BIOLOGIA PLANTARUM 48 (3): 461-464, 2004

BRIEF COMMUNICATION

Influence of salt stress on growth, ion accumulation and seed oil content in sweet fennel

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Abstract

A greenhouse experiment was conducted to assess the effect of 25, 50, 75, and 100 mM NaCl on growth, ion accumulation, seed yield, and seed oil content in 67-d-old plants of *Foeniculum vulgare* Mill. Increasing NaCl concentration caused a significant reduction in fresh and dry masses of both shoots and roots as well as seed yield. Na^+ and Cl^- in both shoots and roots increased, whereas K^+ and Ca^{2+} decreased consistently with the increase in NaCl concentration. Plants maintained markedly higher Ca^{2+}/Na^+ ratios in the shoots than those in the roots, whereas that of K^+/Na^+ ratios remained almost uniform in both shoots and roots. Proline content in the shoots increased markedly at the highest NaCl concentration. Oil content in the seed decreased progressively with increase in salinity.

Additional key words: *Foeniculum vulgare*, medicinal plant, proline, salinity.

Fennel (*Foeniculum vulgare* Mill.) is an important medicinal plant. The essential constituent of fennel fruit is a volatile oil (3 - 4 % in best cultivars; Bentely and Trimen 1999). Although fennel occurs worldwide, it is widely grown in arid and semi-arid regions where high concentration of salts is an important characteristic of the soils (Ashraf 2002, Munns 2002, Qasim *et al*. 2003). Keeping this in mind the present study was carried out to assess the response of this crop to salt stress.

The experiment was carried out in the netting house supplied with natural sunlight in the Botanic Garden, University of Agriculture, Faisalabad, Pakistan, from October 2002 to May 2003. The average day and night temperatures were 29 ± 9 °C and 15 ± 7 °C, respectively, average relative humidity ranged from 41 to 69 %, and natural photoperiod from 10 to 12.5 h. The experiment was laid out in a completely randomized design with four replications. The salt treatments used were: 0 (control), 25, 50, 75, and 100 mM NaCl. The earthen pots of 30 cm diameter lined with polythene sheets were filled with 9.0 kg of well washed pure river sand. Six plants of a land race of *Foeniculum vulgare* were raised in each pot. Full strength Hoagland's nutrient solution was applied every week until 67th day of germination before the start of salt treatments. NaCl treatment in Hoagland's nutrient solution was initiated step-wise in an aliquot of 25 mM every day.

Two plants were harvested from each pot 46 d after the start of NaCl treatments. Plants were uprooted carefully and washed with distilled water. After recording the fresh masses of both shoots and roots, they were oven-dried at 65 °C for one week and dry masses recorded. Remaining four plants were used for recording seed yield.

Proline in fresh shoot samples was determined according to Bates *et al.* (1973). Fresh leaves (1.0 g) were homogenized in 10 cm^3 of 3 % sulfosalicylic acid and the homogenate filtered. The filtrate (2.0 cm^3) was reacted with 2.0 cm^3 of glacial acetic acid, and then with 4.0 cm^3 of toluene. Absorbance was read at 520 nm using a spectrophotometer (*Hitachi-220*, Tokyo, Japan). To determine ions in shoots and roots the dried ground material (0.1 g) was digested with sulphuric acid and hydrogen peroxide according to the method of Wolf (1982). Cations such as Na^+, K^+ and Ca^{2+} were determined with a flame photometer (*Jenway PFP7*, *Gransmore Green*, Dunmow, UK). For the determination of Cl^-, shoot and root samples of 100 mg were ground and extracted in 10 cm^3 of distilled water by heating at 80 °C for 3 h. Cl^- content in the extracts was determined

Received 19 January 2003, *accepted* 15 April 2004.
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with a chloride meter (*Sherwood Chloride Analyzer 926*, Tokyo, Japan).

At maturity all plants were harvested and seed yield per plant was recorded for each salt treatment. For the extraction of oil from seed, the dried seeds were ground to a uniform powder and oil was extracted in *n*-hexane. A weighed quantity (15 g) of the seed powder was placed in an extraction thimble and extraction was carried out for 6 h in a Soxhlet extractor, after which time the oil containing solvent was filtered through *Whatman No. 42* filter paper. The oil was heated in an oven at 60 °C to remove last traces of the solvent.

Analyses of variance of data for all attributes were computed using the *COSTAT* computer package (*Cohort Software*, Berkeley, USA). The mean values were compared with the least significant difference test (LSD) following Snedecor and Cochran (1980).

Salt stress applied for 46 d to 67-d-old plants of *F. vulgare* decreased fresh and dry masses of both shoots and roots consistently with increase in NaCl concentration of the growth medium (Table 1). Maximum reduction in biomass was observed at 100 mM NaCl. Salt stress also caused a marked reduction in seed yield per plant (Table 1). Such an adverse effect of salt stress on the growth and seed yield has earlier been observed in a number of crops, *e.g.*, alfalfa (Serraj and Drevon 1988, Esechie *et al*. 2002), carrot (Gibberd *et al.* 2002), and kalonji (Hajar *et al*. 1996). However, the reduction in seed yield at varying NaCl concentrations was higher than that in vegetative growth: 50 % reduction in biomass occurred at 90 mM of NaCl and that in seed yield at 86 mM of NaCl. In view of Maas and Hoffman (1977) this crop can be categorized as moderately salt sensitive.

Salt stress significantly ($P < 0.001$) increased Na^+ and Cl^- concentrations of both shoots and roots (Table 1). The concentration of both ions in shoots and roots increased with increasing NaCl concentration and they were maximal at 100 mM NaCl (Table 1). However, concentration of Na^+ was higher in the shoots than that in the roots. A consistent decrease in concentrations of K^+ and Ca^{2+} in shoots and K^+ in roots was found with increasing concentration of NaCl in the rooting medium, and maximum reduction in both ions was again observed at 100 mM NaCl (Table 1). Such pattern of accumulation of the toxic ions has earlier been reported in a number of plant species referred to as salt accumulators (Greenway and Munns 1980, Munns *et al*. 2000). It has been generally observed that plants exposed to NaCl, take up high amounts of Na^+, whereas the uptake of K^+ and Ca^{2+} is significantly reduced. However, reasonable amounts of both K^+ and Ca^{2+} are required by plants to maintain the integrity and functioning of cell membranes (Marschner 1995, Davenport *et al*. 1997, Wenxue *et al*. 2003). The underlying phenomenon for maintenance of adequate

Table 1. Growth, seed oil, shoot proline content, and nutrient concentrations of *Foeniculum vulgare* when 67-d-old plants were subjected for 46 d to varying concetrations of NaCl in sand culture. Means ± SE, means with the same letters within each row do not differ significantly at the 5 % level.

Attributes	0	25	50	75	100	LSD 5%
Shoot fresh mass [g plant^{-1}]	33.71 ± 4.13a	28.44 ± 6.10ab	25.14 ± 1.93ab	20.32 ± 1.83bc	12.58 ± 0.95c	10.65
Shoot dry mass [g plant^{-1}]	1.89 ± 0.37a	1.73 ± 0.17a	1.42 ± 0.01ac	1.18 ± 0.06bc	0.74 ± 0.08b	0.50
Root fresh mass [g plant^{-1}]	1.89 ± 0.27a	1.42 ± 0.19b	1.25 ± 0.11bc	0.98 ± 0.01cd	0.76 ± 0.03d	0.42
Root dry mass [g plant^{-1}]	0.57 ± 0.01a	0.35 ± 0.02b	0.29 ± 0.01c	0.17 ± 0.01d	0.06 ± 0.01e	0.05
Seed yield [g plant^{-1}]	4.49 ± 0.41a	3.41 ± 0.39b	3.16 ± 0.22b	2.62 ± 0.20b	1.68 ± 0.22c	0.80
Seed oil [%]	4.35 ± 0.06a	3.64 ± 0.55ab	2.44 ± 0.35bc	0.99 ± 0.07c	0.85 ± 0.12c	1.84
Shoot proline [mg g^{-1}(f.m.)]	0.51 ± 0.11a	0.86 ± 0.05b	0.57 ± 0.06a	0.86 ± 0.10b	1.08 ± 0.17b	0.28
Shoot Na^+ [mg g^{-1}(d.m.)]	12.00 ± 1.26a	23.80 ± 2.89b	28.80 ± 1.23b	30.25 ± 2.11b	39.50 ± 4.74c	7.24
Root Na^+ [mg g^{-1}(d.m.)]	10.70 ± 1.06a	13.20 ± 2.19a	23.00 ± 4.48b	27.50 ± 2.63bc	31.20 ± 3.27c	7.72
Shoot Cl^- [mg g^{-1}(d.m.)]	15.60 ± 1.75a	20.80 ± 1.67a	35.05 ± 3.67b	51.00 ± 5.19c	58.55 ± 6.42c	10.93
Root Cl^- [mg g^{-1}(d.m.)]	13.30 ± 3.23a	18.76 ± 0.76ab	21.80 ± 1.33b	22.00 ± 4.08b	23.04 ± 1.95b	6.74
Shoot K^+ [mg g^{-1}(d.m.)]	58.75 ± 2.50a	53.12 ± 4.13ab	46.87 ± 3.41b	35.00 ± 1.17c	33.75 ± 3.00c	7.99
Root K^+ [mg g^{-1}(d.m.)]	27.20 ± 1.79a	50.80 ± 7.50b	41.65 ± 2.65b	29.25 ± 1.25a	26.70 ± 4.43a	10.94
Shoot Ca^{2+} [mg g^{-1}(d.m.)]	15.25 ± 1.09a	15.00 ± 2.52a	13.13 ± 1.44ab	9.50 ± 1.69b	8.75 ± 1.80b	4.64
Root Ca^{2+} [mg g^{-1}(d.m.)]	5.72 ± 1.12a	5.25 ± 1.57a	5.00 ± 1.22a	6.00 ± 0.52a	3.87 ± 0.54a	2.82
Shoot K^+/Na^+ ratio	5.06 ± 0.73a	2.33 ± 0.39b	1.62 ± 0.07c	1.17 ± 0.07d	0.87 ± 0.03d	0.32
Root K^+/Na^+ ratio	2.56 ± 0.15a	3.98 ± 0.71b	1.93 ± 0.42c	1.09 ± 0.11d	0.89 ± 0.18d	0.33
Shoot Ca^{2+}/Na^+ ratio	1.31 ± 0.17a	0.67 ± 0.18b	0.45 ± 0.04c	0.31 ± 0.04d	0.22 ± 0.02e	0.10
Root Ca^{2+}/Na^+ ratio	0.52 ± 0.09a	0.41 ± 0.13b	0.26 ± 0.1c	0.22 ± 0.03c	0.13 ± 0.03d	0.07

K^+ in plant tissue under saline conditions seems to be dependent upon selective K^+ uptake and selective cellular K^+ and Na^+ compartmentation and distribution in the shoots (Munns *et al*. 2000, Carden *et al.* 2003).

As expected, there was a consistent decrease in K^+/Na^+ and Ca^{2+}/Na^+ ratios in the shoots and roots of *F. vulgare* with increase in NaCl concentration of the rooting medium (Table 1). Plants maintained considerably higher Ca^{2+}/Na^+ ratios in the shoots as compared to those in the roots, whereas K^+/Na^+ ratios in both shoots and roots were similar. High K^+/Na^+ selectivity in plants under saline conditions is considered as one of the important selection criteria for salt tolerance (Gorham *et al*. 1997, Ashraf 2002, Wenxue *et al*. 2003). At 100 mM NaCl the shoot and root K^+/Na^+ ratio was less than 1, a minimum level suggested for the normal functioning of most mesophytes under saline conditions (Wyn Jones 1981).

The change in leaf proline concentration was not consistent up to 75 mM, but there was a sharp rise in its concentration at 100 mM NaCl (Table 1). Such rise in proline content in the shoots of *F. vulagre* shows the positive role of proline in the salt tolerance of this crop, inasmuch as proline is known to contribute to membrane stability (Hanson and Burnet 1994, Gadallah 1999), and mitigates the effect of NaCl on cell membrane disruption (Mansour 1998). In addition, proline may act as a signaling/regulatory molecule able to activate multiple responses that are component of the adaptation to abiotic stresses including salt stress (Maggaio *et al*. 2002). The results reported here for proline can be explained in view of some earlier reports that proline accumulation is one of the common characteristics in many plants under saline conditions (Storey *et al.* 1977, Wyn Jones and Storey 1978). Increase in proline due to salinity has also been reported in some medicinal plants, *e.g*., black cumin (Hajar *et al*. 1996), anise and coriander (Zidan and Elewa 1995).

There was a progressive decrease in seed oil content with increasing salt level of the rooting medium, and the maximum reduction in oil was found at 100 mM of NaCl (Table 1).

Overall, *F. vulgare* is a moderately salt sensitive crop whose response to salinity is associated with a considerable decrease in K^+/Na^+ and Ca^{2+}/Na^+ ratios.

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