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# Nitrogen fixation by alfalfa as affected by salt stress and nitrogen levels

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# NITROGEN FIXATION BY ALFALFA AS AFFECTED BY SALT STRESS AND NITROGEN LEVELS

by

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**ABSTRACT**

The yield of alfalfa (Medicago sativa L.) in Arizona is generally much higher than yields in other states. Nitrogen (N) fertilizer is rarely applied to alfalfa fields because high yields are attainable with symbiotic N fixation between alfalfa and Rhizobium meliloti. The growth and N fixation by one low salt tolerant alfalfa and two germination salt tolerant selections were investigated at two salt levels (0, -0.6 Mpa) and two N rates (1, 5ppm) using a system which automatically recirculates a nutrient solution.

The high level of salinity (-0.6 Mpa osmotic potential of culture solution) resulted in substantial reduction in the N fixation percentage and total fixed N. The effect of salinity was more pronounced for later cuttings than for the earlier cutting.

The N fixation percentages were substantially decreased by increasing N level and the reduction was enhanced by time. The N treatment levels did not exhibit a significant effect on total fixed N.

Cultivars did not differ in either growth or N fixation. However, the interaction of N and salinity significantly decreased the percentage and amount of N fixation.

# CHAPTER 1

## INTRODUCTION

Alfalfa (Medicago sativa L.) is one of the legumes grown in large areas of arid and semiarid environments, not only because of its ability to fix N and moderate salt tolerance of some varieties, but also it is well suited to the sunny dry climate. Alfalfa is the third most economically important crop in Arizona, and produces high annual yields, ranging from 9.0 to 27.0 metric tons/ha in contrast to the national average of 7.6 metric tons/ha (USDA, 1987). The ability of alfalfa to fix N in symbiotic association with bacteria Rhizobium meliloti has played an important role in the high production of alfalfa as has the favorable climatic conditions.

The continuous depletion of soil N and the necessity for higher crop yields have led to ever increasing emphasis on means of conserving the limited supply of this element. Because only a fraction of total agricultural need for N comes from synthetic and natural fertilizers, the remaining portion must be satisfied from the soil reserves and through the biological fixation of atmospheric N.

Biological N fixation by all the different types of organisms in the world has been estimated to fix 80 million

metric tons annually from the vast supply in the atmosphere. Crop legumes account for about 35 million tons. In contrast, only 50 to 60 million metric tons of N are produced annually by fertilizer manufactures at a high cost (Nitrogen fixation for tropical agricultural legumes project, 1984). The effective utilization of the biological N fixation is an alternative means for satisfying the increasing demand for N fertilizer, and to overcome the problem of high cost of fertilizer and pollution problems.

As the biological N fixation is such an important process, the quantity of N fixation should be a criterion for evaluating alfalfa and other legumes in current use as well as new genetic materials under salt and drought stress. However, very little information is available about the salinity effect on quantitative N fixation of alfalfa. Cepeda (1987) showed that N fixed in the first harvest was from 61 to 27 % of total shoot N in the plant when salinity levels increased from 0 to -1.2 Mpa with 5 ppm N in the nutrient media. In the second harvest, fixed N percentages were from 94 to 57 % of the total shoot N in the same range of salinity as the first harvest except only 1 ppm N was applied. The causes of the different N fixation percentages under the same osmotic potential in two harvests need to be investigated correlating to plant stages and N rates.

The objectives of this study were:

1. To compare the growth and development of alfalfa cultivars under various N levels and salt stress.

2. To evaluate the effect of increasing salinity levels and N rate on N fixation by three alfalfa cultivars.

2. To identify whether the growth process or the different N levels cause different rate of N fixation by the above alfalfa cultivars.

# CHAPTER TWO

## LITERATURE REVIEW

More than 4 million $km^2$ of potential arable land are affected by excessive soluble salt in the world (Flowers et al., 1977). If these lands are managed properly, increasing productivity of these agricultural areas can be very significant. Also as saline water irrigation and inorganic fertilization increase dramatically, the secondary salinity problems of productive agricultural lands become more serious in irrigated areas. The detrimental effects of salinity are due to the influence of ions on the water activity external to the root which affect the water status of the plant. The salt stresses also affect directly physiological and biochemical functions of the ions in the plant (Flowers et al., 1977; Greenway et al., 1980; Hasegawa et al., 1986). These can result in induction of ion deficiency due to inadequate transport and selectivity mechanisms (Jeschke, 1984), inhibition of photosynthesis (Shone and Gale, 1983), or increased use of metabolic energy for non-growth process involved in maintaining tolerance to salinity (Hasegawa et al., 1986). As a result, the number of alfalfa shoots, root nodules, the dry weight of shoot, root, whole plant or root

nodules (Noble et al., 1984; Mikkelson et al., 1988), the concentration of total N, the amount of N fixation (Cepeda, 1987), or the percentage of seed germination declined as the salt stress increased (Dotzenko and Haus, 1960; Allen et al., 1986).

## General Effects of Stress on Alfalfa Growth

Genetic variation for salt tolerance among species and within cultivars may be important sources of salt tolerant genotypes. In plant breeding, it is essential to identify the stage most sensitive to salinity effects that may be utilized in improving the tolerance at this sensitive stage. A number of reports show that alfalfa (Medicago sativa L.) is more sensitive to salt stress at the stage of germination (Uhvits, 1946; Forsberg, 1953; Chang, 1961; Robinson et al., 1986) or in young seedlings (Forsberg, 1953; Chang, 1961) than later stages. Therefore, considerable research has focused on increasing salt tolerance at the germination stage by selection of germination salt tolerant cultivars (Dotzenko and Haus, 1960; Carlson et al., 1983; Allen et al., 1985). Dotzenko and Haus (1960) observed alfalfa seeds that germinated at -12 atm (-1.2 Mpa) of mannitol and found that the capacity of alfalfa to germinate under high osmotic stress had a high heritability. Allen et al. (1985) released five

Arizona Salt Tolerant germplasm sources 'AZST 1978' through 'AZST 1982' and reported broad sense heritability for germination salt tolerance in alfalfa of 50% and the germination salt tolerant cultivar at -1.3 Mpa had increased germination from 3% (germination rate of parent materials) to 86%. Carlson et al. (1983) identified that one cycle of phenotypic selection in a 1.75% NaCl agar mixture increased germination of the resulting generation by 3.75 times.

The evaluation of the effects of salt stress on seed germination of alfalfa cultivars showed many interesting results. Allen et al.(1986) reported no difference was found between the parent cultivar Mesa-Sirsa and the most salt tolerant selection of germination stage AZST 1982 in $Na^+$ and $Cl^-$ during germination. Selection of germination tolerant alfalfa can increase tolerance of toxic effects of NaCl and osmotic effects of other osmotic agents. Stone et al.(1979) determined the germination percentages of 'U. C. Salton' and 'Ladak 65' alfalfa cultivars using nine concentrations of salt and four temperatures. They found a highly significant interaction effect on alfalfa seed germination between temperature and osmotic potentials. Robinson et al.(1986) reported the seven cycles of recurrent selection for germination salt tolerance Cycle 7 Syn-1 had extended its germination range of 1 Mpa and its seeds germinated more than

two times as fast as Mesa-sirea at -1.90 Mpa. Different aged seeds from the same germplasm source differed significantly in germination salt tolerance when expressed in the percentage of its nonsaline germination rate. The germination salt tolerant rate was adversely correlated to solute leakage of stressed seed (Smith and Dobrenz, 1987). Assadian and Miyamoto (1987) indicated irrigation with saline water more than 6 $dSm^{-1}$ significantly reduced the rate of alfalfa 'Moapa' and 'Mesilla' emergence, but the emergence rate was least affected by salinity at deepest seedling depth of 15 mm.

Abel and MacKenzie (1964) found that salt tolerance at maturity and during seed germination were unrelated in soybean (Glycine max (L) Merr.). Similarly, the germination salt tolerant selection of alfalfa cultivars showed no difference in salt tolerance in later stages compared with its germplasm sources (Allen, 1984; Cepeda, 1987). Therefore, selection of overall salt tolerance must embrace tolerance at several growth stages. Noble et al.(1984) conducted an experiment to select salt tolerance alfalfa cultivars using the criteria of percentage leaf damage and length of the main shoot after alfalfa grew for 70 days under 250 mM NaCl. They found that the shoot and root dry matter production as well as the shoot number and shoot length of the alfalfa selections were significantly higher than their parent alfalfa cultivar.

Both sodium and chloride concentration of the shoot in the tolerant alfalfa selection were lower than in the salt sensitive one, whereas Na and Cl level did not change in the root with the degree of salt tolerance.

The application of tissue culture to plant breeding provides an alternative means and the most important potential for contributing new genetic variability to plant breeders. This method involves cultured plant cells in culture solution with certain levels of salt to select salt tolerant mutants from the cell culture. This approach permits characterization at cellular levels of the process involved in salt tolerance. Croughan et al.(1978 ) cultured alfalfa plant cells in culture medium with NaCl added. They observed a salt tolerant line of cultured alfalfa cells. These cells grew better than unstressed cultured cells at high levels of NaCl, but performed poorly in the no saline culture. They suggested that the salt tolerance of alfalfa mutant was a consequence of a shift towards a true halophytic nature.

Only few studies showed salt effects on the growth of alfalfa at later stages. Bower (1969) found that alfalfa yield decreased 10% at 5 dS $m^{-1}$ of electrical conductivity (EC) of soil saturation-extraction value and the yield declined 50% with 11 dS $m^{-1}$ of EC. Brown and Hayward (1956) studied six alfalfa cultivars at four salinity levels for growth under

field conditions. Results showed no difference among the six cultivars and the average yield for all the cultivars was reduced to 42% of the control at EC 15.93 dS $m^{-1}$. Studies indicated the yield of both shoots and roots were reduced by addition of salinity as either a chloride salt or sulfate salt (Mikkelsen et al., 1988) while the growth of alfalfa shoots was affected more than roots by salinity (Keck et al., 1984).

Researches have shown that alfalfa yield did not respond to soil water salinity averaged by depth, but related to the calculated mean salinity against which water was absorbed (Bernstein and Francois, 1973). Bower et al.(1969) pointed out that alfalfa yield was closely related to average salinity in the root zone. Ingvalson et al.(1976) concluded that the soil water salinities integrated over time was the best alfalfa yield indicator among irrigation salinities; averaged profile salinity and soil salinities weighted on the basis of water uptake by crop at the rootzone depth.

## The Physiology and Mechanism of Alfalfa Salt Tolerance

One of the major salt effects on the plant is that a high salt concentration will depress plant growth by osmotic effects. To study the salt stress and osmotic effects, sodium chloride (NaCl) and polyethylene glycols (PEG) have been widely used (Jensen, 1981; Lawlor, 1970; West et al., 1980;

Sanchez-Diaz et al.; 1982). Several studies showed the effects of NaCl and PEG on N metabolism (Frota and Tucker, 1978), the absorption of $NO_3^-$ and $NH_4^+$ (Frota and Tucker, 1978) and ion relations (Storey et al., 1978). Sanchez-Diaz et al.(1982) showed that the effects of N fixation and other physiological parameters of alfalfa were always greater in plants in PEG than in NaCl solution. They concluded that the result was probably induced by penetration of PEG which caused plant damage. Kawasaki et al.(1983) reported NaCl treatment depresses K, Ca and Mg content more severely than PEG treatment, even though PEG has a slightly larger inhibitory effect on plant growth than NaCl.

Allen et al.(1986) found that the average seed respiration of Mesa-Sirsa was higher than the selection of salt seed germination tolerance AZST 1982 during germination under salt stress conditions. Shone and Gale (1983) reported that salt stress increased the respirations of alfalfa at low salt concentration, whereas the high salt stress inhibited the respiration during a short day length. They also reported that salt stress decreased photosynthesis without stimulating respiration during a very short day length. Increase in respiration under salt stress may be a characteristics of salt tolerance which may suggest an ability to divert assimilates and respiratory energy to the repair of tissues and to active

transport process.

The major inhibitory effects of salinity on alfalfa growth also include imbalance of nutritional ions and toxic effects caused by the ions. Sodium and chloride contents of alfalfa plants increased greatly under salt stress as shown in many studies (Bernstern and Pearson, 1956; Chang, 1961; Bernstein and Francois, 1973; Smith, 1981; Noble, 1984). The sodium and chloride concentration in the shoot was inversely correlated to plant tolerance but chloride was a better indication of salt tolerance than sodium (Noble, 1984). The plant damage caused by chloride salt was more severe than that caused by sulfate salt or phosphate salt which may be due to $Cl^-$ toxic effects. This effect was enhanced by increasing temperature and moisture. The leaflet accumulated the highest chloride concentration (Smith, 1981); on the other hand, the levels of chloride in alfalfa roots were relatively unchanged with increased salt or salt tolerance (Smith, 1981; Noble, 1984). The reduction of N, P, Ca, Mg, S, B, Mn and the increased Na, Cu, Fe, Al concentrations have been reported for alfalfa under high salinity (Smith, 1981) while the K, Ca, Mg concentrations dropped in plant under increased salt stress (Kawasaki, 1983).

The mechanism of ion exclusion is effective at low to moderate levels of salinity (Hasegawa et al., 1986), and has

been related to salt tolerance in plant growth in several glyphytic crops. Abel and MacKenzie (1964) reported that salt tolerant cultivars had a greater capacity to exclude $Cl^-$ compared with salt sensitive soybean. This restricted accumulation of both sodium and chloride is a common response to salt stress by salt tolerant plants.

Accumulation of organic solutes is an important salt tolerance mechanism found in both plants and some bacteria. In salt adapted cells, the amount of sugars, free amino acid, glycine betaine and proline increased with the levels of adaption. The function of organic solutes in salt tolerance has been hypothesized to balance the osmotic potential difference between cell cytoplasm and cell vacuoles (Steward and Lee, 1974) or it may protect the enzyme activity when high electrolytes are present in the cytoplasm (Pollard and Wyn Janes, 1979).

A notable number of papers indicate that glycine betaine or proline, normally minor constituents of the amino acid pool in many organisms, may play an important role in plant salt tolerance. Bar-Nun et al.(1977) showed proline content in the free amino acid pool increased following the salt stress rise and proline could mediate the inhibitory effects of NaCl on pea germination and root growth. Also, Steward and Lee (1974) found the enzyme activity in vitro was not reduced

when proline reached the level of 600 mM. Addition of glycine betaine can counteract the reduction of N fixation under high osmotic stress of Klebsiella pneumoniae (Bouillard and Le Rudulier, 1983) and Rhizobium mililoti (Pocard et al., 1984). The function of glycine betaine and proline are not very clear. Some hypotheses have been presented in the literature. One is that glycine betaine probably balances the different osmotic potentials between cytoplasm and vacuoles (Rataeli-Eshkol and Avi-Dor, 1968; Bouillard and Le Rudulier, 1983). It also might interact with NaCl, protein, and water (Pollard and Wyn Jones, 1979); Or its effect could be exerted on membrane stability (shkedy-Vinkler and Avi-Dor, 1975).

Membrane permeability is closely correlated to the tolerance of salt stress. One study (Shkedy-Vinkler and Avi-Dor, 1975) suggested that membrane permeability and stability were positively related to Na/Ca ratio and the content of glycine betaine, respectively. Membrane lipids may also adjust the solute permeability of plant cellular membrane (Muller, 1978). Hirayama and Mihara (1987) showed the ratio of glycolipid/phospholipid in roots increased when the plant salt tolerance increased. A slight increase in the ratio was also found with increasing external salinity of a salt sensitive plant. They concluded that the glycolipid/ phospholipid ratio directly regulated the membrane permeability.

## Nitrogen Rate and Salt Stress Effects on Symbiotic Nitrogen Fixation of Alfalfa and Rhizobium

A number of researchers (Richardson et al., 1957; Weber and Leggett, 1966; Fishbeck and Phillips, 1981) when studying the N effects on alfalfa which showed growth, N fixation and nodulation of alfalfa (Medicago sativa L.) were enhanced by various levels of N fertilization under certain greenhouse conditions. Fishbeck and Phillips (1981) indicated dry matter weight and N concentration of alfalfa were increased by N fertilization in early growth stages. Eardly et al.(1985) found $NH_4NO_3$ application did not affect alfalfa dry matter weight significantly but the nodulation and acetylene reduction diminished in seedling alfalfa under field conditions. Fishbeck et al.(1987) reported low levels of N application increased both dry matter yield and N uptake (mg/plant) of ineffective symbioses, whereas the N fixation by effective symbioses were decreased significantly by N fertilization. Aparicio-Tijo and Sanchez-Diaz (1982) also showed N fixation decreased in the presence of $NO_3$. Applied N did not affect the preference of alfalfa for a certain Rhizobium strain. The N inhibition of N fixation may contribute to decreased nodule formation (Hardarson et al., 1981) or may inhibit nitrogenase activity (Becana et al., 1986). Information is not available about the N effects on

symbiotic N fixation under salt stress.

Studies of salt stress should not only emphasize their effects on the growth and physiology of legumes, but also the salt effects on the symbiotic N fixation. However, the area of salt effects on N fixation has been largely ignored by scientists. Nitrogen fixation by various crops under normal conditions has been reported quantitatively. Broadbent et al. (1982) showed that clover could fix about 85-100% of its N under field condition. El-Hassan and Focht (1986) showed cowpea obtained 60 to 98% of shoot N from N fixation and the Rhizobium partner effectiveness affected the N fixation rate dramatically. George et al.(1988) found N fixation provided 66 to 97% of its N in soybean at different elevations and the highest elevation showed the largest fixation rate. They concluded low temperature may have decreased root growth and soil N uptake, or reduced available soil N more than it reduced N fixation. At this point, the question should be raised as to how the N fixation of legume crops is affected by salt stress.

Saline conditions might affect the N fixation symbiosis in several aspects: (1) inhibiting survival of Rhizobium spp. in the soil and rhizosphere, (2) diminishing plant growth and photosynthesis and (3) affecting the interaction of the infection process and root nodule function (Parker et al.,

1977). Therefore, a salt tolerant alfalfa cultivar should be accompanied by a salt tolerant Rhizobium mililoti stain with highly infective and effective characteristics for growth in saline soils.

The effect of salinity on Rhizobium varies from species to species and from strain to strain (Yadav and Vyas, 1971). Douka et al.(1978) reported that Rhizobium isolated from salt affected soil can survive under high salinity with no history of previous alfalfa growth. Pillai and Sen (1973) found the growth rate of Rhizobium spp. increased with 1% NaCl added. Stenborne and Roughley (1975), and Singleton et al.(1982), on the other hand, showed a reduction of growth rate of Rhizobium when salt was added. The later also showed that there were no consistent salt tolerant differences between isolates from salty soil and the isolate from non-saline environments. Furthermore, many Rhizobium can grow and survive at higher salt levels than most legumes can tolerance. Raman and Prasad (1983) obtained a Rhizobium mutant which can tolerate 1.5% of NaCl While Yadav and Vyas (1971) showed the salt resistant strain could grow and survive in up to 3.0% salt concentration.

The salt tolerance of alfalfa for N fixation varies among cultivars. Bekki et al.(1987) reported that the $C_2H_2$ reduction activity of Medicago ciliaris nodules was still 50% of the

control under 200 mM NaCl, whereas the activity of nodules of Medicago sativa ceased by 100 mM NaCl. Brown and Hayward (1956) showed that alfalfa yield decreased 42% at 15.93 dS $m^{-1}$ of salinity.

Tan and Tan (1986) reported that the interaction between alfalfa cultivars and Rhizobium strains presented the largest percentage of variation for total dry matter and acetylene reduction rate. Therefore, the salt effect on the infection process and nodule function can directly reduce the amount of N fixation. Lakshmi-Kumari et al.(1974) indicated that increasing salt levels reduced the number of root hairs and formed a mucilaginous layer around roots. Root hair infection and the number of nodules were reduced at 0.2% NaCl. Subba Rao et al.(1972) found that 0.4% NaCl delayed the initial nodulation and diminished the number of nodules. When 0.7% of NaCl was present, the plant could not form nodules. Keck et al.(1984), and Bernstein and Ogata (1966) showed that the nodule weight was insensitive to salt stress. However, the salt treatments in these experiments were initiated at 60 days and 4 weeks, respectively. It could be inferred that the root nodules may be already formed or partially formed before the salt treatment began.

# Chapter 3

## Material and Methods

The experiments were conducted under greenhouse conditions at the University of Arizona campus. One low salt tolerant alfalfa (Medicago sativa L.) and two germination salt tolerant alfalfa selections) were used in sand culture systems which automatically recirculated a nutrient solution to study N fixation under various N levels and salinity stress. Alfalfa was selected for this study primarily because new genetic materials were available that are known to have varying degrees of germination salt tolerance as well as the original line from which the selections were made; alfalfa also has the highest N fixation rate among legume crops, and is generally grown as a major forage crop in both saline and non-saline soils. Alfalfa has also been studied extensively in regard to its response to either N or salt stress. In order to measure the N fixation by alfalfa, $^{15}N$ labelled N was used in nutrient solution to allow $^{15}N$ measurement of fixed amount of plant N.

### Environmental Conditions

During the experiment, the light was provided by a combination of greenhouse natural light at approximately 12

hours photoperiod with supplemental light to a total of 14 hours photoperiod. The supplemental light was provided by 4 cool white fluorescent bulbs which were turned on and off automatically by a time clock.

The air temperature and relative humidity were recorded on a hygrothermograph and summarized in Table 3.1.

Table 3.1. Average maximum and minimum temperature (temp) and relative humidity during alfalfa growth.

| | First cutting (40 days) | Second cutting (29 days) | Third cutting (22 days) |
|---|---|---|---|
| max. temp. | 29.6 °C | 32.7 °C | 34.4 °C |
| min. temp. | 11.9 °C | 13.7 °C | 14.2 °C |
| max. RH | 70.2 % | 66.5 % | 58.8 % |
| min. RH | 29.4 % | 25.4 % | 31.8 % |

## Experimental Procedures

Nitrogen fixation rate by alfalfa plants was studied at two different NaCl salinity levels and two N levels. The combination of three alfalfa cultivars with two salinity levels and two N rates resulted in 12 treatments, which were arranged in a Randomized Complete Block Design with 6 replications. The alfalfa plants were harvested three times. The experimental treatments were arranged as follows:

| Cultivar | N level ppm | Osmotic potential Mpa |
|---|---|---|
| Mesa Sirsa 83 cl | 1 | 0.0 |
| | 5 | 0.0 |
| | 1 | -0.6 |
| | 5 | -0.6 |
| Mesa-Sirsa $C_3$ | 1 | 0.0 |
| | 5 | 0.0 |
| | 1 | -0.6 |
| | 5 | -0.6 |
| Mesa-Sirsa $C_5$ | 1 | 0.0 |
| | 5 | 0.0 |
| | 1 | -0.6 |
| | 5 | -0.6 |

The plants were grown in sand culture and half strength Hoagland solutions which were automatically recirculated. The

salt stress level was achieved by adding NaCl to the culture solution. Nitrogen was provided to plants by the addition of $^{15}N$ labelled potassium nitrate (5.1 atom % $^{15}N$) to the nutrient solution.

Preparation of the experiments

Alfalfa (Medicago sativa L., 'Mesa-Sirsa 83 CL') used in this study was from the Mesa-Sirsa population (Schonhorst et al., 1968). The two germination salt tolerant selections (Mesa-Sirsa cycle 3 and cycle 5 ) are populations selected from 3 and 5 cycles of recurrent selection for NaCl tolerance during germination, respectively. These are non-dormant cultivars.

Approximately 3,500 $cm^3$ of silica sand (California Silica Products Co.) was placed in each plastic tube (45 cm long x 10 cm in diameter). The sand in the tube was watered with deionized water by a drip irrigation system for three days to clean the sand culture system. Eight alfalfa seeds from each cultivar were planted in each plastic tube on 14 Jan., 1988. The alfalfa seeds were inoculated by adding PELLNOC, a commercial inoculant ( The Nitragen Company, Milwaukee, WI) over the seed and covering with approximately of 0.8 cm of sand. All plants were watered with a half strength Hoagland's solution lacking N (Hoagland and Arnon, 1950). This nutrient solution was prepared by mixing 25 liters of the full strength

Table 3.2 Nutrient content of a full strength Hoagland's solution lacking nitrogen (Hoagland and Arnon, 1950)

Solution 1. Macronutrients

| Substances | ml/liter of nutrient solution |
|---|---|
| 0.5 M $K_2SO_4$ (87.3 g/l) | 5 |
| 1.0 M $MgSO_4$ (120.36 g/l) | 2 |
| 0.05 M $Ca(H_2PO_4)_2$ $H_2O$ (12.61 g/l) | 10 |
| 0.01 M $CaSO_4$ $2H_2O$ (1.72 g/l) | 200 |

Solution a: Micronutrients

| Substances | grams/ liter of water |
|---|---|
| $H_3BO_3$ | 2.86 |
| $MnCl_2$ $4H_2O$ | 1.81 |
| $ZnSO_4$ $7H_2O$ | 0.22 |
| $CuSO_4$ $5H_2O$ | 0.08 |
| $H_2MoO_4$ $H_2O$ | 0.02 |

Solution b: A 1300 ppm solution was prepared by adding 21.67 g/liter of iron chelate (EDDHA, 6% of Fe).

Final N free solution: One ml of solution a and one ml of solution b were added to each liter of nutrient solution to prepare N free Hoagland's solution.

Hoagland's solution with 25 liters of deionized water to produce a half strength nutrient solution lacking N. Description of the full strength Hoagland's solution lacking N is presented in Table 3.2.

The sand culture system which recirculated the nutrient solution can be described as following: Fifty liters of prepared half strength nutrient solution were placed in each of the four reservoirs. A drip irrigation system was used to deliver the solution to each individual plastic tube which containing one of the planted alfalfa cultivars. This process was accomplished by placing submergible pumps (Little Giant Pump Co., model 3E-12N. Oklahoma City, OK) to the reservoir and connecting to the plastic pipe line for each solution reservoir. Small plastic lines connected the plastic lines and the Agrifin emitter which irrigated at the rate of 45 to 55 ml per minute by actual measurement. The system was controlled by a electrical timer set for a 20 minute irrigation period every 2 hours. The irrigation solution drained from bottom of the plastic tube and returned to the original reservoir by a drainage pipe line.

The alfalfa cultivars were completely germinated on the fourth day after planting. Five days after germination, the alfalfa plants were thinned to four plants in each tube. For two weeks following planting, the alfalfa plants were grown

with a normal half strength Hoagland's solution lacking N. The solution in the reservoirs were monitored daily and water was added to keep a constant volume in the reservoirs.

Nitrogen and salt stress treatments

The salinity levels were achieved by adding NaCl to nutrient solution. Salt treatments were made by adding the first salt two weeks following planting. The osmotic potential of the nutrient solutions was decreased by addition of 24 meq NaCl/liter of nutrient solution for -0.1 Mpa of stress (O'Leary, 1969; Forta and Tucker, 1978). The osmotic potential was decreased -0.3 Mpa every other day by added total 210 g NaCl until -0.6 Mpa osmotic potential was reached. The measured pH in all solutions were around 6.6 to 6.7 while the measured osmotic potential in salt treatments were 16 dS $m^{-1}$.

Nitrogen level of the treatments was achieved by adding potassium nitrate with the $^{15}N$ enriched ($K^{15}NO_3$) source to culture solution at the beginning of salt addition. The atom percent of $^{15}N$ in the potassium nitrate was 5.1.

After the N and osmotic potential reached the required level, the reservoirs were monitored and water was added as needed to maintain a constant volume and therefore to insure a constant osmotic potential in the solution during the experimental period.

The first cutting was made 40 days after alfalfa

germination. On the same day, new solutions were added after the old solutions were drained from the reservoirs. The salinity and N treatments were achieved simultaneously by adding NaCl and $^{15}N$ labelled $KNO_3$. The second cutting was made 29 days after the first cutting. Similarly, the nutrient solution with N and salt added was renewed in the reservoirs as before for the alfalfa regrowth. The third growth period was 22 days.

All the procedures described for the first cutting were valid for the second and the third cutting, however, with the following exceptions : 1) salt treatments were renewed immediately after each cutting (for the first cutting, salt treatments started 10 days after germination), 2) the second and third cuttings were from regrowth of axillary stems rather than the primary stems of the first cutting which originated from seed, 3) the third cutting was made immediately at the surface in contrast to other cuttings which were made about 3 cm above the surface.

In the first and second cuttings, plants were dried in an oven at 70°C for 48 hours, then plant dry matter weights were determined by weighing after samples cooled to room temperature. The plant materials were ground in a Wiley mill with a 30 mesh screen, and saved for chemical analysis. After the third cutting, plants were separated into roots and

shoots. The roots were removed from the plastic tube and washed in water for about 30 seconds to insure that the roots were free of sand. Then the roots and shoots were dried, weighed and ground in the same way as other cuttings and saved for chemical analysis.

## Chemical Analysis

Total Nitrogen Determination:

The total N in shoots and roots was analyzed by the micro-Kjeldahl procedure. Samples of 100 mg of dry plant materials were placed in digestion tubes and digested with 5 ml of concentrated $H_2SO_4$ and 1.5 g of mixture catalyst (15 g of $K_2SO_4$ plus 0.7 g of HgO), until a transparent solution appeared. After the digestion tubes cooled, water was added to the tubes and the solutions were transferred to micro-Kjeldahl flasks. The sample was steam distilled after addition of approximately 20 ml 50% NaOH. The ammonia liberated was collected in a 5 ml boric acid indicator solution. The collected solution was titrated with 0.01 N $KH(IO_3)_2$ and the total N percent was calculated from the amount of $KH(IO_3)_2$. After the titration, the samples were redistilled and collected in 0.5 ml of 0.5 N $H_2SO_4$, and stored in cold a room for atom percent $^{15}N$ determination.

$^{15}N$ determination

The atom percent in plant samples was calculated from 28 and 29 mass ratio determined by a mass spectrometer (Consolidated Electrodynamics Corporation Model 21-621), following the method described by Bremner (1965) and the procedures by Frota and Tucker (1978) and Pessarkli (1981).

Solution samples saved from the total N determination which contained $NH_4^+$ labelled $^{15}N$ were used to determine $^{15}N$ according to the above procedures. The main steps of the procedure can be summarized as follows (Pessarkli, 1981):

1. Dried about 2 ml of solution samples completely in a sample tube.
2. Cooled the dried residue and repelled the air with argon gas.
3. Added about 0.3 ml of argon saturated deionized water into each sample tube held in dry ice, and connected to the argon gas system. The frozen water layer was formed immediately over the sample and kept it free of air.
4. Imparted a similar amount of argon saturated sodium hypobromite (NaOBr) on the top of the completely frozen layer in each tube and allowed to freeze.
5. Attached the sample tube to the inlet system of a mass spectrometer followed by rough and diffusion pumping to insure that the samples were air free. Then samples

were thawed the to permit sodium hypobromite to react with ammonium and converted it to $N_2$. The reaction is presented as follow (Bremner, 1965):

$$2NH_3 + 3NaOBr = 3NaBr + 3\ H_2O + N_2$$

6. Determined and calculated atom percent of $^{15}N$ by the recording the mass 28 and 29 peak height.

## Calculation of Nitrogen Fixation

The fraction of N fixed by Rhizobium was calculated from percent of N in the plant tissue derived from the nutrient solution (Cepeda, 1987). This percent of N is also called "percent of N in plant from source salt " (% NPSS) and it is calculated from data derived from the $^{15}N$ analysis with a mass spectrometer. The follow equation was employed to calculate the percentage of N fixed (% NF) when the percentage of N recovered was known.

$$\% \text{ NF} = 100 - \% \text{ NPSS}$$

The percent of N in plant from source salt (% NPSS) was determined as follows:

Percent of N in plant from source salt (% NPSS): The atom percent $^{15}N$ in all sample solutions was computed from 28 to 29 mass to charge ratios determined by a mass spectrometer. From the atom percent $^{15}N$ in each sample, % NPSS was calculated by using the equation below:

$$\% \text{ NPSS} = \frac{(\text{Atom } \%\ ^{15}\text{N in sample} - \text{Normal})}{(\text{Atom } \%\ ^{15}\text{N in fertilizer} - \text{Normal})} \times 100$$

Where:

Atom % $^{15}$N in sample = sample atom % $^{15}$N calculated from the 28 and 29 m/e peak height,

Atom % $^{15}$N in fertilizer = Atom % $^{15}$N of a solution containing the fertilizer used in the experiment.

Normal = natural abundance atom % $^{15}$N as given for the mass spectrometer (values close to 0.366).

## Statistical Analysis

An analysis of variance (ANOVA) for a Completely Randomized Design was employed to analyze all data obtained in this experiment. The Duncan Multiple Range Test was used to compare the means for cultivars, nitrogen rates and osmotic potentials. The following transformation was done for data presented in percent (% total-N and % N - fixed) prior to the ANOVA procedure:

$Y_1$ = arc sine (SQRT (y/100))

y = % data

The $Y_1$ value was assigned to each experimental unit and the ANOVA procedure was executed. These transformations were

preformed to satisfy one of the requirements for analysis of variance (i.e. treatment variances must be homogeneous). The arc sine transformation has been suggested by Gomez and Gomez (1983) and has been used by others. All the ANOVA and Duncan tests in this research were executed through SAS software for analysis of statistical data (1985).

# CHAPTER 4

## RESULTS AND DISCUSSION

This research was designed to evaluate the effects of different NaCl salinity levels and N rate on N fixation by various alfalfa cultivars. For this purpose, the plant samples of several alfalfa cuttings at bud stage (50% plants have first flower bud) was used for determining the total dry matter production (g/pot), total Kjeldahl-N percentage, total Kjeldahl-N content (mg/pot), fixed fraction of N content (mg/pot), and concentration of fixed N (Fixed N%) in shoots and roots. The results of measured and calculated parameters in this experiment are given in the appendices.

### Dry Matter Production

The standard alfalfa, 'Mesa Sirsa 83CL' and the two germination salt tolerant selections were compared by using dry matter production. The analysis of variance for dry matter production of the first, second and third cuttings in Table 4.1 shows that only the dry matter production of the first cutting had statistically significant differences among cultivars, whereas, no differences were found for other cuttings. The Duncan test for dry matter production of shoots

Table 4.1. Analysis of variances for dry matter production of shoots and roots.

| | FCS | SCS | TCS | TCR |
|---|---|---|---|---|
| Cult | * | ns | ns | ns |
| OP | ** | ** | ** | ** |
| N | ** | ns | * | ** |
| Cult*OP | ns | ns | ns | ns |
| Cult*N | ns | ns | ns | ns |
| OP*N | ns | ns | ns | ns |
| Cult*OP*N | ns | ns | ns | ns |

ns = non significant F-test; * = significant F-test at 5% level

** = significant F-test at 1% level; Cult = Cultivars;

N = Nitrogen rate; OP = osmotic potential;

FCS = First cutting shoots; SCS = second cutting shoots;

TCS = third cutting shoots; TCR = third cutting roots.

Table 4.2. Mean value per cultivar for dry matter production at different cuttings.

| Cultivar | Dry weight of plant (g/pot) | | | |
|---|---|---|---|---|
| | FCS | SCS | TCS | TCR |
| MS-cycle 3 | 0.39a | 1.04a | 1.51a | 0.53a |
| MS-83CL | 0.35ab | 0.95a | 1.22a | 0.47a |
| MS-cycle 5 | 0.33b | 0.98a | 1.45a | 0.52a |

* Values are the means for twenty four experimental units (two osmotic potentials, two N rates and six replications.)

* Means with the same letter within a column are not significantly different at 5% probability level.

Table 4.3. Effects of salt stress and N rate on dry matter production of alfalfa shoots and roots.

| | | Dry weight of plants (g/pot) | | | |
|---|---|---|---|---|---|
| | | FCS | SCS | TCS | TCR |
| OP | 0 Mpa | 0.50a | 1.65a | 2.32a | 0.77a |
| | -0.6 Mpa | 0.21b | 0.33b | 0.46b | 0.24b |
| N rate | 5 ppm | 0.41a | 1.08a | 1.54a | 0.58a |
| | 1 ppm | 0.30b | 0.90a | 1.24b | 0.44b |

*Values are the means for thirty six experimental units (three cultivars, two salt stress or N rate and six replications).

* Means followed by a common letters within a column are not significantly different at the 5% level.

and roots during various cuttings (Table 4.2) indicated that a significant difference between the two new selections was where the new selection MS cycle 3 had higher dry matter production than selection MS cycle 5 in first cutting. No differences were shown between the standard cultivar and either of two selections. The results supported the conclusion that germination salt tolerance does not necessarily lead to salt tolerance at later stages of alfalfa (Allen, 1984) and of soybean growth (Abel and MacKenzie, 1964).

Table 4.3 shows the effects of NaCl salinity and N rate on dry matter production of alfalfa plants in three different cuttings. In all three cuttings of alfalfa, salt stress drastically reduced the amount of dry matter production. The dry matter production of alfalfa in the second and third cuttings was more severely affected by salt stress than in the first cutting. Salt stress depressed plant growth by osmotic effects as well as specific ion toxicity and nutritional imbalance. Osmotic adjustment occurred when plants were exposed to salt stress. The osmotic adjustment was achieved by uptake and accumulation of electrolytes such as Na, K and Cl, or synthesizing and accumulating neutral organic solutes such as sugar and amino acids (Greenway and Munns, 1980). Plant growth was retarded because more Na and Cl ions entered the plant cell or extra energy was consumed by

synthesizing organic solutes. The increased respiration and decreased photosynthesis under salt stress also inhibited plant growth.

Nutritional imbalance as well as specific ion toxicities could also retard plant growth resulting in low dry matter production. Smith et al. (1981) reported that alfalfa plants exposed to high salt stress result in increasing Na, Cu, Al and Fe while decrease N, P, Ca, Mg and S concentrations. Plant survival was much higher with application of high rates of $K_2SO_4$ and $Na_2SO_4$ than with KCl or NaCl due to Cl toxicity.

The amount of dry matter production of the three alfalfa cultivars in the three cuttings under salt stress with two N levels are presented in Figs.4.1, 4.2 and 4.3. The shoots and of three alfalfa cultivars in three cuttings as well as the roots in the third cutting were affected by salt stress to nearly the same degrees. The N levels increased the amount of dry matter production in first and third cuttings (Table 4.3), but the effects on shoot growth in the first cutting and root growth in the third cutting were more evident by N level than shoots in other cuttings. The higher N level resulted in a relatively higher shoot dry matter production in first cutting than other cuttings for higher N level. This was probably due to the energy used in nodule formation and N fixation. The energy is provide by respiration. The roots in the third

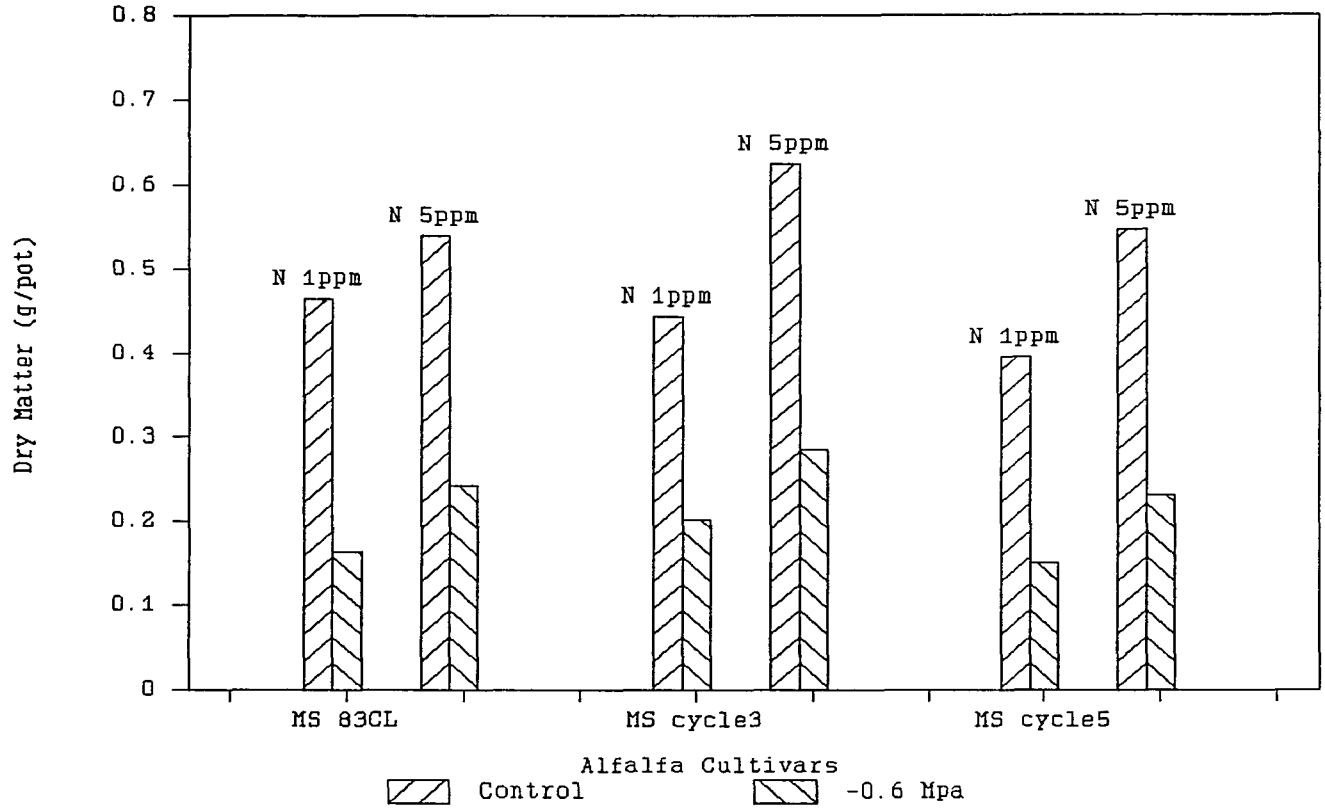


Fig.4.1 Amount of dry matter production in three cultivars of alfalfa shoots in cutting 1 under salt stress with 2 N levels

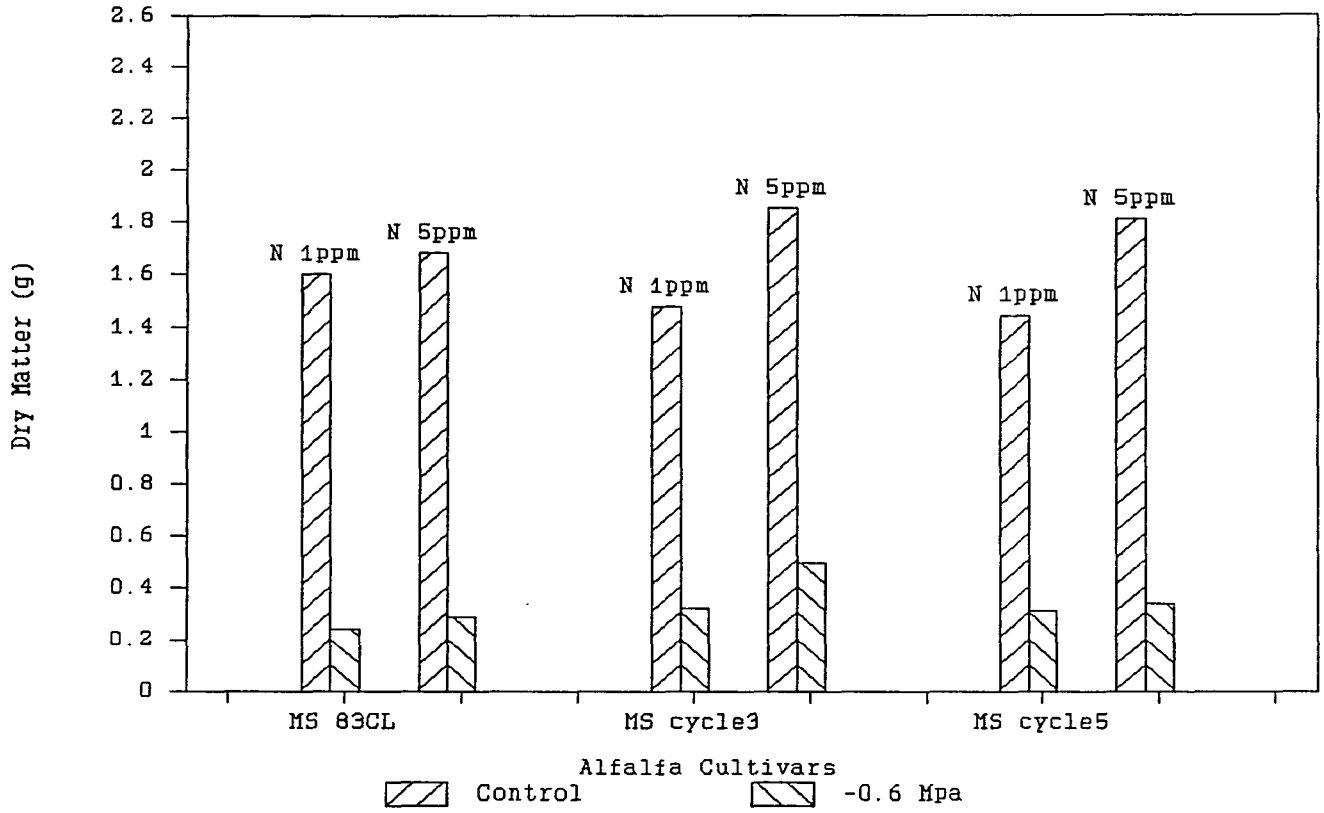


Fig.4.2 Amount of dry matter production in three cultivars of alfalfa shoots in cutting 2 under salt stress with 2 N levels

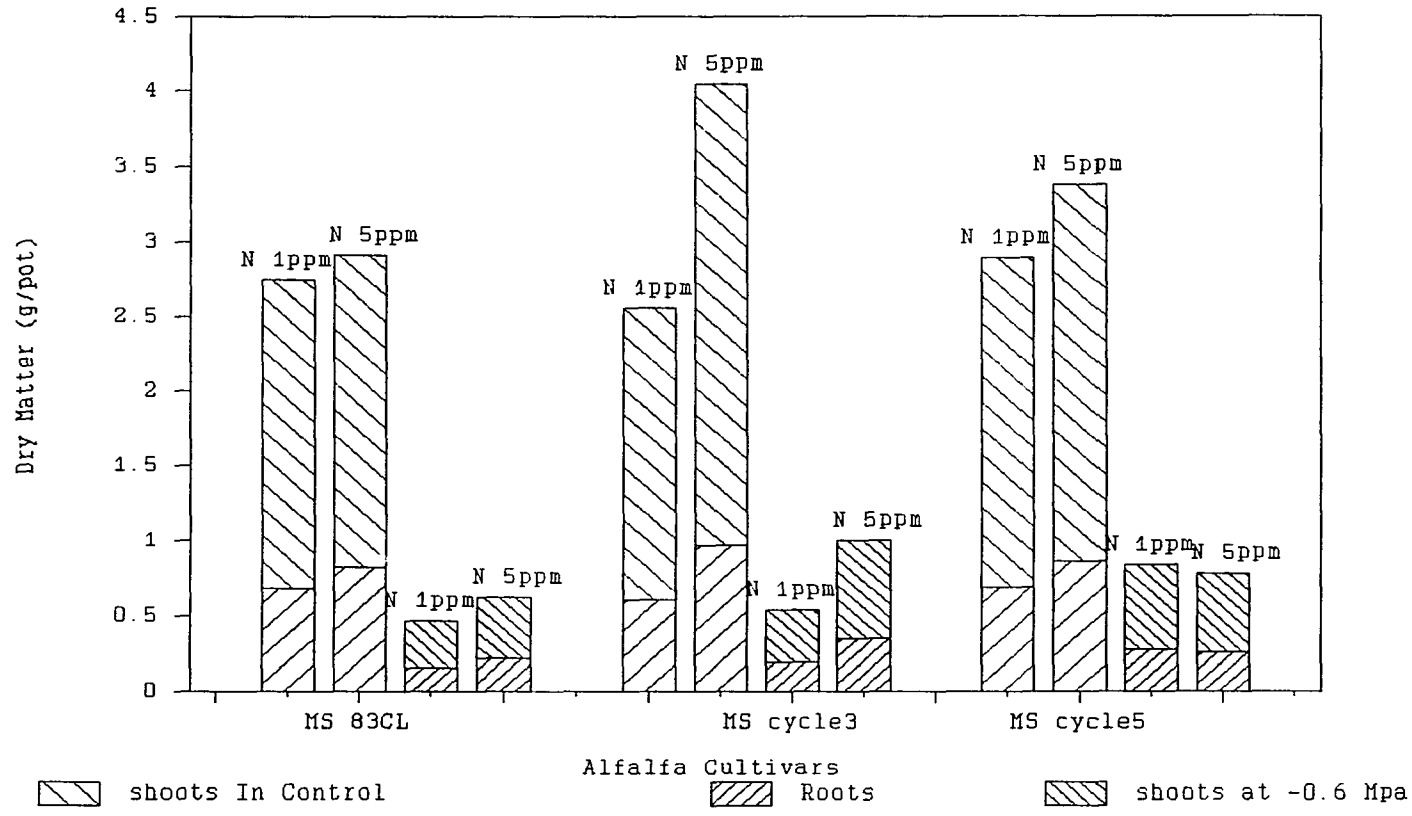


**Fig.4.3 Amount of dry matter production in three cultivars of alfalfa shoots and roots in cutting 3 under salt stress with 3 N levels**

cutting showed the cumulative effects of N on roots during the growth period. Less N applied to alfalfa cultivars resulted in smaller amount of root dry matter production.

The complete picture of the salt stress and N levels effect on each individual alfalfa cultivar can be observed clearly (Figs.4.1, 4.2 and 4.3). Salt stress affected the alfalfa cultivars in a similar way at both 1 and 5 ppm N levels. The dry matter production of three cultivars was reduced sharply by salt stress and these effects were more obvious in the later cutting than the early cutting. However, the salt effects on different cultivars appeared slightly different. The dry matter production of cultivar 'MS cycle 3' was relatively higher than alfalfa 'MS 83CL' at the higher N level.

These figures also indicate that increasing the N supply from 1 to 5 ppm increased the dry matter production of alfalfa plants under both normal and salt stress conditions. This result agrees with Fishbeck and Phillips (1981) who showed dry matter production of alfalfa increased by N fertilization. The N level effects on the dry matter production are less evident in later cuttings except for cultivar 'MS cycle 3'. Although no significant differences were found among alfalfa cultivars, the cultivars responded to N levels in slightly different tendency. High N level increased the dry matter

production of standard cultivar 'MS 83CL' only in cutting 1 for both normal and salt stress conditions. The dry matter production of new selection MS cycle 5 increased in all three cuttings with high N for normal conditions and only in the first cutting under salt stress. The effects of high N on dry matter production in later cuttings under salt stress disappeared. The reason for less degree of N effects in later cuttings could be the salt stress became the most limiting growth factor of MS cycle 5. The dry matter production of new selection (MS cycle 3), on the other hand, increased for higher N rate during all three cuttings for both normal and salt stress conditions. This could mean the N levels in growth medium play a important role in alfalfa cultivar selection for salt tolerance.

## Total N Content of Plants

The total N concentration of each sample was measured by a semimicrio-Kjeldahl method. Table 4.4 shows the analysis of variance for the N concentration (N%) and total N content of three alfalfa cultivars in different cuttings under salt stress with two N levels. The results indicate that there is a statistical difference among cultivars only for percent N in the shoots of the third cutting. Highly significant differences were shown between normal and salt stress conditions for percent N in the second and third cuttings,

Table 4.4. Analysis of variances of N percentage and N content in shoots and roots.

| | N percent | | | | N content | | | |
|---|---|---|---|---|---|---|---|---|
| | FCS | SCS | TCS | TCR | FCS | SCS | TCS | TCR |
| | ---------- % ---------- | | | | -------mg/pot ------- | | | |
| Cult | ns | ns | * | ns | ns | ns | ns | ns |
| OP | ns | ** | ** | ** | ** | ** | ** | ** |
| N | ns | ns | ** | ** | ** | * | ns | ns |
| Cult*OP | ns | ns | ns | ns | ns | ns | ns | ns |
| Cult*N | ns | ns | ns | ns | ns | ns | ns | ns |
| OP*N | ns | ns | ** | ** | ns | ns | ns | ns |
| Cult*OP*N | ns | ns | ns | * | ns | ns | ns | ns |

ns = non significant F-test; * = significant F-test at 5% level

** = significant F-test at 1% level; Cult = Cultivars;

N = Nitrogen rate; OP = osmotic potential;

FCS = First cutting shoots; SCS = second cutting shoots;

TCS = third cutting shoots; TCR = third cutting roots.

Table 4.5. Mean value per cultivar for N percentage and N content (mg/pot) in different cuttings.

| Cultivar | N percent | | | | N content | | | |
|---|---|---|---|---|---|---|---|---|
| | FCS | SCS | TCS | TCR | FCS | SCS | TCS | TC |
| | ---------- % ---------- | | | | -------mg/pot ------- | | | |
| MS-cycle3 | 3.54a | 3.15a | 2.95b | 1.90a | 13.8a | 31.8a | 45.1a | 11.1a |
| MS-83CL | 3.59a | 3.23a | 3.05ab | 1.92a | 12.6ab | 27.6a | 36.7a | 9.72a |
| MS-cycle5 | 3.62a | 3.30a | 3.10a | 1.97a | 11.9b | 31.4a | 45.3a | 10.9a |

* Values are the means for twenty four experimental units (two osmotic potentials, two N rates and six replications.)

* Means followed by the same letter within a column are not significantly different at 5% probability level.

Table 4.6. Effects of salt stress and N rate on N percent and N content of alfalfa shoots and roots.

| | N percent | | | | N content | | | |
|---|---|---|---|---|---|---|---|---|
| | FCS | SCS | TCS | TCR | FCS | SCS | TCS | TCR |
| | ---------- % ---------- | | | | -------mg/pot ------- | | | |
| OP 0.0 Mpa | 3.56a | 3.12b | 3.12a | 2.25a | 17.9a | 49.4a | 71.2a | 17.3a |
| -0.6 Mpa | 3.60a | 3.35a | 2.95b | 1.55b | 7.64b | 11.2b | 13.5b | 3.80b |
| N 5 ppm | 3.56a | 3.23a | 2.91b | 1.85b | 14.6a | 33.8a | 46.1a | 11.5a |
| 1 ppm | 3.60a | 3.22a | 3.15a | 2.02a | 10.9b | 26.7b | 38.6a | 9.63a |

*Values are the means for thirty six experimental units (three cultivars, two salt stress or N rate and six replications).

* Means followed by a common letters within a column are not significantly different at the 5% level.

and for total N content in all three cuttings. Highly significant differences were also observed between high and low N levels for percent N in shoots and roots of the third cutting, and for N content in shoots of the first and second cutting. Interaction between osmotic potential and N rate had highly significant effects on both the shoots and roots in the third cutting, while interaction among cultivar, osmotic potential and N rate was significantly different for percent N in the roots of the third cutting. High N in nutrient solution enhanced the salt effects on total N concentration.

Table 4.5 shows the mean values per cultivar for N percentage and N content of three different cultivars in different cuttings. The results of the Duncan test indicated that the only significant difference occurred between two selections where selection MS cycle 5 had a higher percent N than the selection MS cycle 3.

Table 4.6 presents the effects of osmotic potentials on total N concentration and N content in shoots and roots of alfalfa for different cuttings. The effects of salt stress on percent N of the first cutting alfalfa showed a significant difference between the normal and salt stress conditions. Salt stress increased the N concentration of the second cutting shoots but decreased the shoots and roots N concentration of the third cutting. Salt stress decreased the N content

dramatically in all three cuttings and this effect became more serious as more cuttings were made.

The N levels effects on percent N and N content of alfalfa in different cuttings are also presented in Table 4.6. Increased N in the solution did not affect shoot N for the first cutting or second cutting, but decreased N significantly for shoots and roots in the third cutting. In the case of N content, high N level increased the N content significantly in the first and second cutting but no change was found in the third cutting. The salt stress and N levels on total N content was similar to their effects on dry matter production of alfalfa.

Figures 4.4 and 4.6 shows the percent N of shoots and roots of alfalfa cultivars in the third cutting under salt stress with two N rates. The interaction between salt stress and N levels in the shoots of the third cutting is presented in Fig. 4.4. When 1 ppm N was applied, increased salt stress normally increased the percent N of alfalfa, whereas, with 5 ppm N supplied, salt stress decreased the percent N in the shoot of alfalfa. The interaction between osmotic potential and N levels, and among alfalfa cultivars, osmotic potential and N levels were shown in Fig. 4.5. In the control, the N effects on the alfalfa cultivar MS cycle 3, MS cycle 5 and MS 83CL showed no difference, slight difference and highly

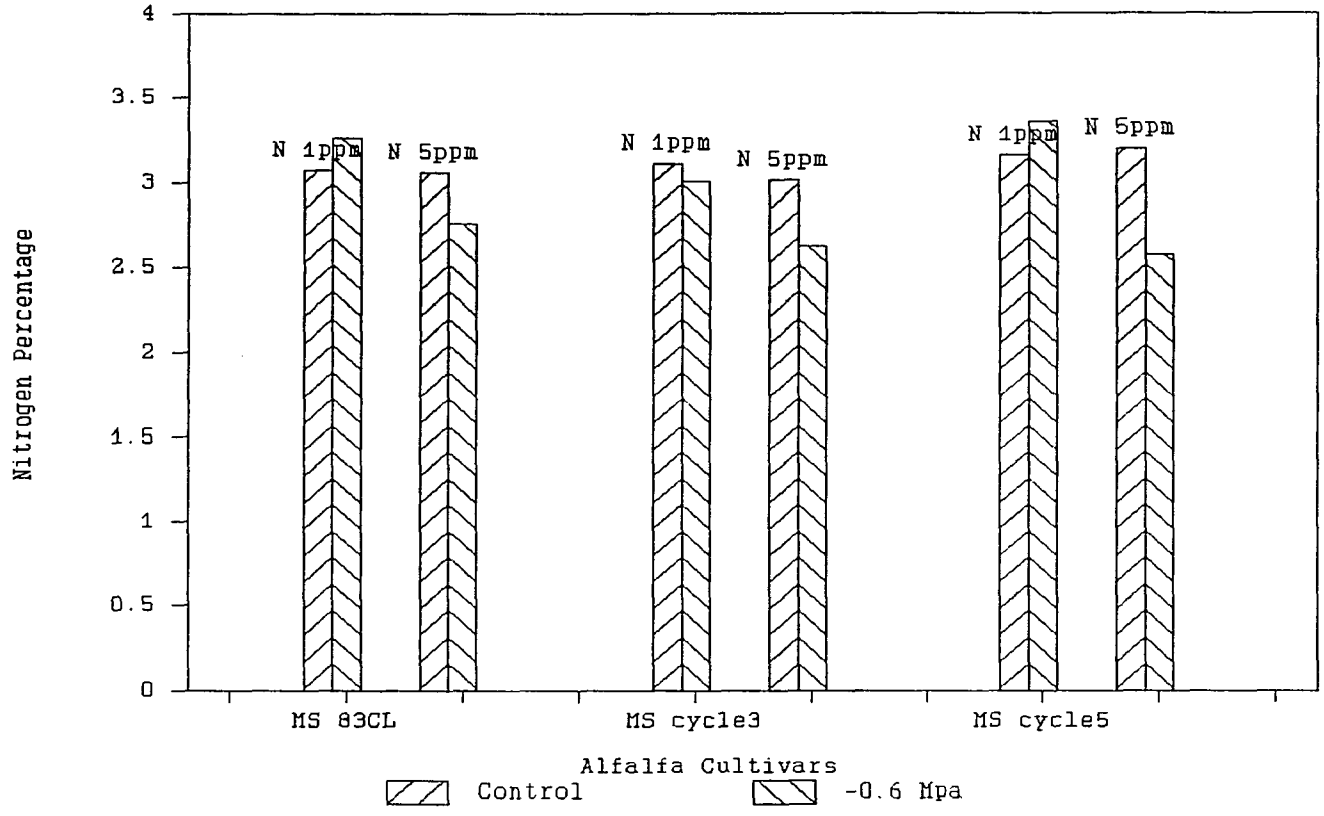


Fig.4.4 Total N percentage in three cultivars fo alfalfa shoots in cutting 3 under salt stress with 2 N levels

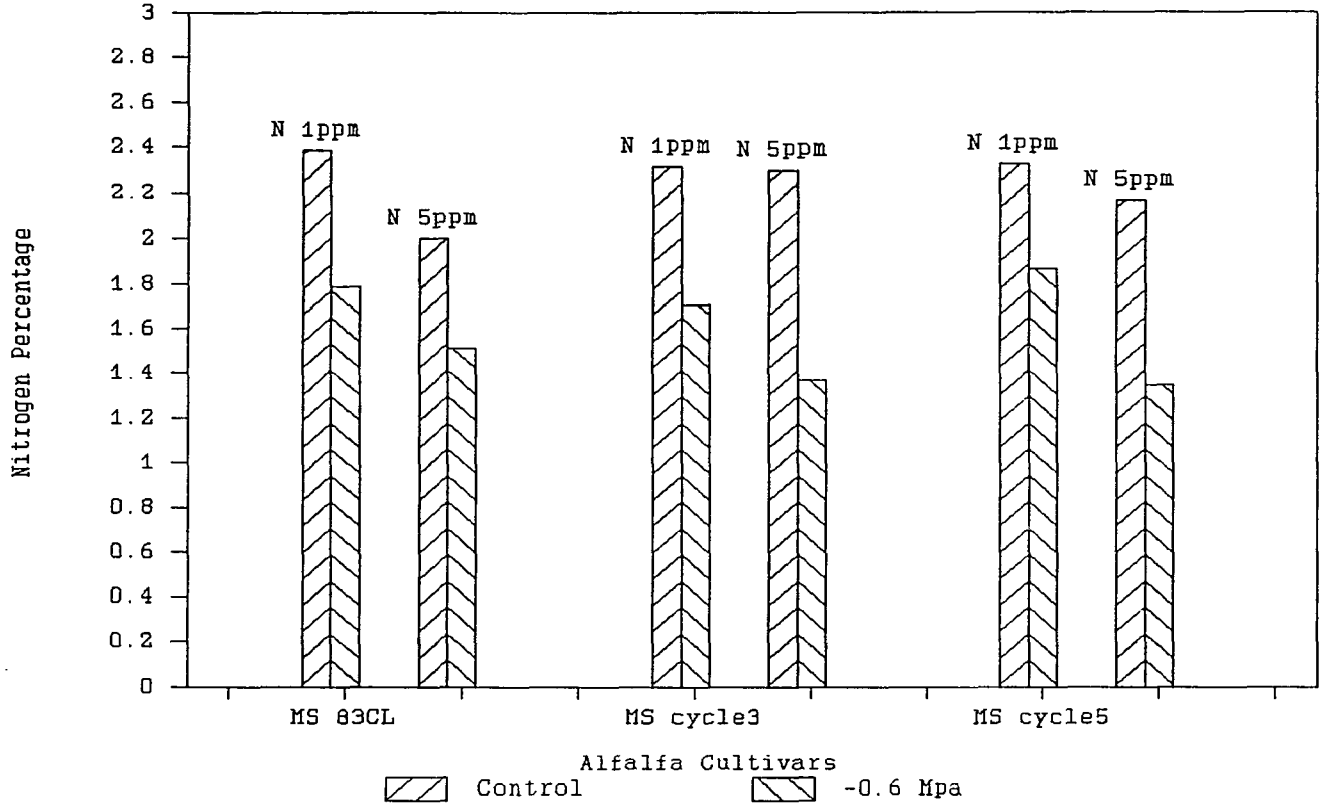


Fig.4.5 Total N percentage in three cultivars of alfalfa roots in cutting 3 under salt stress with 2 N levels

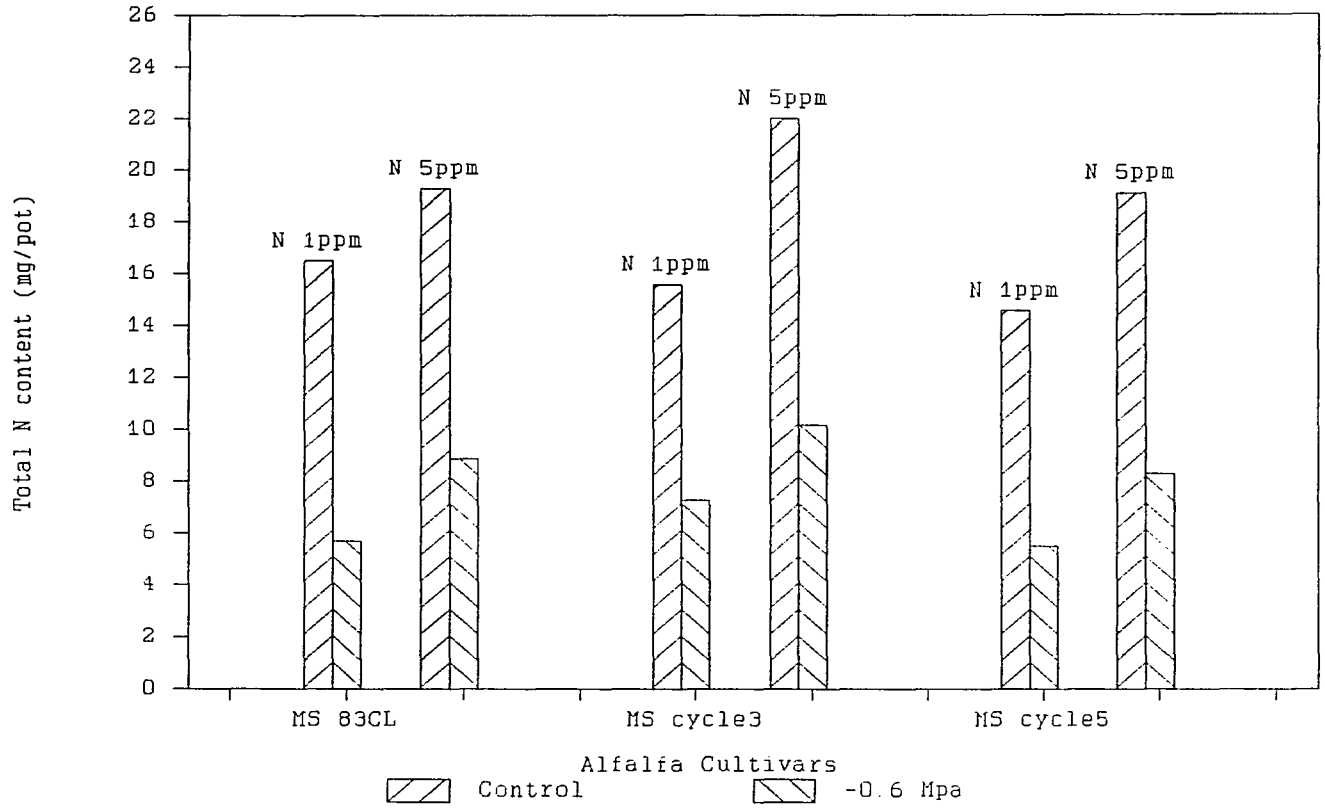


**Fig.4.6 Amount of total N in three cultivars of alfalfa shoots in cutting 1 under salt stress with 2 N levels**

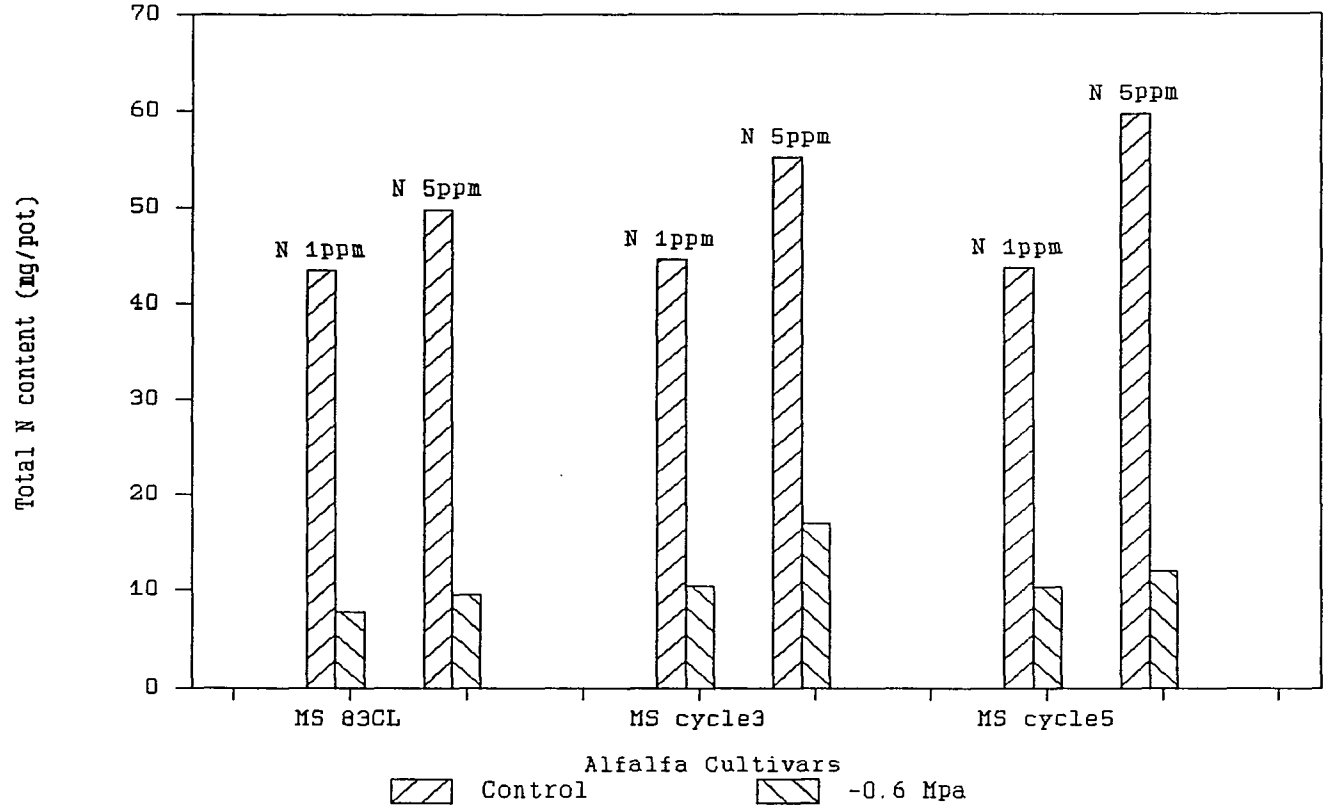


Fig.4.7 Amount of total N in three cultivars of alfalfa shoots in cutting 2 under salt stress with 2 N levels

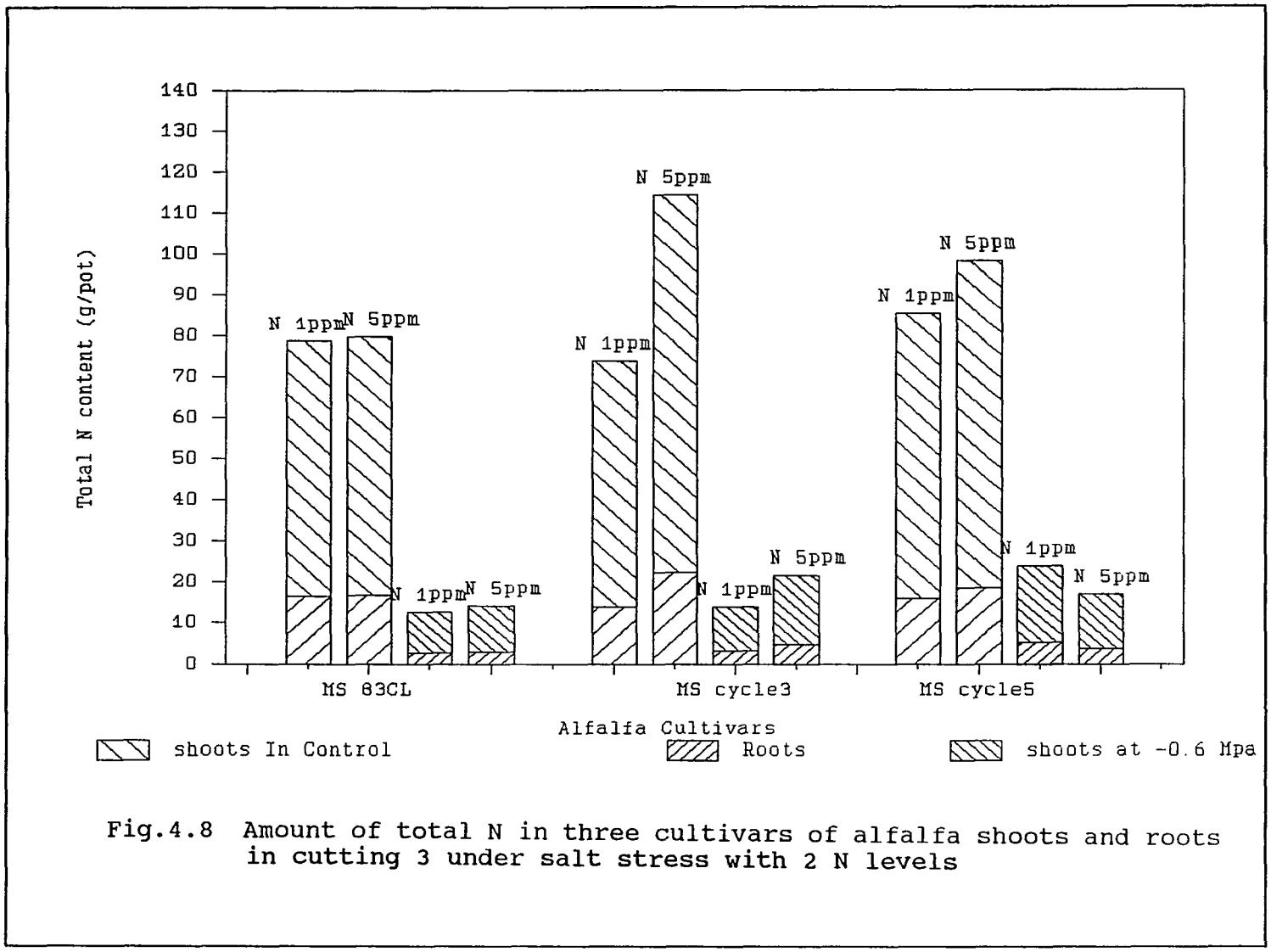


Fig.4.8 Amount of total N in three cultivars of alfalfa shoots and roots in cutting 3 under salt stress with 2 N levels

significant difference, respectively. Under salt conditions, the N effects on the roots of the three cultivars in the third cutting was as MS cycle 5 > MS cycle 3 > MS 83CL.

Figures 4.6, 4.7 and 4.8 show the N content of the three alfalfa cultivars in the first cutting, second cutting and third cutting as affected by osmotic potential with various N levels, respectively. In the first cutting, a significant difference exists between MS cycle 3 and MS cycle 5 where N content of MS cycle 3 is higher than MS cycle 5 (Fig. 4.6). Although no significant differences were found among the the three alfalfa cultivars in the second cutting, the figures show a tendency for the high level of N to increase N content more for the MS cycle 5 than the other cultivars under normal conditions. Under salt stress the N content increased for MS cycle 3 with no changes in the other cultivars (Fig. 4.7). The N levels and salt stress had slightly different effects on the three cultivars. The selection MS 83CL was not affected by N levels but the high N level increased the N content of MS cycle 3 under both normal and salt stress conditions; high N increased N content of MS cycle 5 for normal condition but decreased the N content under salt stress (Fig. 4.8).

## Nitrogen Fixation by Plants

The proportion of fixed N in the plants was calculated

from the $^{15}N$ data which allowed partitioning of plant N from fixed or nutrient solution sources. Table 4.7 shows the analysis of variances for the fixed fraction of N (Fixed N %) and the fixed content in the mg/pot of three cultivars for each cutting by salt stress with various N levels. These results indicate highly significant differences among cultivars only for the fixed fraction of N in both shoots and roots of the third cutting. Highly significant differences were also found between normal and salt stress conditions in both the fixed fraction of N and fixed N content for all cuttings. The percentage fixed was highly significantly different between 1 and 5 ppm N, and the interaction of osmotic potential and N levels was highly significant. The total N fixed was not significantly affected by N level and the interaction of N and salt level was only significant for the third cutting shoots and roots.

Data in Table 4.8 are the mean values for the fixed fraction and total content of fixed N at different cuttings. The results of the Duncan test showed that the selection MS cycle 3 had a higher fixed fraction of N than MS 83CL in the second cutting, whereas, the standard cultivar MS 83CL and selection MS cycle 5 had a higher fraction of fixed N in shoots and roots in the third cutting than MS cycle 3. A significant difference in the amount of fixed N was not found among all three cultivars for three cuttings.

Table 4.7. Analysis of variances for the fixed fraction of N and the fixed N percent in the shoots and roots.

| | Fixed N | | | | Fixed N content | | | |
|---|---|---|---|---|---|---|---|---|
| | FCS | SCS | TCS | TCR | FCS | SCS | TCS | TCR |
| | ---------- % ---------- | | | | -------mg/pot ------- | | | |
| Cult | ns | ns | ** | ** | ns | ns | ns | ns |
| OP | * | ** | ** | ** | ** | ** | ** | ** |
| N | ** | ** | ** | ** | ns | ns | ns | ns |
| Cult*OP | ns | ns | ns | ns | ns | ns | ns | ns |
| Cult*N | ns | ns | ns | ns | ns | ns | ns | ns |
| OP*N | ** | ** | ** | ** | ns | ns | * | * |
| Cult*OP*N | ns | ns | ns | ns | ns | ns | ns | ns |

ns = non significant F-test; * = significant F-test at 5% level

** = significant F-test at 1% level; Cult = Cultivars;

N = Nitrogen rate; OP = osmotic potential;

FCS = First cutting shoots; SCS = second cutting shoots;

TCS = third cutting shoots; TCR = third cutting roots.

Table 4.8. Mean value per cultivar for the fixed fraction of N and the content of the fixed N at different cutting.

| Cultivar | Fixed N | | | | Fixed N content | | | |
|---|---|---|---|---|---|---|---|---|
| | FCS | SCS | TCS | TCR | FCS | SCS | TCS | TCR |
| | ---------- % ---------- | | | | -------mg/pot ------- | | | |
| MS-cycle3 | 77.7a | 72.7a | 74.7b | 76.8b | 10.2a | 25.6a | 38.0a | 9.25a |
| MS-83CL | 76.4a | 69.8b | 78.5a | 79.6a | 9.50a | 22.6a | 31.5a | 8.36a |
| MS-cycle5 | 76.1a | 72.0ab | 78.6a | 80.3a | 8.85a | 25.5a | 40.6a | 9.41a |

* Values are the means for twenty four experimental units (two osmotic potentials, two N rates and six replications.)

* Means followed by the same letter within a column are not significantly different at 5% probability level.

Table 4.9. Effects of salt stress and N rate on the fixed N percentage and fixed N content of alfalfa shoots and roots.

| | Fixed N | | | | Fixed N content | | | |
|---|---|---|---|---|---|---|---|---|
| | FCS | SCS | TCS | TCR | FCS | SCS | TCS | TCR |
| | ---------- % ---------- | | | | -------mg/pot ------- | | | |
| OP 0.0 Mpa | 78.7a | 89.5a | 91.1a | 90.2a | 13.7a | 44.0a | 65.7a | 15.6a |
| -0.6 Mpa | 74.4b | 50.5b | 59.5b | 64.7b | 5.38b | 5.16b | 7.71b | 2.37b |
| N 5 ppm | 59.9b | 52.7b | 55.5b | 61.8b | 9.95a | 24.8a | 37.1a | 9.09a |
| 1 ppm | 93.2a | 86.5a | 92.1a | 90.6a | 9.10a | 24.4a | 36.3a | 8.91a |

*Values are the means for thirty six experimental units (three cultivars, two salt stress or N rate and six replications).

* Means followed by a common letters within a column are not significantly different at the 5% level.

The effects of osmotic potential and N levels on the fixed N percentage and fixed N content of alfalfa shoots and roots for three cuttings are shown in Table 4.9. Salt stress reduced the fixed fraction of N and fixed N content significantly. This reduction of N fixation was much more apparant in later cuttings than in the first cutting. It also can be seen that at the higher N level the fixed fraction of N decreased enormously in all three cuttings. The effects of N levels on the fixed fraction of N changed little for the different cuttings. The total N fixed was not affected by N levels indicating that the higher nutrient N level was responsible for increased growth.

The effects of salt stress and N levels on the fixed N percentage for each individual alfalfa cultivar are shown in Figs. 4.9, 4.10, 4.11 and 4.12 for shoots of the first, second and third cutting and roots of the third cutting, respectively. The pattern is essentially the same for all cultivar cuttings and plant parts. The interaction between osmotic potential and salt stress can be seen in these figures. The figures showed that salt stress reduced the fixed fraction of N at 5 ppm N for all cultivars at the first cutting. A more drastic reduction in the fixed fraction occurred at cutting two and three with small reductions in the fixed percentage with 1 ppm N. The reduction in fixed N was

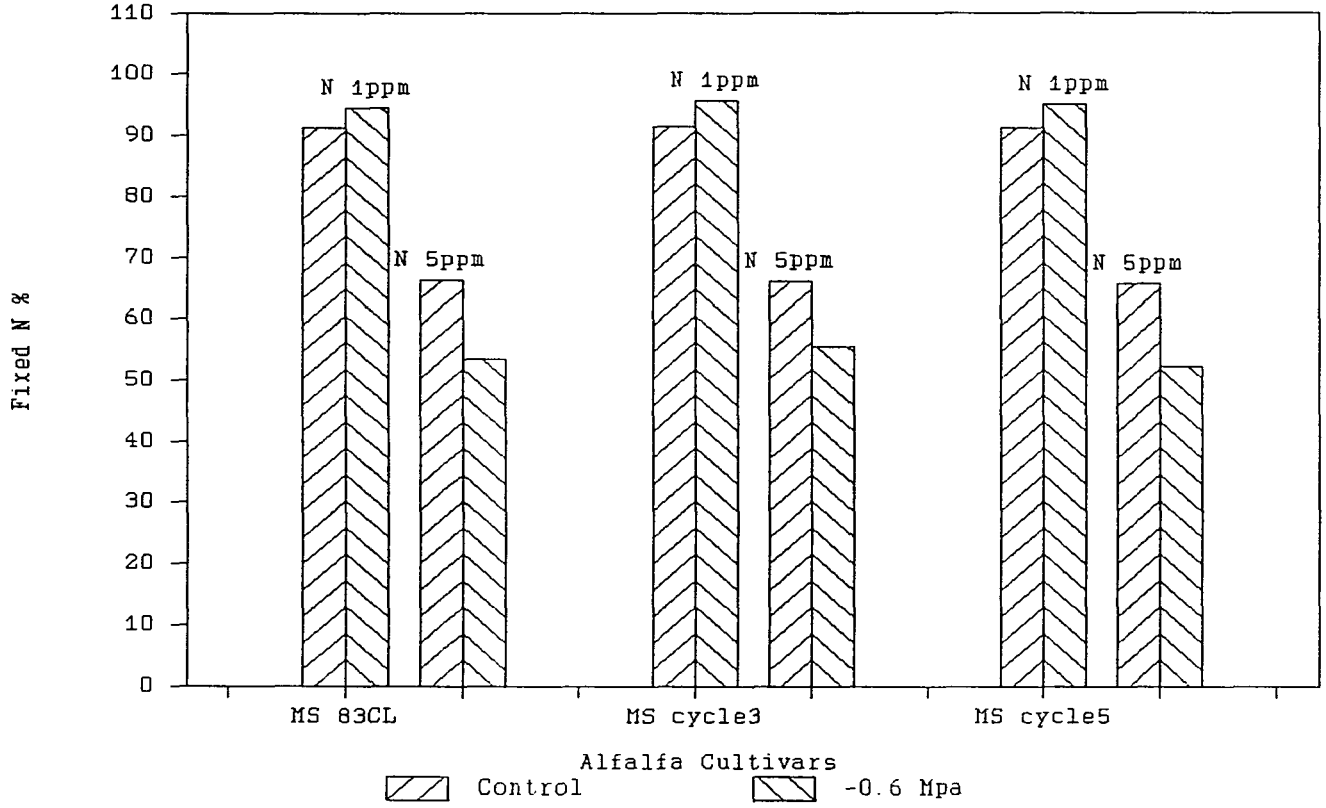


Fig.4.9 The fraction of N fixed in three cultivars of alfalfa shoots in cutting 1 under salt stress with 2 N levels

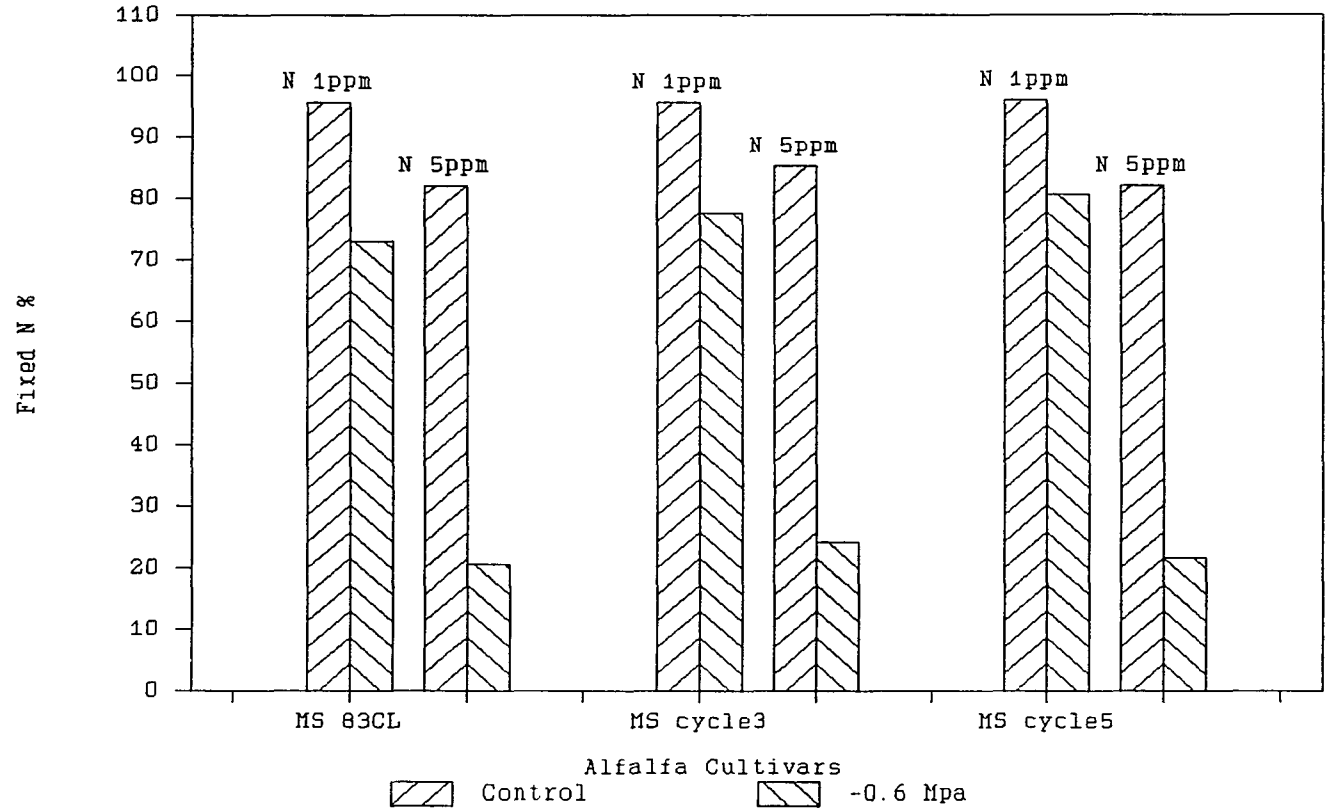


**Fig.4.10 The fraction of N fixed in three cultivars of alfalfa shoots in cutting 2 under salt stress with 2 N levels**

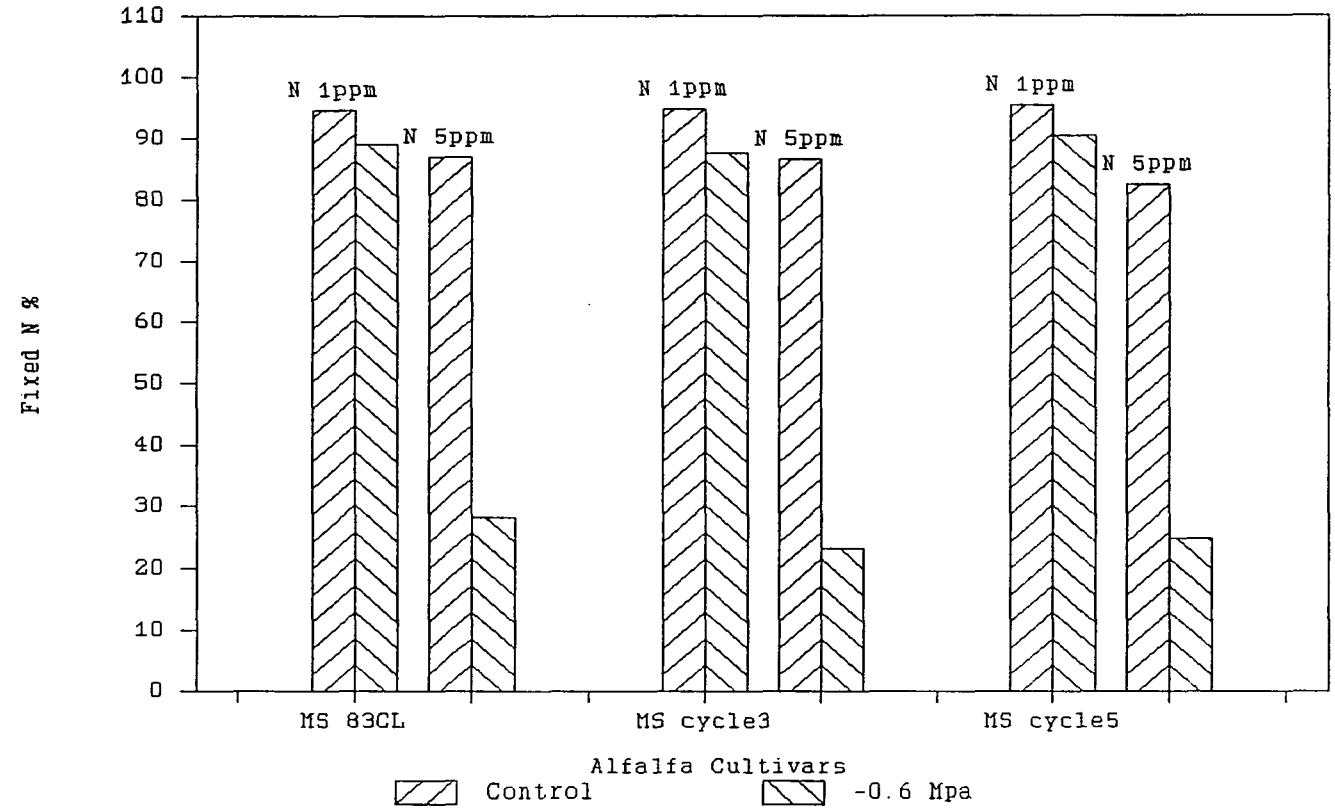


**Fig.4.11 The fraction of N fixed in three cultivars of alfalfa shoots in cutting 3 under salt stress with 2 N levels**

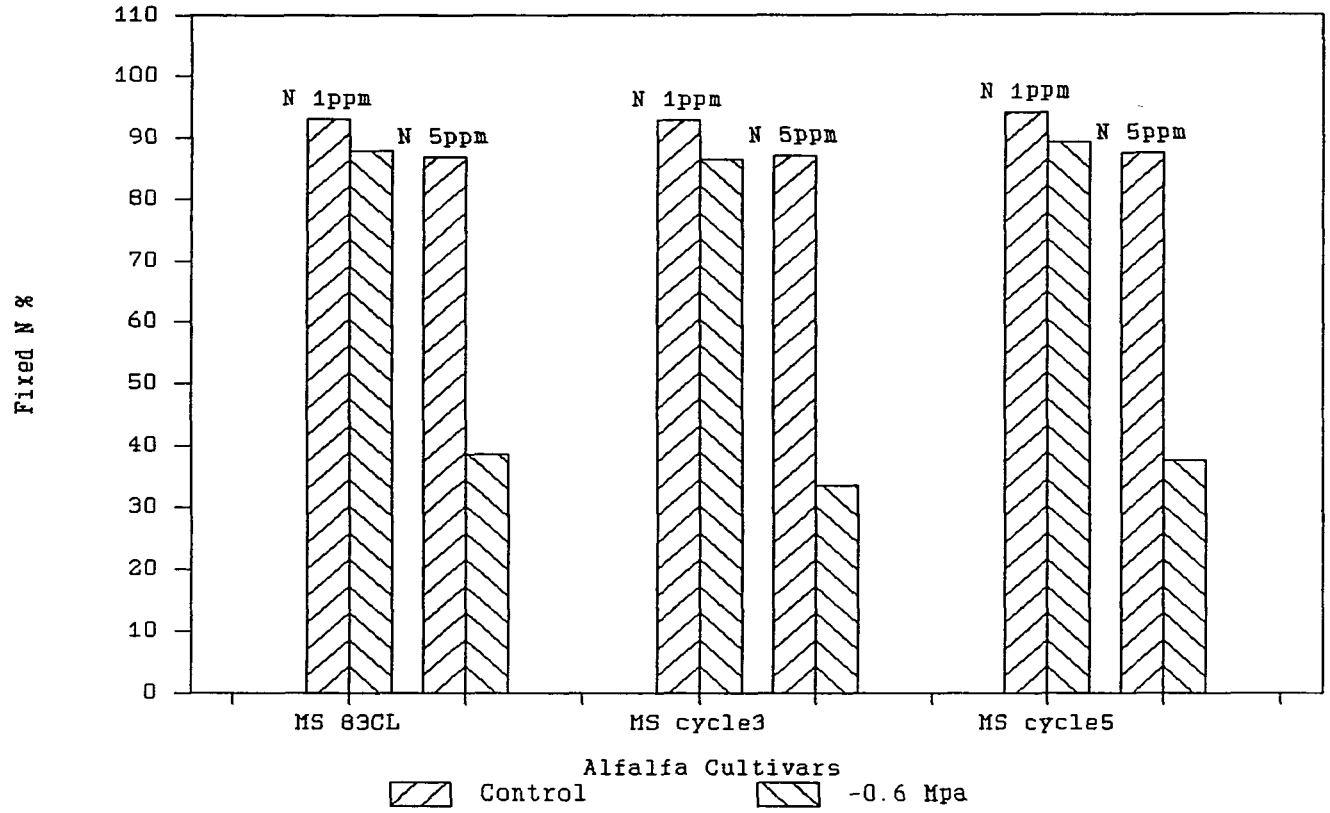


**Fig.4.12 The fraction of N fixed in three cultivars of alfalfa roots in cutting 3 under salt stress with 2 N levels**

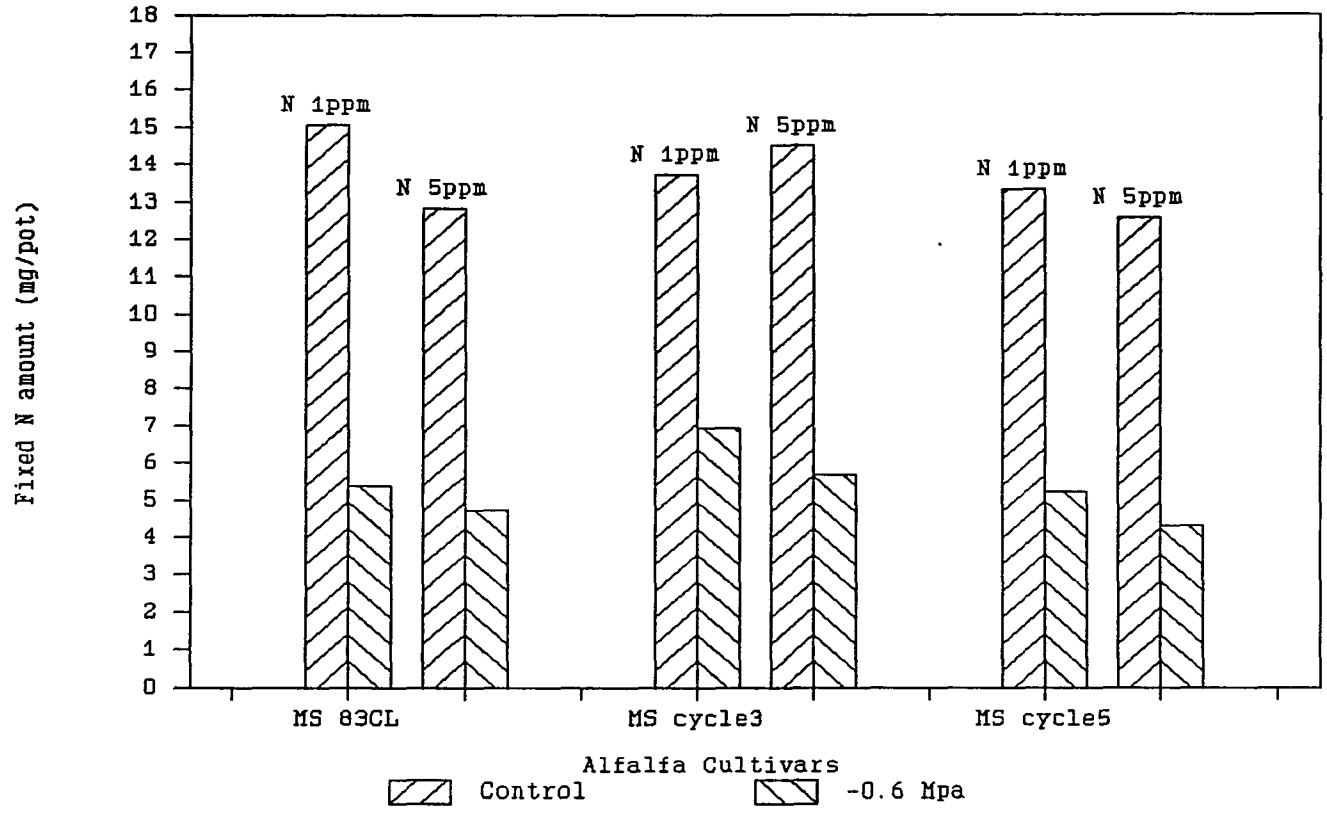


**Fig.4.13** Amount of N fixed in three cultivars of alfalfa shoots in cutting 1 under salt stress with 2 N levels

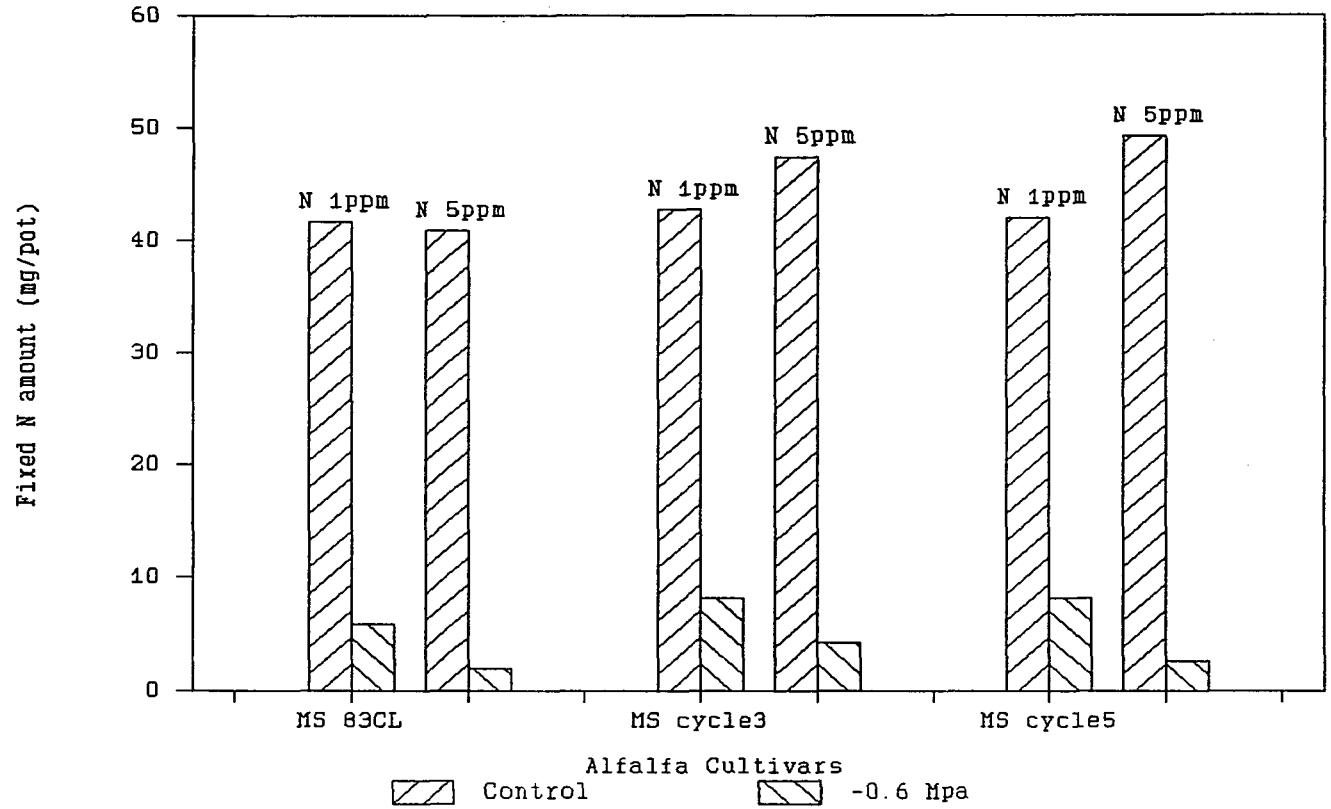


**Fig.4.14 Amount of N fixed in three cultivars of alfalfa shoots in cutting 2 under salt stress with 2 N levels**

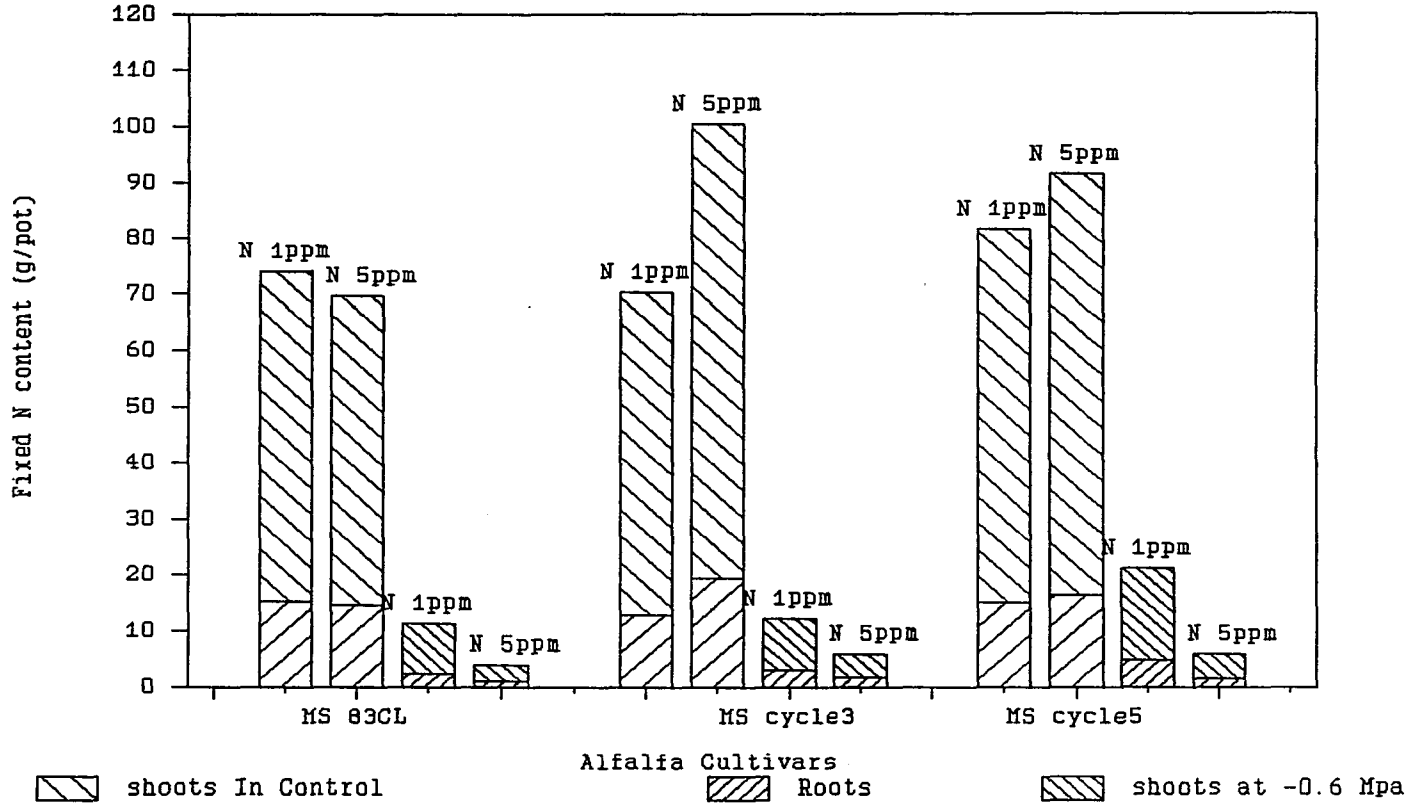


**Fig.4.15 Amount of N fixed in three cultivars of alfalfa shoots and roots in cutting 3 under salt stress with 2 N levels**

much greater for salt stress at 5 than 1 ppm N.

Figures 4.13, 4.14 and 4.15 present the effects of salt stress and N levels on fixed N content for the first, second and third cuttings, respectively. The fixed N content of the cultivar and selections reflect the difference in growth among the selection and the response to salt stress. Primary differences, then, are for the combined reduction of growth and fixed N percentage caused by salt stress.

The fixed N fraction of alfalfa plants under non-saline condition ranged from 66 to 96% of their N requirement depending on the concentration of N in the nutrient medium. Other reported fixed N values were 61 to 94% of alfalfa shoots N from N fixation (Cepeda, 1987) and 85 to 100% of Ladino clover N from N fixation (Broadbent el al., 1986). The fixed N fraction under saline conditions was largely dependent upon the N concentration in the nutrient solution. When 1 ppm N was in the solution, the alfalfa plants fixed 73 to 95% of their N requirement whereas the alfalfa plants only fixed 20 to 55% of total N required when solution N was at 5 ppm. High N levels reduced N fixation by inhibiting nitrogenase activity (Becana et al., 1986) or reducing nodule formation and decreasing N fixation efficiency of Rhizobium (Hardarson et al, 1981). The effects of salt stress on N fixation could result from inhibition survival of Rhizobium spp. in the soil

or rhizosphere, diminished plant growth and photosynthesis, and/or effect on the interaction of the infection process and root nodule function (Parker et al., 1977).

# CHAPTER 5

## SUMMARY AND CONCLUSIONS

The effects of NaCl salt levels (0, -0.6 Mpa) and N rate (1 ppm, 5 ppm) on N fixation by one low salt tolerant alfalfa cultivar 'Mesa-Sirsa 83CL' and two germination salt tolerant selections were investigated in three cuttings in this experiment. The evaluation of the experimental results was accomplished through the consideration of total dry matter production, total N concentration and content, and the fixed N fraction and amount. The $^{15}N$ technique was used to evaluate the N fixation of alfalfa.

Statistical analyses of the results were made on all variables separately. The results showed that the dry matter production and total N content of alfalfa MS cycle 3 were higher than MS cycle 5 in the first cutting. No difference in total N concentration was found between alfalfa MS 83CL and the two selections. The difference in total N content between MS cycle 3 and MS cycle 5 resulted from the difference in dry matter production of alfalfa cultivars.

The fixed N fraction of MS cycle 3 was higher than MS cycle 5 in the shoots of the second cutting, but much lower

in shoots and roots of the third cutting than MS cycle 5 and MS 83CL. There were no significant differences in amount of total fixed N. These results indicate that the two new selections were not significantly different from their parent alfalfa MS 83CL with respect to total N fixing capacity.

The salinity affected almost all parameters significantly. The results showed that total dry matter production, total N content, fixed N fraction and total fixed N of all cuttings and total N concentration in the last two cuttings were reduced significantly by salt added to the media. The reduction rate in these parameters was more pronounced in later than in earlier cuttings.

The different parameters were affected by N levels to different degrees. The higher N level increased total dry matter production of alfalfa highly significantly in shoots of the first cutting and roots of the third cutting. The dry matter production from the low N treatment was probably because the N level was the major growth limiting factor as large amounts of photosynthetic products are used to form nodules and to fix N.

The results also showed that the higher level of N in the nutrient solution only increased the total N content in the first two cuttings (the nodulation stages or stage of less fixation efficiency). As the alfalfa nodules formed and fixed

N became available in the plants, the effects of N levels on total N content gradually decreased.

When N levels in nutrient solution increased from 1 to 5 ppm, the fixed fraction of N decreased about 30%. The large percentage reduction of fixed N by increased N levels indicates that N level was the major factor causing the different fixed N percentages between two cuttings in the experiment done by Cepeda (1987). However, N levels had no effect on total fixed N content.

The highly significant interaction between osmotic potential and N levels was found in N fixation percentage in all cuttings and total N concentration in the third cutting, while a significant N rate and salt level interaction was observed on total fixed N content in the third cutting. The high salinity level reduced the fixed N fraction in all cuttings, and total N percentage and total fixed N content in the third cutting to a much greater degree with 5 ppm N than 1 ppm N in the nutrient solution.

# APPENDIX A

# EXPERIMENTAL DATA

Table A.1. Dry matter production of shoots in g/pot for the first cuting .

| Cultivar | OP Mpa | N Rate ppm | Block 1 | Block 2 | Block 3 | Block 4 | Block 5 | Block 6 | Totals | Average |
|---|---|---|---|---|---|---|---|---|---|---|
| MS 83CL | 0.0 | 1 | .427 | .618 | .314 | .483 | .510 | .436 | 2.787 | .465 |
| | 0.0 | 5 | .628 | .458 | .459 | .560 | .643 | .488 | 3.237 | .540 |
| | -0.6 | 1 | .200 | .153 | .156 | .128 | .212 | .131 | .980 | .163 |
| | -0.6 | 5 | .169 | .269 | .178 | .310 | .258 | .269 | 1.453 | .242 |
| MS cycle 3 | 0.0 | 1 | .507 | .495 | .358 | .570 | .444 | .292 | 2.665 | .444 |
| | 0.0 | 5 | .411 | .832 | .658 | .526 | .718 | .600 | 3.745 | .624 |
| | -0.6 | 1 | .124 | .273 | .169 | .245 | .198 | .197 | 1.205 | .201 |
| | -0.6 | 5 | .323 | .370 | .193 | .236 | .365 | .222 | 1.709 | .285 |
| MS cycle 5 | 0.0 | 1 | .484 | .505 | .247 | .437 | .394 | .312 | 2.378 | .396 |
| | 0.0 | 5 | .516 | .486 | .601 | .617 | .417 | .654 | 3.292 | .547 |
| | -0.6 | 1 | .153 | .171 | .142 | .132 | .082 | .218 | .898 | .150 |
| | -0.6 | 5 | .280 | .202 | .158 | .261 | .273 | .211 | 1.387 | .231 |

Table A.2. Dry matter production of shoots[1] in g/pot for the second cutting.

| Cultivar | OP Mpa | N Rate ppm | Block 1 | Block 2 | Block 3 | Block 4 | Block 5 | Block 6 | Totals | Average |
|---|---|---|---|---|---|---|---|---|---|---|
| MS 83CL | 0.0 | 1 | .917 | 2.915 | .870 | 1.737 | 1.704 | 1.477 | 9.620 | 1.603 |
| | 0.0 | 5 | 1.480 | 1.780 | 1.235 | 2.495 | 1.634 | 1.458 | 10.08 | 1.680 |
| | -0.6 | 1 | .159 | .300 | .336 | .000 | .449 | .195 | 1.439 | .240 |
| | -0.6 | 5 | .348 | .000 | .035 | .388 | .832 | .133 | 1.796 | .289 |
| MS cycle 3 | 0.0 | 1 | 2.306 | 1.952 | 1.335 | 1.703 | .875 | .686 | 8.857 | 1.476 |
| | 0.0 | 5 | 1.163 | 1.954 | 1.366 | 2.087 | 2.505 | 2.045 | 11.12 | 1.854 |
| | -0.6 | 1 | .366 | .444 | .407 | .232 | .366 | .121 | 1.936 | .323 |
| | -0.6 | 5 | .743 | .813 | .372 | .246 | .799 | .000 | 2.972 | .495 |
| MS cycle 5 | 0.0 | 1 | 1.330 | 1.545 | 0.855 | 2.275 | 1.593 | 1.060 | 8.659 | 1.443 |
| | 0.0 | 5 | 1.705 | 1.315 | 1.805 | 1.676 | 1.198 | 3.170 | 10.87 | 1.811 |
| | -0.6 | 1 | .000 | .393 | .369 | .258 | .289 | .550 | 1.859 | .310 |
| | -0.6 | 5 | .346 | .106 | .000 | .485 | .634 | .463 | 2.035 | .339 |

[1]Zero dry matter production in some pots indicates the plants in these pots died at the beginning of the second cutting.

Table A.3. Dry matter production of shoots[1] in g/pot for the third cutting .

| Cultivar | OP Mpa | N Rate ppm | Block 1 | Block 2 | Block 3 | Block 4 | Block 5 | Block 6 | Totals | Average |
|---|---|---|---|---|---|---|---|---|---|---|
| MS 83CL | 0.0 | 1 | 1.259 | 1.648 | 1.849 | 2.495 | 2.692 | 2.444 | 12.39 | 2.065 |
| | 0.0 | 5 | 1.703 | 2.076 | 1.155 | 2.179 | 2.691 | 2.734 | 12.54 | 2.090 |
| | -0.6 | 1 | .090 | .405 | .622 | .000 | .456 | .282 | 1.855 | .309 |
| | -0.6 | 5 | .221 | .000 | .000 | .617 | 1.339 | .259 | 2.436 | .406 |
| MS cycle 3 | 0.0 | 1 | 2.626 | 2.072 | 2.536 | 2.019 | 1.406 | 1.004 | 11.66 | 1.944 |
| | 0.0 | 5 | 1.584 | 3.465 | 2.721 | 3.122 | 4.152 | 3.546 | 18.50 | 3.083 |
| | -0.6 | 1 | .218 | .494 | .499 | .216 | .653 | .012 | 2.097 | .350 |
| | -0.6 | 5 | .839 | .937 | .785 | .487 | .821 | .000 | 3.868 | .645 |
| MS cycle 5 | 0.0 | 1 | 1.851 | 1.715 | 1.418 | 3.314 | 3.219 | 1.730 | 13.25 | 2.208 |
| | 0.0 | 5 | 2.332 | 2.021 | 2.436 | 2.300 | 1.544 | 4.478 | 15.17 | 2.518 |
| | -0.6 | 1 | .000 | .450 | .624 | .349 | .488 | 1.416 | 3.326 | .554 |
| | -0.6 | 5 | .829 | .000 | .000 | .734 | .695 | .858 | 3.116 | .519 |

[1]Zero dry matter production in some pots indicates the plants in these pots died at the beginning of the second or third cutting.

Table A.4. Dry matter production of roots[1] in g/pot for the third cutting .

| Cultivar | OP Mpa | N Rate ppm | Block 1 | Block 2 | Block 3 | Block 4 | Block 5 | Block 6 | Totals | Average |
|---|---|---|---|---|---|---|---|---|---|---|
| MS 83CL | 0.0 | 1 | .333 | .643 | .602 | .814 | .891 | .812 | 4.094 | .682 |
| | 0.0 | 5 | .515 | .701 | .496 | 1.010 | 1.115 | 1.089 | 4.927 | .821 |
| | -0.6 | 1 | .080 | .191 | .318 | .000 | .214 | .134 | .937 | .156 |
| | -0.6 | 5 | .150 | .000 | .000 | .295 | .749 | .089 | 1.283 | .214 |
| MS cycle 3 | 0.0 | 1 | .919 | .748 | .756 | .669 | .340 | .228 | 3.661 | .610 |
| | 0.0 | 5 | .564 | 1.066 | 1.014 | .835 | 1.270 | 1.065 | 5.815 | .969 |
| | -0.6 | 1 | .150 | .222 | .281 | .118 | .331 | .048 | 1.149 | .192 |
| | -0.6 | 5 | .492 | .435 | .471 | .306 | .395 | .000 | 2.099 | .350 |
| MS cycle 5 | 0.0 | 1 | .622 | .629 | .458 | 1.087 | .854 | .491 | 4.141 | .690 |
| | 0.0 | 5 | .751 | .679 | .893 | .815 | .518 | 1.504 | 5.161 | .860 |
| | -0.6 | 1 | .000 | .268 | .405 | .177 | .242 | .593 | 1.685 | .281 |
| | -0.6 | 5 | .365 | .000 | .000 | .444 | .342 | .424 | 1.575 | .262 |

[1]Zero dry matter production in some pots indicates the plants in these pots died at the beginning of the second or third cutting.

Table A.5. Total nitrogen percentage (N%) per experimental unit for first cutting shoots.

| Cultivar | OP Mpa | N Rate ppm | Block 1 | Block 2 | Block 3 | Block 4 | Block 5 | Block 6 | Totals | Average |
|---|---|---|---|---|---|---|---|---|---|---|
| MS 83CL | 0.0 | 1 | 3.560 | 3.738 | 3.856 | 3.447 | 3.201 | 3.582 | 21.38 | 3.564 |
| | 0.0 | 5 | 3.331 | 3.974 | 3.899 | 3.637 | 3.330 | 3.472 | 21.64 | 3.607 |
| | -0.6 | 1 | 3.098 | 3.680 | 3.950 | 3.036 | 3.431 | 3.873 | 21.07 | 3.511 |
| | -0.6 | 5 | 3.920 | 4.004 | 3.498 | 3.709 | 3.667 | 3.185 | 21.98 | 3.664 |
| MS cycle 3 | 0.0 | 1 | 3.422 | 3.883 | 3.574 | 3.273 | 3.535 | 3.329 | 21.02 | 3.503 |
| | 0.0 | 5 | 3.452 | 3.955 | 3.695 | 3.004 | 3.542 | 3.331 | 20.98 | 3.497 |
| | -0.6 | 1 | 3.618 | 3.867 | 3.777 | 3.504 | 3.648 | 3.344 | 21.76 | 3.626 |
| | -0.6 | 5 | 3.472 | 3.639 | 3.653 | 3.485 | 3.678 | 3.242 | 21.17 | 3.528 |
| MS cycle 5 | 0.0 | 1 | 3.565 | 3.767 | 3.922 | 3.717 | 3.506 | 3.653 | 22.13 | 3.688 |
| | 0.0 | 5 | 3.258 | 3.625 | 3.589 | 3.107 | 3.883 | 3.571 | 21.03 | 3.506 |
| | -0.6 | 1 | 3.594 | 3.896 | 3.672 | 3.907 | 4.037 | 3.306 | 22.41 | 3.735 |
| | -0.6 | 5 | 3.612 | 3.415 | 3.174 | 3.889 | 3.774 | 3.439 | 21.30 | 3.511 |

Table A.6. Total nitrogen percentage (N%) per experimental unit for second cutting shoots[1].

| Cultivar | OP Mpa | N Rate ppm | Block 1 | Block 2 | Block 3 | Block 4 | Block 5 | Block 6 | Totals | Average |
|---|---|---|---|---|---|---|---|---|---|---|
| MS 83CL | 0.0 | 1 | 3.274 | 2.915 | 3.444 | 3.257 | 3.535 | 3.267 | 19.69 | 3.282 |
| | 0.0 | 5 | 2.758 | 2.804 | 3.061 | 2.747 | 3.530 | 3.024 | 17.92 | 2.987 |
| | -0.6 | 1 | 3.771 | 3.299 | 2.967 | - - | 3.182 | 3.444 | 16.66 | 3.333 |
| | -0.6 | 5 | 3.095 | - - | 3.362 | 3.579 | 3.206 | 3.574 | 16.82 | 3.363 |
| MS cycle 3 | 0.0 | 1 | 2.881 | 3.048 | 3.092 | 2.961 | 3.258 | 3.160 | 18.40 | 3.067 |
| | 0.0 | 5 | 2.707 | 3.130 | 3.098 | 3.025 | 2.840 | 3.014 | 17.81 | 2.969 |
| | -0.6 | 1 | 3.046 | 3.107 | 3.496 | 3.467 | 3.121 | 3.240 | 19.48 | 3.246 |
| | -0.6 | 5 | 3.903 | 3.610 | 2.916 | 3.081 | 3.124 | - - | 16.62 | 3.325 |
| MS cycle 5 | 0.0 | 1 | 2.807 | 3.211 | 3.401 | 2.841 | 2.982 | 3.224 | 18.47 | 3.078 |
| | 0.0 | 5 | 3.367 | 3.517 | 3.510 | 3.266 | 3.220 | 3.078 | 19.96 | 3.326 |
| | -0.6 | 1 | - - | 3.577 | 3.323 | 3.503 | 3.568 | 2.875 | 16.85 | 3.369 |
| | -0.6 | 5 | 3.565 | 3.126 | - - | 3.503 | 3.527 | 3.674 | 17.40 | 3.479 |

- -Undetermined value due to no shoot dry matter production.

Table A.7. Total nitrogen percentage ( N% ) per experimental unit for third cutting shoots[1]

| Cultivar | OP Mpa | N Rate ppm | Block 1 | Block 2 | Block 3 | Block 4 | Block 5 | Block 6 | Totals | Average |
|---|---|---|---|---|---|---|---|---|---|---|
| MS 83CL | 0.0 | 1 | 3.335 | 3.479 | 2.929 | 3.093 | 2.738 | 2.827 | 18.40 | 3.067 |
| | 0.0 | 5 | 3.352 | 2.719 | 3.320 | 2.778 | 3.243 | 2.918 | 18.33 | 3.055 |
| | -0.6 | 1 | 3.344 | 3.146 | 3.320 | - - | 3.305 | 3.192 | 16.31 | 3.261 |
| | -0.6 | 5 | 2.729 | - - | - - | 2.727 | 2.797 | 2.775 | 11.02 | 2.754 |
| MS cycle 3 | 0.0 | 1 | 3.080 | 3.138 | 3.364 | 2.932 | 2.703 | 3.448 | 18.67 | 3.111 |
| | 0.0 | 5 | 3.326 | 2.817 | 2.987 | 3.045 | 3.120 | 2.782 | 18.08 | 3.013 |
| | -0.6 | 1 | 2.859 | 3.138 | 3.191 | 2.938 | 3.059 | 2.827 | 18.01 | 3.002 |
| | -0.6 | 5 | 2.654 | 2.674 | 2.596 | 2.449 | 2.742 | - - | 13.12 | 2.623 |
| MS cycle 5 | 0.0 | 1 | 3.075 | 3.446 | 3.027 | 3.159 | 3.055 | 3.176 | 18.94 | 3.156 |
| | 0.0 | 5 | 2.882 | 3.278 | 3.340 | 3.193 | 3.420 | 3.074 | 19.18 | 3.198 |
| | -0.6 | 1 | - - | 3.427 | 2.945 | 3.678 | 3.310 | 3.441 | 16.80 | 3.360 |
| | -0.6 | 5 | 2.434 | - - | - - | 2.487 | 2.694 | 2.690 | 10.31 | 2.576 |

- -Undetermined value due to no shoot dry matter production.

Table A.8. Total nitrogen percentage (N%) per experimental unit for third cutting roots.

| Cultivar | OP Mpa | N Rate ppm | Block 1 | Block 2 | Block 3 | Block 4 | Block 5 | Block 6 | Totals | Average |
|---|---|---|---|---|---|---|---|---|---|---|
| MS 83CL | 0.0 | 1 | 2.286 | 2.200 | 2.514 | 2.152 | 2.682 | 2.489 | 14.32 | 2.387 |
| | 0.0 | 5 | 1.884 | 1.984 | 1.793 | 1.892 | 2.303 | 2.151 | 12.01 | 2.001 |
| | -0.6 | 1 | 1.662 | 1.666 | 1.841 | - - | 1.651 | 1.967 | 8.920 | 1.784 |
| | -0.6 | 5 | 1.547 | - - | - - | 1.672 | 1.233 | 1.613 | 6.065 | 1.516 |
| MS cycle 3 | 0.0 | 1 | 2.224 | 2.409 | 2.179 | 2.240 | 2.250 | 2.589 | 13.89 | 2.315 |
| | 0.0 | 5 | 2.171 | 2.339 | 2.308 | 2.366 | 2.199 | 2.413 | 13.80 | 2.299 |
| | -0.6 | 1 | 1.666 | 1.950 | 1.629 | 1.692 | 1.796 | 1.495 | 10.23 | 1.705 |
| | -0.6 | 5 | 1.328 | 1.352 | 1.289 | 1.459 | 1.502 | - - | 6.930 | 1.386 |
| MS cycle 5 | 0.0 | 1 | 1.970 | 2.284 | 2.333 | 2.354 | 2.441 | 2.576 | 13.96 | 2.326 |
| | 0.0 | 5 | 2.093 | 2.117 | 2.208 | 2.067 | 2.349 | 2.160 | 13.00 | 2.166 |
| | -0.6 | 1 | - - | 1.858 | 1.884 | 1.807 | 1.775 | 1.993 | 7.452 | 1.863 |
| | -0.6 | 5 | 1.249 | - - | - - | 1.357 | 1.338 | 1.447 | 5.392 | 1.348 |

- -Undetermined value due to no root dry matter production.

Table A.9. Total nitrogen content in mg/pot. First cutting shoots.

| Cultivar | OP Mpa | N Rate ppm | Block 1 | Block 2 | Block 3 | Block 4 | Block 5 | Block 6 | Totals | Average |
|---|---|---|---|---|---|---|---|---|---|---|
| MS 83CL | 0.0 | 1 | 15.19 | 23.08 | 12.10 | 16.65 | 16.33 | 15.63 | 99.0 | 16.50 |
| | 0.0 | 5 | 20.93 | 18.21 | 17.90 | 20.37 | 21.42 | 16.95 | 115.8 | 19.30 |
| | -0.6 | 1 | 6.19 | 5.61 | 6.16 | 3.89 | 7.29 | 5.07 | 34.2 | 5.70 |
| | -0.6 | 5 | 6.62 | 10.76 | 6.22 | 11.51 | 9.47 | 8.56 | 53.1 | 8.86 |
| MS cycle 3 | 0.0 | 1 | 17.34 | 19.21 | 12.78 | 18.65 | 15.71 | 9.70 | 93.4 | 15.57 |
| | 0.0 | 5 | 14.20 | 32.90 | 24.30 | 15.79 | 25.42 | 20.00 | 132.6 | 22.01 |
| | -0.6 | 1 | 4.48 | 10.54 | 6.36 | 8.59 | 7.23 | 6.49 | 43.7 | 7.28 |
| | -0.6 | 5 | 11.22 | 13.48 | 7.05 | 8.21 | 13.44 | 7.67 | 61.1 | 10.18 |
| MS cycle 5 | 0.0 | 1 | 17.24 | 19.01 | 9.68 | 16.25 | 13.81 | 11.40 | 87.4 | 14.57 |
| | 0.0 | 5 | 16.81 | 17.60 | 21.56 | 19.22 | 16.20 | 23.35 | 114.7 | 19.12 |
| | -0.6 | 1 | 5.49 | 6.64 | 5.20 | 5.15 | 3.34 | 7.21 | 33.0 | 5.51 |
| | -0.6 | 5 | 10.12 | 6.91 | 5.03 | 10.16 | 10.30 | 7.27 | 49.8 | 8.30 |

Table A.10. Total nitrogen content in mg/pot. Second cutting shoots.

| Cultivar | OP Mpa | N Rate ppm | Block 1 | Block 2 | Block 3 | Block 4 | Block 5 | Block 6 | Totals | Average |
|---|---|---|---|---|---|---|---|---|---|---|
| MS 83CL | 0.0 | 1 | 30.02 | 36.09 | 29.97 | 56.56 | 60.25 | 48.24 | 261.1 | 43.52 |
| | 0.0 | 5 | 40.83 | 49.92 | 37.79 | 68.53 | 57.68 | 44.10 | 298.8 | 49.81 |
| | -0.6 | 1 | 6.01 | 9.90 | 9.96 | 0.00 | 14.27 | 6.73 | 46.9 | 7.81 |
| | -0.6 | 5 | 10.76 | 0.00 | 1.19 | 13.90 | 26.66 | 4.77 | 57.3 | 9.55 |
| MS cycle 3 | 0.0 | 1 | 66.44 | 59.50 | 41.26 | 50.44 | 28.50 | 21.67 | 267.8 | 44.64 |
| | 0.0 | 5 | 31.49 | 61.16 | 42.32 | 63.12 | 71.15 | 61.65 | 330.9 | 55.15 |
| | -0.6 | 1 | 11.16 | 13.79 | 14.23 | 8.04 | 11.41 | 3.92 | 62.6 | 10.43 |
| | -0.6 | 5 | 29.01 | 29.34 | 10.81 | 7.57 | 24.95 | 0.00 | 101.7 | 16.95 |
| MS cycle 5 | 0.0 | 1 | 37.34 | 49.62 | 29.07 | 64.64 | 47.52 | 34.16 | 262.4 | 43.73 |
| | 0.0 | 5 | 57.39 | 46.25 | 63.34 | 54.74 | 38.56 | 97.57 | 357.9 | 59.64 |
| | -0.6 | 1 | 0.00 | 14.05 | 12.27 | 9.04 | 10.32 | 15.80 | 61.5 | 10.25 |
| | -0.6 | 5 | 12.35 | 3.30 | 0.00 | 16.99 | 22.37 | 17.02 | 72.0 | 12.01 |

[1]zero values indicats no dry matte production in these pots, therefore, zero amount of total N.

Table A.11. Total nitrogen content in mg/pot. Third cutting shoots.

| Cultivar | OP Mpa | N Rate ppm | Block 1 | Block 2 | Block 3 | Block 4 | Block 5 | Block 6 | Totals | Average |
|---|---|---|---|---|---|---|---|---|---|---|
| MS 83CL | 0.0 | 1 | 41.99 | 57.35 | 54.17 | 77.17 | 73.72 | 69.08 | 373.5 | 62.25 |
| | 0.0 | 5 | 57.07 | 56.44 | 38.35 | 60.53 | 87.26 | 79.78 | 379.4 | 63.24 |
| | -0.6 | 1 | 2.99 | 12.74 | 20.64 | 0.00 | 15.08 | 9.01 | 60.4 | 10.07 |
| | -0.6 | 5 | 6.03 | 0.00 | 0.00 | 16.82 | 37.45 | 7.19 | 67.5 | 11.25 |
| MS cycle 3 | 0.0 | 1 | 80.88 | 65.02 | 85.31 | 59.20 | 37.99 | 34.61 | 363.0 | 60.50 |
| | 0.0 | 5 | 52.68 | 97.60 | 81.26 | 95.05 | 129.54 | 96.14 | 552.3 | 92.05 |
| | -0.6 | 1 | 6.24 | 15.51 | 15.91 | 6.33 | 19.97 | 0.00 | 64.0 | 10.66 |
| | -0.6 | 5 | 22.25 | 25.04 | 20.38 | 11.94 | 22.50 | 0.00 | 102.1 | 17.02 |
| MS cycle 5 | 0.0 | 1 | 56.91 | 59.09 | 42.93 | 104.68 | 98.34 | 54.93 | 416.9 | 69.48 |
| | 0.0 | 5 | 67.20 | 66.25 | 81.35 | 73.42 | 52.82 | 137.65 | 478.7 | 79.78 |
| | -0.6 | 1 | 0.00 | 15.42 | 18.36 | 12.83 | 16.16 | 48.73 | 111.5 | 18.58 |
| | -0.6 | 5 | 20.19 | 0.00 | 0.00 | 18.24 | 18.72 | 23.08 | 80.2 | 13.37 |

[1]Zero values indicats no dry matte production in these pots, therefore, zero amount of total N.

Table A.12. Total nitrogen content in mg/pot. Third cutting roots.

| Cultivar | OP Mpa | N Rate ppm | Block 1 | Block 2 | Block 3 | Block 4 | Block 5 | Block 6 | Totals | Average |
|---|---|---|---|---|---|---|---|---|---|---|
| MS 83CL | 0.0 | 1 | 7.61 | 14.14 | 15.14 | 17.52 | 23.86 | 20.21 | 98.5 | 16.41 |
| | 0.0 | 5 | 9.70 | 13.91 | 8.41 | 19.11 | 25.69 | 23.43 | 100.3 | 16.71 |
| | -0.6 | 1 | 1.34 | 3.18 | 5.85 | 0.00 | 3.53 | 2.64 | 16.5 | 2.76 |
| | -0.6 | 5 | 2.32 | 0.00 | 0.00 | 4.94 | 9.24 | 1.43 | 17.9 | 2.99 |
| MS cycle 3 | 0.0 | 1 | 20.44 | 18.01 | 16.48 | 14.99 | 7.66 | 5.89 | 83.5 | 13.91 |
| | 0.0 | 5 | 12.24 | 24.93 | 23.41 | 19.74 | 27.93 | 25.70 | 134.0 | 22.33 |
| | -0.6 | 1 | 2.49 | 4.32 | 4.57 | 2.00 | 5.95 | 0.72 | 20.1 | 3.34 |
| | -0.6 | 5 | 6.53 | 5.88 | 6.07 | 4.47 | 5.94 | 0.00 | 28.9 | 4.82 |
| MS cycle 5 | 0.0 | 1 | 12.25 | 14.37 | 10.69 | 25.58 | 20.84 | 12.64 | 96.4 | 16.06 |
| | 0.0 | 5 | 15.71 | 14.37 | 19.71 | 16.81 | 12.17 | 32.50 | 111.3 | 18.55 |
| | -0.6 | 1 | 0.00 | 4.98 | 7.62 | 3.20 | 4.30 | 11.81 | 31.9 | 5.32 |
| | -0.6 | 5 | 4.56 | 0.00 | 0.00 | 6.02 | 4.74 | 6.13 | 21.5 | 3.58 |

Zero values indicats no dry matte production in these pots, therefore, zero amount of total N.

Talbe A.13. Fixed fraction of plant nitrogen (fixed N %), per experimental unit. First cutting Shoots.

| Cultivar | OP Mpa | N Rate ppm | Block 1 | Block 2 | Block 3 | Block 4 | Block 5 | Block 6 | Totals | Average |
|---|---|---|---|---|---|---|---|---|---|---|
| MS 83CL | 0.0 | 1 | 89.56 | 92.61 | 90.76 | 90.51 | 93.55 | 90.21 | 547.2 | 91.20 |
| | 0.0 | 5 | 73.46 | 60.49 | 58.31 | 68.27 | 63.62 | 74.21 | 398.3 | 66.39 |
| | -0.6 | 1 | 92.54 | 95.96 | 94.05 | 91.72 | 95.41 | 96.67 | 566.4 | 94.40 |
| | -0.6 | 5 | 52.92 | 56.01 | 55.01 | 47.78 | 58.74 | 50.34 | 320.8 | 53.47 |
| MS cycle 3 | 0.0 | 1 | 93.28 | 90.11 | 91.84 | 90.99 | 92.22 | 90.00 | 548.5 | 91.41 |
| | 0.0 | 5 | 61.85 | 59.22 | 65.06 | 71.25 | 69.68 | 69.95 | 397.0 | 66.17 |
| | -0.6 | 1 | 97.38 | 95.63 | 96.22 | 94.69 | 94.80 | 94.83 | 537.5 | 95.59 |
| | -0.6 | 5 | 51.72 | 56.88 | 58.24 | 45.67 | 61.54 | 59.10 | 333.2 | 55.53 |
| MS cycle 5 | 0.0 | 1 | 93.70 | 92.62 | 92.18 | 90.91 | 88.20 | 89.92 | 547.6 | 91.26 |
| | 0.0 | 5 | 68.45 | 60.87 | 62.55 | 70.12 | 64.27 | 67.47 | 397.8 | 65.63 |
| | -0.6 | 1 | 91.47 | 96.29 | 95.25 | 96.85 | 96.52 | 93.94 | 570.3 | 95.05 |
| | -0.6 | 5 | 50.22 | 58.32 | 48.82 | 50.58 | 46.38 | 59.88 | 314.2 | 52.37 |

Table A.14. Fixed fraction of plant nitrogen (fixed N %), per experimental unit. Second cutting shoots.

| Cultivar | OP Mpa | N Rate ppm | Block 1 | Block 2 | Block 3 | Block 4 | Block 5 | Block 6 | Totals | Average |
|---|---|---|---|---|---|---|---|---|---|---|
| MS 83CL | 0.0 | 1 | 95.26 | 95.93 | 95.72 | 96.13 | 96.57 | 95.02 | 574.6 | 95.77 |
| | 0.0 | 5 | 82.93 | 76.99 | 81.61 | 84.38 | 80.25 | 86.12 | 492.1 | 82.05 |
| | -0.6 | 1 | 58.15 | 74.58 | 69.80 | - - | 81.31 | 81.49 | 365.4 | 73.07 |
| | -0.6 | 5 | 14.64 | - - | 27.04 | 25.01 | 19.89 | 16.42 | 103.0 | 20.60 |
| MS cycle 3 | 0.0 | 1 | 96.40 | 96.11 | 94.43 | 95.32 | 96.02 | 96.02 | 574.3 | 95.72 |
| | 0.0 | 5 | 78.71 | 79.16 | 81.79 | 87.59 | 84.74 | 99.36 | 511.4 | 85.23 |
| | -0.6 | 1 | 83.60 | 77.59 | 79.60 | 78.91 | 76.03 | 69.63 | 465.4 | 77.56 |
| | -0.6 | 5 | 29.01 | 16.50 | 26.67 | 18.86 | 33.16 | - - | 121.6 | 24.31 |
| MS cycle 5 | 0.0 | 1 | 96.59 | 95.32 | 95.98 | 97.78 | 94.66 | 95.86 | 576.2 | 96.03 |
| | 0.0 | 5 | 81.89 | 81.21 | 78.38 | 83.43 | 85.34 | 84.93 | 495.2 | 82.53 |
| | -0.6 | 1 | - - | 78.89 | 80.53 | 81.36 | 85.22 | 77.53 | 403.5 | 80.71 |
| | -0.6 | 5 | 11.94 | 25.34 | - - | 19.34 | 25.68 | 25.80 | 108.1 | 21.62 |

- -Undetermined value due to no shoot dry matter production.

Table A.15. Fixed fraction of plant nitrogen (fixed N %), per experimental unit. Third cutting shoots.

| Cultivar | OP Mpa | N Rate ppm | Block 1 | Block 2 | Block 3 | Block 4 | Block 5 | Block 6 | Totals | Average |
|---|---|---|---|---|---|---|---|---|---|---|
| MS 83CL | 0.0 | 1 | 95.77 | 94.63 | 96.03 | 93.94 | 92.76 | 95.33 | 568.5 | 94.75 |
| | 0.0 | 5 | 86.29 | 87.22 | 85.00 | 88.57 | 85.56 | 89.32 | 522.0 | 87.00 |
| | -0.6 | 1 | 90.15 | 86.91 | 90.18 | - - | 87.29 | 90.69 | 445.3 | 89.05 |
| | -0.6 | 5 | 18.66 | - - | - - | 38.64 | 18.58 | 37.54 | 113.4 | 28.36 |
| MS cycle 3 | 0.0 | 1 | 96.23 | 95.20 | 94.22 | 94.86 | 95.15 | 94.15 | 569.8 | 94.97 |
| | 0.0 | 5 | 87.02 | 88.14 | 84.56 | 88.22 | 83.94 | 84.19 | 519.5 | 86.58 |
| | -0.6 | 1 | 86.83 | 90.44 | 88.38 | 86.13 | 86.45 | - - | 438.3 | 87.65 |
| | -0.6 | 5 | 22.64 | 21.26 | 17.55 | 9.02 | 45.63 | - - | 116.1 | 23.22 |
| MS cycle 5 | 0.0 | 1 | 96.33 | 93.33 | 95.02 | 96.46 | 96.61 | 95.65 | 573.4 | 95.56 |
| | 0.0 | 5 | 88.76 | 87.35 | 87.85 | 86.40 | 86.07 | 89.19 | 525.6 | 82.53 |
| | -0.6 | 1 | - - | 91.27 | 88.69 | 89.07 | 91.53 | 91.99 | 452.6 | 90.51 |
| | -0.6 | 5 | 13.08 | - - | - - | 26.22 | 19.71 | 40.90 | 99.8 | 24.98 |

- -Undetermined value due to no shoot dry matter production.

Table A.16. Fixed fraction of plant nitrogen (fixed N %), per experimental unit. Third cutting roots.

| Cultivar | OP Mpa | N Rate ppm | Block 1 | Block 2 | Block 3 | Block 4 | Block 5 | Block 6 | Totals | Average |
|---|---|---|---|---|---|---|---|---|---|---|
| MS 83CL | 0.0 | 1 | 90.92 | 93.01 | 95.20 | 93.75 | 92.12 | 93.44 | 558.4 | 93.07 |
| | 0.0 | 5 | 87.70 | 83.91 | 82.23 | 87.68 | 88.31 | 90.78 | 520.6 | 86.77 |
| | -0.6 | 1 | 83.77 | 87.17 | 86.26 | - - | 86.83 | 94.80 | 438.8 | 87.76 |
| | -0.6 | 5 | 27.62 | - - | - - | 47.56 | 29.40 | 49.79 | 154.4 | 38.59 |
| MS cycle 3 | 0.0 | 1 | 94.41 | 93.18 | 94.20 | 90.60 | 91.78 | 93.19 | 557.3 | 92.89 |
| | 0.0 | 5 | 85.94 | 86.32 | 85.46 | 89.90 | 86.92 | 88.30 | 522.8 | 87.14 |
| | -0.6 | 1 | 86.42 | 89.29 | 84.22 | 84.96 | 89.37 | 84.35 | 518.6 | 86.44 |
| | -0.6 | 5 | 40.22 | 31.06 | 28.16 | 14.81 | 53.23 | - - | 167.5 | 33.50 |
| MS cycle 5 | 0.0 | 1 | 93.61 | 93.23 | 93.82 | 94.22 | 93.68 | 96.51 | 565.1 | 94.18 |
| | 0.0 | 5 | 86.42 | 87.39 | 86.64 | 87.38 | 84.86 | 91.88 | 524.5 | 87.42 |
| | -0.6 | 1 | - - | 85.35 | 89.39 | 87.37 | 92.60 | 91.57 | 446.3 | 89.25 |
| | -0.6 | 5 | 24.88 | - - | - - | 41.19 | 32.17 | 52.35 | 150.6 | 37.65 |

- -Undetermined value due to no root dry matter production.

Table a.17. Fixed amount of plant nitrogen (mg/pot), per experimental unit. First cutting shoots.

| Cultivar | OP Mpa | N Rate ppm | Block 1 | Block 2 | Block 3 | Block 4 | Block 5 | Block 6 | Totals | Average |
|---|---|---|---|---|---|---|---|---|---|---|
| MS 83CL | 0.0 | 1 | 13.60 | 21.38 | 10.99 | 15.07 | 15.27 | 14.09 | 90.40 | 15.07 |
| | 0.0 | 5 | 15.37 | 11.02 | 10.44 | 13.90 | 13.62 | 12.58 | 76.93 | 12.82 |
| | -0.6 | 1 | 5.73 | 5.39 | 5.80 | 3.57 | 6.96 | 4.90 | 32.35 | 5.39 |
| | -0.6 | 5 | 3.51 | 6.03 | 3.42 | 5.50 | 5.56 | 4.31 | 28.33 | 4.72 |
| MS cycle 3 | 0.0 | 1 | 16.17 | 17.31 | 11.73 | 16.97 | 11.49 | 8.73 | 82.40 | 13.73 |
| | 0.0 | 5 | 8.78 | 19.48 | 15.81 | 11.25 | 17.71 | 13.99 | 87.02 | 14.50 |
| | -0.6 | 1 | 4.37 | 10.08 | 6.12 | 8.13 | 6.86 | 6.15 | 41.71 | 6.95 |
| | -0.6 | 5 | 5.80 | 7.66 | 4.11 | 3.75 | 8.27 | 4.54 | 34.13 | 5.69 |
| MS cycle 5 | 0.0 | 1 | 16.15 | 17.60 | 8.92 | 14.77 | 12.18 | 10.25 | 79.87 | 13.31 |
| | 0.0 | 5 | 11.50 | 10.71 | 13.48 | 13.49 | 10.41 | 15.76 | 75.35 | 12.56 |
| | -0.6 | 1 | 5.05 | 6.40 | 4.95 | 4.98 | 3.22 | 6.78 | 31.35 | 5.23 |
| | -0.6 | 5 | 5.08 | 4.03 | 2.45 | 5.14 | 4.78 | 4.35 | 25.83 | 4.31 |

Table A.18. Fixed amount of plant nitrogen (mg/pot), per experimental unit. Second cutting shoots.

| Cultivar | OP Mpa | N Rate ppm | Block 1 | Block 2 | Block 3 | Block 4 | Block 5 | Block 6 | Totals | Average |
|---|---|---|---|---|---|---|---|---|---|---|
| MS 83CL | 0.0 | 1 | 28.59 | 34.62 | 28.69 | 54.37 | 58.18 | 45.83 | 250.3 | 41.71 |
| | 0.0 | 5 | 33.86 | 38.43 | 30.84 | 57.83 | 46.29 | 37.98 | 245.2 | 40.87 |
| | -0.6 | 1 | 3.49 | 7.38 | 6.95 | 0.00 | 11.60 | 5.48 | 34.9 | 5.82 |
| | -0.6 | 5 | 1.57 | - - | 0.32 | 3.48 | 5.30 | 0.78 | 11.5 | 1.91 |
| MS cycle 3 | 0.0 | 1 | 64.05 | 57.18 | 38.96 | 48.08 | 27.37 | 20.81 | 256.5 | 42.74 |
| | 0.0 | 5 | 24.78 | 48.41 | 34.61 | 55.29 | 60.30 | 61.26 | 284.6 | 47.44 |
| | -0.6 | 1 | 9.33 | 10.70 | 11.32 | 6.35 | 8.68 | 2.73 | 49.1 | 8.19 |
| | -0.6 | 5 | 7.64 | 4.84 | 2.88 | 1.43 | 8.27 | 0.00 | 25.1 | 4.18 |
| MS cycle 5 | 0.0 | 1 | 36.07 | 47.29 | 27.90 | 63.21 | 44.98 | 32.75 | 252.2 | 42.03 |
| | 0.0 | 5 | 47.00 | 37.56 | 49.65 | 45.67 | 32.91 | 82.86 | 295.7 | 49.28 |
| | -0.6 | 1 | 0.00 | 11.08 | 9.88 | 7.35 | 8.80 | 12.25 | 49.4 | 8.23 |
| | -0.6 | 5 | 1.47 | 0.84 | 0.00 | 3.29 | 5.74 | 4.39 | 15.7 | 2.62 |

- -Undetermined value due to no root dry matter production.

Table A.19. Fixed amount of plant nitrogen (mg/pot), per experimental unit. Third cutting shoots.

| Cultivar | OP Mpa | N Rate ppm | Block 1 | Block 2 | Block 3 | Block 4 | Block 5 | Block 6 | Totals | Average |
|---|---|---|---|---|---|---|---|---|---|---|
| MS 83CL | 0.0 | 1 | 40.21 | 54.27 | 52.01 | 72.50 | 68.38 | 65.86 | 353.2 | 58.87 |
| | 0.0 | 5 | 49.24 | 49.23 | 32.60 | 53.61 | 74.66 | 71.27 | 330.6 | 55.10 |
| | -0.6 | 1 | 2.70 | 11.07 | 18.62 | 0.00 | 13.16 | 8.17 | 53.7 | 8.95 |
| | -0.6 | 5 | 1.13 | 0.00 | 0.00 | 6.50 | 6.96 | 2.70 | 17.3 | 2.88 |
| MS cycle 3 | 0.0 | 1 | 77.83 | 61.90 | 80.38 | 56.16 | 36.15 | 32.59 | 345.0 | 57.50 |
| | 0.0 | 5 | 54.84 | 86.03 | 68.72 | 83.85 | 108.73 | 84.19 | 486.4 | 81.06 |
| | -0.6 | 1 | 5.42 | 14.02 | 14.06 | 5.46 | 17.26 | 0.00 | 56.2 | 9.37 |
| | -0.6 | 5 | 5.04 | 5.32 | 3.58 | 1.08 | 10.27 | 0.00 | 25.3 | 4.22 |
| MS cycle 5 | 0.0 | 1 | 54.82 | 55.14 | 40.80 | 100.98 | 95.00 | 52.54 | 399.3 | 66.55 |
| | 0.0 | 5 | 59.64 | 57.86 | 71.47 | 93.44 | 45.46 | 122.77 | 450.6 | 75.11 |
| | -0.6 | 1 | 0.00 | 11.04 | 16.29 | 11.42 | 11.79 | 44.83 | 98.4 | 16.40 |
| | -0.6 | 5 | 2.64 | 0.00 | 0.00 | 10.78 | 3.69 | 9.44 | 26.6 | 4.43 |

- -Undetermined value due to no root dry matter production.

Table A.20. Fixed amount of plant nitrogen (mg/pot), per experimental unit. Third cutting roots.

| Cultivar | OP Mpa | N Rate ppm | Block 1 | Block 2 | Block 3 | Block 4 | Block 5 | Block 6 | Totals | Average |
|---|---|---|---|---|---|---|---|---|---|---|
| MS 83CL | 0.0 | 1 | 6.92 | 13.15 | 14.41 | 16.42 | 22.00 | 18.89 | 91.79 | 15.30 |
| | 0.0 | 5 | 8.51 | 11.67 | 6.92 | 16.76 | 22.68 | 21.27 | 87.81 | 14.64 |
| | -0.6 | 1 | 1.11 | 2.77 | 5.05 | 0.00 | 3.08 | 2.50 | 14.50 | 2.42 |
| | -0.6 | 5 | 0.64 | 0.00 | 0.00 | 2.35 | 2.72 | 0.71 | 6.42 | 1.07 |
| MS cycle 3 | 0.0 | 1 | 19.30 | 16.79 | 15.52 | 13.58 | 7.03 | 5.49 | 77.71 | 12.95 |
| | 0.0 | 5 | 10.52 | 21.53 | 20.00 | 17.75 | 24.28 | 22.64 | 116.70 | 19.45 |
| | -0.6 | 1 | 2.15 | 3.86 | 3.85 | 1.70 | 5.31 | 0.61 | 17.48 | 2.91 |
| | -0.6 | 5 | 2.63 | 1.83 | 1.71 | 0.66 | 3.16 | 0.00 | 9.99 | 1.67 |
| MS cycle 5 | 0.0 | 1 | 11.47 | 13.40 | 10.03 | 24.10 | 19.52 | 12.19 | 90.71 | 15.12 |
| | 0.0 | 5 | 13.58 | 12.56 | 17.08 | 14.69 | 10.33 | 29.86 | 98.10 | 16.35 |
| | -0.6 | 1 | 0.00 | 4.25 | 6.82 | 2.80 | 3.89 | 10.82 | 28.67 | 4.78 |
| | -0.6 | 5 | 1.13 | 0.00 | 0.00 | 2.48 | 1.53 | 3.21 | 8.35 | 1.39 |

- -Undetermined value due to no root dry matter production.

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