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Ameliorating effect of triacontanol on salt stressed *Erythrina variegata* seedlings. Changes in composition and activities of chloroplasts

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Abstract

The detrimental effect of NaCl was found at the oxidation site of photosystem 2 (PS2). An impairment of PS2 was caused by damage of the oxygen evolving polypeptides (33, 23 and 20 kDa) of thylakoid membranes as well as by changes in the unsaturated and saturated fatty acids. Application of triacontanol, TRIA (1 mg kg^{-1}) ameliorated the effect of NaCl and promoted the ratio of unsaturated to saturated fatty acids, the rate of $^{14}CO_2$ fixation and the activity of ribulose 1,5-bisphosphate carboxylase.

Additional key words: $^{14}CO_2$ fixation, electron transport activities, polypeptides, ribulose-1,5-bisphosphate carboxylase activity, thylakoid lipids.

Introduction

The inhibitory effect of NaCl salinity on photosynthesis is exerted partly through a stomatal control mechanism (*e.g.* Downton *et al.* 1985, Seemann and Critchley 1985) and partly through effects on the capacity of CO_2 fixation (Yeo *et al.* 1985), reduced activity of ribulose 1,5-bisphosphate carboxylase (Stiborová *et al.* 1987), and inhibition of electron transport system (Gilmour *et al.* 1984). Recently, studies have been focussed on understanding the differential action of triacontanol [$CH_3(CH_2)_{28}CH_2OH$] and its second messenger L(+)-adenosine on physiological functions in plants (Ries 1991). TRIA promotes photosynthesis in *Erythrina*

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Abbreviations: BQ - p-benzoquinone; Chl - chlorophyll; DCPIP - 2,6-dichlorophenol indophenol; DPC - diphenyl carbazide; DTT - dithiothreitol; MV - methyl viologen; PS1, PS2 - photosystems 1 and 2; RuBPC - ribulose 1,5-bisphosphate carboxylase; TRIA - triacontanol.

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(Muthuchelian *et al.* 1994) and *Rhizophora* (Moorthy and Kathiresan 1993) seedlings. In this paper, we tried to elucidate the influence of TRIA on photosynthesis in salt-stressed seedlings of the leguminous tree species *E. variegata.*

Materials and methods

Plants: Seedlings of *Erythrina variegata* Lam. were grown at SSFRD Nursery Centre, Madurai Kamaraj University, Madurai [temperature 30 ± 5 °C, relative humidity 65 ± 5 %, maximum irradiance (PAR) 1800 μmol m^{-2} s^{-1}, photoperiod 13 h]. Forty day after sowing, the seedlings (five per pot) were divided into six equal groups. Plants in the first group (control) were maintained at soil moisture close to the field capacity throughout their growing period. Plants in the second and third groups were grown at two different concentrations of NaCl (100, 250 mM). Seedlings of the fourth group were sprayed with growth stimulator TRIA [1 mg $kg^{-1}(H_2O)$] (*NOCIL*, India) with *Tween 20* [0.1 % (m/v)] added as a surfactant by hand pump sprayer. Care was taken to wet both sides of the leaf. The fifth and sixth groups of seedlings were treated by NaCl (100 or 250 mM) and simultaneously were sprayed with TRIA.

Fatty acid analysis: Fatty acids were analysed by GLC at 180 °C using a *Girdel 30* gas chromatograph equipped with a flame ionization detector as described by Metcalfe *et al.* (1966).

Activities of electron transport: Chloroplasts were isolated from fresh leaves as described by Reeves and Hall (1973). PS1 and PS2 activities were measured as described by Noorudeen and Kulandaivelu (1982). Whole chain electron transport was measured according to Armond *et al.* (1978). The rate of DCPIP photoreduction was determined from the decrease in absorbance at 590 nm using a *Gilford 250* spectrophotometer. The reaction mixture (3 cm^3), unless otherwise mentioned, contained 20 mM Tris-HCl, pH 7.8, 100 mM saccharose, 50 μM DCPIP, and chloroplasts or PS2 particles equivalent to 20 μg of chlorophyll (Chl). Where mentioned, the concentrations of $MnCl_2$, DPC and NH_2OH were 5, 0.5 and 5 mM, respectively. Chl content was determined spectrophotometrically according to Arnon (1949).

$^{14}CO_2$ fixation: Leaves were circulated for 5 min under saturating (100 W m^{-2}) irradiance (40 W "white" fluorescent tubes) in 5 cm^3 of 50 mM KH_2PO_4-KOH buffer (pH 7.5) containing 50 mM $MgCl_2$, and 35 mM NaCl. 10 mM $NaH^{14}CO_3$ (1850 kBq) was injected into the reaction medium and irradiated for 30 min. The reaction was arrested by cold acetic acid (final concentration 10 %). The leaf segments were washed and ground in an incubation medium and the volume was made up to 3 cm^3. Aliquots of 10 mm^3 of the homogenate were loaded on to the *Whatman No. 1* filter paper discs and dried at room temperature under incandescent lamps. The radioactive carbon fixed was measured using the liquid scintillation counter (*Packard 2425*).

Extracts and assay of RuBPC: Fully expanded leaves were cut into small pieces and homogenized in a grinding medium of 50 mM Tris-HCl (pH 7.8), 10 mM $MgCl_2$, 5 mM DTT or 10 mM 2-mercaptoethanol, and 0.25 mM EDTA. The extract was clarified by centrifugation at 20 000 *g* for 10 min. The clear supernatant was decanted slowly and used as the crude RuBPC source. All these steps were carried out at 4 °C. RuBPC activity was determined by a modified method of Bowes and Ogren (1972). The incubation mixture contained 50 mM DTT and 10 mM $NaH^{14}CO_3$ (1850 kBq) in a total volume of 2.0 cm^3. The reaction mixture was placed in *Pyrex* tubes after flushing with N_2 for 3 min. Aliquots of 0.2 cm^3 of the enzyme extract were then injected through the serum cap into the mixture to initiate the reaction. After 3 min at 32 °C the reaction was stopped by injecting 0.2 cm^3 of 6 M glacial acetic acid. The known aliquots were transferred to *Whatman No. 3* filter paper discs, dried under infrared lamp, and the radioactivity was determined using *Packard 2425* liquid scientillation counter. Soluble proteins were estimated by the procedure of Lowry *et al.* (1951).

SDS-PAGE was performed as described by Laemmli (1970) using a polyacrylamide gradient of 8 - 16 % gel.

Results and discussion

TRIA induced differences in fatty acid profiles of thylakoid membranes of *Erythrina* grown under NaCl of 100 and 250 mM (Fig. 1). The amounts of short chain fatty acids C14:0 and C14:1 decreased by 37 and 55 %, respectively, under low salinity (100 mM NaCl). Contents of palmitic (16:0) and stearic (18:0) acids remained approximately the same in both salt stresses. Increase in the degree of saturation of membrane associated fatty acids may make the membrane less permeable to NaCl (Kuiper 1984). But the amount of palmitoleic acid (C16:1) decreased by 45 and 47 % in both salt stresses, whereas the decrease in linolenic acid (18:3) was parallel to the corresponding increases in linoleic (18:2) and oleic (18:1) acids. Similar changes have been found in other plant species in response to high concentrations of NaCl (*e.g.* Harwood 1984). The extensive change in thylakoid membrane fatty acid composition correlating with electron transport activitites is an essential part of the processes by which cells control membrane function and stability (Huflejt *et al.* 1990). Significant decrease in the ratio of unsaturated to saturated fatty acids in salt-stressed seedlings perhaps reflects depletion of unsaturated fatty acids by lipid peroxidation due to salinity (Chrominski *et al.* 1986). TRIA application rapidly increases the unsaturated fatty acid contents (18:1, 18:2, 18:3) which would help to maintain the thylakoid membrane in an appropriate state.

The overall photosynthetic electron transport and PS2 activity were significantly inhibited (24 and 43 %, respectively) by high salinity (250 mM NaCl). TRIA application reduced the inhibition by NaCl in both whole chain and PS2 activities (Table 1). Salt stress had little effect on PS1 activity.

Addition of DPC partially restored the inhibited PS2 electron transport in chloroplasts from salt stressed seedlings. Hydroxylamine (NH_2OH), an immediate

electron donor to PS2 reaction centre, restored the DCPIP reduction more efficiently in NaCl treated seedlings than in the control (Fig. 2). $MnCl_2$, an electron donor on the water oxidation site, was inefficient. Hence, the site of NaCl effect was probably

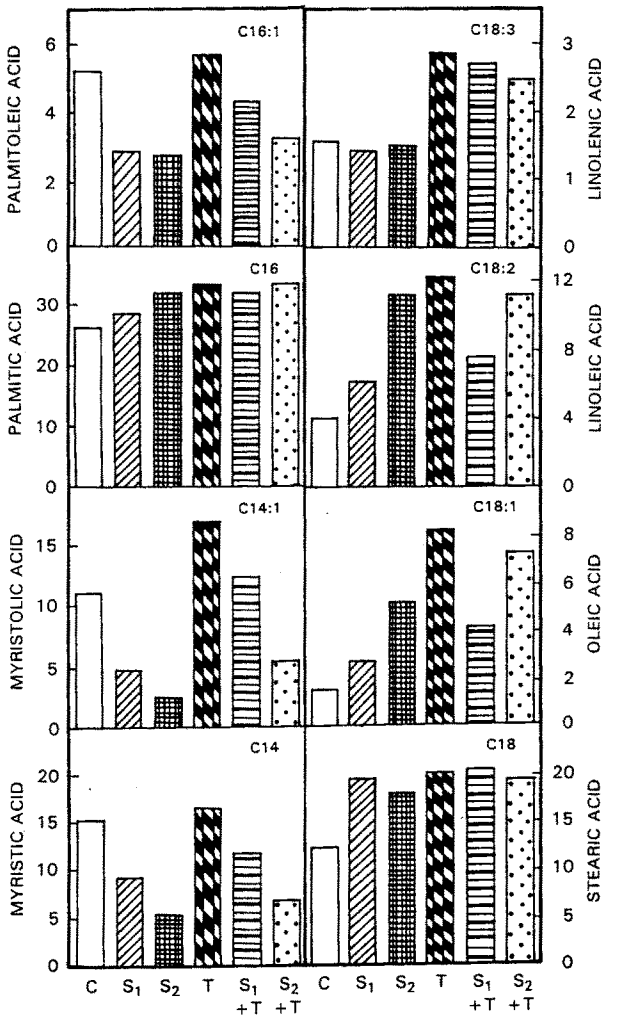

Fig. 1. Changes in the thylakoid fatty acid contents [mol %] of *Erythrina* seedlings treated with NaCl/triacontanol and deionized water (control). C - control, S_1 - 100 mM NaCl, S_2 - 250 mM NaCl, T - TRIA, S_1 + T - 100 mM NaCl + TRIA, S_2 + T - 250 mM NaCl + TRIA.

at the oxidation site of PS2 prior to the hydroxylamine donating site and perhaps enough close to alter the DPC donating site. Similar results have been observed in *Dunaliella* PS2 activity of which was inhibited by increasing the external concentration of NaCl (Gilmour *et al.* 1984).

Table 1. Effect of TRIA [1 mg $kg^{-1}(H_2O)$] on whole chain, PS2 and PS1 activities [nmol(O_2) kg^{-1} s^{-1}], $^{14}CO_2$ fixation and RuBPC activity [nmol(CO_2) kg^{-1}(protein) s^{-1}] in NaCl stressed *Erythrina variegata* seedlings (n = 3).

Treatments	Whole chain $H_2O \rightarrow MV$	PS2 $H_2O \rightarrow BQ$	PS1 DCPIP→MV	$^{14}CO_2$ fixation	RUBPC activity
Control	83	96	124	68	114
100 mM NaCl	71	73	119	48	91
250 mM NaCl	63	55	115	37	81
TRIA	102	125	138	86	147
100 mM NaCl + TRIA	75	83	128	58	112
250 mM NaCl + TRIA	68	68	119	50	92
LSD 5 %	1.68	1.19	1.45	1.51	1.19
LSD 1 %	2.35	1.66	2.04	2.12	1.67

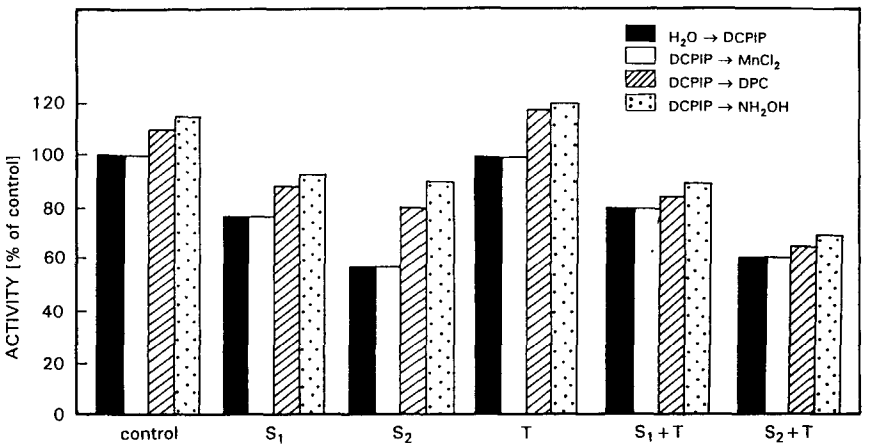

Fig. 2. Effect of various exogenous electron donors on PS2 activity ($H_2O \rightarrow$ DCPIP) in NaCl/triacontanol affected and control *Erythrina* seedlings. For symbols see Fig. 1.

Leaves collected from seedlings treated with TRIA showed additional evidence on structural changes in thylakoid polypeptides by SDS-PAGE analysis. In both the salt treatments, we found a specific loss in the contents of 55, 43, 33, 25, 23 and 20 kDa polypeptides (Fig. 3). Only marginal loss in the contents of polypeptides was found on salt-stressed TRIA treated seedlings. Both 43 and 25 kDa polypeptides belong to the reaction centre and LHCP of the PS2 complex, while the 33, 23 and 17 kDa polypeptides participate in photosynthetic O_2 evolution (Murata and Miyao 1987).

Marked changes were also observed in the RuBPC activity in leaf extracts when expressed on protein basis (Table 1). The reduction of CO_2 fixation (46 %) and RuBPC activity (29 %) under high salinity and the improvement by TRIA treatment were concomitant. The reduction in CO_2 fixation on the salt-stressed seedlings was

probably an indirect effect due to the destruction of photosynthetic pigments (Downton *et al.* 1985). It was also a consequence of stomatal closure (Seemann and Critchley 1985). The lowering of RuBPC activity by salinity may be attributed to proteolytic degradation of the enzyme (Swirshi and Gepstein 1985) or to competitive inhibition of RuBPC by anions (Seemann and Critchley 1985). TRIA application reduced the inhibition of RuBPC activity under salt stress. The increase in RuBPC activity by TRIA treatment may be due to increase in the substrate supply, the molecule of which gets bound to the enzyme site and increases the rate of reaction (Sharkey 1985). Similar increase in CO_2 fixation and RuBPC activity has been reported in *Erythrina* seedlings treated with TRIA (Muthuchelian *et al.* 1994).

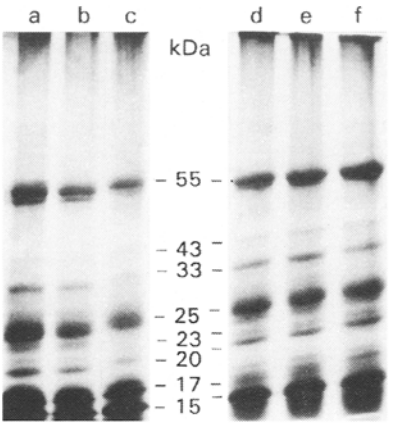

Fig. 3. Changes in the thylakoid polypeptides of *Erythrina* seedlings treated with NaCl/TRIA and deionized water (control). *a* - control, *b* - 100 mM NaCl, *c* - 250 mM NaCl, *d* - TRIA, *e* - 100 mM NaCl + TRIA, *f* - 250 mM NaCl + TRIA.

These observations indicate that the loss of photosynthetic capacity in the salt-stressed seedlings could be related to induced senescence, which in turn changes the overall photosynthetic electron transport, and to the reduction in unsaturated to saturated fatty acids ratio. The damaged thylakoid polypeptides could be restored by foliar application of TRIA.

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