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Salinity and Nitrogen Level Affect Agronomic Performance, Leaf Color and Leaf Mineral Nutrients of Vegetable Amaranth

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ABSTRACT

A study was undertaken to determine the level of salt tolerance of vegetable amaranth (*Amaranthus tricolor* L.) on a Leadvale soil amended with NaCl to give salinity levels of 0.17, 0.34, 0.74, and 3.11 dS/m (0-15 cm depth). Nitrogen, as NH_4NO_3, was split-applied at 0, 135, and 270 kg/ha. Twenty-one day old plants of USDA PI 2153 were transplanted on 17 August and harvested 13 Sept. 1988. Increasing soil salinity increased soil NO_3, K, Mn, and Zn, quadratically (Q), NH_4 and Na linearly (L), and decreased Fe, Mg, and Ca (L) in soil sampled 14 September. Adding N increased residual soil NH_4, NO_3, K, Fe, Mn and Zn (L). Addition of NaCl and N decreased soil pH (Q) and (L), respectively. Plants in 3.11 dS/m soil had reduced stand, yield, height, leaf area, hue, and leaf chlorophyll and total carotenoids. At the highest soil salinity level, leaf chlorophyll a:b ratio increased and chlorophyll:carotenoid ratio decreased in plants grown. Increasing N rates increased leaf area, pigments, and chlorophyll:carotenoid ratio (L), but decreased plant stand (Q) and chlorophyll a:b ratio (L). Increased soil salinity resulted in increased leaf blade Na and Cl (Q) and B (L), but decreased leaf blade NO_3 (Q) and Ca and Mg (L). Applying increased N increased leaf blade TKN, NO_3, Fe, and B (L), but decreased Ca, Mg, and Cl (L). In conclusion, *A. tricolor* accession PI 2153 performed well in soil which had received up to 2660 kg NaCl/ha (0.7 dS/m) and benefited by the application of 135 kg N/ha.

RESUMEN

Se realizó un estudio para determinar el nivel de tolerancia a sal del amaranto (*Amaranthus tricolor* L) en un suelo Leadvale al que se le incorporó NaCl para obtener niveles de salinidad de 0.17, 0.34, 0.74 y 3.11 dS/m (0-15 cm de profundidad). Se aplicaron un total de 0, 135, y 270 kg/ha de N, en forma de NH_4NO_3, dividido en dos aplicaciones. Las plantas del material USDA PI 2153, de 21 días de edad, se transplantaron el 17 de agosto y se cosecharon el 13 de septiembre de 1988. El aumento en la salinidad del suelo, incrementó el NO_3, K, Mn, y el Zn cuadráticamente (Q), el NH_4 y el Na linealmente (L) y disminuyó el Fe, Mg, y el Ca (L) en el suelo muestreado el 14 de septiembre. La adición de N incrementó (L) el NH_4, el NO_3, el K, el Fe, el Mn y Zn. La adición de NaCl y la disminución de N disminuyeron el pH del suelo (Q) y (L) respectivamente. La plantas en suelo con 3.11 dS/m presentaron reducción del vigor, rendimiento, altura, área foliar, color, y clorofila y carotenoides totales de la hoja. En el nivel mas alto de salinidad, la proporción clorofila a:b se incrementó y la proporción clorofila:carotenoides disminuyó en la plantas. El aumento en las tazas de N aumentó el área foliar, los pigmentos y la proporción clorofila: carotenoides (L), pero disminuyó el vigor de las plantas (Q) y la proporción clorofila a:b (L). El aumento en la salinidad del suelo resultó en un incremento del Na y del Cl (Q) y del B (L), pero en una reducción del NO_3 (Q) y del Ca y Mg (L) en la lámina foliar. El incremento en la aplicación de N aumentó (L) el TKN, el NO_3, el Fe, y el B en la lamina foliar, pero disminuyó (L) el Ca, el Mg, y el Cl. En conclusión, el material PI 2153 de *A. tricolor* creció bien en suelo que recibió hasta 2660 kg de NaCl/ha (0.7 dS/m) y se benefició con la aplicación de 135 kg de N/ha.

Additional index word: Amaranthus tricolor, pigments, mineral nutrition, leafy greens

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Vegetable amaranth has been grown domestically in the mid-south, Southeast USA, and the Caribbean, where soils are generally leached of salts (Makus, 1990). However, in other areas, such as the Southwest USA, Northern Mexico, and areas on the subcontinent of India, soil salinity and water quality are issues for concern in the production of vegetable amaranth.

Amaranth is in the botanical family Amaranthaceae, one of three families of higher plants which are natrophilic,

Table 1. Soil nutrients (0-15 cm depth) sampled day after harvest are affected by salt and nitrogen amendments.

Main effect[z]	pH	Electrolytes 0-15 cm	Electrolytes 15-30 cm	NH_4	NO_3	K	Ca	Mg	Na	Fe	Mn	Zn
		dS/m		kg/ha								
NaCl applied (kg/ha)												
0	5.3a	0.17b	0.21b	56a	38c	81c	1160a	106a	236c	158a	177c	1.6c
739	5.1b	0.34b	0.22b	61a	79c	96c	1085a	104a	430bc	166a	215b	1.9c
2,660	5.0b	0.74b	0.44b	78a	141b	121b	1055ab	98ab	989b	156ab	234b	2.5c
13,150	5.0b	3.11a	1.47b	83a	256a	183a	973b	85b	3985a	143b	261a	3.4a
	**[y]	**	**	NS	**	**	**	**	**	*	**	**
polynomial fit	Q**	L**	L**	L*	Q**	Q**	L**	L**	L**	L**	Q**	Q**
Nitrogen level (kg/ha)												
0	5.3a	1.03a	0.54a	38b	68c	111b	1038a	93a	1416a	146b	203b	2.2a
135	5.1b	1.06a	0.59a	66b	119b	122ab	1061a	100a	1416a	158ab	225ab	2.4a
270	4.9b	1.18a	0.62a	104a	197a	127a	1160a	102a	1398a	164a	237a	2.4a
	**	NS	NS	**	**	*	NS	NS	NS	**	**	NS
polynomial fit	L**	-	-	L**	L**	$L^{.07}$	-	-	-	L**	L**	L*

[z]There were no interactions.
[y]*, **, NS = significant (P=0.05), highly significant (P=0.01) and not significant, respectively. Means not followed by the same letter are significantly different at the significance level shown. L, Q, C = linear, quadratic, and cubic, respectively.

having a sodium requirement for growth (Brownell and Bielig, 1996; Match, et al., 1986; Marschner, 1995). However, excess amounts of sodium, usually in conjunction with chloride, can reduce plant growth through many physiological and biochemical processes. Most experiments which deal with sodium nutrition or sodium salinity involve hydroponic or 'challenge' concentrations of NaCl for relatively brief periods of time. Few, if any, growth chamber or greenhouse studies consider the dynamics between Na and Ca which occur in sodic and calcareous soils (Marschner, 1995). Reports that *A. tricolor* can germinate at concentrations of 250 mM NaCl [electrical conductivity (EC) of ~25 dS/m] were not done under field conditions (Macler, et al., 1990). However, in sand culture greenhouse experiments, *A. tricolor* has been judged 'relatively salt-tolerant' when grown with nutrient solutions containing 0 to 60 meq. / l NaCl (EC ~0 to 6 dS /m) (Shimose, et al, 1991).

In contrast to other greens, such as spinach, *A. tricolor* has a high ash content (Makus, 1984). The potential for amaranth to remove soil salts would be an advantage in any cropping system where soils are high in salts.

A. tricolor can respond favorably to supplemental N

Table 2. Effect of salinity and nitrogen level on amaranth field performance.

Main effect[z]	Surviving plants	Yield	Plant Height	Total leaves Produced	Total leaves At harvest	Leaf area
	%	t/ha	cm			cm^2
NaCl applied (kg/ha)						
0	96a[y]	12.9a	14.7a	15.7ab	11.6ab	91.9a
739	93a	13.3a	15.0a	16.2a	12.1a	106.0a
2,660	93a	12.1a	14.2a	16.3a	12.3a	95.1a
13,150	30b	7.2b	9.4b	14.7b	10.4b	57.7b
	**	**	**	**	**	**
polynomial fit	Q**	-	-	-	C**	C*
Nitrogen level (kg/ha)						
0	81a	9.5b	13.3ab	15.4a	11.4a	77.8b
135	81a	12.1a	13.9a	16.0a	11.6a	92.7a
270	73b	12.5a	12.8b	15.6a	11.8a	92.5a
	**	**	0.08[x]	NS	NS	*
polynomial fit	Q*	L**	-	-	-	L*

[z]There were no interactions.
[y]Means ranked by LSD at the level of significance shown. L, Q, C = linear, quadratic, and cubic, respectively.
[x]Probability of a greater 'F' value.

(Makus and Hettiarachchy, 2001) when adequate P and K are present (Makus, 1992).

The objective of this experiment was to determine the effect of soil salinity and supplemental nitrogen fertilizer on vegetable amaranth performance, biomass partitioning, leaf blade constituents (protein-N, mineral nutrients, nitrate, and chloride), and leaf color.

MATERIAL AND METHODS

Three-week-old, greenhouse-grown, containerized plants of vegetable amaranth 'RRC 241', now USDA PI 2153, were transplanted into a Leadvale silt loam soil (fine-silty, siliceous, thermic, Typic Fragiudults) at a site near Booneville, Ark. (35º 06' N Lat. and 93º 55' W Long.; 155 m elevation) on 28 July 1988. Plants were spaced 15x15 cm apart in 4 rows on 0.12 high X 0.6 wide x 1.35 m long raised beds. Beds were on 1.2 m centers. Plant density was equivalent to 215,270 plants/ha. The beds received 90 kg P and K/ha and one-half the N rate of 0, 135 or 270 kg/ha (broadcast basis) on the day of transplanting. The remaining 0, 67.5 or 135 kg N/ha was applied one week later. Prior to transplanting, four soil salinity levels were established by surface application of 0, 740, 2660, or 13,150 kg NaCl/ha. The experimental design was a randomized complete block with 5 replications. Factorial arrangements of salinity (4) and nitrogen level (3) were randomized within each replication. Trickle irrigation was used to restore soil moisture to field capacity when soil moisture fell below -20 kPa, as measured by 30-cm tensiometers (Irrometer Co., Riverside, CA).

Forty-eight days after planting, 12 plants, selected from the middle two rows of each four-row plot, were used to determine yield, plant height, and to supply leaf blades for nutrient analysis. Approximately 20 leaf blades located 1/3 the distance from the base of each plant were removed and composited from each plot and then washed with distilled water, frozen, lyophilized, ground through a 40 mesh (0.36 mm^2) screen and stored at -20º C until analyzed. A single representative plant from each treatment was used to determine the following plant biomass attributes: fresh and dry weight of the stem, petiole, leaf blade, and corresponding fresh and dry weights of these plant parts as a percent of the total plant fresh and dry weights. Stem length and leaf area were also determined from these plants. Leaf area was determined with a CID Model 201 area meter (CID, Vancouver, Wash.) for a main-axis leaf located 1/3 the distance from the base of the stem.

On 14 Sept., the day after plant sampling and harvest, soil samples were composited from each plot at the 0-15 and 15-30 cm depths and dried at 70º C for subsequent soil nutrient analysis.

Nitrate-nitrogen was determined by specific ion electrode (after aluminum-sulfate extraction), ammonical-nitrogen by distillation, soil Cl^- by a Buchler Chloridometer (after acetic acid extraction), P colorimetrically, protein-N (TKN) and SO_4-S by the Leco combustion method, and mineral elements (K, Ca, Mg, Na, Fe, Al, Mn, Zn, Cu, and B) by ICP spectrometry (Plank, 1992). Leaf chlorophylls 'a' and 'b' and total caroteniods were determined by the method of Welburn and Lichtenthaler (1984). Leaf color was measured with a CR100 Chroma Meter (Minolta Corp., Ramsey, N.J.) and calibrated against a white standard.

Means of the responses were compared by 'LSD' tests using the TEST option in PROC ANOVA of SAS (Ver. 6.04, Cary, N.C.). PROC GLM was used to determine polynomial fits and regression equations based on the rate of added amendments.

In this report, the term 'salinity' will be used interchangeably with 'NaCl amended soils'.

Table 3. Effect of salinity and nitrogen level on amaranth plant water content and plant components (dry wt. basis).

Main effect[z]	Plant water content	Stem	Petiole	Leaf Blade	Whole Plant	Stem	Petiole	Leaf Blade	Total
	%	dry wt (g)				as % of plant dry wt			
NaCl applied (kg/ha)									
0	87.3b[y]	0.89a	0.53a	5.34b	6.75b	13.0a	7.9a	79.1b	87.0ab
739	88.1ab	1.02a	0.63a	6.36a	8.01a	12.6a	7.8a	79.5ab	87.3b
2,660	88.8a	0.85a	0.57a	5.38b	6.80ab	12.4ab	8.3a	79.2b	87.6ab
13,150	88.6a	0.42b	0.31b	3.17c	3.90c	11.1b	8.0a	80.8a	88.8a
	**	**	**	**	**	**	NS	*	**
polynomial fit	Q**	C**	C*	C**	C**	-	-	-	-
Nitrogen Level (kg/ha)									
0	88.2a	0.70a	0.45b	4.22b	5.38b	12.8a	8.4a	78.8b	87.2b
135	88.1a	0.89a	0.56a	5.60a	7.04a	12.4ab	7.8b	79.8a	87.6ab
270	88.3a	0.79ab	0.52ab	5.36ab	6.68a	11.8b	7.8b	80.4a	88.2a
	NS	**	**	**	**	0.08[x]	**	**	0.08
polynomial fit	-	Q*	Q*	Q**	Q**	-	-	-	-

[z]There were no interactions.
[y]Means ranked by LSD at the level of significance shown. Q and C = quadratic and cubic, respectively.
[x]Probability of a greater 'F' value.

RESULTS AND DISCUSSION

Environment. From 17 Aug. until 13 Sept., mean temperature and cumulative rainfall were 27.8º C and 56 mm, respectively.

Soil. There were no interactions between salinity and N application for the soil attributes. Soils amended with 0, 740, 2660, or 13150 kg NaCl/ha resulted in EC values of 0.17, 0.34, 0.74, and 3.11 dS/M, respectively, at the 0-15 sampling depth 49 days after application (Table 1). EC values were lower in magnitude and difference at the 15-30 cm depth. Increasing salt application resulted in linear (L) increases in soil salinity at both sampling depths. At the 0-15 cm depth, with increasing salinity, there were linear increases in NH_4 and Na, quadratic increases in NO_3, K, Mn, and Zn, whereas Ca, Mg, and Fe were all decreased (L), and pH decreased quadratically (Q). Increases in Mn and perhaps other soil nutrients were probably brought about by salt-induced solubilization (Khattak and Jarrell, 1988). Increasing N application reduced soil pH (L) and increased soil NH_4, NO_3, K, Fe, Mn, and Zn levels (L), but had no significant effect on soil electrolytes, Ca, or Mg concentrations. Soil P and Cu values were 29 and 2.0 kg/ha, respectively, and were not affected by treatments. Soil SO_4-S values were all <5 kg/ha and organic matter of 0.99% was not affected by any treatment.

Agronomic. There were no salinity X nitrogen interactions in any of the agronomic responses. At the highest level of soil salinity, yield, plant height, leaf area, and the final plant stand were significantly reduced (Table 2). There were no differences in the number of leaves produced or retained at harvest between plants grown in 0 and 13,150 kg NaCl /ha rates, but more leaves were retained at the 739 and 2660 kg NaCl/ha rates than at the highest NaCl/ha rate. Increasing the application of supplemental N from 0 to 270 kg/ha increased yield and leaf area (L), but decreased plant stand at harvest (Q).

Because plant water content was affected by salinity level, plant component parts were expressed on a dry wt. basis (Table 3). Total plant, stem, petiole, and leaf blade weights decreased (C) as NaCl application rate increased. The cubic decreases in plant component performance are probably because the 2660 kg NaCl/ha rate represents the threshold for plant tolerance to added soil salts. Based upon regression analysis and as a percentage of total plant weight, stem weights declined and leaf blade and leaf weights increased with increased NaCl application rate. Increasing N levels increased total plant, stem, petiole and leaf blade weights (Q). As a percentage of total plant weight, leaf blade weight increased at the expense of petiole mass and possibly stem weight (P=0.08). Plant height was also smaller as N application levels increased (Table 2).

Leaf blade mineral constituents. Leaf blade K, P, Mn, Cu, and Zn levels were 28.7 and 3.46 mg/kg and 462, 112, and 90 *u*g/kg, respectively, and were not affected by either soil salinity or nitrogen level. Increasing soil salinity had no effect on leaf blade TKN, but decreased Ca (L), Mg (L), and increased Na (Q), Cl (Q), and B (L) leaf blade concentrations (Table 4). Increasing N application rate increased leaf blade TKN (L), Fe (L), and B (L), but decreased Ca (L), Mg (L), and Cl (L), and had no effect on leaf blade Na and Al. Increasing salinity reduced the leaf NO_3 concentration at higher N application levels (Fig. 1A). Low NaCl levels (0.5 mM), in the presence of NO_3 and NH_4 salts, can stimulate *A. tricolor* nitrogen reductase activities by 2.6 fold compared to plants grown in the absence of Na and with 0.5 mM KCl (Ohta, et al.,1989). Shimose, et al. (1991) reported that the absorption of K, Ca, and Mg in *A. tricolor* leaves decreased with increasing salinity. Potassium levels were not effected by salinity in this study, but the K/Na ratio was reduced by increased salinity. Shimose, et al. (1991) observed that the ratio of the decrease in

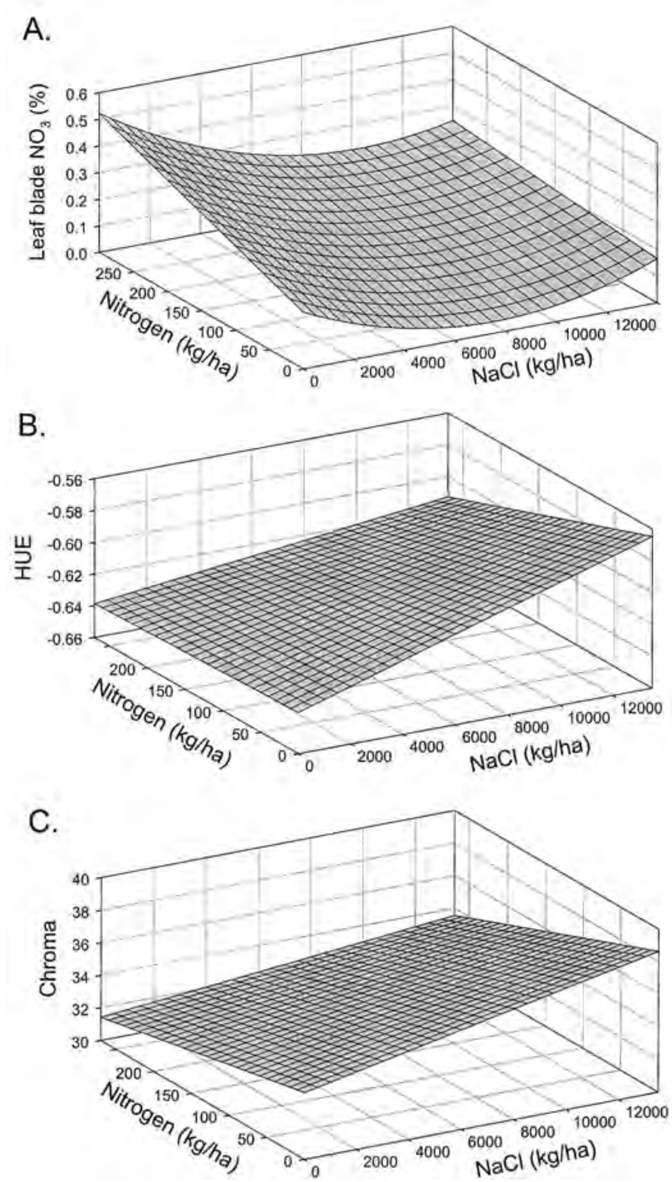

Fig. 1. Interactions between salinity and nitrogen level are shown for leaf blade nitrate (A), leaf Hue (B), and leaf Chroma (C). Corresponding regression equations are: Leaf blade $NO_3 = 212.96 + 1.0298\ N - 0.0445\ S + 0.000002938\ S^2 - 0.00005421\ NxS$; $Hue= -0.634 + 0.00000514\ S - 0.0000177\ N - 0.0000000119\ SxN$; $Chroma= 34.108 + 0.000336\ S - 0.00997\ N - 0.00000066\ SxN$; where N= nitrogen applied (in kg/ha) and S= NaCl applied (in kg/ha).

Table 4. Foliar leaf blade nutrients at harvest as affected by salinity and nitrogen level.

Main effect[z]	TKN	Ca	Na	Mg	CL	Al	Fe	B	K/Na ratio
	———	———	%	———	———	———	mg/kg	———	
NaCl applied (kg/ha)									
0	4.4a	2.45a	0.15c	1.06a	0.80c	958ab	555a	35b	18.3c
739	4.4a	2.19a	0.31c	1.01ab	1.16c	869b	519ab	36ab	9.4b
2,660	4.4a	2.27a	0.56b	0.91b	1.84b	850b	490b	36ab	5.4c
13,150	4.3a	1.74b	1.20a	0.72c	2.63a	1012a	558a	38a	2.7d
	NS[y]	**	**	**	**	*	*	*	**
polynomial fit	-	L**	Q**	L**	Q**	-	-	L*	C**
Nitrogen level (kg/ha)									
0	4.0c	2.51a	0.52a	1.02a	1.93a	880a	491b	35b	9.2ab
135	4.4b	2.18b	0.60a	0.92ab	1.63a	917a	534ab	36b	7.9b
270	4.7a	1.79c	0.54a	0.84b	1.26b	970a	568a	38a	9.9a
	**	**	NS	**	**	NS	**	**	**
polynomial fit	L**	L**	-	L**	L**	-	L*	L**	Q**

[z]There were no interactions.

[y]*, **, NS = significant (P=0.05), highly significant (P=0.01) and not significant, respectively. Means not followed by the same letter are significantly different at the significance level shown. L, Q, C = linear, quadratic, and cubic, respectively.

Table 5. Amaranth leaf blade pigments and color as affected by salinity and nitrogen level.

	Chlorophyll		Chlorophyll: Carotenoid Ratio	Total carotenoids	Hue	Chroma
	mg/g	a:b Ratio		mg/g	a/b	$(a^2+b^2)^{1/2}$
NaCl applied (kg/ha)						
0	8.46a	4.0b	2.14a	3.4a	-0.62b	33.4b
739	8.60a	3.9b	2.20a	3.4a	-0.64b	32.3b
2,660	8.08a	4.0b	2.09a	3.4a	-0.64b	33.2b
13,150	7.10b	4.2a	1.91b	3.3b	-0.59a	36.1a
	**[z]	**	**	**	**	**
polynomial fit	L**	L**	L**	L**	Q*	-
Nitrogen level (kg/ha)						
0	7.02b	4.1a	1.86c	3.3b	-0.61a	35.9a
135	8.28a	4.0ab	2.14b	3.4ab	-0.63b	33.0b
270	8.88a	4.0b	2.26a	3.4a	-0.63b	32.4b
	**	*	**	**	0.07	**
polynomial fit	L**	L*	L**	L**	-	L**
Interaction:						
S x N	NS	NS	NS	NS	*	*

[z]*, **, NS = significant (P=0.05), highly significant (P=0.01) and not significant, respectively. Means not followed by the same letter are significantly different at the significance level shown. L and Q = linear and quadratic, respectively.

K absorption to the increase in Na absorption was found to be a good indicator of salt tolerance in *A. tricolor*, *Artemisia princeps*, *Solanum melongena*, and *Perilla frutescens*. In the case of *A. tricolor*, these authors reported increases in plant (top part) Na and Cl and decreases in S, N, P, K, Ca, and Mg from added NaCl (to ~ 60 meq. / L, which is equivalent to ca. 6 dS / m). In a companion study of salt tolerance in 39 different species, Shimose and Sekiya (1991) determined that *A. tricolor* had 'strong tolerance' (not less than 75% growth of the control plants) to 60 meq. / L NaCl. The high tissue Na and Cl levels reported in Table 4 would indicate that *A. tricolor* is an 'includer' (vs 'excluder') of these salts and can tolerate their presence by osmotic adjustment.

Leaf color. Leaf blade chlorophyll (chloro), total carotenoids (carot), and chloro:carot ratio declined (all L) with increasing soil salinity (Table 5). The chlorophyll a:b ratio increased (L) with increasing soil salinity. When Na nutrient levels increased from insufficiency to sufficiency, chlorophyll a:b ratios in *A. tricolor* were reported to increase (Johnston, et al., 1984). Increasing N application increased (L) chlorophyll, total carotenoids, and chloro:carot ratio. The differences in hue (color or greenness) and chroma (color intensity) due to increasing salinity were the greatest when no nitrogen was applied compared to when the highest amounts of N were applied (Fig. 1B, 1C). Chlorophyll, which is represented by 'a' or 'greenness' values in the SI color space concept, is more sensitive to soil salinity changes than are the carotenoids, which are represented by 'b' or 'yellowness' values. Since hue and chroma are computed from 'a' and 'b' values, this generates the interaction shown in Fig. 1B and C.

Sodium ions can stimulate the regeneration of phosphoenolpyruvate in mesophyll chloroplasts of *A. tricolor* (Murata et al., 1992). The lower 739 kg/ha NaCl rate appeared to be 'beneficial' to yield, leaf area, plant dry wt and total chlorophyll (Tables 2, 3, 5). In other field-grown *Amaranthus* spp., increasing the salinity of irrigation water from 4 to 6 dS/m was observed to reduce plant carbohydrates, soluble and total oxalates, and nitrates at both vegetative and flowering stages of growth (Gaikwad and Chavan, 1995; Gaikwad and Chavan, 1999). Increasing the salinity of irrigation water to 16 dS/m resulted in decreased leaf transpiration, diffusive resistance to CO_2, and more negative osmotic potential in field-grown *A. caudatus*, *A. hypochondriacus*, and *A. paniculatus* (Gaikwad and Chavan, 1998). Chloride ions are known to participate in the splitting of water (Photosystem II) in chloroplasts. Under oxidatative stress, such as intense sunlight, destruction of the chloroplasts, seen as a loss in chlorophyll, was probably due to excessive Cl^- in the leaf tissue. Supplemental N reduced Cl tissue levels (Table 4) and improved leaf chlorophyll (Table 5).

Although the soil electrolytes in this study were determined within 7 weeks of application, relatively high quality water (< 300 ppm salt content) was used to irrigate with and to maintain high (low tension) soil moisture. Initial and mean season soil salt levels were not monitored, but may have been different (higher) and may have been reduced by leaching as the season progressed.

Results from this study suggest that *A. tricolor*, grown under simulated saline soil conditions, will tolerate moderate levels of salinity and do well in the presence of 400 to 1000 kg / ha of soil test Na in a silt loam soil. Supplemental N improves yield and leaf greenness in response to higher soil salinity.

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