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PHYSIOLOGICAL RESPONSES OF *AMARANTHUS TRICOLOR* L. TO NaCl STRESS

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Saline soil is a serious problem which affects the yields of commercial crops in the most part of the world. To reduce the devastating effects of salinity on crops, many trials have been conducted. *Amaranthus tricolor* L., a member of Amaranthus family, has been used as a promising plant to ameliorate the salt affected area. Until now, very little is known about mechanisms of salt tolerance in this plant. Salt-tolerant mechanisms of *A. tricolor* to NaCl were investigated and compared with those of salt-sensitive species, *Phaseolus vulgaris* L. cv. Meal (kidney bean). In this study, all experiments were conducted in a growth chamber at 25/20°C day/night temperatures, with a 14 h photoperiod at 280-290 μE/m²/s, and relative humidity of 70-80%. The experiments were designed with three replications. Seedlings of both species were grown hydroponically in 0, 50, 100, 150, 200 and 250 mM NaCl and harvested 12-18 days after starting NaCl treatment. Effects of NaCl on seed germination, biomass production, chlorophyll content, fluorescence yield (Fv/Fm), ion distribution, change of amino acid and proline contents, ion uptake and translocation were determined.The results showed that tolerance to NaCl was clearly different between the two species. *A. tricolor* could normally grow in 0.9 - 1.2% of NaCl-solution, while 0.3% NaCl-solution caused drastic leaf injury in *P. vulgaris*. The concentrations of 0-200 mM NaCl was not lethal to germination rate of *A. tricolor* seeds. Increasing NaCl concentrations reduced the content of chlorophyll b only in the leaves of *A. tricolor*, but severely reduced total chlorophyll content and chlorophyll fluorescence in *P. vulgaris*. Under NaCl stress, a higher accumulation of Na^+ and Cl^- was found in shoots of *A. tricolor*. It showed that 60- 70% of Na^+ and Cl^- were translocated and accumulate in the shoots. Moreover, the high accumulation of these ions tended to accumulate in the older leaves of *A. tricolor*. While, *P. vulgaris* responded to fairly low salinities by accumulating Na^+ in the roots. Phosphorus content increased with increasing NaCl in both species but potassium content was reduced. Magnesium and calcium contents showed no noteworthy changes in both species. Higher accumulation of amino acids and proline contents was observed with increasing NaCl in the species.

Keywords : *Amaranthus;* Biomass; NaCl; Pigments; Proline; Salinity stress.

Introduction

Excessive salinity and drought are the most important environmental factors that greatly affect plant growth and productivity worldwide. Osmotic and water stresses cause pleiotopic effects, and stress tolerance is a complex and polygenic trait that involves morphological, physiological, as well as biochemical changes. Salinity impairs seed germination, reduces nodule formation, retards plant development and reduces crop yield[1]. The plants that grow in saline soils have diverse ionic compositions and a range in concentrations of dissolved salts. These concentrations fluctuate because of changes in water source, drainage, evapotranspiration, and solute availability. Successful seedling establishment depends on the frequency and the amount of precipitation as well as on the ability of the seed species to germinate and grow while soil moisture and osmotic potentials decrease. These salts interfere with seed germination and crop establishment[2]. Germination and seedling characteristics are the most viable criteria used for selecting salt tolerance in plants. Salinity stress can affect seed germination through osmotic effects. It is generally accepted that salinity stress causes a decrease

both in growth and in the photosynthesis of higher plant species. The subsequent evaluation of biomass production, which forms a majority of plant growth, will indicate the capacity with which each plant tolerates salinity. It was found that in general, the production of fresh and dry matter is severely reduced with the low concentration of salinity in glycophytes, but is still maintained in halophytes. Reduction in the photosynthetic rate as a result of NaCl exposure is observed in many species. The evaluation on the photosynthesis in leaves was extensively carried out by determining chlorophyll content, the major photosynthetic pigments that absorb light energy and initiate electron transport, and/or the kinetics of chlorophyll fluorescence that indicate the function of the photosynthetic electron-transport[3]. Survey of tolerant varieties is often carried out by determining their growth and photosynthesis.

Amaranthus tricolor generally grows in dry arid waste lands in Kerala. Due to its vigorous growth and drought tolerance, this plant has been used as a potential green manure plant. Until now, a few studies have been conducted on the mechanism of salt tolerance. The objective of this study was to investigate effects of NaCl on seed germination, fresh and dry matter production, chlorophyll content and chlorophyll fluorescence in *A. tricolor* comparing with *Phaseolus vulgaris.*

Material and Methods

Seeds of *A. tricolor* (obtained from the Department of Agricultural University, Trichur, Kerala) were soaked in concentrated H_2SO_4 for 30 min to break dormancy. The seeds were surface-sterilized with NaClO (1.25% active chlorine) under vacuum for 15 min and rinsed several times with sterile distilled water. 15 seeds were placed in a petri dish with two layer filter papers. 10 ml of 0, 50, 100, 150, 200, 250 and 300 mM NaCl solution was put into petri dish. Germination rate were determined 7 days after treatment.

Seedling preparation and NaCl treatment -Seeds of *A. tricolor* were prepared as described earlier. *Phaseolus vulgaris* L. seeds were incubated on moist filter paper at 30°C for 2 days in darkness. Germinated seeds were planted in a container containing vermiculite and grown at 25°C for 2 days in darkness with aluminum foil cover. Then a half strength of modified MS medium[4] was added to the vermiculite. After 5 days, seedlings were transferred to a hydroponic culture with the same nutrient solution. *A. tricolor* was grown to the 3rd to 4th and *P. vulgaris* to the 1st to 2nd leaf stage. Salt treatment was started by adding 50 mM NaCl to the solution. For the treatments with higher concentrations, plants were transferred to 100, 150, 200, 250 and 300 mM NaCl at two-day intervals. NaCl-solution containing nutrient was renewed every 4 days. Plants were grown in a growth chamber at 25/20°C day/night temperatures, with a 14 h photoperiod at 280- 290 $\mu E/m^2/s$, and relative humidity of 70-80%. Seedlings were harvested 14 days after starting NaCl treatment. All experiments were designed with three replications.

Fresh and dry matter production: The test seedlings were harvested and separated into roots and shoots. Each part was then weighed for the determination of fresh matter mass. They were then dried at 80°C for 48 hours for the measurement of dry matter mass.

Measurement of chlorophyll contents- All leaves were excised from the test seedlings for the measurement of chlorophyll content. Chlorophyll contents were determined according to Chappelle *et al.*[5].

Determination of amino acid and proline contents- The test seedlings were harvested and separated into roots and shoots. The total free amino acid and proline were estimated as per the methods of Desmaison *et al.*[6], Moore and Stein[7] and Bates *et al.*[8] in fresh roots and shoots.

Quantification of ion content- Ion contents were measured according to the method described by Kim *et al.*[9]. Roots and leaves of the test seedlings were divided and dried at 80°C for 2 days. Dried samples were then ground into a fine powder for wet digestion and dry ashing.

Results and Discussion

Germination rate of *A.tricolor* did not reduce much up to 200 mM NaCl. Their germination rate showed 86.6% control at 200 mM compared to control. At higher concentrations (250 and 300 mM), the germination was reduced to 50%. Moreover, the plant's root became shorter and hypocotyls turned pale (Table 1a).

Increase of NaCl concentration reduced biomass production in both species (Table 1a and b). However, at the highest concentration of NaCl (150 mM), salinity affected the fresh and dry matter slightly in shoots and roots of *A. tricolor.* In contrast, the shoots of *P. vulgaris* were markedly inhibited by more than 50% of the control value at the lowest concentration of NaCl (50 mM). Visible leaf injury (chlorosis and necrosis) were very apparent even at fairly low concentrations of NaCl in the 1st and 2nd leaves of *P. vulgaris,* while this level caused no symptoms in *A.tricolor.* Furthermore, the highest dose of NaCl (150 mM) caused death in *P.vulgaris.*

Decrease of total chlorophyll content was observed with increasing NaCl concentration in both species but its reduction was more obvious in *P. vulgaris.* It showed that both chlorophyll a and b decreased and consequently affected chlorophyll a+b and a/b ratio (Table

2a). For *A. tricolor,* increase of NaCl concentration decreased the content of chlorophyll a slightly, but the loss of chlorophyll b was evident. This resulted in the enhancement of the chlorophyll a/b ratio. The determination of quantum yield (Fv/Fm) showed that chlorophyll fluorescence was unaffected in *A.tricolor* but very severe in *P. vulgaris* at lowest concentration of NaCl (50 mM) (Table 2b). Furthermore, their levels were undetectable in the quantum yield at the higher concentrations of NaCl (100 and 150 mM) due to necrosis.

Decrease in fresh and dry weights of shoots undoubtedly indicated the difference of salt tolerance between the species. *A. tricolor* could tolerate 150 mM NaCl or about 0.8% NaCl (w/v). Moreover, this concentration of NaCl inhibit seed germination. This finding is similar to the case of previous studies which classified such plant as a moderately salt tolerant species[10]. On the other hand, *P. vulgaris* was very sensitive to the salt and could survive under 0.2- 0.3% NaCl (w/v), as previously reported[11]. In the investigation on salt tolerant mechanism in *A. tricolor,* comparison of its characteristics to *P. vulgaris* seemed to be useful. The decreasing tendency of chlorophyll content and chlorophyll fluorescence corroborates with the biomass production in shoots of both species.

In *A. tricolor,* the decrease in Chl a+b content was mainly attributed to the destruction of Chl b, which is more sensitive to salinity than Chl a[12]. In *P. vulgaris,* chlorophyll a+b decreased drastically as reported previously by Singh and Dubey[13]. So it can be attributed that NaCl stress decrease total chlorophyll content of the plant by increasing the activity of Chl degrading enzyme chlorophyllase, inducing the destruction of chloroplast structure and instability of pigment-protein complexes[13]. Results obtained from this study indicate that chlorophyll b is more susceptible to NaCl stress than chlorophyll a and it will be an ideal marker of salt stress. The quantum yield (Fv/Fm) is an indicator of potential yield of photochemical reaction of Photosystem II. Moreover, the reduction of chlorophyll fluorescence is associated with increased Na^+ accumulation[14]. Under NaCl stress, the quantum yields were unaffected in *A.tricolor,* but greatly reduced in *P. vulgaris.* This indicates that photosynthetic electron transport normally works in *A. tricolor* but disorders in *P. vulgaris.* The evaluation of chlorophyll fluorescence could be used for screening of the salt sensitive and tolerant species.

Na^+ and Cl^- contents increased with increasing salinity in roots and shoots of *A.tricolor.* However, their accumulations were noted as being greater in shoots than in roots. In roots of *P. vulgaris,* the Na^+ and Cl^- contents also increased similarly as in the shoots of *A. tricolor* (Table 3 and 4). A difference of Na^+ distribution in the shoots of *P. vulgaris* was observed. At the high concentration of NaCl (100 and 150 mM), Na^+ content tended to decrease, but Cl^- content increased slightly. Furthermore, these doses of NaCl caused wilting on the shoots of this plant and eventual death.

Instability of Potassium (K^+) content in shoots and roots was observed in *P. vulgaris,* while it is slightly reduced in *A. tricolor.* Phosphorus (P) content tended to increase in roots and shoots with the increasing concentration of NaCl, but there was no difference in concentrations of this element between the two species. The change of Mg^{++} and Ca^{++}content by salinity in the roots and shoots of the two species was not observed (Table 3 and 4). In *A.tricolor,* the distribution patterns of Na^+ and Cl^- were further investigated in stem and leaf. The result also showed that the high level of NaCl treatment caused greater accumulation of Na^+ and Cl^- ions in leaves and stem of *A.tricolor* (Figs.1a and b). The ion analyses in individual leaf showed that Na^+ tended to accumulate in the older leaves with higher concentration. The chlorine ion showed the same tendency although it was not clear when compared with Na^+ (Figs.1 c and d).

Total amino acid and proline contents in *A.tricolor* and *P. vulgaris* are shown in table 5 and table 6. The amount of soluble amino acids increased in both plants at par with increasing NaCl concentration. At the highest concentration, although the total amino acid and proline were clearly built up in both the shoots and roots of *A. tricolor,* their accumulation was more obvious in shoots. Similar trend was noticed in *P. vulgaris* at the higher NaCl concentrations (100 and 150 mM).

In plant species, higher accumulation of Na^+ and Cl^- requires compatible solutes for osmotic adjustment under salinity stress[15]. In this experiment, accumulations of the ions are found in *A.tricolor* and *P. vulgaris* as a response to changing salinity stress levels. However, a different pattern of Na^+ and Cl^- distribution in each part of the two species was clearly seen with increasing salinity. *A.tricolor* has a higher accumulation of the ions in shoots, but *P. vulgaris* in roots. It may likely indicate that *A.tricolor* has an ability to translocate the ions and hold them in the shoots. This physiological process may be important to reduce the salt toxicity in and away from the root cells. As a result the plant could survive even at the higher salinity levels to which were subjected during this study.

Some halophytes are known to accumulate the salts in their leaves under salinity stress[16]. Glycophytes,

Table 1a. Effect of NaCl on germination rate and fresh and dry weights (g/plant) of 14 days old *A.tricolor* seedling.

NaCl Conc.		0 mM	50 mM	100 mM	150 mM	200mM	250*mM	300*mM
Germ. Rate		20±0.35	19±0.53	19±0.13	18±0.67	17±0.2	9±0.34	8±0.89
Root	Fresh weight	4.1±2.6	4.3±3.5	2.6±2.4	2.1±2	1.5±1.3	1±0.3	D
	Dry weight	0.38±0.67	0.4±0.9	0.3±2.2	0.3±1	0.25±0.3	0.2±1	D
Shoot	Fresh weight	3.8±6	4.2±5	2.8±2.2	2.6±1.2	1.7±1	1.2±0.7	D
	Dry weight	0.76±1	0.75±0.9	0.4±0.5	0.38±0.4	0.33±2	0.3±0.8	D

Table 1b. Effect of NaCl on germination rate and fresh and dry weights (g/plant) of 14 days old *P.vulgaris* seedling.

NaCl Conc.		0 mM	50 mM	100 mM	150 mM	200mM	250*mM	300*mM
Germ.Rate		20±0.6	15±0.2	9±0.9	6±1.3	1±0.7	0	0
Root	Fresh weight	8.2±0.1	5.9±0.8	4.8±1.4	D			
	Dry weight	0.6±0.89	0.4±0.13	0.3±0.22	D			
Shoot	Fresh weight	7.2±2.6	1.9±1.5	1±1.1	D			
	Dry weight	0.86±3	0.38±1	0.37±2	D			

Table 2a. Salinity stress on chlorophyll content (µg/g FW) in the *A.tricolor* leaves.

NaCl	Chl a	Chl b	Chl a+b	Chl a/b	Fv/Fm
0mM	149.8± 1.4	225± 2.1	374.8± 0.6	0.67± 0.2	0.74±0.001
50mM	152.2± 1.3	134.5± 1.9	272.2± 2.4	1.0± 1.2	0.75±0.003
100mM	120.6 ± 0.54	38.1± 2	166.7± 3.2	3.3±3.2	0.75±0.003
150mM	102.4± 2.0	35.2± 1	160.6± 0.4	3.2± 0.6	0.76±0.002
200mM	96.5± 2	40± 0.4	136.5± 0.89	2.41± 0.76	0.77±0.001

Table 2b. Salinity stress on chlorophyll content (µg/g FW) in the *P. vulgaris* leaves.

NaCl	Chl a	Chl b	Chl a+b	Chl a/b	Fv/Fm
0mM	68.5± 1	35± 3.1	103.5± 2.6	1.96± 0.02	0.68±0.001
50mM	61.2± 0.5	33± 2	94.2± 1.9	1.9± 0.2	0.05±0.001
100 mM	**143 ± 0.4**	**11 ± 2.9**	**25.3 ± 0.2**	**1.3 ± 0.8**	**0.01±0.001**

Table 3a. Ion contents (μmol/g DW) in root of *P. vulgaris* 14 days after starting NaCl treatment.

Root NaCl Conc.	0 mM	50 mM	100 mM	150 mM
Na	182±2.5	1436±4	2409±13	2718±9
K	689±3.3	597±3	491±4	411±4
Mg	168±1.67	255±9	233±2	190±2
Ca	70±3.7	138±7	113±0.6	87±17
P	357±5	701±5	618±12	602±1.2
Cl	416±12	1905±2.9	2424±4	2638±9

Table 3b. Ion contents (μmol/g DW) in shoot of *P. vulgaris* 14 days after starting NaCl treatment.

Shoot NaCl Conc.	0 mM	50 mM	100 mM	150 mM
Na	98±5	1304±11	816±0.13	931±17
K	698±2	1010±3	754±2	731±2
Mg	198±17	193±19	175±12	181±4
Ca	210±11	319±17	399±7	390±2
P	394±5	511±11	520±11	560±12
Cl	567±12	1068±5	1460±5	1667±23

Data the means of 3 replicates ± S.E.
Shoots of *P. vulgaris* wilted and died at 100 and 150 mM NaCl.

on the other hand, respond to salinity basically by ion inclusion. The majority of these species accumulate high levels of Na+ in their roots and stems[17]. At lower NaCl concentration, an ability to localize Na^+ and Cl^- ions in root cells seemed to be important to alleviate salinity stress in *P. vulgaris*. At higher doses of NaCl, however, they could not resist toxicity of salt resulting in wilting and death of the plant. The higher Na^+ and Cl^- accumulation in shoot of *A. tricolor* than *P. vulgaris* may indicate that shoot cells of *A.tricolor* has mechanism to tolerate higher ion concentrations.

The replacement of K^+ by Na^+ observed in *P. vulgaris* was considered to be similar with that reported previously in tomato in which the replacement occurred progressively with increasing absorption of Na^+, and absorbed Na^+ and was transported to the stem[18]. NaCl did not increase P uptake in roots and shoots of both species as was found by Tattini *et al.*[19] in aeroponically grown olive plants. As plant nutrients, calcium (Ca) and magnesium (Mg) play the same role by acting as a buffer system of the plant cells[20]. NaCl did not affect on the Ca++ and Mg++ uptake in roots and shoots of *A.tricolor* as was found in Sesbania[21].

From this study, using 50, 100 and 150 mM NaCl concentrations, it was difficult to discuss the pattern of ion distribution in *P. vulgaris*. The highest concentration of NaCl (150 mM) is not a lethal dose of *A.tricolor*, whilst the lowest concentration (50 mM) proved too strong for the shoots of *P. vulgaris*. Furthermore, 14 days of NaCl exposure might be too long for the seedlings to discuss the effect of NaCl on the uptake of these ions because this species is very susceptible to the stress. More precise determinations of the distribution of the ions in shoot parts showed that *A.tricolor* accumulated more Na^+ and Cl^- ions

Table 4. Ion contents in root and shoot (μmol/g DW) of *A. tricolor* 14 days after starting NaCl treatment.

Root NaCl Conc.	0 mM	50 mM	100 mM	150 mM
Na	388 ±13	498 ± 1.2	648 ± 12	696 ± 3.8
K	497 ±26	297 ± 1.7	334 ± 7	302 ± 4
Mg	123 ±10	102 ± 1.8	128 ± 3.4	131 ± 4
Ca	45±7	29 ± 13	30 ± 3	29 ± 1
P	323±34	302 ± 11	362 ± 4	395 ± 7
Cl	341±14	368 ± 5.7	598 ± 14	522 ± 5
Shoot **NaCl Conc.**	**0 mM**	**50 mM**	**100 mM**	**150 mM**
Na	416 ± 4	1548 ± 2	1923 ± 4	2522 ± 4
K	998 ± 13.2	602 ± 3.2	589 ± 3.2	577 ±13
Mg	197 ± 0.4	202 ± 1.3	211 ± 3.6	203 ± 10
Ca	166 ± 2.1	122 ± 4.4	159 ± 7.3	212 ± 12.7
P	432 ± 2.5	639 ± 5.3	788 ± 9.3	804 ± 13
Cl	389 ± 4	1002 ± 2	1311 ± 12	2021 ± 9

Table 5. Total free aminoacids and proline content in *A.tricolor* after 14 days of NaCl treatment.

	Roots		Shoot	
	Free aminoacids	Proline	Free aminoacids	Proline
0	2.5 ±0.9	0.4±1	3.8.2±1.9	0.53±0.01
50	5.9±0.29	0.42±0.29	12.7±2	1.2±0.03
100	9.2±0.35	0.9±0.38	16.9±3.2	1.5±1.9
150	11.4±2.9	1.4±0.7	23±0.99	2.9±2.3
200	12±2.6	2.3±0.5	27.8±0.65	3.3±3.9

Table 6. Amino acid and proline contents (mg/g DW) in root and shoot of *P. vulgaris* 14 days after starting NaCl treatment.

Roots			Shoot	
	Free aminoacids	Proline	Free aminoacids	Proline
0	3.5±.91	0.2±0.45	8.8±1	0.5±0.2
50	8.3±0.8	0.3±0.12	11.3±2.1	0.8±0.34
100	13.1±0.45	0.81±0.33	19.2±2.7	1.6±0.5
150	22.4±0.78	1±0.65	23±2.9	2.7±0.8

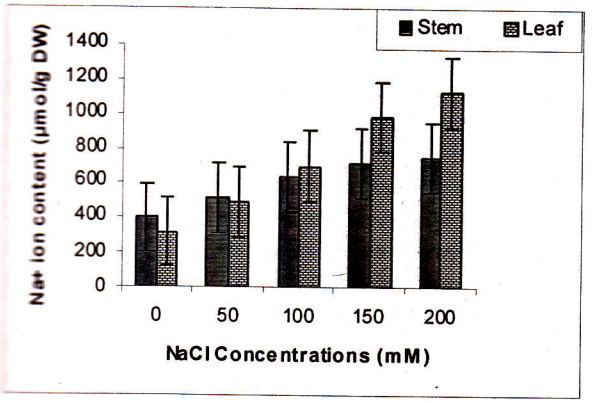

Fig.1a. Distribution of Na^+ in stem and leaves of *A.tricolor* 14 days after starting NaCl treatment.

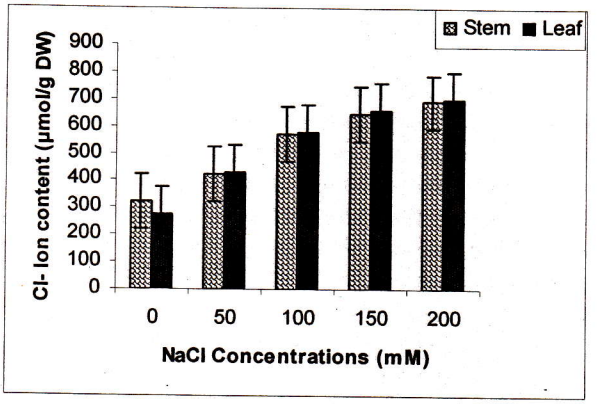

Fig. 1b. Distribution of Cl^- in stem and leaves of *A.tricolor* 14 days after starting NaCl treatment.

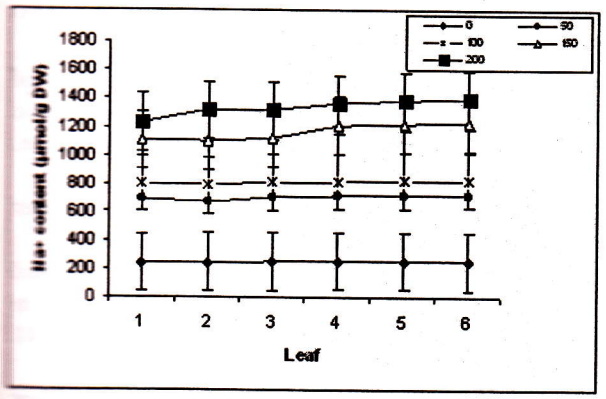

Fig.1c. Distribution of Na^+ in 1st - 6th leaves of *A.tricolor* 14 days after starting NaCl treatment.

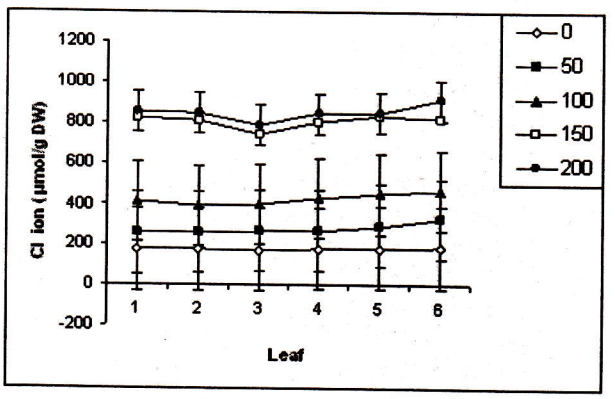

Fig.1d. Distribution of Cl^- in 1st - 6th leaves of *A.tricolor* 14 days after starting NaCl treatment.

particularly in the old leaves. Greater accumulation of these ions in the older leaves has been reported in several other plant species and this function is considered to be effective in avoiding huge accumulations of the toxic salts in the growing young leaves[22].

In some plant species, salt tolerance associates with the capacity of a species to accumulate proline, which acts as a compatible solute involved in osmotic adjustment at the plant cell level[3]. Proline accumulates in the cytoplasm without having any detrimental effects on cytosolic enzymes activities, while toxic ions, mainly Na^+, are sequestered in vacuole[23]. It has been suggested that the proline accumulation is due primarily to the function of both gene encoding Δ1-pyrroline-5-carboxylate reductase, and Δ1-pyrroline-5-carboxylate synthetase[3]. Hsiao *et al.*[24] reported that decreased protein synthesis and/ or increased protein hydrolysis in pearl millet seedling by salinity could lead to the accumulation of free amino acids and proline. In the present study, proline accumulation was observed in the roots and shoots of both species. However, at higher NaCl (100 and 150 mM) seedlings of *P. vulgaris* wilted or died; this suggests the amount of proline does not help reducing salt damage in this plant. On the contrary, these concentrations had no detrimental effect on the seedlings of *A.tricolor;* this indicates that higher proline accumulation may contribute to the alleviation of NaCl stress in the plant. Another compatible solute, glycinebetaine also acting as osmoprotectant between the cytoplasm and vacuole[25]. Furthermore, this compound can reduce lipid peroxidation and protect mitochondria electron transport reactions from salt damage[26]. Previous studies have reported that increased glycinebetaine contributed to overcome water and salt stress in leguminous plants[27]. Until now, the accumulation of glycinebetaine in *A.tricolor* has not been reported. For a better understanding the role of this compatible solute in osmotic maintenance in *A.tricolor,* further studies are warranted.

Accumulation of Na^+ and Cl^- in the old leaves appears to be part of the mechanism with which *A.tricolor* derives its salt tolerance. Other elements, P, Mg and Ca showed no noteworthy change in either two species. The higher accumulation of proline with increasing NaCl was found in the roots and shoots in both species. According to biomass production, the involvement of NaCl alleviation is considered only in *A.tricolor.*

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References

1. Kavi Kishor P B S, Sangam R N, Amrutha P, Sri Laxmi K R, Naidu K R, Rao S S, SreenathRao, Reddy K J, Theriappan P and Sreenivasulu N 2005, Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. *Curr. Sci.* **88** 3.
2. Jaleel C A, Manivannan P, Lakshmanan G M A, Sridharan R and Panneerselvam R 2007, NaCl as a physiological modulator of proline metabolism and antioxidant potential in Phyllanthus amarus. *Comptes Rendus Biologies.* **330** 806-813.
3. Delauney A J and Verma D P S 1993, Proline biosynthesis and osmoregulation in plants. *Plant J.* **4** 215-223.
4. Murashige T and Skoog F 1962, A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* **15** 473-497.
5. Chappelle E W, Kim M S and McMurtrey J E 1992, Ratio analysis of reflectance spectra (RARS): an algorithm for the remote estimation of the concentrations of chlorophyll a, chlorophyll b and carotenoids in soybean leaves. *Remote Sens. Environ.* **39** 239-247.
6. Desmaison A M, Marcher M H and Tixier M 1984, Changes in the free and total amino acid composition of ripening chestnut seeds. *Phytochemistry* **23** 2453-2456.
7. Moore S and Stein W H 1948, Partition chromatography of amino acids on starch. *Ann. N T Acad. Sci.* **49** 265
8. Bates L S, Waldern R P and Teare I D 1973, Rapid determination of free proline for water stress studies. *Plant and Soil* **39** 205-207.
9. Kim Y H, Shim I S, Kobayashi K and Usui K 1999, Relationship between Na content or K/Na ratio in shoots and salt tolerance in several Gramineous plants. *J. Weed Sci. Tech.* **44** 293-299.
10. Wongwattana C, Na Nakorn M, Surawattananon S and Kobayashi K 1998, Effect of sodiumchloride on growth and root nodulation in some leguminous plants. *J. Weed Sci. Tech.* **43** 129-133.
11. Gouia H, Ghorbal M H and Touraine B 1994, Effects on NaCl on flows of N and mineral ions and on NO_3^- reduction rate within whole plants of saltsensitive bean and salt-tolerant cotton. *Plant Physiol.* **105** 1409-1418.
12. Ma H C, Fung L, Wang S S, Altman A and Huttermann A 1997, Photosynthetic response of *Populus euphratica* to salt stress. *For. Ecol. Manage.* **93** 55-61.
13. Singh A K and Dubey R S 1995, Changes in chlorophyll a and b contents and activities of photosystems 1 and 2 in rice seedlings induced by NaCl. *Photosynthetica* **31** 489-499.
14. Dionisio-Sese M L and Tobita S 2000, Effects of salinity on sodium content and photosynthetic responses of rice seedlings differing in salt tolerance. *J. Plant Physiol.* **157** 54-58.
15. Zhu J K 2003, Regulation of ion homeostasis under salt stress. *Curr. Opin. Plant Biol.* **6** 441-445.
16. Scholander P F, Bradstreet E D, Hammel H T and Hemmingsen E A 1966, Sap concentrations in halophytes and some other plants. *Plant Physiol.* **41** 529-532.
17. Flowers T J 1985, Physiology of halophytes. *Plant Soil.* **89** 41-56.
18. Besford R T 1978, Effect of replacing nutrient potassium by sodium on uptake and distribution of sodium in tomato plants. *Plant Soil* **50** 399-409.
19. Tattini M, Leonardo Lombardini and Riccardo Gucci 1997, The effect of NaCl stress and relief on gas exchange properties of two olive cultivars differing in tolerance to salinity. *Plant and Soil.* **197** 87-93.
20. Clarkson D T and Hanson J B 1980, The mineral nutrition of higher plants. *Ann. Rev. Plant Physiol.* **31** 239-298.
21. Chavan P D and Karadge B A 1986, Growth, mineral nutrition, organic constituents and rate of photosynthesis in Sesbania grandiflora L. grown under saline conditions. *Plant Soil* **93** 395-404.
22. Wahome P K, Jesch H H and Grittner I 2001, Mechanisms of salt stress tolerance in two rose rootstocks: *Rosa chinensis* 'Major' and *R. rubiginosa. Sci. Hortic.* **87** 207-216.
23. Barkla B J, Zingarelli L, Blumwald E and Smith JAC 1995, Tonoplast Na^+/H^+ antiport activity and its

energization by the vacuolar H^+-ATPase in the halophytic plant *Mesembryanthemum crystallinum* L. *Plant Physiol.* **109** 549-556.

24. Eder A, Huber W and Sankhla N 1977, Interaction between salinity and ethylene in nitrogen metabolism of Pennisetum typhoides seedlings. *Biochem. Physiol. Pflanzen.* **171** 93-100.
25. Venkatesan A and Chellappan K P 1998, Accumulation of proline and glycine betaine in *Ipomoea pes-caprae* induced by NaCl. *Biol. Plant.* **41** 271-276.
26. Chen T H H and Murata N 2002, Enhancement of tolerance of abiotic stress by metabolic engineering of betaines and other compatible solutes. *Curr. Opin. Plant Biol.* **5** 250-257.
27. Girija C, Smith B N and Swamy P M. 2002, Interactive effects of sodium chloride and calcium chloride on the accumulation of proline and glycinebetaine in peanut (*Arachis hypogaea* L.). *Environ. Exp. Bot.* **47** 1-10.