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BRIEF COMUNICATION

# Salt-induced changes in two canola cultivars differing in salt tolerance

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## Abstract

Responses of 20 d-old plants of two *Brassica napus* L. cultivars Dunkeld and Cyclon to NaCl salinized soil [electrical conductivity 2.4 (control), 4.0, 8.0 or 12.0 dS $m^{-1}$] were examined. The salt tolerant line Dunkeld had significantly higher fresh and dry masses of shoots, and seed yield than salt sensitive line Cyclon in all salinities. The effect of salt stress on reduction in total leaf soluble sugars was markedly greater in Dunkeld as compared to that in Cyclon. No effect of salt stress was observed on leaf soluble proteins but there was a slight increase in total free amino acids of both cultivars. Leaf proline content increased markedly in both cultivars and Dunkeld had greater proline content than Cyclon at all salinities. Salt stress had no significant effect on seed oil content and erucic acid content of seed oil, however, content of glucosinolates in the seed meal increased and Cyclon had greater content of glucosinolates than Dunkeld.

*Additional key words*: *Brassica napus*, proline, soluble proteins, soluble sugars.

Salt tolerance in plants is a complex phenomenon, which depends on a number of inter-related factors based on morphological, biochemical and physiological processes (Greenway and Munns 1980, Jacoby 1999, Dash and Panda 2001, Arshi *et al.* 2002, Ashraf 2002). The organic solutes such as sugars, organic acids, polyols, and many nitrogen containing compounds such as amino acids, amides, imino acids, ectoine, proteins, and quaternary ammonium compounds have been found to be helpful in osmoregulation (Grumet *et al.* 1985) and in tolerance of toxicity of ions under salt stress (Greenway and Munns 1980). For example, it was investigated that NaCl salinity reduced the contents in total lipids, monogalactosyl-diacylglycerol, digalactosylglycerol, and phospholipids in *B. napus*. In contrast, the neutral lipid contents increased with increasing NaCl concentration (Najine *et al.* 1995).

Since the canola crop has been primarily developed for its low erucic acid in seed oil and low glucosinolates content in seed meal (Francois 1994), it may be possible that salinity of the growth medium may influence the contents of these two undesirable biochemical variables. Thus the effect of salt stress on these two biochemical variables in addition to some other organic compounds that play important role in salt tolerance was assessed in this study.

The seeds of two canola (*Brassica napus* L.) cultivars, Dunkeld (originally from Australia) and Cyclon (from Denmark) were obtained from the Oilseed Botanist, Ayub Agricultural Research Institute Faisalabad, Pakistan. All seed samples were surface sterilized with 10 % sodium hypochlorite solution for 5 min and washed three times with distilled water. The experiment was conducted in a glasshouse at the Ayub Agricultural Research Institute, Faisalabad, where the average photosynthetic photon flux density (PPFD) measured at noon ranged from 425 to 1360 μmol $m^{-2}$ $s^{-1}$, day/night relative humidity 58/74 % and temperature 24/8 °C. Twenty seeds were sown in pot with soil. The experiment comprised four treatments with four replications and two canola cultivars. At the seedling stage (20 d after germination) salt treatment was started by adding appropriate amount of NaCl in half strength Hoagland's nutrient solution to attain electrical conductivity 2.4 (control), 4, 8, and 12 dS $m^{-1}$. The salt was applied gradually in aliquot of 4 dS $m^{-1}$ every day.

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Every day 200 $cm^3$ of distilled water was added to each pot to compensate for evapotranspiration loss. This was sufficient to moisten the soil but did not cause leaching of salts from the pots.

Fifty days after the start of salt treatment two plants from each pot were harvested. The fresh leaf material was used to determine some of the organic compounds mentioned below. Plants were separated into shoots and roots and washed with distilled deionized water. After recording fresh mass, the plant samples were dried in an oven at 64 °C.

Total amino acids were estimated according to the method of Moore and Stein (1957). Fresh leaves (1.0 g) were chopped in 10 $cm^3$ of citrate buffer (pH 5.0) and incubated for 1 h at room temperature and centrifuged at 23 700 *g* at 15 °C for 10 min. The supernatant was separated and used to measure the total free amino acids with ninhydrin reagent. The absorbance was read at 570 nm on a spectrophotometer (*Model-220, Hitachi Instruments*, Tokyo, Japan). Total soluble proteins were determined as described by Lowry *et al.* (1951). 0.2 g of fresh leaf material was homogenized in 4 $cm^3$ of sodium phosphate buffer (pH 7.0) and centrifuged. The absorbance of the reaction solutions was read at 620 nm. Total soluble sugars of dry leaf tissues were determined following Yemm and Willis (1954). 0.1 g of well-ground dry leaf material was homogenized in hot 80 % ethanol and centrifuged at 2 900 *g*. The residue was retained and was repeatedly washed with 80 % ethanol to remove all the traces of soluble sugars. The filtrate thus obtained was used for the determination of soluble sugars. The extracts were treated with the anthrone reagent and absorbance was read at 625 nm.

Oil contents of the seeds were determined using NMR (*Nuclear Magnetic Resonance 4000, Oxford Analytical Instruments*, Newport, UK). The samples were run, at two point calibration (highest and lowest standards were selected as two point and other standards were checked on the agreement between NMR and calibration method). The sample holder used was of 40 $cm^3$. The programme mode was 1954 with the following specifications: Rf 200, amplification 300×, gate width 1.5 guall, analysis time 30 s, calibration time 60 s. Glucosinolates in seed meal were determined following Olsen and Sorensen (1981). Seed meal (200 mg) was taken into 7 $cm^3$ plastic scintillation vials containing steel rod. Myosinase activation buffer (3 $cm^3$) was added and vials were capped. Vials were shaken for 20 min and then 1 $cm^3$ charcoal suspension was added. Sample (0.01 $cm^3$) from each vial was taken into semi-micro cuvette, and centrifuged at 1 700 *g* for 10 min along with the standard solution of glucose. Then 1 $cm^3$ of coloured reagent (50 mg glucose oxidase + 75 mg horseradish peroxidase + 100 mg 4-aminoantipyrine + 500 mg phenol + 500 $cm^3$ pH 7.0 Myosinase activation buffer) was added to each cuvette and incubated at room temperature (25 °C) for > 45 min. Absorbance was read at 510 nm. For determining erucic acid of seed oil, a loop of oil was mixed with 1 $cm^3$ methylating solution and stirred, then allowed to stand at room temperature (25 °C) for 30 min. 1.5 $cm^3$ distilled water was added and mixed thoroughly. The upper layer of the sample (0.001 $cm^3$) was injected into gas chromatograph (*Perkin Elmer Model 3920*, Norwalk, USA) under the following conditions: column 3 mm × 2.1 m *Chromosorb WAW*, injector temperature 280 °C; detector temperature 280 °C; column temperature 230 °C; carrier 50 $cm^3$ $min^{-1}$ hydrogen.

Data for all the parameters were subjected to two-way analysis of variance following Steel and Torrie (1980). Differences between mean values were compared by the Least Significant Difference (LSD) test at 5 % level.

Fresh and dry masses of shoots, and seed yield of two canola cultivars differing in salt tolerance declined with increased soil salinity (Table 1). The salt tolerant line Dunkeld had significantly greater shoot mass and seed yield than those of the salt sensitive line Cyclon at all salinities. These results are in agreement with those of Qasim (2000) in which he ranked Dunkeld as the most salt tolerant and Cyclon the most salt sensitive of all eight cultivars of canola.

The effect of salt stress on total leaf soluble sugars was markedly greater in Dunkeld as compared to that on that of Cyclon since in the former line soluble sugars reduced considerably at the higher salinities. Overall, Cyclon had significantly greater content of leaf soluble sugars than Dunkeld (Table 1). Cram (1976) was of the view that of the various organic osmotica, sugars contribute up to 50 % of the total osmotic potential in glycophytes subject to salinity. However, in the present study Cyclon accumulated more soluble sugars in its leaves as compared to Dunkeld under salinity. These results are partially in line with the earlier findings of Ashraf and Fatima (1995) who reported that in safflower the pattern of accumulation of soluble sugars was different even within salt tolerant accessions. A similar pattern of sugar accumulation was found within some salt tolerant accessions of *Lens culinaris* (Ashraf and Waheed 1993).

Soluble proteins generally accumulate in plants grown under saline conditions and they may provide a storage form of nitrogen which is re-utilized when stress is over (Singh *et al.* 1987), and may play a role in osmotic adjustment. Proteins may be synthesized *de novo* in response to salt stress, or may be present constitutively in low concentration and are increased when plants are exposed to salt stress (Pareek *et al.* 1997). However, in the present study neither salt stress had any significant effect on the soluble protein content of both cultivars nor the cultivars differed significantly in this variable. These results are similar to those of Ashraf and Fatima (1995) who found that salt tolerant and salt sensitive accessions of safflower did not differ significantly in leaf soluble proteins. Similarly, comparison of salt tolerant wild populations with cultivated populations of *Melilotus*

Table 1. Fresh and dry masses of shoots, seed yield, and different organic compounds determined in leaf or seed of two cultivars of canola grown at different salinities. Electrical conductivity of soil 2.4 (control), 4.0, 8.0, and 12 dS $m^{-1}$. Means ± SE; those with the same letters within each column do not differ significantly at the 5 % level ($n = 4$).

| Parameter | Cultivar | 2.4 dS $m^{-1}$ | 4.0 dS $m^{-1}$ | 8.0 dS $m^{-1}$ | 12.0 dS $m^{-1}$ |
|---|---|---|---|---|---|
| Shoot fresh mass [g $plant^{-1}$] | Dunkeld | 44.60 ± 5.20 a | 32.20 ± 3.70 a | 29.40 ± 3.00a | 27.10 ± 4.30a |
| | Cyclon | 24.20 ± 3.40 b | 21.30 ± 2.30 b | 13.20 ± 2.60b | 8.40 ± 1.90b |
| Shoot dry mass [g $plant^{-1}$] | Dunkeld | 3.74 ± 0.42a | 2.36 ± 0.21a | 2.82 ± 0.26a | 2.22 ± 0.31a |
| | Cyclon | 1.92 ± 0.32b | 1.61 ± 0.06b | 0.94 ± 0.04b | 0.68 ± 0.05b |
| Seed yield [g $plant^{-1}$] | Dunkeld | 5.36 ± 0.61a | 4.78 ± 0.52a | 3.56 ± 0.42a | 3.24 ± 0.46a |
| | Cyclon | 3.64 ± 0.42b | 3.43 ± 0.39b | 2.76 ± 0.19b | 2.52 ± 0.36b |
| Leaf soluble sugars [mg $g^{-1}$(d.m.)] | Dunkeld | 41.30 ± 4.80a | 51.40 ± 3.90a | 30.50 ± 4.30a | 17.90 ± 2.70a |
| | Cyclon | 51.60 ± 4.40b | 56.60 ± 4.90b | 41.90 ± 5.10b | 46.80 ± 5.70b |
| Leaf soluble proteins [mg $g^{-1}$(d.m.)] | Dunkeld | 22.10 ± 1.07 | 21.40 ± 2.05 | 20.10 ± 2.56 | 20.90 ± 2.11 |
| | Cyclon | 25.90 ± 2.12 | 23.70 ± 2.14 | 22.40 ± 1.08 | 18.50 ± 2.07 |
| Leaf free amino acids [mg $g^{-1}$(d.m.)] | Dunkeld | 15.70 ± 1.54 | 19.10 ± 2.09 | 19.20 ± 1.43 | 25.90 ± 2.87 |
| | Cyclon | 15.30 ± 1.09 | 21.30 ± 2.12 | 25.20 ± 2.98 | 23.70 ± 2.13 |
| Leaf proline [µmol $g^{-1}$(d.m.)] | Dunkeld | 62.60 ± 5.30a | 132.40 ± 6.90a | 221.90 ± 10.1a | 407.20 ± 20.8a |
| | Cyclon | 55.40 ± 4.30a | 116.30 ± 5.40a | 247.90 ± 12.6b | 341.90 ± 24.3b |
| Seed oil content [% of seed m.] | Dunkeld | 41.10 ± 3.20a | 41.20 ± 4.30a | 41.20 ± 2.90a | 39.80 ± 3.40a |
| | Cyclon | 36.50 ± 2.90b | 35.20 ± 2.80b | 37.50 ± 3.20b | 36.70 ± 3.80a |
| Erucic acid [% of oil] | Dunkeld | 1.82 ± 0.04 | 2.22 ± 0.12 | 2.18 ± 0.06 | 2.24 ± 0.11 |
| | Cyclon | 1.79 ± 0.09 | 2.13 ± 0.07 | 2.04 ± 0.08 | 1.64 ± 0.09 |
| Glucosinolates [µg $g^{-1}$(seed meal)] | Dunkeld | 12.31 ± 1.03a | 14.22 ± 1.32a | 15.36 ± 1.69a | 16.42 ± 1.54a |
| | Cyclon | 16.42 ± 0.96b | 17.16 ± 1.24b | 21.43 ± 2.51b | 22.86 ± 2.02b |

*indica* and *Eruca sativa* (Ashraf 1993, 1994b) showed that the salt tolerant populations did not differ from salt sensitive populations in contents of soluble proteins in their leaves at varying salt concentrations in the growth medium.

Amino acids and amides have been reported to accumulate in higher plants under salinity stress (Dubey and Pessarakli 1995, Mansour 2000). Such pattern of accumulation of free amino acids was found in both the canola cultivars examined in this study, although the cultivars showed similar response to salt stress (Table 1). These results are not in agreement with some earlier studies in which it was found that salt tolerant cultivars/cultivars of different plant species had significantly higher total free amino acids in the leaves than those in salt sensitive cultivars, *e.g.*, sunflower (Ashraf and Tufail 1995), safflower (Ashraf and Fatima 1995), *Melilotus indica* (Ashraf 1993), *Eruca sativa* (Ashraf 1994b), and *Lens culinaris* (Ashraf and Waheed 1993).

Of the different amino acids, proline accumulates in large amounts in salt stressed plants (Rains 1989, Ashraf 1994a, Ali *et al.* 1999). Similarly, in the present investigation, salt stress caused an increase in leaf proline content of both cultivars, but Dunkeld accumulated significantly higher amount of proline in the leaves as compared with Cyclon (Table 1). The role of this amino acid in enzyme and membrane stabilization has been reported (Hanson and Burnet 1994, Gadallah 1999). Although the role of proline in osmoregulation has been questioned, its concentration in most salt tolerant plants has been found to be higher than that in salt sensitive ones. For example, Petrusa and Winicov (1997) found that salt tolerant alfalfa plants rapidly doubled their proline content in the roots, whereas in salt sensitive plants such response was slow. Similar results were also reported in alfalfa by Fougere *et al.* (1991). *In vitro* studies with *Brassica juncea* showed that salt adapted calluses had high accumulation of free proline compared with the non-stressed calluses (Madan *et al.* 1995, Gangopadhyay *et al.* 1997).

Glucosinolates content of the seed meal of both cultivars increased significantly with increase in salinity, and Cyclon had more glucosinolates than Dunkeld at all NaCl concentrations (Table 1). Although there was no significant effect of salt stress on seed oil content, Dunkeld had significantly greater oil content as compared with Cyclon (Table 1). Erucic acid content of seed oil was not affected due to salt stress in both cultivars and the cultivars also did not differ significantly in this variable (Table 1). Similar resutls have been reported by Francois and Kleiman (1990), who observed that increased salinity did not significantly affect the erucic acid content of the seed oil in crambe.

Based on all biochemical attributes measured in this study, it can be concluded that high accumulation of proline in leaves and low accumulation of glucosinolates in seed meal are important adaptive components of salt tolerance in the two canola cultivars examined.

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