

## Salt Tolerance Assessment in Alfalfa (*Medicago sativa L.*) Ecotypes

<sup>1</sup>Shaily Bhardwaj, <sup>2</sup>N.K. Sharma, <sup>2</sup>Prashant Kumar Srivastava and <sup>1</sup>Gaurav Shukla

<sup>1</sup>Department of Botany, Bareilly College, Bareilly, U.P., India

<sup>2</sup>Department of Biological and Environmental Science, NVPAS, Anand, Gujarat, India

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**Abstract:** Salt stress is a serious environmental problem throughout the world which may be partially relieved by breeding cultivars that can tolerate salt stress. Plant breeding may provide a relatively cost effective short-term solution to the salinity problem by producing cultivars able to remain productive at low to moderate levels of salinity. Two alfalfa cultivars, IGFRI-S-54 and Anand II were assessed for salt tolerance at three stages leaf, flowering and mature plant stage. A petriplates screening system was used to evaluate individual alfalfa plants grown in per lit medium and irrigated with water containing different amounts of NaCl. Soil sample analysis, seed germination, growth rate (root length and shoot length) fresh weight and dry weight were determined. Almost both the variety showed reduction in root length and shoot length with increasing regimes of salinity. The increase in ECE level showed inverse relation with the plant height. The analysis of both the cultivar indicates that ANAND II was better adapted to salinity condition as compared to IGFRI-S-54.

**Key words:** Alfalfa, salinity, growth rate, stress, salt tolerance, root length

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### INTRODUCTION

Nearly 10% of the earth's land is salt-affected and an estimated 10 million ha of agricultural land are lost annually due to salinization and water logging. Salinity of soil and water resources is a serious threat (Amer, 2010). The estimated land area affected by salinity varies between 16-23 Mha. These figures include both cultivated and barren lands. No exact data is available on intensity of this problem in the arable lands. The main difficulty in this regard is the temporal variations of salinity during the growing season due to the effects of irrigation water which add or leach the salts (Bezborodov *et al.*, 2010; Pang *et al.*, 2009). Reclamation, drainage and improved irrigation practices might reduce the severity and spread of salinization in some regions but costs of these practices are generally prohibitive (Johnson *et al.*, 1992). Plant breeding may provide a relatively cost effective short-term solution to the salinity problem by producing cultivars able to remain productive at low to moderate levels of salinity. However, breeding for improved salt tolerance in many crop plants, including alfalfa has progressed slowly (Blum, 1988; Johnson *et al.*, 1992; Noble *et al.*, 1984). Germination is one of the most critical periods for a crop subjected to salinity. Soil salinity may influence the germination of alfalfa seeds either by creating an osmotic potential-external to the seed, preventing water uptake or by the toxic effects of Na and Cl ions on the germinating seeds (Bybordi and

Tabatabaei, 2009). Nutrition can significantly influence a plant's response to saline conditions (Grattan and Grieve, 1994). The interaction between salinity and phosphorus nutrition is particularly complex and plant responses can vary according to which species or cultivar is being examined, the stage of plant growth as well as the level and form of NaCl and P and the specific conditions of the experiment (Champagnol, 1979). Lucerne (*Medicago sativa L.*) is a species whose tolerance to NaCl alone has been well-studied (Johnson *et al.*, 1992), however its performance under saline conditions and varying P nutrition is unknown. Salinity research in alfalfa has focused primarily on germination (Carlson *et al.*, 1983; Allen *et al.*, 1986; Dobrenz *et al.*, 1989) and seedling establishment (Ashraf *et al.*, 1987; McKimmie and Dobrenz, 1987) in the presence of NaCl. Currently, research interest is growing in mapping soil Electrical Conductivity (EC) as a surrogate for soil salinity (Eigenberg *et al.*, 2002; McCutcheon *et al.*, 2006) and agricultural researchers have widely used soil EC survey data to measure various soil physicochemical properties. Many findings have demonstrated that diverse types of spatial and temporal information can be derived from EC survey data and that this information can be readily used to help improve the overall management of agricultural fields (Lesch *et al.*, 2005; Li *et al.*, 2007).

Development of salt tolerance in crops depends ultimately on two factors. Availability of genetic variation by screening and selection of those plants with superior

performance when exposed to such stress is very important (Epstein *et al.*, 1980). The presence of phenotypic variation for salt tolerance was reported for alfalfa cultivars (Al-Khatib *et al.*, 1993; Noble *et al.*, 1984). Monirifar *et al.* (2004) reported the presence of phenotypic variation between some Azarbaijan alfalfa cultivars at different salinity levels.

Hence, the purpose of this study was to investigate the response of lucerne to the combined effects of NaCl salinity to determine how these two effects interact to influence salt tolerance. This information may be of importance when deciding fertilizer applications in saline areas or in areas where wastewaters (containing high salinity levels) are used for irrigation.

## MATERIALS AND METHODS

Seeds of alfalfa (*Medicago sativa* L.) of two varieties Anand II and IGFRI-S-54 were procured from Genetic Resource Unit, International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru (AP) and G.B. Pant University of agriculture and technology, Pantnagar (UA) India. Twelve soils samples were analyzed for electrical conductivity, sulphate and ESP (Exchangeable Sodium Percentage), SAR (Sodium Absorption Ratio), sodium, potassium, calcium and magnesium by using spectrophotometer and chloride by silver nitrate titration using an ion selective electrode. Plants were grown hydroponically in lab as well as field, Rohelkhand region (Bareilly), India. Seedlings of two cultivars of lucerne (Anand II and IGFRI-S-54) were grown in petridishes containing modified nutrient solution. The experiment was a factorial design with three salinity treatments and six replicates placed in a split block structure. Plants were harvested destructively at weekly intervals and there were four harvests in total. At harvest, five plants of each cultivar from each treatment were removed and divided into roots and shoots. Height of plants, fresh weight, dry weight (dried at 70°C for 48 h), relative growth rate, net assimilation rate and leaf area ratio were measured on each sample.

## RESULTS AND DISCUSSION

**Physico-chemical analysis:** The results from soil analysis indicated that pH of soil samples ranged from 4.0-5.8; the electrical conductivity of soil saturation extract was found to be about 4 mS cm<sup>-1</sup> at 25°C and ESP (Exchangeable Sodium Percentage) did not exceed 14.95 accomplished with almost corresponding SAR (Sodium percentage ratio). As is obvious from the results among different cations investigated the maximum was contributed by sodium ions i.e., some of the samples

exceeded 60 meq L<sup>-1</sup>, followed by calcium and magnesium displaying between 33.25 and 53.05 meq L<sup>-1</sup>. However, potassium ions were recorded to be in least concentrations i.e., between 0.33 and 2.37 meq L<sup>-1</sup>. On the other hand, the maximum was contributed to the extensively accumulated soluble salts by chloride anions measuring 13.5-29.3 meq L<sup>-1</sup>. Thus aforesaid findings related to the inorganic analysis revealed that the composition of soil collected from different 12 sites of Uttar Pradesh (India) was found saline. The correlation analyses of data indicate a significant coefficient of correlation existed between SAR and ESP, EC and SAR (Table 1).

**Seed germination:** Salinity is known to affect adversely the germination of seeds and early seedling growth (Fig. 1). It was observed that with increasingly levels of salinization (NaCl salt treatment) variety IGFRI-S-54 and germination percentage decrease more than in variety Anand II. The percent germination decreased gradually with an increase in electrical conductivity (0-8 mS cm<sup>-1</sup>). However, the trend of reduction was not found identical. The seed of var. Anand II behaved relatively more favorably under all EC levels tested whereas the seeds of var. IGFRI-S-54 behaved sensibly to the saline conditions. As regards the magnitude of reduction in percentage seed germination, it was observed only 2.5% in var. IGFRI-S-54 under lowest salinity (2 mS cm<sup>-1</sup>). On the contrary var. IGFRI-S-54 suffered up to 50% under 8 mS cm<sup>-1</sup> EC level and seeds of var. Anand II showed germination reduced only by 6.7% under same treatment.

**Early seedling growth, root length and shoot length:** The observations on early seedling growth (root and shoot length) were taken after 24 h of planting the seeds and continued up to 144 h.

The results have been expressed for length of root and shoot as averages of transformed variables. Significant effect of salinity was observed with respect to root elongation. Almost both the variety showed reduction in root length over control variety IGFRI-S-54 suffered relatively more than Anand II. IGFRI-S-54 variety proved more susceptible exhibiting 83.34% reduction over control at 8 mS cm<sup>-1</sup> EC and 25% reduction over control under 4 mS cm<sup>-1</sup> EC (Table 2).

The finding is shown in Table 2 for variety; treatment and variety X treatment were significant at 5% P for critical differences. It was experienced from the results that like root growth and shoot growth also reduced markedly with increasing regimes of salinity (0-8 mS cm<sup>-1</sup> EC). The seedlings of var. IGFRI-S-54 did not show the emergence of shoot under highest salinity level (8 mS cm<sup>-1</sup> EC) used. Shoot growth suffered lesser (7.15% over control) in var. Anand II.

Table 1: Correlation analysis between estimated soil parameters Seed germination

| Parameters     | ESP    | SAR     | pH     | Ec      | Na+    | K+     | Total hardness | Chloride | Sulphate |
|----------------|--------|---------|--------|---------|--------|--------|----------------|----------|----------|
| ESP            | 1.000  | 0.611*  | 0.427  | 0.402   | 0.497  | 0.200  | -0.295         | -0.368   | 0.110    |
| SAR            | 0.611* | 1.000   | 0.192  | 0.742** | 0.496  | 0.086  | -0.225         | -0.006   | 0.279    |
| pH             | 0.427  | 0.192   | 1.000  | 0.106   | -0.072 | 0.443  | -0.014         | 0.016    | 0.338    |
| Ec             | 0.402  | 0.742** | 0.106  | 1.000   | 0.095  | 0.454  | -0.187         | -0.074   | 0.530    |
| Na+            | 0.497  | 0.496   | -0.072 | 0.095   | 1.000  | -0.181 | -0.435         | 0.101    | -0.044   |
| K+             | 0.200  | 0.086   | 0.443  | 0.454   | -0.181 | 1.000  | -0.071         | -0.219   | 0.447    |
| Total hardness | -0.295 | -0.225  | -0.014 | -0.187  | -0.435 | -0.071 | 1.000          | 0.054    | 0.001    |
| Chloride       | -0.368 | -0.006  | 0.016  | -0.074  | 0.101  | -0.219 | 0.054          | 1.000    | -0.181   |
| Sulphate       | 0.110  | 0.279   | 0.338  | 0.530   | -0.044 | 0.447  | 0.001          | -0.181   | 1.000    |

\*Correlation is significant at the 0.05 level (2-tailed); \*\*Correlation is significant at the 0.01 level (2-tailed)

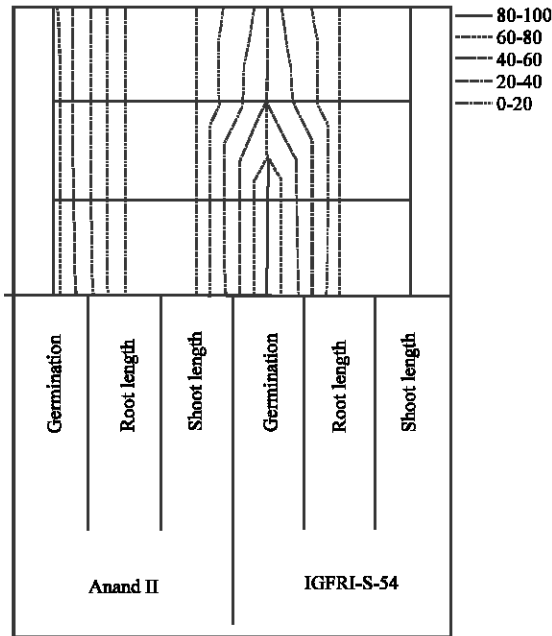


Fig. 1: Trend of germination in two varieties with salinity treatment

Under 4 mS cm<sup>-1</sup> EC treatment, exhibition of trends 14.3% in variety Anand II. As regards the lowest treatment (2 mS cm<sup>-1</sup> EC) shoot growth maintained almost 100% in var. Anand II. The growth of var. IGFRI-S-54 suffered more i.e., 66.67%. Thus keeping in to consideration the percentage germination of seeds and early seedling growth behavior of lucerne varieties, Anand II and IGFRI-S-54 were selected for further experimentation being salinity tolerant.

**Height of plants, fresh weight of plants and dry weight of plants:** The differences among varieties, treatment, stages and variety X treatments, varieties X stages, treatments X stages and varieties X treatments X stages were significant. The result shown in Table 3 revealed that the height of plants of both lucerne varieties decreased markedly with an increase in ECe levels (4-8 mS cm<sup>-1</sup>) at all three stages of growth. The plants of leafy stage in var.

IGFRI-S-54 showed 77.8% reduction under 8 ECe treatments as compared to 74.5% reduction in height in var. Anand II. At flowering stage, the reduction in height was recorded to be 62.0% over control in var. IGFRI-S-54 as compared to only 29.6% reduction in var. Anand II under same ECe level. Likewise, at maturity stage 72.2% reduction in height of plants of var. IGFRI-S-54 was compared >34.4% reduction in plants of var. Anand II (Table 3).

Differences among varieties, treatment, stages and variety X treatments, varieties X stages, treatments X stages and varieties X treatments X stages were significant. In general, the fresh weight of plants of both lucerne varieties was affected adversely by salinity stress (0-8 mS cm<sup>-1</sup>) at all the three stages of growth (Table 4). The fresh weight suffered relatively more in var. IGFRI-S-54 than the var. Anand II. Similarly the magnitude of reduction over control was greater in former variety. For instance 79.5 reductions against 45.4% of var. Anand II took place under 8 mS cm<sup>-1</sup> level in var. IGFRI-S-54 at leafy stage. It was observed 57.5 against 23.7% at flowering stage and 63.8% against 24.8% at maturity stage under same ECe level. Differences among varieties, treatment, stages and variety X treatments, varieties X stages, treatments X stages and varieties X treatments X stages were significant.

Dry weight was also affected adversely due to increasingly salinity at all three stages. Dry matter suffered relatively more in var. IGFRI-S-54. Moreover, the magnitude of reduction over control also kept pace with. Plants of leafy stage demonstrated 76.1 reductions over control against 36.0% reduction in var. IGFRI-S-54. It was recorded 62.9% against 36.6% at flowering stage and 69.8% against 46.6% at maturity stage under same ECe level (Table 5).

**Relative Growth Rate (RGR), Net Assimilation Rate (NAR) and Leaf Area Ratio (LAR):** The results shown in Table 6 reveal that the values of relative growth rate were observed to limit with an increase in soil salinity during both first and second periods of growth in salt tolerant var. Anand II and salt susceptible var. IGFRI-S-54. Parallel trend was recorded in relation to growth periods also.

Table 2: Germination percentage of seeds, roots length and shoot length of Lucerne varieties in relation to salinity in petri plate culture (Means of three replicates)

| Varieties  | Parameters        | Electrical conductivity (mS cm <sup>-1</sup> ) |       |      |       | SoV        | CD at 5% P |       |       |  |
|------------|-------------------|--|-------|------|-------|------------|------------|-------|-------|--|
|            |                   | DW   | 2     | 4    | 8     |            | G          | R     | S     |  |
| Anand II   | Germination       | 90.0   | 88.00 | 86.0 | 84.00 | Varieties  | 1.500      | 0.152 | 0.197 |  |
|            | Root length (cm)  | 1.0  | 0.90  | 0.7  | 0.00  | Treatment  | 1.400      | 0.113 | 0.112 |  |
|            | Shoot length (cm) | 0.7  | 0.70  | 0.6  | 0.65  | Replicates | 0.900      | 0.114 | 0.117 |  |
| IGFRI-S-54 | Germination       | 98.5   | 97.50 | 60.2 | 50.20 | V×T        | 1.200      | 0.011 | 0.091 |  |
|            | Root length (cm)  | 1.2  | 1.00  | 0.9  | 0.20  | T×R        | 1.100      | 0.121 | 0.212 |  |
|            | Shoot length (cm) | 0.8  | 0.80  | 0.6  | 0.40  | V×R        | 1.900      | 0.230 | 0.090 |  |

Table 3 : Effect of NaCl salinity on height (cm) of plants of two Lucerne varieties

| No. | Treatments of (mS cm <sup>-1</sup> ) | Leafy stage | Flowering stage | Maturity stage | SoV       | CD at 5% P | SE    |
|-----|--------------------------------------|-------------|-----------------|----------------|-----------|------------|-------|
| 1   | Control (1.2)                        | 6.720       | 32.00           | 46.50          | Varieties | 1.990      | 0.050 |
|     | 4                                    | 5.450       | 27.50           | 41.50          | Treatment | 1.006      | 0.008 |
|     | 6                                    | 4.320       | 24.31           | 38.00          | Stages    | 1.020      | 1.930 |
|     | 8                                    | 1.710       | 22.50           | 30.50          | V×T       | 2.710      | 1.980 |
| 2   | Control (1.2)                        | 5.900       | 29.50           | 40.30          | V×S       | 2.000      | 2.100 |
|     | 4                                    | 4.770       | 26.30           | 31.10          | T×S       | 1.871      | 1.991 |
|     | 6                                    | 4.000       | 20.10           | 20.50          | V×T×S     | 2.931      | 2.825 |
|     | 8                                    | 1.310       | 11.20           | 11.20          |           |            |       |

Table 4: Effect of salinity on fresh weight (gms) of aerial shoot of two lucerne varieties

| No. | Varieties  | Treatments of (mS cm <sup>-1</sup> ) | Leafy stage | Flowering stage | Maturity stage | SoV        | CD at 5% P | SEM±  |
|-----|------------|--------------------------------------|-------------|-----------------|----------------|------------|------------|-------|
| 1   | Anand II   | Control (1.2)                        | 5.500       | 18.500          | 24.500         | Varieties  | 1.305      | 0.035 |
|     |            | 4.000                                | 5.000       | 17.000          | 23.900         | Treatments | 1.002      | 0.093 |
|     |            | 6.000                                | 4.500       | 15.500          | 20.100         | Stages     | 1.008      | 0.765 |
|     |            | 8.000                                | 3.000       | 14.100          | 18.400         | V×T        | 0.759      | 0.932 |
| 2   | IGFRI-S-54 | Control (1.20)                       | 4.900       | 16.500          | 23.500         | V×S        | 1.201      | 1.732 |
|     |            | 4.000                                | 3.000       | 14.000          | 20.000         | T×S        | 2.051      | 3.061 |
|     |            | 6.000                                | 2.100       | 14.000          | 14.000         | V×T×S      | 3.591      | 3.105 |
|     |            | 8.000                                | 1.000       | 7.000           | 8.500          |            |            |       |

Table 5: Effect of salinity on dry weight (gs) of aerial shoot of two lucerne varieties

| No. | Varieties  | Treatments of (mS cm <sup>-1</sup> ) | Leafy stage | Flowering stage | Maturity stage | SoV       | CD at 5% P | SEM±  |
|-----|------------|--------------------------------------|-------------|-----------------|----------------|-----------|------------|-------|
| 1   | Anand II   | Control (1.2)                        | 2.500       | 6.000           | 7.500          | Varieties | 0.092      | 0.003 |
|     |            | 4                                    | 2.100       | 4.600           | 6.000          | Treatment | 1.009      | 0.923 |
|     |            | 6                                    | 1.700       | 4.000           | 5.200          | Stages    | 1.031      | 0.593 |
|     |            | 8                                    | 1.600       | 3.800           | 4.000          | V×T       | 1.731      | 1.710 |
| 2   | IGFRI-S-54 | Control (1.2)                        | 2.100       | 5.400           | 6.300          | V×S       | 1.003      | 1.735 |
|     |            | 4                                    | 1.200       | 2.800           | 3.200          | T×S       | 0.591      | 0.152 |
|     |            | 6                                    | 0.900       | 2.600           | 2.900          | V×T×S     | 3.492      | 1.731 |
|     |            | 8                                    | 0.500       | 2.000           | 1.900          |           |            |       |

RGR values were computed lesser in salinity susceptible variety IGFRI-S-54 than salinity tolerant var. Anand II. It is exhumed from the data shown in Table 6. That net assimilation rate decreased not only with the onset of growth. The enormity of decrement was observed more at second period than at first period of growth in both varieties e.g., NAR reduced 2/3rd at first period and almost 3/4th at second period of growth over control under 8 mS cm<sup>-1</sup> level of salinity in var. Anand II. In the contrary in var. IGFRI-S-54 the reduction was recorded more pronounced at second period than at first period of growth under same level of salinity. The results are shown in Table 6 reveal that LAR reduced markedly with enhancing growth periods. In var. Anand II, 20.7% increment over control was recorded under 8ECe at I period while in var. IGFRI-S-54 it was noted to be 24.6% over control under same treatment.

Table 6: Effect of salinity on Relative Growth Rate (RGR); Net Assimilation Rate (NAR) and Leaf Area Ratio (LAR) of two lucerne varieties

| Parameters | Treatments | Anand II |           | IGFRI-S-54 |           |
|------------|------------|----------|-----------|------------|-----------|
|            |            | I period | II period | I period   | II period |
| RGR        | Control    | 0.0586   | 0.0163    | 0.0423     | 0.0124    |
|            | 4          | 0.0564   | 0.0148    | 0.0415     | 0.0129    |
|            | 6          | 0.0487   | 0.0135    | 0.0403     | 0.0121    |
|            | 8          | 0.0422   | 0.0127    | 0.0380     | 0.0117    |
| NAR        | Control    | 0.0191   | 0.0112    | 0.0148     | 0.0098    |
|            | 4          | 0.0183   | 0.0094    | 0.0137     | 0.0069    |
|            | 6          | 0.0178   | 0.0081    | 0.0121     | 0.0042    |
|            | 8          | 0.0063   | 0.0026    | 0.0110     | 0.0018    |
| LAR        | Control    | 6.7900   | 4.3200    | 8.0300     | 4.3300    |
|            | 4          | 7.0100   | 6.3300    | 8.0300     | 4.9800    |
|            | 6          | 7.9200   | 7.2900    | 9.3500     | 5.7100    |
|            | 8          | 8.2000   | 7.9100    | 10.0100    | 6.1400    |

On the other hand at II period of growth corresponding increasing percentages were recorded 83.1 and 41.8% (Table 6).

Thus it may be inferred that both RGR and NAR decrease while LAR increase consistently due to salinity revealing that there is a direct relationship between chlorophyll pigments and both NAR and RGR irrespective of LAR in both salinity tolerant and susceptible varieties of lucerne.

The findings are in accordance with those of Goel (1986). The adverse effects of salinity on RGR and NAR in plants of both lucerne varieties may be attributed to the process of senescence since LAR demonstrated increasing trend. The depleted RGR can successfully be ascribed more to LAR than to lack of intrinsic ability to assimilate. Previous study carried out with in soyabean and alfalfa also showed inhibition in growth parameters by NaCl salinity (Bernstein and Ogata, 1986). Mumms (2002) has recently suggested that excessive quantities of salt enter the plants and eventually rise to toxic levels in the transpiring leaves causing senescence and overall growth and development.

Much of the cropland affected by salinity is in traditional alfalfa growing regions of the world. Forage yield of alfalfa decreases 7.3% for each  $\text{dS m}^{-1}$  increase above a threshold of  $2.0 \text{ dS m}^{-1}$  (Johnson *et al.*, 1992). Maas and Hoffman reported alfalfa seedling yield is decreased by 50% at  $8.9 \text{ dS m}^{-1}$ . It might therefore be that selection of highly salt tolerant genotypes between and within cultivars could be expected to provide useful material for further breeding and for experimental comparisons (Al-Khatib *et al.*, 1993).

It was seen that percent germination, seedling length decreased with increase in salt concentration. Misra and Dwivedi (2004) studied the effect of salinity levels on green gram. It was observed that in the absence of salinity almost 100% germination was observed from day 1 onwards.

However, in the presence of salinity the seed germination decreased. The decrease was more prominent at the beginning which progressively became less prominent during subsequent days of germination at all salinity levels. Furthermore with increasing salt concentration the germination of seeds decreased progressively. The seedling vigour (root and shoot length) increased gradually with 1-5 days of seed germination under the conditions of absence and presence of various levels of salinity. However, salinity treatment resulted in decrease in the root and shoot length as compared to control values. Inhibition of germination due to salinity has been reported earlier (Ghoulam *et al.*, 2002; Niazi *et al.*, 2004; De Lacerda *et al.*, 2003; Hassan and Maryam, 2009).

## CONCLUSION

In conclusion, plant breeding may provide a relatively cost effective short-term solution to the salinity problem by producing cultivars able to remain productive at low to moderate levels of salinity. Salinity effects the growth, development and germination of alfalfa adversely. Out of the two varieties recently developed, the germination, growth (flowering, root and shoot) showed a better development of Anand II variety as compared to IGFRI-S-54. RGR, NAR and LAR analysis indicate that salinity has direct relationship with NAR and RGR irrespective of LAR in both salinity tolerant and susceptible varieties of Lucerne.

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