Organic.substances responsible for salt tolerance in *Eruca sativa*

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Abstract

Responses of a salt tolerant and a normal population of an oilseed crop, *Eruca sativa* Mill. were assessed after four weeks growth in sand culture salinized with 0 (control), 100, 200, or 300 mol m^{-3} NaCl. The salt tolerant plants produced significantly greater dry biomass than the normal population. The populations did not differ significantly in leaf osmotic potential, relative water content and leaf soluble proteins. However, the tolerant population accumulated significantly greater amounts of soluble sugars, proline and free amino acids in the leaves compared with the non-tolerant population. It is established that leaf soluble sugars, proline, and free amino acids are important components of salt tolerance in *Eruca sativa.*

Introduction

Taramira (*Eruca sativa* Mill.) is an important conventional oilseed crop of arid and semi-arid regions. The significance of the crop lies in its high degree of salt tolerance compared with other oilseed crops. The physiological/biochemical characteristics responsible for its salt tolerance are not known.

The vital role of organic osmotica such as proline, soluble sugars, free amino acids in salinity tolerance of different crops has been highlighted in a number of studies (*e.g.* Greenway 1973, Flowers *et al.* 1977, Maas and Nieman 1978, Greenway and Munns 1980, Dikshit and Pathak 1992). Of these the soluble sugars are considered as the principal organic osmotica in a number of glycophytes subjected to saline conditions (Maas and Nieman 1978, Greenway and Munns 1980). It has been found that sugars contribute up to 50 % of the total osmotic potential in glycophytes growing under natural environments (Cram 1976). Proline plays an important role in osmoregulation and acts as a nitrogen reserve in plants subject to stress conditions such as salinity and drought (*e.g.* Hsiao 1973, Rains 1981, Wyn Jones 1981), although a negative correlation between proline content and salt tolerance in *Glycine max* (Moftah and Michel 1987) and *Vigna mungo* (Ashraf 1989) has been observed.

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Similarly Ashraf and Naqvi (1992) could not find any positive relationship between the accumulation of sugars, free amino acids, soluble proteins, and proline and salt tolerance of four *Brassica* species subjected to varying Na/Ca ratios. Thus the major objective of the work reported here was to establish the role of soluble sugars, proline, free amino acids and soluble proteins in salinity tolerance of two *Eruca sativa* populations.

Materials and methods

Seed of a salt tolerant population of *Eruca sativa* Mill. was collected from a salt-affected field (EC_e 7.86, pH 7.5, SAR 9.6) and that of a normal line was obtained from a local seed supplier.

All seed samples were surface sterilized and sown in plastic Petri dishes. After two weeks ten seedlings of comparable size of each population were transplanted equidistant from each other into 24 cm size plastic pots containing 5.0 kg well washed and dried sand. The experiment was placed in a randomized complete block design in a glasshouse with 12 h photoperiod, and day/night temperature 26 ± 4/14 ± 3 °C. The salt treatments used were 0, 100, 200, and 300 mol m^{-3} of NaCl in full strength Hoagland nutrient solution (Epstein 1972).

Four weeks after the initiation of salt treatments all plants from each pot were harvested. Plant roots were removed carefully from the sand and then were washed in cold $LiNO_3$ solution containing 1 mol m^{-3} $Ca(NO_3)_2.4H_2O$ isotonic with the corresponding treatment in which the plants were growing.

Fresh masses of the shoots and roots were recorded. Shoot and root materials were dried at 70 °C for one week and dry masses recorded. Fully expanded youngest leaf was excised from each plant at 09.00. It was frozen for two weeks, after which time it was thawed and sap was extracted for osmotic potential determination in an osmometer (*Wescor 5500*). Relative water content was determined gravimetrically.

Proline was determined spectrophotometrically (spectrophotometer *Hitachi U-2000*) following the ninhydrin method described by Bates *et al.* (1973). Total soluble proteins were determined following Lowry *et al.* (1951). Total free amino acids were determined following Hamilton and Van Slyke (1943). Soluble sugars were determined following Malik and Srivastava (1979).

The results of all growth and physiological variables were subjected to two-way analysis of variance using a computer package *MStat 87*. The least significant differences (LSD) were calculated following Snedecor and Cochran (1980) for comparing mean values.

Results

The salt tolerant plants had significantly greater ($P < 0.05$) dry mass than the normal plants at all NaCl concentrations of the growth medium (Fig. 1).

The tolerant population accumulated significantly greater amounts of proline in the leaves (Fig. 1) than the non-tolerant population at 100 and 200 mol m^{-3} NaCl, whereas the populations did not differ significantly in proline content at the highest salt concentration (300 mol m^{-3} NaCl). Leaf soluble sugars (Fig. 1) increased significantly in the tolerant population with the increase in salt level, whereas in the non-tolerant population soluble sugars increased at 100 mol m^{-3} NaCl but thereafter they declined considerably. Thus the tolerant line had significantly greater ($P < 0.05$) leaf soluble sugars than the non-tolerant population at the highest salt treatment. Leaf free amino acids were greater in the tolerant population compared with those in the non-tolerant population at the two higher salt concentrations (Fig. 1).

The results for leaf soluble proteins, relative water content and leaf osmotic

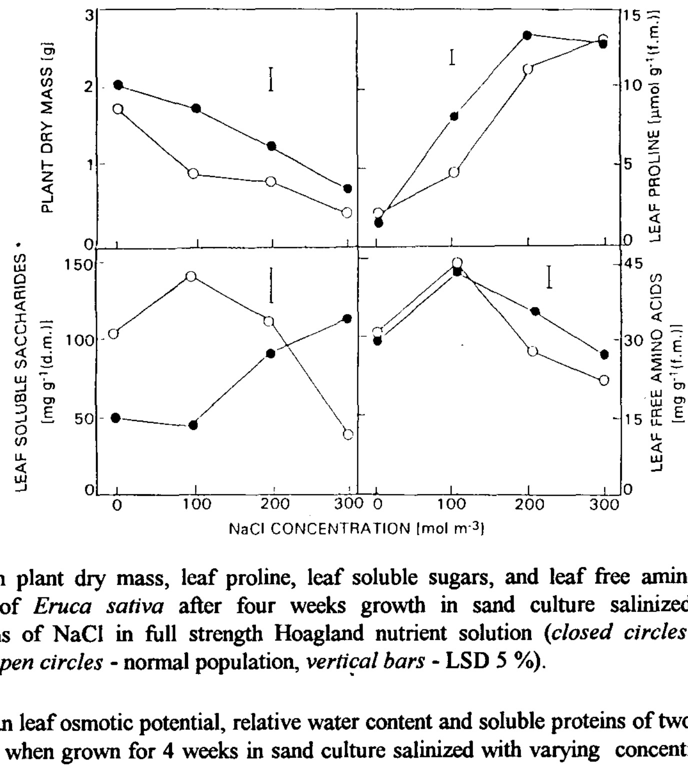

Fig. 1. Mean plant dry mass, leaf proline, leaf soluble sugars, and leaf free amino acids of two populations of *Eruca sativa* after four weeks growth in sand culture salinized with varying concentrations of NaCl in full strength Hoagland nutrient solution (*closed circles* - salt tolerant population, *open circles* - normal population, *vertical bars* - LSD 5 %).

Table 1. Mean leaf osmotic potential, relative water content and soluble proteins of two populations of *Eruca sativa* when grown for 4 weeks in sand culture salinized with varying concentrations of NaCl in full strength Hoagland nutrient solution.

NaCl concentration [mmol dm^{-3}]	Leaf osmotic potential [-MPa]		Relative water content [%]		Soluble proteins [mg g^{-1}(f.m.)]	
	tolerant	non-tolerant	tolerant	non-tolerant	tolerant	non-tolerant
0 (control)	0.85	0.93	93.4	82.0	2.68	2.21
100	1.19	1.14	83.1	87.9	2.69	2.96
200	1.41	1.39	80.2	82.2	3.00	2.19
300	1.77	1.84	93.7	95.4	2.06	2.09

LSD 5 % - non significant

potential presented in Table 1 show that the populations did not differ significantly for either parameter. However, leaf osmotic potential declined linearly in both populations with the increase in salt concentration of the growth medium. Leaf soluble proteins remained almost unchanged in both populations at all external NaCl concentrations.

Discussion

It has been established that plants subjected to saline conditions maintain their intracellular solute potential lower than that of the soil solution to avoid physiological drought (Maas and Nieman 1978, Greenway and Munns 1980) and salt tolerant plants are generally more capable of osmoregulation than non-tolerant plants (Ahmad *et al.* 1981, Rains 1981, Levitt 1980). Lack of difference between the two populations for leaf osmotic potential is thus surprising. Since other parameters of water relations such as leaf water potential and turgor potential were not measured in this study therefore a clear picture of osmoregulation in these populations is not possible to be drawn.

It is not possible to draw any parallel between leaf osmotic potential and the different organic osmotica determined in this study. For instance the tolerant population under saline conditions had considerably greater soluble sugars, proline and free amino acids in the leaves compared with the non-tolerant population. It is possible that reduction in some other organic or inorganic solutes that were not determined in this study, might have played role in maintaining the leaf osmotic potential of the tolerant population equivalent to that of the non-tolerant population. These results are in close conformity with those of Ashraf and Naqvi (1992) in which they could not find any relationship between leaf osmotic potential and different organic osmotica determined in four *Brassica* species subjected to varying Na/Ca ratios.

Lack of any significant effect of salt on the soluble protein content of the two populations of *Eruca sativa* does not support the early findings of Langdale *et al.* (1973) and Helal *et al.* (1975) that salinity enhance protein synthesis.

The results for high proline content of the tolerant population are in close agreement with a number of workers (Storey *et al.* 1977, Wyn Jones and Storey 1978, Rains 1981, Wyn Jones 1981, Ashraf and Naqvi 1992). The high concentrations of both free amino acids and soluble sugars in the leaves of the tolerant population compared with those in the non-tolerant ones are quite parallel to what was found in some salt tolerant accessions of *Lens culinaris* (Ashraf and Waheed 1993). Thus these two physiological variables coupled with proline could be used as selection criteria for salt tolerance in *Eruca sativa* as were proposed for *L. culinaris*.

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