at 75 mM NaCl. Another fate of SO₄2⁻ is that it can be stored in the vacuole, possibly for later incorporation into organic-S compounds. In this study, bulb SO₄2⁻ was significantly influenced by NaCl levels (P ≤ 0.05). A linear increase was found for bulb SO₄2⁻ responding to increasing NaCl levels (Bulb SO₄2⁻ = 0.6516 + 0.009 NaCl, r² = 0.30) (Fig. 3). Bulb SO₄2⁻ levels ranged from 0.68 mg g⁻¹ DW with no NaCl to 1.76 mg g⁻¹ DW at 100 mM NaCl concentration. Organic-S can be estimated by subtracting bulb SO₄2⁻ from
total bulb S (Randle et al., 1999). The amount of organic-S was also significantly affected by NaCl treatments ($P \leq 0.01$) and the response was similar to the bulb S (organic-S = 4.4277 – 0.0406 NaCl, $R^2 = 0.55$) (Fig. 3). The amount of organic-S ranged from 4.36 mg·g$^{-1}$ DW with no NaCl to 2.16 mg·g$^{-1}$ DW at 100 mM NaCl. Therefore, as NaCl increased, the percentage of absorbed S that entered the organic pathway decreased.

**Soluble solids content and total pyruvate.** Bulb SSC was not significantly affected by increasing NaCl levels and no statistically significant trend could be fitted to the data (Fig. 4). Within treatment variability may have contributed to this. Bulb pungency (TPY), on the other hand, was affected by NaCl concentrations ($P \leq 0.001$) (Fig. 4), but no statistically significant trend could be found. Pungency changed little up to 75 mM NaCl levels, but increased sharply at the 100 mM level. Similarly, onions grown in low water potential conditions resulted in high TPY (Freeman and Mossadeghi, 1973). One-µmole differences in TPY can be perceived when eaten up to about 8 µmol TPY (Wall and Corgan, 1992; personal observations). Above 8 µmol, the sensation is too intense to discriminate single increment changes.

**Flavor precursors and intermediates.** To understand the influence of NaCl on changes in flavor development and potential quality, total and individual flavor precursors and their related peptide intermediates were measured. Different flavor precursors are decomposed to produce various flavor attributes. Thus, changing the concentrations or ratios of individual flavor precursors can affect flavor quality (Randle et al., 1994). Total flavor precursors were significantly affected by NaCl concentrations ($P \leq 0.001$) (Fig. 5). ACSO content ranged from 2.45 mg·g$^{-1}$ FW to 3.32 mg·g$^{-1}$ FW. However, ACSO accumulation was relatively unresponsive to increasing NaCl levels through 75 mM NaCl in solution. It was only at the 100 mM NaCl treatment that ACSO content significantly increased. This result was similar to the TPY curve and could be expected because pyruvic acid should be produced proportionally to the amounts of ACSOs hydrolyzed (Schwimmer and Weston, 1961), although this is not always the case (Lancaster et al., 1998).

MCSO was influenced by NaCl concentrations and was found in highest concentration among the individual flavor precursors ($P \leq 0.001$). However, no significant trend was found in response to increasing NaCl (Fig. 5) The concentration of MCSO ranged from 1.49 to 2.15 mg·g$^{-1}$ FW and the response was similar to total ACSO accumulation. Generally, MCSO is a less important flavor precursor in onion, but can accumulate at high levels under certain conditions (Kopsell and Randle, 1999; Randle, 2000; Randle et al., 1995). The thiosulfimates that result from decomposition of MCSO cause a fresh onion and chive-like taste (Randle et al., 1994).

1-PRENSO was generally unresponsive to NaCl treatment and no significant trend was found (Fig. 5). The decomposition of 1-PRENSO produces the lacrimary factor which results in mouth burning and tearing sen-

sations (Randle et al., 1994). PCSO was found in the lowest concentrations among the individual ACSOs and was affected significantly by NaCl concentrations ($P \leq 0.05$). The concentrations of PCSO ranged from 0.10 mg·g$^{-1}$ FW to 0.27 mg·g$^{-1}$ FW, and the response was quadratic (PCSO = 0.1776 – 0.004 NaCl + 0.00004826 NaCl$^2$, $R^2 = 0.40$) (Fig. 5). The decomposition of PCSO produces thiosulfimates that impart fresh onion and chive-flavor attributes (Randle et al., 1994).

Two measurable peptide intermediates in ACSO synthesis were also affected by NaCl concentration. 2-Carboxypropyl glutathione (2-CARB) ($P = 0.002$) ranged from 0.48 mg·g$^{-1}$ FW to 0.29 mg·g$^{-1}$ FW, and the decreased response was linear with increasing NaCl levels (2-CARB = 0.4754 – 0.0005924 NaCl, $R^2 = 0.62$) (Fig. 6). A decrease in 2-CARB would support the result that more S was metabolized via the MCSO pathway rather than the 1-PRENSO pathway, a result reported by Randle (2000). 2-CARB is not thought to be a part of the MCSO biosynthetic pathway. γGlutamyl propenyl cysteine sulfoxide (γGPRECSO) decreased with increasing NaCl concentrations ($P \leq 0.05$). γGPRECSO levels ranged from 0.63 mg·g$^{-1}$ FW to 1.17 mg·g$^{-1}$ FW, with a decreasing linear response to increasing NaCl concentrations (γGPRECSO = 1.1154 + 0.00338 NaCl, $R^2 = 0.37$) (Fig 6). γGPRECSO concentrations were higher than 2-CARB at all NaCl treatment levels. A similar relationship was
found among these peptides when onions were grown with increasing S and N, respectively (Randle et al., 1995; Randle, 2000). At severe NaCl stress, 2-CARB and γGPRECSO were at relatively low concentrations relative to ACSO and PCSO. Similar results were found when onions were grown under stress conditions (Randle et al., 1995).

As millions of irrigation lands are subjected to salinization, it was necessary to understand the interaction between onion flavor synthesis, onion growth, and NaCl levels. When plants were exposed to NaCl from a period of transplanting to maturity, NaCl affected onion physiology as reflected by changes in leaf and bulb FW, sodium and chloride accumulation, total ACSO accumulation, the concentration of individual flavor precursors and their peptide intermediates. Although onions could survive NaCl concentrations up to 100 mM, reduced growth, even at moderate NaCl levels, would hinder onion production under similar saline conditions. Because flavor intensity and quality were only affected at severe salt stress levels, however, NaCl should not be considered an issue when assessing onion flavor development and accumulation. The possibility of applying NaCl at specific growth stages to affect flavor accumulation and development, however, still needs to be determined.

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