# SCREENING OF SOME CHICKPEA GENOTYPES FOR SALINITY TOLERANCE IN A MEDETERRANEAN ENVIRONMENT

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## ABSTRACT

To understand the mechanisms that affect the response to salinity of legumes, six chickpea genotypes were tested in sand-filled pots in the greenhouse. The chickpea seeds were irrigated with water of four different salinity levels (0.5, 2, 4, 6 dS/m). Physiological and phenological observations were made. The results indicated that the varieties responded differently to different salinity levels. The salinity had little effect on germination. Genotypes F.97-74, F.87-59 and ILC 3279 were found to have higher salt tolerance and produced more dry matter than genotype F.97-265.

### INTRODUCTION

Water has always been a central concern in many Mediterranean countries (Hamdy, 1995). Some of the countries in the region experience presently severe water scarcity. With populations growing and standards of living increasing, all countries are facing a trend of declining water availability. Agriculture is likely to be forced to use more and more marginal quality water, either brackish water or treated sewage effluent. High salt concentrations inhibit crop growth and yield. The response of cultivated species to salinity in terms of growth and yield are the ultimate expression of several interacting physiological and biochemical processes.

Numerous studies have been conducted on the management and identification of saline tolerant crops such as cotton or cereals (e.g., Leidi and Saiz, 1997; Hoffman and Jobes, 1978; Pessarakali et al., 1991). Limited attention has been given to legumes and forages, which are known to have low tolerance to salinity. As for most legumes, germination of chickpea is relatively less affected by salinity than subsequent seedling growth (Geol and Vashnery, 1987; Yadav et al., 1989). Kumar (1985) also indicated that later stages of chickpea growth were more sensitive than earlier stages. The sensitivity of all chickpea genotypes increases with plant growth and greater salinity (Sheoran and Garg, 1983; Dua, 1992; Gandour, 2002).

Salinity also affects the photosynthetic C14 assimilation in chickpea leaves (Murumakar and Chavan, 1993). Shoot dry mass declines at higher salinity levels (Rao and Sharma, 1995). Salt stress affects growth, nodulation and nitrogen accumulation in legumes (Saxena and Rewari, 1991; 1992; Saxena et al., 1993). Murumkar and Chavan (1989) and Gandour (2002) reported that salt stress caused accumulation of both sodium and chloride in the shoot parts, especially in the leaves. This was accompanied by a decrease in potassium in different plant parts.

The effect of salinity may create two types of osmotic adjustment: the first corresponds with the phenological development of the plant; the second is achieved by physiological adaptation. The objective of the current study was to enhance our understanding of the mechanisms that affect the response of different chickpea varieties to salinity stress.

### MATERIALS AND METHODS

To select the 6 chickpea genotypes, 40 genotypes were re-screened in vitro in the laboratory at ICARDA, before greenhouse study.

During the 2001-2002 season, seeds of different chickpea (Cicer arietinum) genotypes were tested.

The experiments were conducted in the greenhouse at ICARDA, with a day and night temperature of 20-30  $^{\circ}$ C, 60% relative humidity, and a light intensity of 20.000 lux for 12 hr/day. The set-up consisted of 120 plastic pots (6 genotypesX5 replications X4 treatments) with a top diameter of 19.5 cm and a depth of 18 cm, filled with sand. Ten seeds were sown per pot. Water with three different salinity levels, 2, 4, 6 dS/m, was prepared by adding a mixture of NaCl and CaCl<sub>2</sub> in a 3:1 ratio to the water. No salt was added to the control. All experiments were conducted using a randomized block design.

Six chickpea genotypes (FLIP 96-59, FLIP 96-74, ICCV2, FLIP 87-85, ILC 3279, FLIP 97-265) were sterilized with 0.1% mercuric acid for 5 min and washed in sterile water before germination. The chickpea genotypes were sown in April 2002. After the emergence percentage had been determined, the seedlings were thinned out to a number of 5 per pot.

Two inoculums of Rhizobium strain were added immediately to the genotypes. Modified Arnon and Hogland's N free solution was added twice a week. Irrigation with saline water was started 3-5 days after sowing. The pots were weighed after the first irrigation and before each of the following irrigations.

### Growth and yield

The leaf area and dry matter accumulation of leaf, stem, and root of chickpea were determined at the successive phenological stages. The seedlings were first used to measure the leaf area with an AM-Licor 1300 apparatus. The dry matter was determined by oven drying at 85 °C for 48 hours. The water content was determined from the difference between the fresh and dry weight.

#### **Phenological observations**

The emergence and the survival of the seedlings were determined by daily counting of the number of plants during the first 15 days after sowing. To determine the phenological dates and the development of the shoot and root lengths and weights at day 25, 35, 45, 55, 65, and 75;leaf initiation (plastochrom) and numbers, flowers and pods, two plants per pot were marked. Thus, the number of leaves and flowers is the average of 10 observations. When the plants had attained a phenological stage, this date was noted.

#### **Physiological observation**

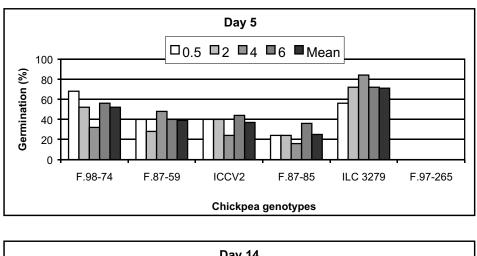
Leaf water potential was measured on one plant in each pot at 100% flowering. The leaf area and the dry matter of leaf and stem were determined at the successive phenological stages (25, 35, 45, 55, 65, and 75 days after sowing) on two plants in each pot, first leaf area and afterwards the dry matter. At harvest the yield components (number of pods per plant, weight of pods) were measured from all plants present in the pot.

## **RESULTS AND DISCUSSION**

#### Germination

At an EC of 6 dS/m germination varied between 20 and 100%. The greenhouse testing of the six selected chickpea genotypes showed that the genotypes responded differently to saline conditions (Fig. 1). Germination capacity decreased with increasing salinity levels for FLIP.92-265. Seeds of FLIP.98-74, FLIP.87-59, FLIP.87-85, and ILC 3279 showed better germination than ICCV2 and F.97-265.

The reduction in germination of the seed (at day 14 after sowing), as compared to the respective controls, was 13% for ICCV2, 18% for FLIP.97-265, and 23% for FLIP.98-74. In general, the germination percentage of the six genotypes of chickpea reduced with increasing levels of salinity. The results of germination indicated that the six genotypes of chickpea differed in their response to different salinity levels. This would suggest the possibility of exploiting genotypes variation in chickpea to specific concentration of salts.



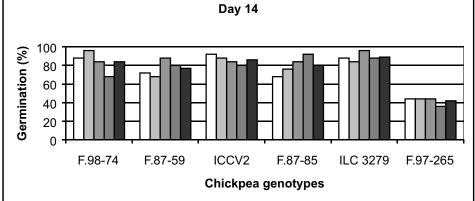


Fig. 1. Effect of salinity on germination of chickpea genotypes at 5 and 14 days after sowing.

## **Growth parameters**

The length of the phenological stages of the chickpea genotypes showed no clear effect of salinity. The average time to ramification varied between 17.9 days at the control to 19.9 days at an EC of 4 dS/m. Start to flowering varied between 42 days at 4 dS/m and 46.8 days at the control. Start to pod setting varied between 47 to 50.8 days at ECs of 4 and 6 dS/m, respectively.

The observed effects of salinity on seedling growth were a function of both salt level and time of exposure. Twenty-five days after sowing, salinity stress did not exert significant effects on shoot and root growth, and fresh and dry weights at salinities up to 4 dS/m for all genotypes, except for FLIP.97-265, and up to 6 dS/m for ILC 3279.

At day 35, seedling growth parameters for FLIP.98-74, FLIP.87-59, ICCV2, FLIP.97-265 demonstrated greater positive response to the inhibitory effect of salinity at 4 dS/m than F. 87-85. There was a gradually delayed shoot growth of FLIP.98-74, FLIP.87-59, ICCV2, and ILC 3279 in relation to increased regimes of salinity levels (0-6 dS/m). At the highest level of salinity (6 dS/m) the reduction in root and shoot lengths relative to the control value was 35, 30, 0, and 26% for the root and 37, 29, 48 and 47% for the shoot of varieties FLIP.98-74, FLIP.87-59, ICCV2, FLIP.97-265, respectively.

As the duration of salinity stress increased a significant reduction in seedling growth and shoot-root ratio was observed (Fig. 2). At day 55 and 65, the growth parameters for all varieties were inhibited at 6 dS/m except for FLIP.87-85. Increasing levels of salinity adversely affected both root and shoot length of chickpea seedling. The shoot-root ratio generally exceeded 1, except for day 25. The ratios decreased with salinity. The reduction in root and shoot lengths of plants, is one of the most commonly observed responses of salinity (Bernstein and Hayward, 1958).

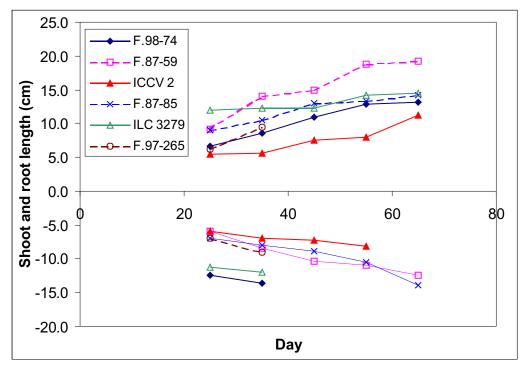
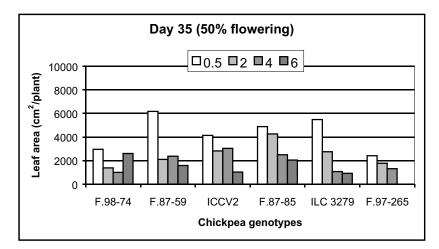
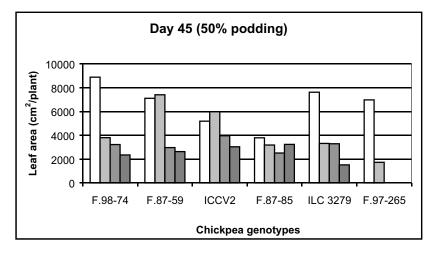


Fig. 2. Shoot and root growth of chickpea genotypes at EC 6 dS/m.

Salinity had effect on the leaf area, but its effect was not strong and decreased with time for the most saline treatment. Figure 3 shows the effect of salinity on the leaf area, determined at 35 days (50% of flowering), 45 days (50% of podding) and 55 days (75% of podding).





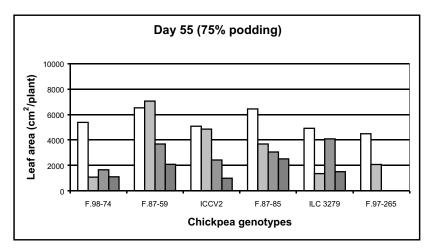


Fig. 3. Effect of four salinity levels on the leaf area of chickpea genotypes at day 35, 45, and 55.

Dry matter accumulation for shoots, leaves and stems developed regularly from transplantation till harvest and was affected by salinity, especially for FLIP.97-265 (Fig. 4). All varieties were affected with time at salinity levels 4 and 6 dS/m. The dry matter seems to be less sensitive to salinity than the leaf area. Similar observations were made by Katerji et al. (2001b).

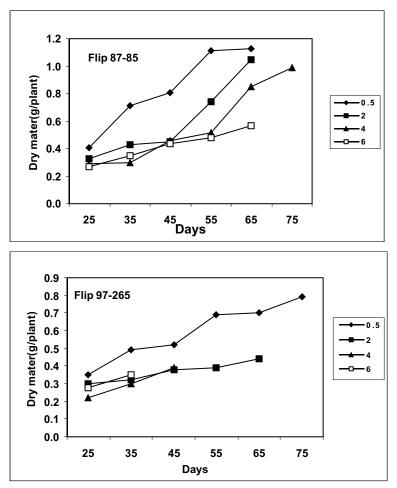


Fig. 4. Effect of four salinity levels on dry matter of shoots for variety F.87-85 and F.97-265.

### Leaf water content and potential

The leaf water potential decreases after dawn, attains a minimum around solar noon, and afterwards increases again. The leaf water potential showed high values for all varieties (Fig. 5). There was no significant response of leaf water potential to salinity.

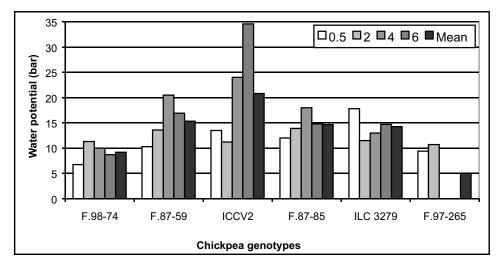
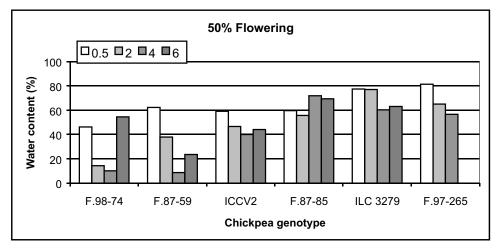


Fig. 5.Effect of salinity on leaf water potential of chickpea genotypes at 100% flowering.

Leaf water content was higher at 50 than at 100% of flowering. The water content (Fig 6) was reduced with increasing salinity level. The reduction values were higher in FLIP.97-265 at 6 dS/m (about 100% at 50% of flowering) than in FLIP.87-59 (24% at 50% flowering and 66% at 100% of flowering). An increase in tissue water content due to salinity was reported in bean plants by Meiri et al. (1971), in lupine and broad bean plants by Shaddad et al. (1990) and in soybean cultivars by Abdel-Samed and Shaddad (1997). Hasegawa et al. (1986) reported that salt tolerance at the whole plant level seems to be related to the capacity of cultivars to resist dehydration. In addition, the ability to accumulate water during the vegetative growth period could be a very important trait to discriminate genotypes by their salt tolerance (Binzel et al., 1985).



(continued Fig.6)

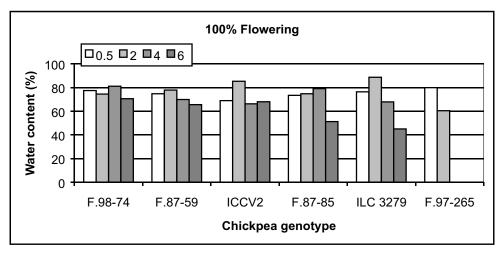


Fig. 6.Effect of salinity on leaf water content of chickpea genotypes at 50 and 100% flowering.

## Plastochron

Plastochron was higher for genotypes FLIP.98-74 and FLIP.87-59 than for the other varieties. There was no significant effect of salinity on plastochron (Fig. 7).

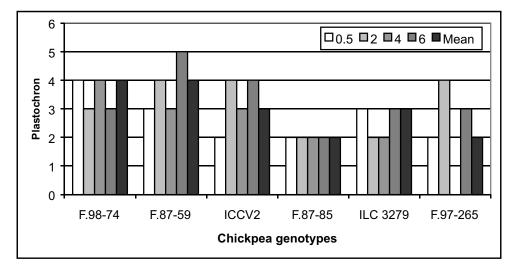


Fig. 7. Effect of salinity on plastochron of chickpea.

## **Yield components**

The results of the growth parameters were confirmed by the yield data. Yield capacity is always one of the primary objectives in crop breeding for increasing crop yield. The decrease in yield is mainly caused by a difference in the grain weight and pods. Flower and pod numbers (Table 1) and pod weights of all chickpea varieties were substantially reduced at a salinity level of 4 dS/m. At 2 dS/m none of the components Of ICCV2 genotype was significantly reduced, as compared with the control. The number of pods decreased from 4 at 0.5 dS/m to 0 at 6 dS/m for all genotypes, except for variety ICCV2, which still averaged 1 pod at 6 dS/m.

Chickpea	0.5 dS/m	2 dS/m	4 dS/m	6 Ds/m
Genotype				
	Number of flowers per plant			
FLIP.98-74	5	0	0	0
FLIP.87-59	9	4	3	2
ICCV2	5	4	2	2
FLIP.87-85	5	5	2	0
ILC 3279	2	1	0	0
FFLIP.97-265	7	2	1	0
	Number of pods per plant			
FLIP.98-74	4	0	0	0
FLIP.87-59	4	1	0	0
ICCV2	4	3	1	1
FLIP.87-85	3	1	0	0
ILC 3279	1	0	0	0
FLIP.97-265	3	1	0	0

Table 1. Effect of salinity on number of flowers and pods of chickpea genotypes.

### CONCLUSIONS

The reduction in seedling survival rates and growth are major causes of the stand loss in salt affected legumes fields. This study indicated that in all genotypes salinity as high as 2dS/m can reduce seedling shoot dry weight and that salinity at 6 dS/m can reduce seedling survival (plant stand). During early seedling, salinity affected the development of the seedlings that showed symptoms of water stress. These symptoms could be observed in the form of leaf water potential, pod number and weights .The six chickpea genotypes studied displayed distinct variation in salinity tolerance during growth. A comparison of the effects of salinity on growth of 6 genotypes indicates that genotypes FLIP.98-74, FLIP.87-59 and ICCV2 seemed to be more salt tolerant than FLIP.97-265, FLIP.87-85 and ILC 3279 at the same salinization levels.

To better understand the difference of responses on osmotic adjustment and water use efficiency, additional greenhouse experiments in sand and soil need to be conducted for chickpea and other legume and forage genotypes. The most promising genotypes of each crop should subsequently be tested in the field. Similar observations should be made in the field as in the greenhouse. The EC and chemical composition of irrigation and soil water should also be analyzed.

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