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# IN VITRO SALINITY TOLERANCE SCREENING OF SOME LEGUMES AND FORAGES CULTIVARS

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## ABSTRACT

The increasing water demand for dmestic and industrial water users in the water-scarce Mediterranean region is effecting the availbility of fresh water supplies for agriculture. Whereas much research has been done on the effect of irrigation with low-quality water on saline tolerant species, little is known about the salinity tolerance of legume and forage cultivars. The salinity tolerance of 200 lentil cultivars(*Lens culinaris*), 205 chickpea cultivars (*Cicer arietinum*) and five tall fescue cultivars (*Fescua Arundinacea* Schreb.) was tested in vitro, using four different salinity levels (0.5, 4, 7,10 dS/m). A second screening was done after pre-soaking the seeds for 24 hours in water (0.5 dS/m). The effect of X-ray (96 Kv, 200 mas,/sec, 5 sec interval) on the germination of lentil under these conditions was also tested. In addition, 21 promising lentil varieties were tested in sand-filled pots in the greenhouse.

Average germination of the 200 lentil cultivars varied between 3% for the dry seeds at an EC of 10 dS/m to 59% for the pre-soaked seeds at an EC of 0.5 dS/m. For the 205 chickpea varieties germination varied between 40% for the dry seeds at an EC of 10 dS/m to 84% for the pre-soaked seeds at an EC of 0.5 dS/m.The experiment indicated that the chickpea cultivars were more saline tolerant than the lentil and tall fescue cultivars. The X-ray treatment had no clear effect on the germination and shoot growth of lentil. Results of the top 50 cultivars of each screening test indicated that even for the more resistant cultivars shoot growth becomes severly impaired at an EC of 4 dS/m and higher. In the greenhouse, 8 of the 21 pre-soaked lentil cultivars survived at an EC of 7 dS/m. The tall fescue cultivars did not survive the saline water treatment (4 dS/m and above). This study indicated that the chickpea cultivars were more saline tolerant than the lentil and tall fescue cultivars.

Keywords: salt tolerance, shoot growth, chickpea, lentil, tall fescue

## INTRODUCTION

The limited water resources in arid and semi arid areas form a major constraint for agricultural and socio-economic development (Hamdy., et al., 2002). In the Mediterranean region, increasing demands from domestic and industrial water users are restricting agricultural expansion. To achieve both water and food security we have to find alternative strategies. One of the options is to increase the use of marginal quality water for irrigation to solve this problem. Marginal quality water could include treated sewage effluent, recycled drainage water, or brackish groundwater. These water resources often have an elevated chloride content. The main management issues involved in using saline water are the control of soil salinity by adequate leaching and drainage, and the selection of crops adapted to the salinity level of the soil.

The use of saline water for irrigation of various food, fuel and fodder crops has been reported by Epstein (1983, 1987), Glenn and O'Leary (1985), Jyengar (1982), Hamdy et al. (1993), Katerji et al. (1997, 2001a, 2001b) and others. Among crop species, legumes are considered salt sensitive (Maas and Hoffman, 1977). Within the legumes, chickpea, faba been and pea are particularly salt sensitive (Maas and Hoffman, 1977; Lauchli, 1984). Lockerman et al. (1983) indicated that faba been may have greater salinity tolerance than the other cool-season food legumes. Food and forage legumes improve soil health and could reduce crop pests and diseases when grown in rotation. These crops are also important protein sources.

Crop salt tolerance depends primarily on (i) morphological features of the plant, (ii) uptake and transport of salt, and (iii) physiological and metabolic processes at the cellular level (Winicov,1993).

Differences in salt tolerance exist not only among different genera and species, but also within certain species. For example, there are reports on the response to salinity of different varieties of chickpea (Katerji et al., 2001a; Gandour, 2002); barley (Touchan and Coons, 1991; Flowers and Hajibagheri, 2001), lentil (Katerji et al., 2001b), and maise (Abdel-Samed and Azooz, 2002) Tall fescue (Wu et al,1996; Leskys et al, 1999). Much less information is available about the effect of X-ray on germination and seedling growth (e.g., Khanna, 1990; Eianev ,1986 for wheat and Abejec ,2003 for barley). Abejec (2003) found that the use of a low dose of X-ray increases field germination, as opposed to the high dose. Hinkovski (1987) reported that increasing the X-ray (10-15 Kr) increased the vegetative growth and the seed yield of alfalfa. Shoot growth, however, was lower for F1X than for F1. This could have been caused by an increase in auxine rate (Khanna, 1990).

Improvement of germination and early growth depend on the dose of mutagen, on the percentage of the morphological variations and on the genetic abnormality. Increasing the mutagen doses increased the genetic abnormality and the number of sterile plantes (Kumar and Shauban, 1979). However, the use of low dose of mutagen chemicals activates the seeds longevity and their tolerance to biotic and abiotic stress (Demchenko, 1987).

The objective of the study was to identify legume and forage cultivars that are tolerant to salinity and to develop an understanding of the mechanisms that affect this tolerance. The effect of X-ray on lentil germination was also studied.

## MATERIALS AND METHODS

During the 1999-2001 seasons, 200 lentil cultivars, 205 chickpea cultivars, and five tall fescue cultivars were screened for salinity tolerance in plastic dishes in the laboratory (in vitro). The seeds were tested at four salinity levels: 0.5 (control), 4, 7, and 10 dS/m. Groundwater with an EC of 0.5 dS/m was mixed with NaCl until the required salinity level was obtained. No salt was added for the control (EC0.5). For chickpea and tall fescue, five seeds were applied per dish. For the lentil cultivars 10 seeds were used. All dishes were regulary filled with water to counter-balance evatranspiration. Each dish received the same amount of water. The seeds were checked for germination every other day. The length of the shoots was measured around day 14 after germination. The relative humidity in the laboratory varied between 33 and 66%; the temperature generally varied between 20 and 30 $^0$C.

The experiments included two phases: seeds that were pre-soaked for 24 hours in groundwater (F1) and dry seeds (F2). The lentil seeds were also treated with X-ray (96 KV, 200 mas, 1 sec shot, 5 sec interval). The screening tests of the X-rayed seeds are referred to as F1X and F2X. After the initial screening, 20 to 40 chickpea cultivars that performed well in the laboratory were tested in the greenhouse. Five seeds were put in 15-cm diameter plastic pots( top diameter of 19.5 cm and a depth of 18 cm) filled with approximately 3 kg sand. Every other day 40 ml water of the selected EC-level was added.

The in-vitro screening of lentil was conducted in April 2000 for phase 1 (F1) and in May, 2000 for phase 2 (F2). The in-vitro screening of the X-rayed lentils was carried out in July and August, 2000. The greenhouse study started on January 4, 2001, with 21 varieties for Phase 1 (F1G) and on March 28, 2001 with 19 cultivars for Phase 2 (F2G).

Phase 1 of the chickpea screening started in the laboratory on January 7, 2001. Phase 2 of the chickpea screening was conducted in February. The in-vitro experiment was repeated with two replicates for the 40 cultivars that were selected for the greenhouse pilot study. Chickpea seeds were planted in the greenhouse in April, 2001 (Phase 1 only). The observations in the greenhouse continued until crop maturity at the end of June. For the Phase 1 greenhouse study (F1G), seven cultivars from the top 10, and 14 cultivars ranking between 11 and 70 were selected. Five seeds of each variety were planted on January 4, 2001. Ten days after planting.

The in-vitro experiment for the tall fescue started on June 26, 2001. Three replicates of fives seeds each were observed.Germination of the seeds was checked for 82 days. Lengths of shoots were measured weekly during 108 days.

A scoring system was used to rank the varieties of each in-vitro screening test for their tolerance to salinity. The aggregated score was computed as:

$$Score = G_{0.5} * L_{0.5} + 2 (G_4 * L_4) + 3*(G_7 * L_7) + 4*(G_{10} * L_{10})$$

where $G_i$ is the number of germinated seeds, $L_i$ is the average length of the shoots of the germinated seeds, and i is the EC-level. The highest score obtained rank number 1, the second highest score rank number 2, and so on and so forth.

## RESULTS AND DISCCUSION

### *Lentil*

Average germination of the 200 lentil cultivars varied between 3% for the dry seeds (F2) at an EC of 10 dS/m to 59% for the pre-soaked seeds (F1) at an EC of 0.5 dS/m (Table 1). The pre-soaking improved germination and shoot growth, especially at the higher salinity levels. The effect of the X-ray treatment on the lentils varied per cultivar. On average, the X-ray had no positive effect on the germination and shoot growth of the pre-soaked seeds. The X-rayed dry seeds (F2X) performed better than the dry seeds without X-ray treatment (F2).

Table 1. Average percentage germination and shoot length of lentil cultivars(at 14 days after germination) under four different treatments and four salinity levels.

| | Germination (%) | | | | Average shoot length (cm) | | | |
|---|---|---|---|---|---|---|---|---|
| | EC0.5 | EC4 | EC7 | EC10 | EC0.5 | EC4 | EC7 | EC10 |
| 200 cultivars | | | | | | | | |
| F1 | 59 | 58 | 51 | 39 | 15.3 | 10.3 | 6.3 | 3.1 |
| F1X | 54 | 50 | 42 | 38 | 9.5 | 6.8 | 3.3 | 1.7 |
| F2 | 48 | 28 | 16 | 3 | 8.1 | 2.6 | 0.5 | 0.0 |
| F2-X | 57 | 47 | 21 | 3 | 8.4 | 3.7 | 0.7 | 0.1 |
| Top 50 cultivars | | | | | | | | |
| F1 | 82 | 81 | 76 | 68 | 19.5 | 14.7 | 10.5 | 5.1 |
| F1X | 93 | 85 | 81 | 74 | 16.0 | 12.1 | 7.3 | 4.7 |
| F2 | 76 | 54 | 31 | 7 | 12.7 | 5.9 | 1.2 | 0.1 |
| F2X | 91 | 85 | 52 | 9 | 12.8 | 8.3 | 1.7 | 0.4 |

The average germination percentage and shoot lengths of the top 50 cultivars of each screening test are also summarized in Table 1. These results indicate that even for the more resistant cultivars shoot growth becomes severely impaired at ECs of 4 dS/m and higher. Interestingly, the top 50 cultivars of the pre-soaked seeds that were treated with X-ray (F1X) had higher germination percentages than the pre-soaked seeds without X-ray treatment (F1). This result conform the findings of Eianev (1986) for wheat and Abejec (2003) for barley. Hinkovski (1987) reported that increasing the X-ray (10-15 Kr) increased the vegetative growth and the seed yield of alfalfa. Shoot growth, however, was lower for F1X than for F1. This could have been caused by an increase in auxine rate (Khanna, 1990).

Increased germination of irradiated seed could be due to the induction role of X-ray in increasing the hydrolysis enzyme such as lipase, protease, B-amylase, and x-amylase, which could accelerate the nutrient reserves' transformation from a complex state to a simple state; and the transport of these reserves to the embryon, which will accelerate the division and elongation of the embryon (Kuzin, 1980). This induction could also cause differences in seeds hormone equilibrium, which will increase the activation hormones such as gebrillic acid and auxine, in comparison with the inhibitors hormones. Grodzinski (1989) reported that the X-ray treatment could activate physical and biochemical processes.

To examine the overall resistance of the tested lentil cultivars against salinity, the top 10 of the four EC levels screening tests were compared. Varieties that occured in more than one top 10 and their associated rankings are sumarized in Table 2. The table also indicate if the varieties were tested in the greenhouse studies.

Table 2. Rankings of the lentil cultivars that occurred in the top 10 of more than one screening test.

| Screening | ILL8010 | ILL8008 | ILL7686 | ILL7520 | ILL2815 | ILL2501 | ILL4402 | ILL7554 | ILL3597 |
|---|---|---|---|---|---|---|---|---|---|
| F1 | 12 | 5 | 13 | 50 | 2 | 6 | 10 | 1 | 166 |
| F1X | 4 | 11 | 9 | 1 | 32 | 24 | 16 | 2 | 3 |
| F2 | 3 | 11 | 18 | 5 | 2 | 1 | 8 | 65 | 7 |
| F2X | 4 | 1 | 6 | 2 | 24 | 34 | 36 | 3 | 51 |
| Total | 23 | 28 | 46 | 58 | 60 | 65 | 70 | 71 | 227 |
| Greenhouse | F2G | | F1G,F2G | | F1G,F2G | F1G,F2G | F1G,F2G | F1G | F2G |

Because not all seeds had germinated, a total of 78 seeds were added on January 16. On January 31, the average length of the shoots varied from 12.5 (at EC 4 dS/m) to 9.7 cm (at EC 10 dS/m). On day 76, all cultivars at the lower two EC levels had survived. The average length of the shoots was 16.1 cm at EC 0.5 dS/m and 15.6 at EC 4 dS/m. At an EC of 7 dS/m only eight cultivars survived, ILL1712, ILL2501, ILL 2815, ILL4402, ILL7521, ILL7522, ILL7553, and ILL7620. Cultivar ILL7521 was also the only surviving cultivar at and EC of 10 dS/m. Of the eight surviving cultivars, ILL2501, ILL2815, and ILL4402 were identified as tolerant cultivars during the initial screening (Table 2).

The greenhouse study for the dry seeds (F2G) included eight cultivars from the top 10 and 11 cultivars that ranked between 11 and 60 during the in-vitro screening of the dry seeds (F2). The seeds were planted on March 28, 2001. After 62 days all varieties had died at the highest three salinity levels. The lenghts of the shoots irrigated with water with an EC of 0.5 dS/m varied between 10 and 22 cm. On day 71 four of the 19 cultivars had also died at the lowest salinity level. Of the six cultivars that were identified in Table 2, Cultivar ILL2501, ILL2815, ILL3597, ILL4402, and ILL7686 survived.

**Chickpea**

Germination of the 205 chickpea cultivars varied between 40% for the dry seeds (F2) at an EC of 10 dS/m to 84% for the pre-soaked seeds (F1) at an EC of 0.5 dS/m (Table 3). As with the lentils, the pre-soaking improved germination and shoot growth at the higher salinity levels. For the top 50 cultivars the length of the shoots of the pre-soaked seeds at the highest salinity level was less than half of the length of the shoots at an EC of 0.5 dS/m. Without pre-soaking (F2) there was almost no shoot growth at the two highest salinity levels. Seed germination was the least affected growth stage. The primary growth stage was slightly affected at a low salt concentration. However, this effect increased with increasing salt concentrations. Similar results were found by Katerji et al. (2001b ) and Gandour (2002).

Table 3. Average percentage germination and shoot length of lentil cultivars under four different treatments and four salinity levels.

| | Germination (%) | | | | Average shoot length (cm) | | | |
|---|---|---|---|---|---|---|---|---|
| | EC0.5 | EC4 | EC7 | EC10 | EC0.5 | EC4 | EC7 | EC10 |
| 205 cultivars | | | | | | | | |
| F1 | 84 | 79 | 71 | 55 | 13.1 | 13.4 | 8.1 | 4.8 |
| F2 | 83 | 74 | 64 | 40 | 16.1 | 4.6 | 0.2 | 0 |
| Top 50 cultivars | | | | | | | | |
| F1 | 94 | 85 | 88 | 73 | 17.2 | 18.2 | 12.7 | 7.8 |
| F2 | 94 | 83 | 81 | 54 | 21.8 | 9.9 | 0.5 | 0 |

Five cultivars ranked in the top 20 of both the F1 and the F2 screening test (Table 4). Of the 40 cultivars that were tested in the greenhouse, FLIP 97-266, FLIP 98-100, FLIP 98-107, FLIP 98-131, FLIP 98-162, FLIP 98-169 performed the best.

Table 4. Rankings of the chickpea cultivars that occurred in the top 40 of both screening tests.

| | FLIP 87-59 | FLIP 97-158 | FLIP 97-205 | FLIP 97-259 | FLIP 98-128 |
|---|---|---|---|---|---|
| F1 | 2 | 3 | 10 | 18 | 19 |
| F2 | 16 | 17 | 12 | 4 | 8 |
| Total | 18 | 20 | 22 | 22 | 27 |

**Tall fescue**

At the lower EC levels (0.5 and 4 dS/m), pre-soaking of the seeds did have little effect on the germination, (Table 5), except for *Centurion* and *Lunibelle* thid result is conform the finding of (Leskeys et al,1999) that thr irrigation of Tall fescue with 2.5 dS/m an acceptable practice. At an EC of 4 dS/m these two species clearly benefitted from pre-soaking. The pre-soaked seeds of *Lunibelle* had consistently higher germination percentages than the other species at the higher EC levels (EC 4-10 dS/m). Without pre-soaking, zero germinated seeds remained after 24 days at an EC of 10 dS/m. None of the species stood out as being saline tolerant.

Table 5. Germination percentage of tall fescue species at four different salinity levels after 24 days, with (F1) and without (F2) pre-soaking.

| | EC0.5 | | EC4 | | EC7 | | EC10 | |
|---|---|---|---|---|---|---|---|---|
| | F1 | F2 | F1 | F2 | F1 | F2 | F1 | F2 |
| *Bariane* | 67 | 87 | 67 | 73 | 67 | 33 | 40 | 0 |
| *Centurion* | 87 | 73 | 80 | 47 | 60 | 20 | 47 | 0 |
| *Lunibelle* | 73 | 67 | 93 | 53 | 80 | 7 | 67 | 0 |
| *Madra* | 53 | 60 | 53 | 60 | 27 | 27 | 60 | 0 |
| *Mylena* | 60 | 80 | 53 | 73 | 67 | 20 | 33 | 0 |

The development of the shoot lengths was similar for all species. The growth of the shoots at the highest two salinity levels was very limited this result is comfrom the finding of (Hoffman et al,1997 and MaAdam et al,1997) the shoot growth of Tall fescue was not affected by irrigation water treatment ranging from 1.2 to 3.6 dS/m. Fot the pre-soaked seeds shoot lengths did not exceed 10-cm and all plants died after 80 days. Without pre-soaking the species performed worse, *Centurion* and *Lunibelle* showed almost no shoot development. The development of the shoots at the lower two salinity levels is illustrated in Figure 1. Clearly, the pre-soaked seeds had a better start, but this advantage dissapeared later on.

At an EC of 0.5 dS/m the average shoot lengths varied between 34.7 and 49.3 for the pre-soaked seeds and 36.3 and 39.3 for the dry seeds, after 108 days. For both pre-soaked and dry seeds, *Centurion* had the shortest shoots and *Lunibelle* the longest.

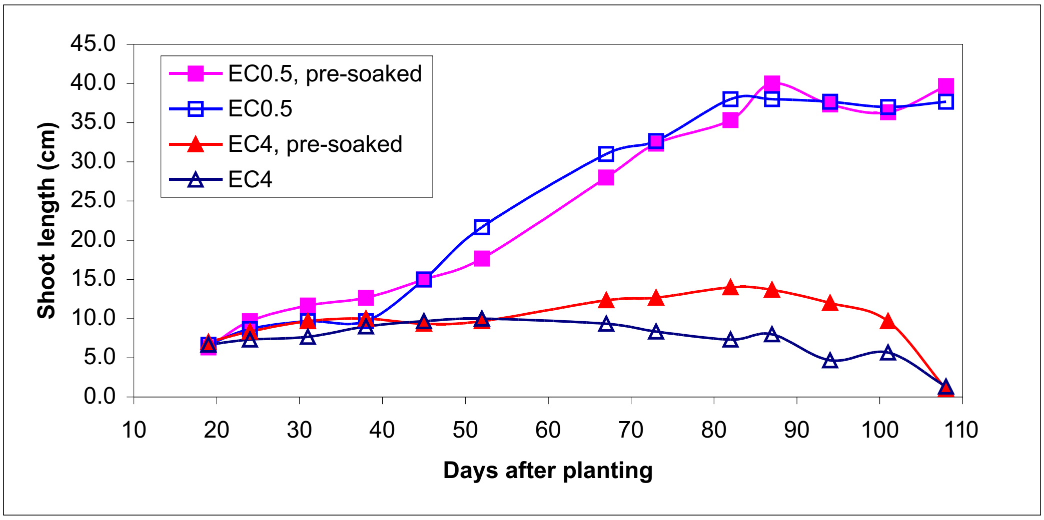


Fig. 1. Average length of *Madra* shoots at EC-levels 0.5 and 4 dS/m

## CONCLUSIONS

The in-vitro screening of 200 lentil, 205 chickpea, and 5 tall fescue cultivars indicated that the chickpea cultivars were more saline tolerant than the lentil and tall fescue cultivars. Pre-soaking of the seeds in water (0.5 dS/m) substantially improved emergence, shoot growth, and survival of the species at the higher salinity levels. The different lentil and chickpea varieties showed a large variability in performance at different salinity levels. Therefore, more testing is required.

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