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BRIEF COMMUNICATION

Ameliorating effect of triacontanol on salt stressed *Erythrina variegata* seedlings. Changes in growth, biomass, pigments and solute accumulation

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Abstract

Erythrina variegata Lam. seedlings were grown under low (100 mM NaCl) and high (250 mM NaCl) salinity. Seedlings exposed to high salinity for 10 d showed significant reduction in growth rate and biomass production while the root/shoot ratio increased. In contrast to pigment and protein contents, starch and saccharide contents increased in salt stressed seedlings. When the seedlings were subsequently sprayed with triacontanol (1 mg kg^{-1}) the salinity effect was partially ameliorated and growth, biomass, chlorophyll and carotenoid contents increased.

Additional key words: carotenoid, chlorophyll, leaf area, protein, relative growth rate, saccharide, starch.

Salinity may affect dry matter allocation, ion relations, water status, physiological processes, biochemical reactions, *etc.* (Greenway and Munns 1980). Decreased growth is often accompanied by reduced rate of photosynthesis, stomatal closure (Walker *et al.* 1983), reduced content of chlorophyll and Hill reaction activity (Reddy and Vora 1986). A common feature of plants, including legumes, is the accumulation of low molecular mass saccharides under salinity (Paul and Cockburn 1989).

Triacontanol [$CH_3(CH_2)_{28}$ CH_2OH] (TRIA) and its second messenger L(+)-adenosine possess a growth stimulating activity in plants (Ries *et al.* 1990). Most profound effect of TRIA is increase in growth, biomass, free amino acids,

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Abbreviations: Car - carotenoids; Chl - chlorophyll; TRIA - triacontanol.
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reducing saccharides and soluble proteins (Ries 1991, Muthuchelian *et al.* 1995). The purpose of the present study was to envisage the alleviating/ameliorating effect of TRIA on various growth and physiological characteristics of salt stressed *E. variegata* seedlings.

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]. 40 d 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.

Fully expanded leaves of plants were harvested 50 d after emergence and growth analysis components were calculated according to Radford (1967). Concentration of Chl, Car and soluble proteins were determined spectrophotometrically by the methods of Arnon (1949), Ridley (1977), and Lowry *et al.* (1951), respectively. Total saccharides was thoroughly extracted with boiling 80 % ethanol and estimated by the anthrone reagent method (Dubois *et al.* 1956). Soluble starch was extracted and its concentration determined following the method of McCready *et al.* (1950).

The major component reduced under salinity was shoot fresh matter which declined to approximately 29 % of the control at 250 mM NaCl (Table 1). Leaf area was reduced by 60 % in a pattern similar to that of the fresh matter of the roots. The root/shoot ratio increased markedly, being more than double of the control (Table 1). High salt stress caused a progressive reduction of specific leaf matter by 64 %, dry matter 46 % and relative growth rate 39 % as compared to the control (Table 1). This is in agreement with the report that the shoot fresh matter declined to 50 % at

Table 1. Effect of TRIA [1 mg $kg^{-1}(H_2O)$] on plant height [cm], leaf area [cm^2], shoot and root fresh matter (f.m.) [g], root/shoot ratio, leaf area index (LAI), relative growth rate (RGR) [g kg^{-1} d^{-1}], specific leaf matter (SLM) [g cm^{-2}] and plant biomass [g $plant^{-1}$] in NaCl stressed *Erythrina variegata* seedlings ($n = 10$).

Treatment	Plant height	Leaf area	Shoot f.m.	Root f.m.	Root/ shoot	LAI	RGR	SLM	Biomass
Control	57.3	0.42	9.5	4.2	0.44	2.06	0.033	0.0036	5.4
100 mM NaCl	44.6	0.27	5.0	3.3	0.66	0.92	0.027	0.0019	4.1
250 mM NaCl	34.0	0.17	2.8	2.6	0.92	0.48	0.020	0.0013	2.9
TRIA	71.0	0.68	13.0	6.0	0.46	3.73	0.044	0.0045	8.2
100 mM NaCl + TRIA	54.3	0.36	7.5	3.8	0.51	1.72	0.032	0.0029	5.1
250 mM NaCl + TRIA	43.0	0.28	4.2	3.1	0.73	1.34	0.028	0.0024	4.2
LSD 5 %	1.78	0.012	0.17	0.19	0.019	0.06	0.002	0.0004	0.23
LSD 1 %	2.49	0.016	0.24	0.26	0.028	0.08	0.003	0.0005	0.31

50 mM NaCl and the root fresh matter and root/shoot ratio are only marginally reduced even at 150 mM NaCl in *Phaseolus vulgaris* (Seemann and Critchley 1985) and in barley and maize (Stiborová *et al.* 1987).

The above mentioned growth rate inhibition by salinity was alleviated by TRIA application (Table 1). Our present findings are in accordance with those that showed TRIA induced increase in biomass content in *Rhizophora* (Moorthy and Kathiresan 1993), in leaf area, relative growth rate and specific leaf matter in *Erythrina* (Muthuchelian *et al.* 1995), and in opium plants (Srivastava and Sharma 1990).

Chl and Car contents in high salinity grown seedlings showed a reduction of 41 and 55 % respectively, than control seedlings (Table 2). Similar conclusions have been drawn in *Phaseolus* (Seemann and Critchley 1985) and spinach (Robinson *et al.* 1983) exposed to NaCl salinity. Reduction in total Chl contents was probably connected with enhanced activity of chlorophyllase (Reddy and Vora 1986). TRIA treatment reduced the inhibition of Chl by about 19 %. Such promotion was probably due to increase in the synthesis of both Chl *a* and Chl *b* (Muthuchelian *et al.* 1995).

Table 2. Effect of TRIA (1 mg kg^{-1}) on chlorophyll, carotenoid, leaf soluble protein, starch and sugar contents [g kg^{-1}(f.m.)] in NaCl stressed *Erythrina variegata* seedlings (n = 3).

Treatment	Chl	Car	Proteins	Starch	Saccharides
Control	1.132	0.361	19.5	13.7	3.08
100 mM NaCl	0.840	0.271	12.5	18.2	4.85
250 mM NaCl	0.673	0.162	6.3	20.5	6.17
TRIA	1.561	0.506	26.8	23.2	10.16
100 mM NaCl + TRIA	1.051	0.349	16.3	19.2	6.30
250 mM NaCl + TRIA	0.885	0.267	13.2	22.5	7.73
LSD 5 %	0.085	0.045	1.37	1.67	0.57
LSD 1 %	0.119	0.062	1.92	2.34	0.80

Salinity induced an increase in both saccharide and starch contents, at high salinity by 100 and 50 %, respectively (Table 2). TRIA treatment lead to further increase in contents of these substances. The difference was most expressed in control plants treated with TRIA. Fougere *et al.* (1991) found that the contents of total saccharides increased 2.1, 1.5 and 1.9 fold, respectively, in cytosol, bacteria and roots of alfalfa subjected to the salt treatment (0.15 M). TRIA application increases the amount of starch, sugar and leaf soluble protein in flooded *Erythrina* seedlings (Muthuchelian *et al.* 1995). Thus total soluble saccharides probably function as a compatible solute in cytosol of *Erythrina*, where they can very significantly contribute to the osmotic adjustment. Saccharides accumulate in leaves of salt stressed *Erythrina* seedlings (Greenway and Munns 1980, Rawson and Munns 1984). Hence the salinity induced inhibition of photosynthesis may be a consequence of an altered source-sink relationship inhibiting certain reactions of photosynthesis, rather than of ion excess directly affecting photosynthetic metabolism. Our experiments also confirmed that the application of TRIA partially ameliorated the salt effect and promoted various growth rates, biomass, Chl, Car and solute accumulations.

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