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VEGETATIVE GROWTH PERFORMANCE OF FIVE MEDICINAL PLANTS UNDER NaCl SALT STRESS

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Abstract

Seeds of *Lepidium sativum* L., *Linum usitatissimum* L., *Nigella sativa* L., *Plantago ovata* Forssk, and *Trigonella foenum-graecum* L. were grown in pots containing loamy soil with 0.21 (Control) 5.0, 7.5, 10.0, 12.5, or 15.0 dS/m concentration of NaCl to see their salinity tolerance.

Various concentrations of salt had a highly significant effect upon the survival %age, plant height, number of branches, shoot fresh and dry weight, root fresh and dry weight and root moisture contents. Number of leaves also varied significantly. However, leaf length and shoot moisture contents exhibited non-significant differences. Differences among the test species for all the parameters under consideration were also highly significant.

The findings suggest that the test species are tolerant to moderate salinity i.e., 7.5 dS/m and might be tried on saline soils to obtain some biomass.

Introduction

Salinity adversely reduces the overall productivity of plants including crops by inducing numerous abnormal morphological, physiological and biochemical changes that cause delayed germination, high seedling mortality, poor crop stand, stunted growth and lower yields. Out of 20.2 million hectares of cultivated land in Pakistan, 6.8 million hectares are affected with some degree of salinity (Anon., 2002). Generally screening and cultivation of crops on the salt affected soil is the main approach. Germination and seedling growth under saline environment are the screening criteria which are widely used to select the salt tolerant genotype (Ashraf *et al*., 1990; Khan *et al*., 1993). Medicinal plants are important source of medicines and livelihood. They are either collected from natural habitat or imported. Salted soils that can not support crops might be used for medicinal plants as novel crops.

Some work on the effect of salinity on germination and growth of medicinal plants include *Linum usitatissimum* (Ashraf & Fatima, 1994; Ashraf & Tufail, 1995; Beke & Graham, 1995), *Plantago* spp. (Boss, 1992; Tanczos *et al*., 1992), *Trigonella foenum–graecum* (Ali *et al*., 1992), *Nigella sativa* (Hajar *et al*., 1996), *Helianthus annuus* (Ashraf & Tufail, 1995; Ashraf *et al*., 2003; Mutlu & Bozcuk, 2007), *Glycine max* (Umezawa *et al.*, 2000; Essa 2002), *Brassica spp.* (Ashraf & Naqvi, 1997; Farhandi & Sharif Zadah, 2006; Gul & Ahmad, 2007; Ulfat *et al.*, 2007; Jenagand *et al.*, 2008), *Ricinus communis* (Raghavaiah, 2002, 2006). It appears that little information is available regarding the effect of salinity on the growth and productivity of medicinal plants. *Lepidium sativum* L., *Linum usitatissimum* L., *Plantago ovata* Forssk and *Trigonella foenum-graecum* L. have been evaluated and proved to be moderately salt tolerant at germination and seedling growth stage (Muhammad & Hussain, 2009). The present study was focused to assess the tolerance of these medicinal plants towards salinity at their vegetative growth. The findings might help in utilizing saline habitats. The successful cultivation of medicinal plants will provide raw material to pharmaceutical companies and for local medicinal uses.

Materials and Methods

Seed of *Plantago ovata* Forssk, *Lepidium sativum* L., *Trigonella foenum-graecum* L., *Linum usitatissimum* L. and *Nigella sativa* L. were obtained from the local market. They were grown in pots salinized with 0.21 (Control), 5.0, 7.5, 10.0, 12.5, or 15.0 dS/m NaCl concentration. There were four replicates for each treatment. Each pot had 5 Kg loamy soil lined with polyethylene bags to avoid salt leaching. The soil used had 8.75% $CaCO_3$, 0.65% organic matter, 0.032% nitrogen, 20.9 ppm phosphorus, 413.5 ppm potassium, 7.6 pH level, 0.21 dS/m electrical conductivity, 0.067% total soluble salts, 5.2% clay, 17.0% silt and 77.8% sand.

Generally, it took 3-4 days for the first emergence of seedlings and within 6-7 days the germination completed in all the species. However, *Nigella* took 14 days to germinate and more than 25 days for the maximum possible germination. Seedlings were thinned to 10 individuals/pot after one month of the maximum germination for each species. Uniform equi-distant and healthy seedlings were left in each pot. Pots were brought to the desired salinity levels by providing 2.5 dS/m salinity levels in 6 equal increments. Salinity was measured with EC meter.

The pots were kept in the net house of Botany Department, University of Peshawar under uniform open environmental conditions. They were maintained at field capacity and protected from rain or dew by polyethylene sheets. Growth parameters such as height of plants, number of leaves, number of branches or tillers and leaf length were recorded at the time of the harvest. Weekly data for the plant survival was also recorded after salt treatment. Crops were harvested when matured. Roots were carefully taken out of soil. Fresh weight of shoots and roots were determined. They were oven dried at 65^oC for 72 hours for dry weight determination.

The moisture contents of shoots and roots were determined on oven dry basis (Hussain, 1989). The results were subjected to ANOVA (Steel & Torie, 1980) and significant differences were accepted at $p = 0.05$.

Results

1. Effect on survival of seedlings: Highly significant differences were observed in the survival % age under different salt treatments and among the species (Table 1). In control the survival was 100%, which decreased gradually with increasing concentration of salt. The differences were non-significant up to 10.0 dS/m salt concentration thereafter the differences were statistically sound at 12.5 dS/m (59.2%) and 15.0 dS/m (33.8%). *Lepidium* exhibited significantly higher survival % age (89.5%), closely followed by *Trigonella* (83.3%) and *Plantago* (83.0%) showing insignificant differences. Survival rate in *Linum* (68.7%) and *Nigella* (68.2%) was statistically at par with each other but significantly different from the aforementioned species (Table 2).

2. Effect on plant height and branching/tillering: ANOVA revealed highly significant differences in the plant height under various applied salt levels and between the test species (Table 1). The plant height was significantly greater in control (40.56 cm), which was closely followed by 5.0 dS/m (31.44 cm). The plant height decreased gradually with every increasing level in the salt concentration reaching to minimum of (7.26 cm) at the highest dose. *Lepidium* exhibited maximum height (32.32 cm), that was respectively followed by *Linum* (26.80 cm), *Trigonella* (23.07 cm), *Nigella* (20.62 cm) and *Plantago* (16.22 cm) (Table 3).

ANOVA showed highly significant differences in the average number of branching both under different salt treatments and between the test species (Table 1). The number of branching/tillering gradually decreased with increasing salt concentration. They were maximum (7.58) in control and minimum (1.86) at 15.0 dS/m treatment. The differences were insignificant within the range of 5.0 to 12.5 dS/m treatments. The number of branches in all the treatments in *Linum* (10.92) was significantly greater than *Plantago* (4.10), *Lepidium* (3.92), *Trigonella* (3.80) and *Nigella* (3.78), which were statistically at par with each other (Table 4).

3. Effect on the number and length of leaves: The number of leaves varied significantly under various salt concentrations and among the species (Table 1). The number of leaves at 7.5 dS/m level (47.14) differed insignificantly from control (45.34) but varied significantly from 15.0 dS/m salt treatment. The number of leaves in *Linum* (78.43) differed significantly from *Trigonella* (33.98), *Nigella* (23.17), *Plantago* (22.13) and *Lepidium* (18.05), which differed non-significantly from each other (Table 5).

Statistically non-significant differences were observed for average leaf length under various salt concentrations but the differences between the test species were highly significant (Table 1). The leaf length showed a declining trend with increasing level of salt concentration as it was maximum at control (33.98 mm) and the minimum (25.80) at 15.0 dS/m. *Nigella* had the maximum leaf length (43.67 mm) in all the salt treatments, which was significantly higher than *Trigonella* (32.78 mm), *Lepidium* (28.22 mm), *Linum* (20.72 mm) and *Plantago* (15.75 mm) (Table 6).

4. Effect on fresh and dry weight of shoot: Differences in the fresh and dry weight of shoots were highly significant both under various levels of salinization and among the species (Table 1). The shoot fresh weight was maximum (2874.4 mg) in control. that decreased gradually to a minimum of 416.0 mg at 15.0 dS/m. Average shoot fresh weight in all the treatments for *Trigonella* (2335.0 mg), *Plantago* (2054.7 mg) and *Linum* (1794.2 mg) differed insignificantly among themselves, while, shoot fresh weight in *Nigella* (743.7 mg) and *Lepidium* (578.8 mg) were statistically at par with each other however, but varied significantly from the aforementioned species (Table 7).

Average dry weight of shoot for all the tested species was maximum (951.0 mg) in control that dwindled to 172.5mg at 15.0 dS/m linearly with increasing salt stress. *Plantago* had the highest weight (780.0 mg), differing significantly from *Lepidium* (298.3 mg) and *Nigella* (201.7 mg) (Table 8).

5. Effect on fresh and dry weight of roots: The differences in fresh and dry weight of roots were highly significant in various salt treatments and among the species (Table 1). The fresh weight of roots was maximum in control (3426.0 mg). It reduced considerably under salt stress, especially under higher concentration, yet the effect was non-significant within various salt concentrations. The fresh weight of *Linum* (3350.0 mg) was significantly higher in all the treatments followed by *Plantago* (2067.9 mg). The root fresh weight in *Nigella* (819.6 mg), *Trigonella* (503.3 mg) and *Lepidium* (502.9 mg) were statistically at par with each other but differed significantly from *Linum* (Table 9).

The dry weight of roots in all the species was significantly greater in control that reduced gradually with increasing salt level with a minimum at 15.0 dS/m. The root dry weight in all the treatments was significantly higher in *Linum* (516.3 mg) which was statistically at par with *Plantago* (347.1 mg), quickly followed by *Nigella* (155.0 mg), *Lepidium* (115.4 mg) and it was the minimum in *Trigonella* (106.1 mg) (Table 10).

6. Effect on shoot and root moisture contents: The differences in the shoot moisture contents in various treatments were non-significant but were highly significant among the species (Table 1). Shoot moisture contents dwindled variously under different levels of salinity. However, it boosted at 10.0 dS/m and higher level of salt. Average moisture contents in all the treatments was significantly higher in *Nigella* (512.7%) as compared to *Trigonella* (240.6%), *Linum* (233.0%), *Plantago* (161.9%) and *Lepidium* (91.0%) (Table 11).

Moisture contents of roots showed highly significant differences under various salt treatments and between species (Table 1). Plants in control (471.4%) had the highest moisture contents that declined steadily under salt stress especially at 15.0 dS/m level (273.5%). Moisture contents in *Linum* (524.7%) were significantly higher and were statistically at par with *Plantago* (505.9%). The root moisture contents in *Lepidium* (325.0%), *Trigonella* (320.8%) and *Nigella* (298.8%) showed non-significant differences among themselves but varied significantly from *Linum* and *Plantago* (Table 12).

Discussion

Salt stress reduces plant growth and productivity by affecting morphological, anatomical, biochemical and physiological characteristics, processes and functions. Disturbed water and nutritional balance of plants may cause reduced crop yield in saline soil. Reduced plant height and other morphological characters are the most distinct and obvious effects of salt stress. Depressed growth due to high salinity is attributed to several factors such as, water stress, specific ion toxicity and ion imbalance stress or induced nutritional deficiency. In the present study, plant height of all the test species decreased at higher salinity levels. Plants growing at 15.0 dS/m were stunted as compared to the control. The reduced plant height might be attributed to the direct effect of excess salt on plant tissues and poor intake of minerals. Reduced plant height under saline conditions has been observed in *Linum usitatissimum* (Singh & Singh, 1991; Kheir *et al*., 1991). Mamo *et al*., (1996) reported reduced plant height in chickpea variety in response to salinity. Essa (2002) reported significantly reduced plant height in soybeen by soil salinity. Mensah *et al*., (2006) reported that plant height tended to decrease with increasing salinity in *Arachis hypogaea*. Sadat-Noori (2006) also reported reduced plant height in wheat under different saline conditions. All these workers support the present findings.

Salt tolerance at vegetative stage is crucial for yielding vigorous plants for tolerating salt stress at later stages of growth. The observed reduced degree of branching at higher salt concentration directly affects the productivity, biomass and seed yield. Decreased branching due to salt stress in different oil seed crops (Singh *et al*., 1988; Narash *et al*., 1993; Mensah *et al*., 2006; Sadat-Noori, 2006) has been reported and our results agree with them.

The number of leaves and leaf length got suppressed in all the test species under higher salinity regimes. Generally, leaf thickness increases under salt stress, which decreases leaf area. Letchamo *et al*., (1993) reported significant decrease in the number of leaves of *Passiflora edulis* under high salinity and our findings agree with them. Likewise, Singh & Singh (1991) observed a considerable reduction in the leaf length of *Linum* under high NaCl stress. Leaf area is a good indicator of water and salinity stress, since leaf expansion generally requires a high turgor pressure for cell enlargement (Krieg, 1983). It is well accepted that osmotic adjustment plays a crucial role in plant adaptation to drought (Quisenberry, 1982). Salinity induced osmotic stress is considered responsible for the reduced leaf area in Canola and wild mustard (Huang & Redmann, 1995). Hajar *et al*., (1996) and Murillo-Amador & Trovo-Dieguez (2002) also reported decreased leaf area under increasing salinity levels in *Nigella sativa* and cowpea, respectively. Our findings agree with the previous workers in this respect.

Reduced fresh weight of shoots under test conditions was noticed in all the test species, except *Lepidium* at 12.5, *Linum* at 10.0, and *Trigonella* at 7.5 dS/m. Fresh weight of roots also dwindled significantly especially at higher salt treatments in all the test species except *Trigonella* at 7.5 and 12.5 dS/m. The reduced fresh weight of shoots and roots could be attributed to low absorption of water from the growth medium as a result of physiological drought (Hussain & Ilahi, 1992). The slight increase in fresh weight of shoots and roots at certain salt concentration might be due to the development of succulence (Ilahi & Hussain, 1990). Bharti & Singh (1994) reported declined fresh weight of roots of *Sesamum indicum* under saline conditions. The present findings are also in line with Hajar *et al*., (1996) who reported decreased fresh weight of shoots and roots in *Nigella sativa* at higher salinity levels. Furthermore, Yagmur *et al*., (2007) has also reported decreased fresh weight in wheat under saline conditions.

Salinity significantly inhibited the growth and resulted in a decrease of dry weight of both shoots and roots of all the tested plants under high salt concentration which could be attributed to the adverse lowering of osmotic potential. The present findings agree with those of Hajar *et al*., (1996) and Mamo *et al*., (1996) who reported decreased dry weight of shoots and roots under increasing salinity levels in *Nigella sativa* and chickpea, respectively. Significantly reduced shoot dry weight have also been reported in cowpea (Murillo-Amador & Trovo-Dieguez, 2000), *Glycine max* (Essa, 2002) and *Lens culinaris* (Turan *et al*., 2007). Moreover, Iqbal *et al*., (2005), Sadat-Noori (2006) and Yagmur *et al*., (2007) recorded reduced dry matter in wheat under increasing salt stress, which also strengthen our findings.

Moisture content plays an important role in the growth and development of plants. The present study shows that shoot moisture contents of all the tested species enhanced with increasing salinity especially at higher levels. Root moisture contents in *Linum* and *Plantago* were significantly greater than *Lepidium, Nigella* and *Trigonella*. In the present study there was a non-significant increase in shoot moisture contents while, root moisture contents decreased significantly with increasing level of salinity which is in contrast to Ibrar & Hussain (2003) who reported enhanced root moisture contents with increasing level of salinity in *Medicago polymorpha*.

The tested species exhibited reasonably good survival percentage up to 10.0 dS/m application of salt. The survival of seedlings declined variously at high salt application. The observed resistance order regarding survival could be *Lepidium* (95%) > *Plantago* (90%) > *Trigonella* (80%) > *Nigella* (75%) > *Linum* (67%). On the basis of survival rate *Linum* and *Nigella* could be regarded as moderately tolerant while *Lepidium, Plantago* and *Trigonella* could be ranked as highly tolerant to salinity. The findings of the present study agree with those of El-Nakhlaway & El-Fawal (1989) who reported that *Linum usitatissimum* was least tolerant to different salinity levels. Ashraf & Fatima (1994) and Ashraf & Tufail (1995) screened linseed and sunflower accessions respectively for salt tolerance and established a great deal of variation for salt tolerance in these species. High or low salt tolerance at germination or vegetative stage in various other medicinal plants like *Cajnus cajan* (Ashraf, 1994), *Nigella sativa* (Hajar *et al*., 1996), *Cicer arietinum* (Mamo *et al*., 1996), *Brasica* sp.(Ashraf & Naqvi, 1997), *Glycine max* (Essa, 2002), *Helianthus annuus* (Ashraf *et al*., 2003), *Medicago polymorpha* (Ibrar & Hussain, 2003), *Ricinus communis* (Raghavaiah *et al*., 2002; 2006), *Arachis hypogaea* (Mensah *et al*., 2006), *Lens culinaris* (Turan *et al*., 2007) and canola cultivers (Jamagard *et al*., 2008) have also been reported at different degree of salinization.

The results indicate the existence of genetic potential for salt tolerance in these medicinal species under field conditions and they are tolerant up to 10 dS/m salinity level during the vegetative stage of growth. The findings suggest that the response of these species to salt stress depends on the species and concentration of the applied salt. Focusing at the survival percentage, growth and biomass production of the test species, it is suggested that the tested plants could be tried on moderately saline habitat. It will not only help in the utilization of the unproductive saline habitats but also provide raw matter for medicinal concerns. Salt tolerant legumes like *Trigonella* can also improve soil fertility through biological nitrogen fixation.

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