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**The Use of plants for Reclamation of Salt Affected Agricultural
Areas of Central Asia**

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Section 1: Technical Progress

A. Research Objectives

Overall aim:

To study the capacity of *Atriplex hortensis* cultivars "Gold Polume" (GOP), "Purple Orach" and "Green Polume" to assimilate allantoin via allantoinase and to study the effect of salinity, nitrate and molybdenum on the level of ureides in shoot and root tissues of these cultivars.

Specific objectives:

- 1) To study ureide accumulation in the roots and shoots of *A. hortensis*-GOP as influenced by the presence of salinity (NaCl), nitrate and molybdenum. Germination rate, total height and percentage of water in *A. hortensis*-GOP will be measured.
- 2) To investigate salt hyper-accumulating halophyte plants surviving in the Aral Sea area despite high salinity. Special attention will be paid to *Aeluropus* as the most effective, at least known, of the salt scavengers.
- 3) To improve seed germination and seedling growth of halophytes under saline conditions by optimizing seed-priming pretreatments with *Aeluropus litoralis*, i.e., by (a) alternating hydration and dehydration treatments (priming); and (b) hydrating seeds in the presence of germination-stimulating and salt-stress-alleviating nitrogen compounds and/or molybdenum in various combinations.
- 4) To study the nitrate, amino-nitrogen, molybdenum, abscisic acid (ABA) and proline content in salt-stressed and non-stressed seeds of *A. litoralis*.

- 5) To study Na^+ and Cl^- uptake mechanisms and their subsequent accumulation in aerial parts of *A. littoralis* and to determine optimal conditions for salt removal from the soil.

B. Research Accomplishments

Scientific Background

All plants are subject to a multitude of stresses throughout their life cycle. Depending on the species of plant and the source of the stress, plant responses will vary. When a certain tolerance level is exceeded, the plant eventually dies. For crop plants, two major environmental factors that reduce productivity are drought and salinity (Serrano, 1999); both these factors lead to water stress. Salt stress can be a major challenge to plants. It limits agriculture all over the world, particularly on irrigated farmlands (Rausch, 1996). To farmers, salt tolerance is important in vegetables because of the cash value of crops (Shannon and Grieve, 1999). As more land becomes salinized by poor irrigation practices, the impact of salinity becomes more important. To date, plant breeding has not provided a solution to overcoming the quantitative trait of salinity resistance (Winicov, 1998).

Salinity induces injury, inhibits seed germination and vegetative and reproductive growth, alters plant morphology and anatomy, and often kills nonhalophytes. In angiosperms, salt injury includes leaf scorching or mottling, leaf shedding, and twig dieback. Salinity inhibits seed germination and adversely influences flowering, pollination, fruit development, yield and fruit quality, as well as seed production. Salinity inhibits vegetative growth of nonhalophytes, with shoot growth typically reduced more than root growth (Maas, 1984).

In nonhalophytes, salt-induced inhibition of plant growth is accompanied by metabolic dysfunctions, including decreased photosynthetic rates, and changes in protein and nucleic acid metabolism and enzyme activity. In halophytes, physiological processes may be stimulated or not altered by salt concentrations that are inhibitory in nonhalophytes (Maas, 1984).

The form in which nitrogen is transported from assimilatory roots to shoots has been shown to vary widely among higher plants (Fugihara and Yamamoto, 1977). A number of legumes, especially tropical species, synthesize ureides (allantoin and allantoic acid) in nodules and use these compounds for transport and storage of nitrogen, whereas legume species in which ureides have not been detected or are present in low amounts typically export organic nitrogen from their roots mainly as amides (asparagines and glutamine) (Butler, 1961).

The ureides allantoin and allantoic acid have been known for many years to be major storage and translocation forms of nitrogen in some plants. The first detailed work of ureide distribution was done by Ishizuka and his colleagues (Ishizuka, 1970). More recent reports have generated considerable interest because of the high allantoin concentrations reported in certain plant parts and because the probable site of allantoin synthesis is the root. High ureide concentrations in shoots might result if ureides are a minor component of the xylem stream but ureide catabolism in the tissue is slow (John.G, Streeter.M, 1978). Allantoin is degraded to allantoate by allantoinase, which has been well characterized from plant extracts (Wells and Lees, 1992; Webb and Lindell, 1993; Bell and Webb, 1995).

Allantoinase catalyzes the hydrolysis of allantoin to allantoic acid in ureide metabolism. These nitrogen-rich organic compounds are used in some plants as nitrogen carriers. Because ureides have a low carbon-to-nitrogen ratio, they are thought to provide efficient transport and storage of nitrogen with minimal expense of reduced carbon (Pate, 1973). The reaction catalyzed by allantoinase plays a dual role in ureide metabolism in plants. It is the final step of ureide biogenesis in tissues that produce allantoic acid and the first step in ureide degradation in tissues that import or store allantoin (Tracey, 1955).

In some legumes, a different nitrogen transport strategy is employed, using molecules with an even higher nitrogen/carbon ratio than the amides. These are the ureides—compounds such as allantoin and allantoic acid—which are all related to urea and have a wide distribution, being found in many seeds and xylem sap. Allantoin and allantoic acid are used for nitrogen transport in a variety of plants, including many nitrogen-fixing legumes. In the majority of ureide-transporting plants, most of the allantoin is converted to allantoic acid before transport by allantoinase, which is associated with the endoplasmic reticulum. Thus, ureide-transporting plants vary in their xylem sap composition, depending on species. The switch from amide to ureide synthesis in the tropical legumes probably involves carbon economy: ureide producers use less organic carbon to transport the same amount of nitrogen than do amide producers (Pate J.S, Atkins S.A 1981). However, the metabolic costs of using these molecules is higher once they reach their destinations: it costs more (in terms of ATP) to degrade ureides than it does to degrade amino acids or amides (Pate and Layzell, 1990). Ureide catabolism seems to be less efficient than that of the amides because some of the ureide carbon is lost as CO₂ and the overall cost of using ureides is metabolically more expensive, almost twice that of using asparagines (Reinbothe and Mothes, 1962).

We also investigated the effect of molybdenum, nitrate and NaCl on germination of *A. hortensis*-GOP seeds. Molybdenum is absorbed by plants as the molybdate ion. Molybdenum is important to legumes because it is a part of two enzyme systems: nitrogenase, which fixes atmospheric nitrogen, and nitrate reductase, which enables plants to utilize the nitrate. Deficiency symptoms for Mo include a general yellowing of the leaves and rolling, curling, and scorching of the leaves (Williams and Frausto da Silva, 2002).

Molybdenum deficiencies have been documented in many plant species. In young plants, visual manifestations—ranging in severity among different phenotypes—include mottling, leaf cupping, grey tinting and flaccid leaves, which are often found on seedlings that remain dwarfed until dying (Hewitt and Bolle-Jones, 1952). In older plants, where deficiencies have been reversed or when deficiency levels are modest, the symptoms appear in younger leaf tissues with the characteristic loss of proper lamina development, leathery leaves and meristem necrosis (Jones and Belling 1967).

Nitrogen is an important constituent of protein and protoplasm and essential for plant growth. Its shortage leads to chlorosis (yellowing of the leaves) and cessation of growth. Nitrogen is given to plants in the forms of organic manure and artificial fertilizer. Most inorganic nitrogen is converted to amino acids and then to proteins. Nitrogen is typically added to plant nutrient media as the nitrate ion (NO_3^- , oxidized) or the ammonium ion (NH_4^+ , reduced), which are added as inorganic salts. In devising media, both the total amounts of nitrogen as well as the relative amounts of NO_3^- and NH_4^+ are important (Hasegawa et al., 1962).

Dormant halophyte seeds are tolerant to high levels of salinity, remaining viable and capable of germination. However, germination of halophyte seeds does not correlate with salt tolerance of adult plants. Increased salinity inhibits seed germination, delays it, and lowers total germination (Waisel, 1991; Ungar, 1995). Thus, the germination stage is significant in determining the ability of halophytes to survive to maturity.

According to our previous results dormant seeds of halophytes can be induced to germinate by supplying them with various nitrogen-containing compounds and molybdenum under laboratory conditions or by the application of nitrogenous

fertilizers in the field. The ability of these treatments to break seed dormancy has also been reported for a wide range of other species. Although this response has been the subject of numerous investigations, there is still no general agreement as to the nature of the physiological mechanism involved. One hypothesis, which has attracted considerable attention, is that oxidized forms of nitrogen (nitrate and nitrite) may promote germination by causing a shift in respiratory metabolism to the pentose phosphate pathway. However, studies on the relationship between the activity of the pentose-phosphate pathway and seed dormancy in halophyte plants have not been carried out. It has also been postulated that nitrates may promote germination by acting as electron acceptors, thereby increasing the respiration rate. This hypothesis is consistent with the increase in respiration rate that precedes nitrate-induced germination but is not supported by evidence that KNO_3 can promote germination without being metabolically reduced. However, nothing has been reported about the effect of nitrate on halophyte seed germination.

Nitrate is known to stimulate the germination of seeds and has received considerable attention as a possible regulator of seed germination in the soil (Bewley and Black, 1994). Moreover, it is generally observed that plants under saline conditions preferentially utilize nitrate as a source of nitrogen and that nitrate-fed plants are more tolerant to salt stress than ammonium-fed plants (Speer et al., 1994; Cordovilla et al., 1996). Inhibition of germination caused by salinity was partially alleviated by nitrate in *Halopyrum* and completely in *Allenrolfea* (Gul and Weber, 1998). Nitrate reductase, a molybdenum containing enzyme, is a key enzyme in nitrate assimilation. This growth-regulation factor plays a role in development, protein production, and yields of plants (Hageman and Lamberti, 1988).

The main objective of the present investigation was to examine the relationship between nitrate- and molybdenum-induced germination and associated changes in the nitrogen content of halophyte seeds. The rationale for this approach was provided by previous evidence that factors affecting nitrate uptake by the seed play a major role in determining the occurrence and degree of dormancy.

One promising approach to improving germination tolerance to salinity is seed priming. The significance of seed priming, which implies controlled seed imbibitions in the presence of an essential element, followed by drying to improve later development of seedlings, has been established by a number of researchers (Bray, 1995; Kohler et al., 1997).

Taken as a whole, in *A. littoralis* the seed coat of the intact seeds prevents the uptake of water by the embryo and endosperm in the amount required for induction of germination. Theoretically, water uptake by the seed will be controlled not only by the hydraulic conductivity of the seed coat but also by the water potential gradient between the embryo and the external water supply. We therefore postulated that application of nitrate and molybdenum to the dormant seeds may promote seed germination. The accumulation of nitrate may stimulate seed germination and molybdenum may play a key role in nitrate reduction during seed germination. In addition, uptake of the cation associated with the nitrate may also have a significant osmotic effect. While these osmotic effects may trigger germination, it is further postulated that nitrate reduction promotes the continued growth of the embryo by a nutritional effect on protein synthesis. The present investigation was conducted to test this hypothesis and also to examine the interaction between the effects of nitrate and molybdenum on halophyte seed germination under normal and saline conditions.

Materials and methods

Three different concentrations of salinity (0 [control], 100 and 200 mM NaCl) were applied to three different cultivars of *A. hortensis* (GOP, PO, GrP) for four weeks. The experiments were conducted in a greenhouse, in which the average daily temperature fluctuated during the growth period between 15°C and 25°C. After four weeks, the plants were harvested to measure ureide levels in their roots and shoots.

The next set of treatments consisted of the following: 0 mM NaCl (control); 100 mM NaCl; 10 mM NO_3^- (as KNO_3); 0.3 μM Mo [as $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$]; 3.0 μM Mo; 100 mM NaCl + 10 mM NO_3^- ; 10 mM NO_3^- + 0.3 μM Mo; 10 mM NO_3^- + 3.0 μM Mo; 100 mM NaCl + 10 mM NO_3^- + 0.3 μM Mo; 100 mM NaCl + 10 mM NO_3^- + 3.0 μM Mo, in half-strength Hoagland's nutrient solution.

A. hortensis-GOP was planted in perlite. The plants were treated daily with a solution containing the following nutrients: 20.22 mM KCl; 15.6 mM $\text{NaH}_2\text{PO}_4\cdot 2\text{H}_2\text{O}$; 49.3 mM $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$; 0.245 mM $\text{MnSO}_4\cdot \text{H}_2\text{O}$; 0.029 mM $\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$; 0.186 mM H_3BO_3 ; 0.0028 mM $\text{CoSO}_4\cdot 7\text{H}_2\text{O}$; 0.024 mM $\text{CuSO}_4\cdot 5\text{H}_2\text{O}$; 29.4 mM $\text{CaCl}_2\cdot 2\text{H}_2\text{O}$; 13.22 mM $(\text{NH}_4)_2\text{SO}_4$; and 6.25mg/l Fe-Sequestrene. The pH of the nutrient solution was 5.5–6. Plants were grown in a greenhouse at a thermo period of 15–25°C and 12 h dark/12 h light (night: day) photoperiod. Three replicate pots for each of the 10 treatments each contained five seedlings. Plants grown in perlite were irrigated once every two days.

Measurement of Allantoic acid

The method used was basically that of Van der Drift and Vogel's (1966). Shoot and root samples from the three varieties of *A. hortensis* were harvested and immediately frozen in liquid nitrogen. Fresh-weight tissue samples (1 g) for ureide assays were extracted in 5 ml 80% ethanol (1:5 w/v). The homogenized plant material was centrifuged at 15000 rpm at 4°C for 5 min with a Hermle Z 233 MK-2 microcentrifuge. To 1 ml of tissue extract 0.15 N HCl was added. Tubes were boiled for 4 min at 100°C then incubated in ice-cold water for 4 min. A solution of 0.4 M Na₂HPO₄ + KH₂PO₄, pH 7, and 23 mM phenylhydrazinium chloride solution was then added and kept for 5 min at room temperature. After this 32% hydrochloric acid and 50.6 mM K-ferricyanide solution were added. The tubes were then kept at room temperature for 15 min, after which allantoic acid was determined spectrophotometrically at 535 nm.

Measurement of Allantoin

Approximately 0.2 g of the plant material (roots and shoots) was mixed with 1 ml of 80% ethanol. The concentrate was dissolved with 2 ml H₂O and 1 ml 0.5 N NaOH, was boiled for 8 min at 100°C, after cooling were added 0.65 N HCl. After 4 min boiled and cooled was added 0.4 M Na₂HPO₄ + KH₂PO₄, pH 7, and 23 mM phenylhydrazinium chloride solution for 5 min at room temperature, submitted again to cooled and determined spectrophotometrically at 535 nm.

Stock solutions of allantoin and allantoic acid (1 mg/ml) were prepared by dissolving these in water and storing them at 4°C. Working standard solutions ranging from 1 to 200 µg/ml were prepared by dilution to establish a calibration curve.

Priming of halophyte seeds with nitrate, molybdate and chloride

Selected halophyte seeds of uniform size were imbibed in glass tubes for 24 h to 30 h in solutions containing different concentrations of KNO_3 , NH_4Cl , and/or Na_2MoO_4 or in distilled water (control) and then dried for 24 h at room temperature. The primed seeds and untreated controls were placed in 9-cm diameter Petri dishes containing two pieces of Whatman No. 3 filter paper and 7 ml of either distilled water or a 25–100 mM solution of KNO_3 or NH_4Cl and/or Na_2MoO_4 (50 mM molybdate was optimum for seed germination). The pH of the water and mineral solutions was adjusted to 6.0 with KOH in all experiments and also with a buffer containing 35 mM KH_2PO_4 and 4 mM Na_2HPO_4 . In all experiments the seeds for the priming procedure were incubated in darkness at 20°C during the treatment period. The Petri dishes were kept in tightly closed plastic trays lined with wet blotting paper to minimize evaporative concentration of the solutions. Seeds were recorded as having germinated when the pericarp had ruptured above the embryo and the tip of the radicle was exposed.

Desalinizing field experiments with *A. litoralis* were carried out in the Aral Sea region for a period of 110 to 115 days, commencing May 20, 2005. One-hundred seedlings grown under laboratory conditions were transplanted from a seedbed into saline soil in each of five plastic pots (diameter 50 cm, height 30 cm). Five pots without seedlings were used as a control. All the pots were irrigated with 2 liters of NaCl solution containing 0.35 g of sodium ions every third day to replace salt absorbed by the plants. The NaCl solution was applied through three glass tubes penetrating the soil to different depths. The pots were placed outside and covered with thin transparent plastic sheets as protection against rain. Temperatures under the plastic sheets were approximately 25 to 35°C. Seeds of *A. litoralis* were also sown in

saline soil in the field. Each treatment was replicated four times. A plastic sheet to a depth of 1 m separated the soil of adjacent plots. The dry weight and Na content of representative samples of plants from pots and the Na content of soil from pots and field plots were measured at the beginning and end of the experiments. Sodium ions were extracted from soil with water and from dried plants by heating with 5 M HCl at 100°C until dry and redissolving in deionized water. Quantitative analysis of sodium ion was carried out in the Al-Farabi National University (in Almaty) with an ASS-N (Germany) atomic absorption spectrophotometer.

Halophyte seeds (1000 seeds) for dry weight and water content determinations were weighed immediately after being excised and surface dried as described above. They were then placed in vials immersed in dry ice, and after being freeze-dried, were equilibrated over anhydrous CaSO₄ in a desiccator for 24 h and their dry weight determined. Both the fresh and dried seed were weighed on a Mettler Toledo electronic microbalance. The seeds were also surface dried with absorbent tissue, placed in vials immersed in dry ice, freeze-dried and stored over CaSO₄ at -15°C until they were analyzed.

Samples for analysis comprised 10 seeds from each treatment and either three or four replicates. Each sample was transferred to a 2-ml conical tissue homogenizer tube and ground up in 1 ml of 80% ethanol, (prepared from 95% ethanol distilled over concentrated H₂SO₄). The homogenate was transferred quantitatively to a 5-ml volumetric flask and made up to volume with 80% ethanol. The extracts were analyzed for amino-nitrogen content by *o*-phthaldehyde derivatization and fluorescence detection using the high-performance liquid chromatographic system in the MA Aitkhozhin Institute of Molecular Biology and Biochemistry (in Almaty). The nitrate contents of the seeds were determined from aliquots of the same extracts used

for the amino-N analysis. The nitrate in the extract was determined using a Waters HPLC system consisting of a model 510 pump operated at 1.0 ml/min, the WISP model 712 autosampler set to inject 60 μg , the model 490 programmable multi-wavelength detector set to monitor 210 nm. A pre-column packed with 37–53 μg silica gels was placed between the pump and autosampler to saturate the mobile phase with silicate ions, thus minimizing dissolution of the packing in the analytical column. A Whatman Partisil 10 SAX anion exchange column was used and was preceded by a guard column packed with pellicular anion exchanger, both of which were maintained at 30°C. The mobile phase, 0.45 mM potassium phosphate buffer at pH 3, was prepared by adding 285 ml of 50 mM H_3PO_4 solution to 1 liter of 50 mM K_2HPO_4 solution and then filtering it through a 0.45 μg nylon filter. Under these conditions, the retention time of the nitrate ion was 7.9 min. The nitrate content of the samples was determined by reference to a series of five standard KNO_3 solutions, with nitrate concentration of 0.5 to 4.0 mg/l. Reagent blanks were included in each experiment. Nitrate content was also determined by the method of Genlin and Lips (2000).

Determination of ABA content in halophytes

ABA content was determined according to Omarov et al. (1998 and 1999). For proline determination, approximately 0.5 g of plant material was homogenized in 10 ml of 3% aqueous sulfosalicylic acid and the homogenate was centrifuged. A 2-ml portion of each extract was reacted with 2 ml of acid-ninhydrin reagent and 2 ml glacial acetic acid in a test tube for 1 h at 100°C and then the reaction was terminated in an ice bath. The reaction mixture was extracted with 4 ml toluene and mixed vigorously for 15 to 20 seconds. The chromophore-containing toluene was separated and absorbance was read at 520 nm. The proline concentration was determined from a standard curve and calculated on a fresh weight basis.

In measuring molybdenum content of the seeds, a 20 ml sample of the alcohol extract was evaporated to dryness, and the residue was dissolved in distilled water. The molybdenum in the aqueous solution was determined with an AAS-N atomic absorption spectrophotometer. All the chemicals including (\pm) ABA were purchased from Sigma.

RESULTS AND DISCUSSION

Availability of reduced nitrogen is an important determinant in the growth and development of plants. Although in most vascular plant species the major transport form of reduced/organic nitrogen is as amino acids (including amides), legumes and halophytes transport large amounts of nitrogenous compounds called ureides. The dominant forms of ureides in these species are allantoin and allantoic acid (Pate et al., 1980). We investigated the effect of salinity on ureide content in halophytes.

Effect of salinity to concentration of ureides in *A.hortensis* (GOP, PO,GrP)

Three varieties of *A. hortensis*—'Gold Plume' (GOP), 'Purple' Orach (PO) and Green Plume (GrP)—were grown in three levels of salinity: 0, 100 and 200 mM NaCl.

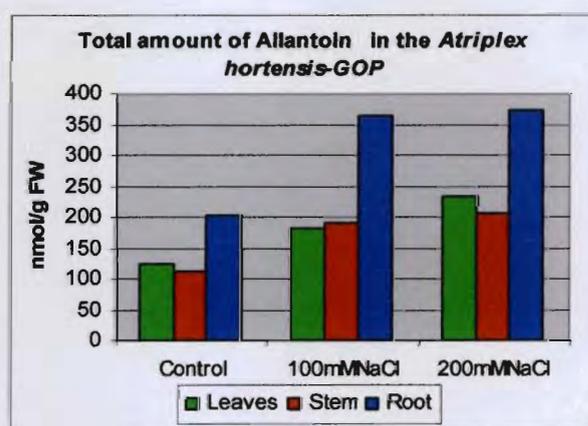


Fig. 1 Total content of allantoin in *A. hortensis*-GOP at different salinities

Figure 1 shows the allantoin content of the leaves, stems and roots of *A. hortensis*-GOP under three conditions of salinity. Increased salinity led to increased allantoin concentrations in all three tissues. Allantoin content in leaf tissue increased incrementally with increased salinity to 43% (se ± 0.02) at 200 mM NaCl, a two-fold increase over the 23% (se ± 0.007) allantoin concentration in the control plants.

Stem and root concentrations were greater with salinity than without (control), yet, while leaf concentrations increased incrementally, stem and root concentrations

increased significantly in the presence of 100 mM NaCl and then remained virtually unchanged when the salinity doubled. Allantoin concentrations in the roots increased most dramatically under salt stress, climbing to 39% (se ± 0.010) under conditions of salinity from 22% (se ± 0.012) in control plants.

High concentrations of ureides have been found in plants exposed to stress (salinity). Recent reports have generated considerable interest because of the high allantoin concentrations reported in certain plant parts, and because the probable site of allantoin synthesis is the root. High ureide concentrations in shoots might result if ureides are a minor component of the xylem stream but ureide catabolism in the tissue is slow (John et al., 1978).

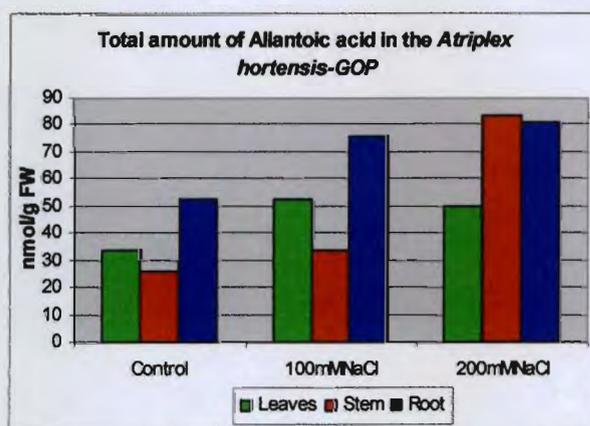


Fig.2 Total content of allantoic acid in *A. hortensis-GOP* at different salinities

Allantoic acid concentration in *A. hortensis-GOP* was higher with salinity than without (control) in all three tissues (Figure 2). Allantoic acid concentration in leaves in salt-stressed plants (100 and 200 mM NaCl) was higher (38%, se ± 0.007) than in control-plant leaves (25%, se ± 0.006). Doubling the level of salinity from 100 to 200 mM NaCl actually led to a slight reduction of allantoic acid concentration in the leaves, while mildly increasing concentrations in the roots.

Concentration in the stems, however, increased dramatically to exceed that of the other tissues at the higher salinity. The total amount of allantoic acid with high

salinity (200 mM NaCl) reached 59% (se ± 0.001), three times greater than that of the control-plants at 18% (se ± 0.001).

Comparing Figure 2 with Figure 1, concentrations of allantoin in the different tissues are three to four times those of allantoic acid.

These studies indicate that stems serve as major repositories for ureide (Fujihara S.K., 1977).

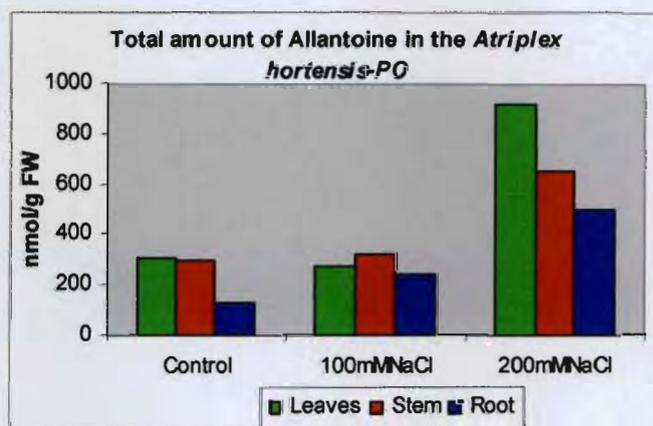


Fig. 3 Total content of allantoin in *A. hortensis-PO* at different salinities

Allantoin concentration in *A. hortensis-PO* was highest at a salinity of 200 mM NaCl (as it had been in *A. hortensis-GOP*). However, in *A. hortensis-PO* the highest concentration of allantoin at this salinity was found in the leaves, with double the concentration of that of the roots. The roots had the lowest allantoin concentration of all three tissues at all three salinities; however, concentration in the roots rose steadily, doubling with each treatment. Allantoin concentration at 100 mM NaCl in the stems and leaves was very similar to that of the control plants, but at 200 mM NaCl, the concentration in the leaves increased dramatically to a concentration three times that found in the control plant leaves (Figure 3). Quantitatively, allantoin levels in *A. hortensis-PO* were twice those of *A. hortensis-GOP*.

Ureide production and degradation also occurs in non-legumes. *A. hortensis* can utilize ureides as the sole source of nitrogen (Desimone et al., 2002).

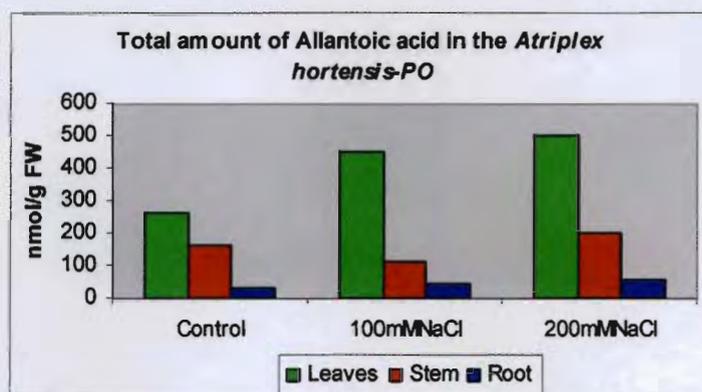


Fig.4 Total content of allantoic acid in *A. hortensis-PO* at different salinities

Allantoic acid content in *A. hortensis-PO* leaves was higher than that of the stems and roots at all three salinities. At both salt-stress levels (100 and 200 mM NaCl), allantoic acid content in the leaves was approximately twice that of the control leaves, increasing slightly as salinity increased. Concentrations of allantoic acid in the stems and leaves were both higher than those in the roots. Allantoic acid concentrations remained low during the salt-stress treatments, although there was an upward trend with increasing salinity (Figure 4). Quantitatively, these values were five times those of *A. hortensis-GOP*, where, although allantoic acid concentrations in *A. hortensis-GOP* leaves were the lowest (Figure 2), *A. hortensis-GOP* root and stem concentrations were no greater in magnitude than those of the lowly concentrations in the *A. hortensis-PO* roots. Comparing allantoin levels in *A. hortensis-PO* (Figure 3) with allantoic acid levels (Figure 4), we see that allantoin concentrations were less than a twice those of allantoic acid in the same tissues.

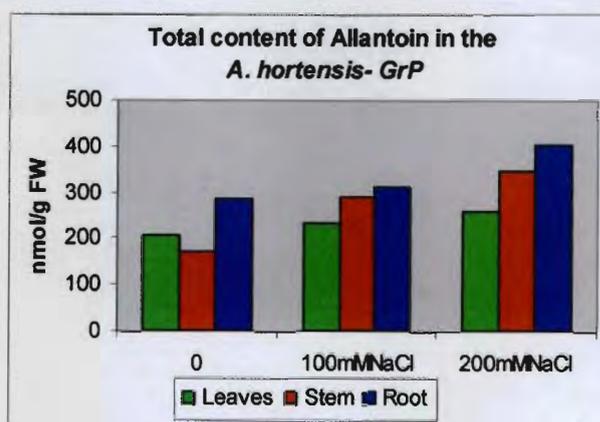


Fig.5 Total content of allantoin in *A. hortensis-GrP* at different salinities

Concentration of allantoin in all three tissues of *A. hortensis-GrP* increased with increasing salinity (Figure 5), similar to that of *A. hortensis-GOP* (Figure 1), however, each increased at a different rate. In control plants, leaf concentrations were higher than stem concentrations, but this was reversed under salt stress. Allantoin concentrations in roots were higher than the other tissues at all concentrations, as had been the case with *A. hortensis-GOP*, and the order of magnitude of allantoin concentration for *A. hortensis-GrP* and for *A. hortensis-GOP* was similar, as well.

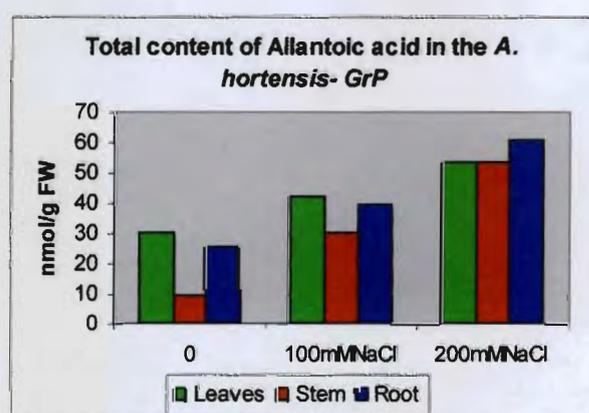


Fig.6 Total content of allantoic acid in *A. hortensis-GrP* at different salinities

Figure 6 shows a trend with allantoic acid content in *A. hortensis-GrP* that is similar to the trend in its allantoin content (Figure 5) i.e. increasing concentrations with increasing salinity. However, allantoin concentration in *A. hortensis-GrP* at 200 mM NaCl was seven times higher than its allantoic acid concentration at the same

salinity (Figure 5). Salt stress resulted in a significant increase in the concentration of these ureides in *A. hortensis*-GrP roots.

During stress treatment, *A. hortensis*-GrP and *A. hortensis*-GOP had increased ureide content, while stressed *A. hortensis*-PO had decreased in stressed plants.

From our results we suggested that with salinity stress content of ureides is increased. Halophytes use ureides as a transport molecule because it takes less carbon to transfer the same amount of nitrogen than the amide transporters, and halophytes in the salinity stress need a lot amount of energy to grow normally, to produce and to survive from stress (Kelly Simpson, 1998).

Salinity, Nitrate and Molybdenum, Ureides

In nodulated soybeans, up to 90% of the fixed nitrogen is transported to the shoots as the ureides allantoin and allantoic acid (Bollard, 1959). We observed that an increase in allantoate concentration in leaves, resulting from the application of potassium nitrate, was accompanied by a substantial decline in activity of allantoate amidohydralase (Hegarty, 1973). Inhibition of allantoate amidohydralase, the enzyme responsible for allantoate degradation in *A. hortensis*-GOP leaves, could cause allantoate accumulation in leaf tissue. Our previous work demonstrated substantial accumulation of allantoin and allantoate in shoot and root of *A. hortensis*-GOP in response to nitrogen, salinity and molybdenum utilization.

We investigated the effect of nitrate, salinity and molybdate on ureide assimilation in *A. hortensis*-GOP shoots and roots.

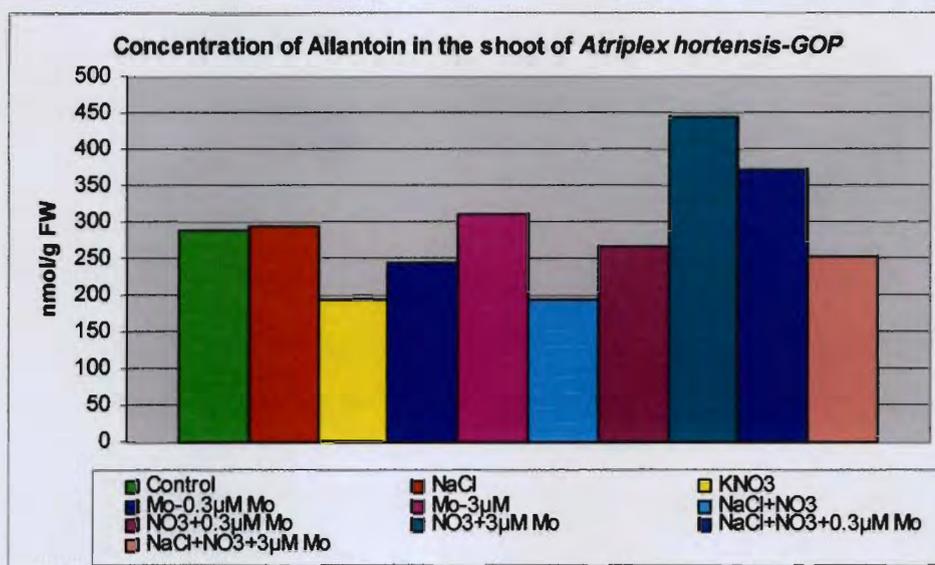


Fig.7 Concentrations of Allantoin in *Atriplex hortensis-GOP* with salinity, nitrate and molybdate

Figure 7 shows that the concentration of allantoin in the shoots of 4-week-old *A. hortensis-GOP* plants grown in untreated water (control) was ~290 nmol/g FW, and that adding 100 mM NaCl to the water had almost no effect (~295 nmol/g FW). Adding 3.0 mM molybdate slightly increased the allantoin concentration to ~310 nmol/g FW, while adding 0.3 mM molybdate reduced the allantoin concentration to ~245 nmol/g FW. Adding nitrate reduced allantoin concentration further to the lowest concentration obtained here (195 nmol/g FW), a concentration that was unaffected by combining NaCl and nitrate. Adding nitrate to 0.3 mM molybdate resulted in an allantoin concentration (~270 nmol/g FW) that was higher than that of either treatment alone (nitrate or 0.3 mM molybdate), and adding nitrate to 3.0 mM molybdate resulted in the highest allantoin concentration obtained here (443.5 nmol/g FW). Adding NaCl to the nitrate + 0.3 mM molybdate combination significantly improved the allantoin concentration obtained to 370 nmol/g FW, but adding NaCl to the nitrate + 3.0 mM molybdate combination significantly reduced the

allantoin concentration obtained to ~252 nmol/g FW. [NaCl + Mo without nitrate were not tested].

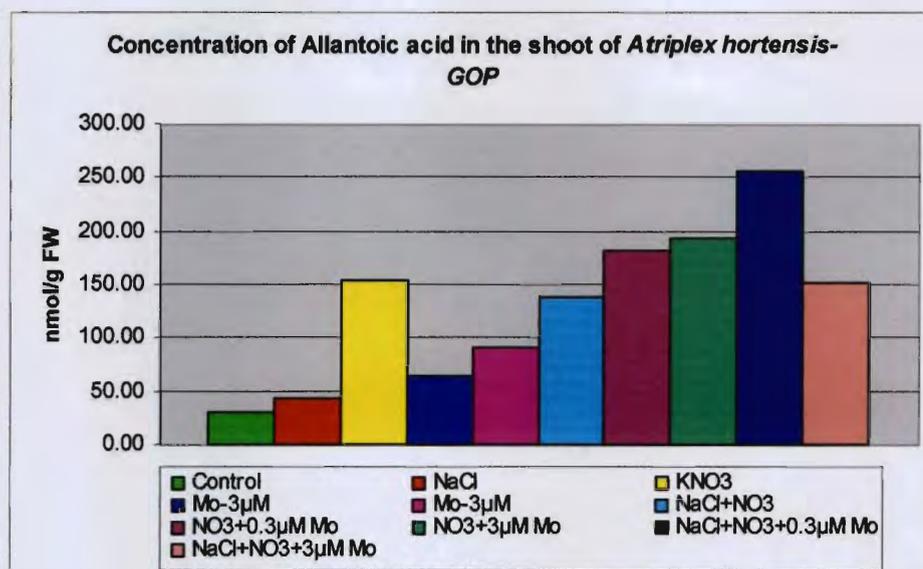


Fig. 8 Concentrations of allantoic acid in shoots of *A. hortensis-GOP* with salinity, nitrate and molybdate

Figure 8 shows that shoot allantoic acid concentration with untreated water (control) was 30 nmol/g FW. Adding 100 mM NaCl resulted in a small increase in allantoic acid concentration to 44 nmol/g FW. Molybdate treatments alone (0.3 mM and 3.0 mM Mo) led to ~55 and ~90 nmol/g FW allantoic acid concentrations (respectively), but both were lower than the concentration obtained from nitrate alone (153.5 nmol/g FW), which was the highest concentration obtained from among single-factor treatments. Combining nitrate and NaCl reduced the results obtained with nitrate alone (139.5 nmol/g FW). However, combining nitrate with molybdate resulted in enhanced allantoic acid concentrations—the stronger the molybdate concentration, the greater the resultant allantoic acid concentration (~180 and ~195 nmol/g FW, respectively). Combining nitrate, NaCl and 0.3 mM Mo resulted in the highest concentration of all (255.5 nmol/g FW), but the enhancement effect was lost when the

stronger concentration of molybdate (3.0 mM Mo) was combined in place of the weaker one (~150 nmol/g FW).

Treatment with molybdate + nitrate positively affected to content of ureides.

The results show that shoots have some capacity to assimilate allantoin to allantoic acid. Allantoin in the shoots of *A. hortensis*-GOP is 3.7 fold higher than allantoic acid.

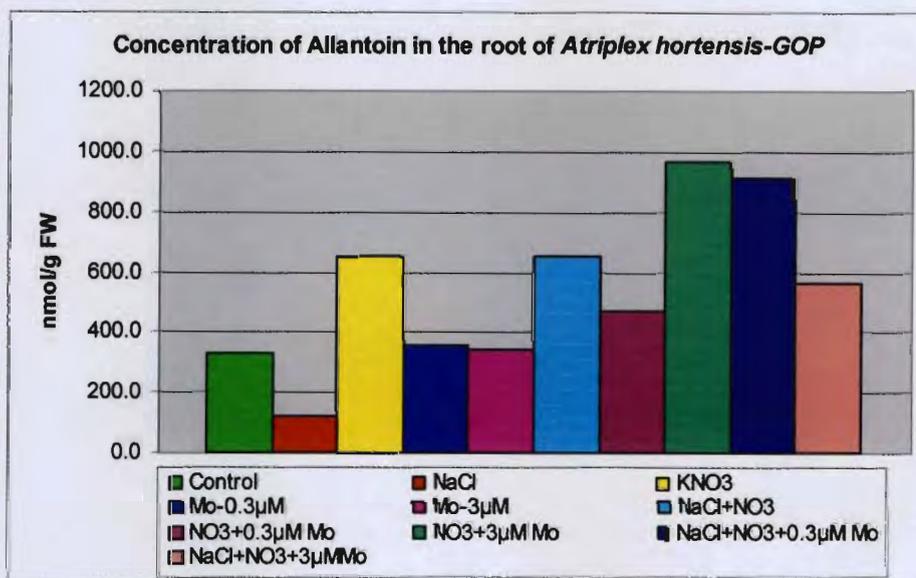


Fig.9 Concentration of allantoin in roots of *A. hortensis*-GOP with salinity, nitrate and molybdate

Allantoin concentration was measured in roots of *A. hortensis*-GOP. Figure 9 shows that with no additives (control), root allantoin concentration was 330.5 nmol/g FW. Adding 100 mM NaCl reduced root allantoin concentration nearly 3-fold to 118.5 nmol/g FW, while adding nitrate increased it nearly 2-fold to 650 nmol/g FW. Neither concentration of molybdate (0.3 or 3.0 nM) had any significant effect on root allantoin concentration over that of the control treatment, nor did combining nitrate and NaCl have a significant effect over that of nitrate alone. Adding the weaker concentration of molybdate (0.3 mM) to nitrate reduced the results obtained from the nitrate treatment alone (~450 nmol/g FW) but adding the stronger concentration of molybdate (3.0 mM) enhanced the allantoin concentration obtained from the nitrate

treatment alone by a factor of 1.5, resulting in the highest concentration of root allantoin obtained here (966 nmol/g FW). By the same token, adding nitrate to the molybdate treatments enhanced the results obtained from both of them. Adding NaCl to the nitrate + 0.3 mM molybdate combination doubled the allantoin concentration obtained (~900 nmol/g FW) from the nitrate + 0.3 mM molybdate treatment, while adding NaCl to the nitrate + 3.0 mM molybdate combination reduced the allantoin concentration obtained (570 nmol/g FW) from the nitrate + 3.0 mM molybdate treatment by a factor of 1.7. This was also lower than the allantoin concentration obtained from the nitrate + NaCl combination treatment.

Figure 7 and Figure 9 together show that allantoin concentrations in the roots were two times greater than in the shoots because the probable site of allantoin synthesis is in the roots.

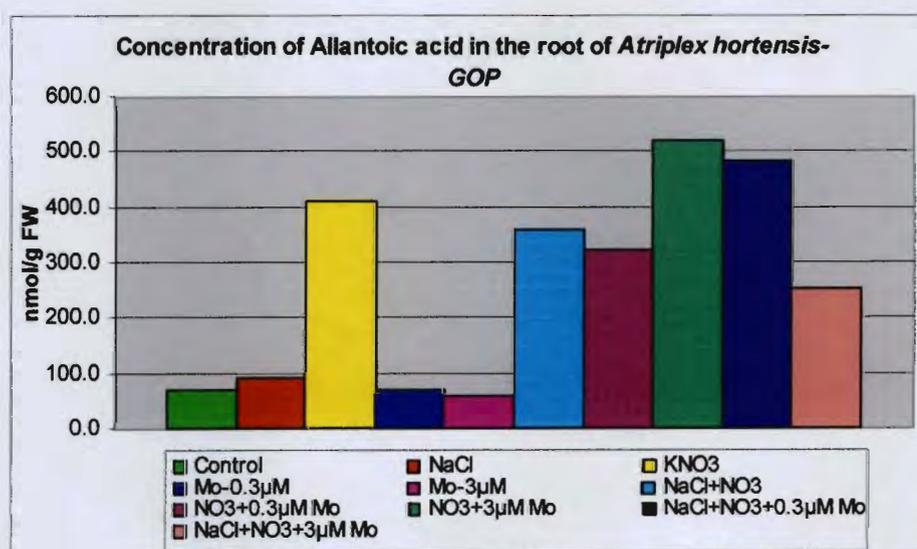


Fig.10 Concentration of Allantoic acid in *A. hortensis*-GOP roots with salinity, nitrate and molybdate

Figure 10 shows the allantoic acid concentrations obtained from roots of *A. hortensis*-GOP. With no additives (control) the allantoic acid concentration was ~75 nmol/g FW. Adding 0.3 mM molybdate had no significant effect, while 3.0 mM molybdate

reduced the allantoin concentration obtained to ~60 nmol/g FW. Adding 100 mM NaCl increased the allantoin acid concentration to ~90 nmol/g FW, but adding nitrate to the water led to a dramatic increase in allantoin acid concentration (to ~410 nmol/g FW), 5.5 times that of the control. Adding NaCl to the nitrate treatment reduced the effect of nitrate alone to obtain ~350 nmol/g FW, and adding 0.3 mM molybdate to the nitrate treatment reduced the effect of nitrate alone even more to obtain an allantoin acid concentration of ~320 nmol/g FW. Adding 3.0 mM molybdate to the nitrate treatment, however, boosted the results to ~520 nmol/g FW. By the same token, adding nitrate to the two molybdate treatments (0.3 and 3.0 mM) improved the allantoin acid concentrations obtained about 4- and 9-fold, respectively. Adding NaCl to the combined 0.3 mM molybdate + nitrate treatment increased the allantoin acid concentration (~480 nmol/g FW) obtained from that combination, but adding NaCl to the combined 3.0 mM molybdate + nitrate treatment reduced the results (~250 nmol/g FW) obtained from that combination by more than half.

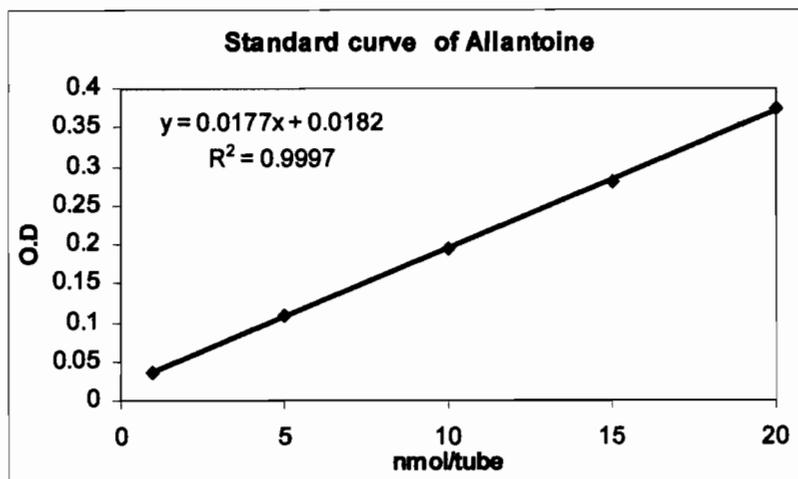


Fig.11 Standard curve for Allantoin

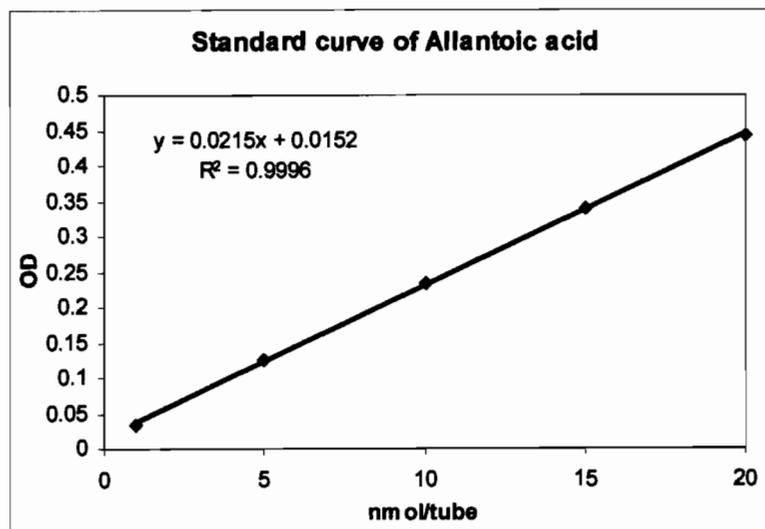


Fig.12 Standard curve for Allantoic acid

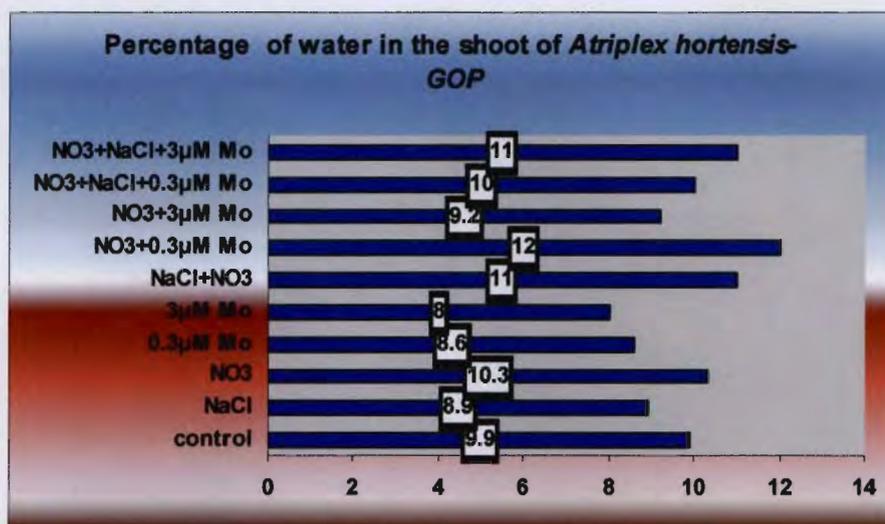


Fig. 13 Percentage of water in salinity, nitrate and molybdate in the *A. hortensis*-GOP.

Shoots of *A. hortensis*-GOP were analyzed for moisture content under the aforementioned treatments: e.g., 0 and 100 mM NaCl; 10 mM KNO₃; 0.3 µM Mo and 3 µM Mo; NO₃ + NaCl; NO₃ + 0.3 µM Mo; NO₃ + 3.0 µM Mo; NO₃ + NaCl + 0.3 µM Mo, and NO₃ + NaCl + 3.0 µM Mo. The results are given in percentage of water (Figure 13). In control plants the percentage of water was 9.9%. Adding NaCl reduced the percentage of water to 8.9%, while adding molybdate reduced the percentage of water to 8.6% (3.0 µM Mo) and 8.0% (3.0 µM Mo), respectively – the lowest percentages obtained. Conversely, adding nitrate increased the percentage of water to 10.3%, and adding nitrate to the other factors improved all their results: nitrate + 3.0 µM Mo improved the percentage of water to 9.2% and nitrate + 0.3 mM Mo improved the percentage of water to 12.0%, – the highest percentage obtained. Nitrate + NaCl resulted in 10.3%, which was better than either factor obtained separately. Adding NaCl to the two-factor molybdate-nitrate combinations produced mixed results: NaCl + NO₃ + 0.3 Mo reduced the percentage of water obtained to 10.0%, while NaCl + NO₃ + 3.0 Mo increased the percentage of water obtained to 11.0%.

Molybdenum is a trace element found in the soil and required for growth by most biological organisms, including plants and animals. When plants are grown under molybdenum deficiency, a number of varied phenotypes develop that hinder plant growth. Plants grown in nutrient solution without molybdenum developed characteristic phenotypes including mottling lesions on the leaves, and altered leaf morphology where the lamellae became involuted, a phenotype commonly referred to as "whiptail" (Arnon and Stout, 1939). Deficiency symptoms for molybdenum include a general yellowing of the leaves and rolling, curling, and scorching of the leaves.

Nitrates are substances that contain nitrogen and oxygen. They are used by the plant to make proteins. Proteins are needed to make new cells as the plant grows. A plant will not grow well without nitrates from the soil. But some plants, such as peas, can live without nitrates in the soil. Their roots contain bacteria that make nitrates from atmospheric nitrogen.

(-Mo)

(-Mo)

(-Mo)



Fig. 14 Total morphology of *A. hortensis*-GOP with salinity and nitrate

Figure 14 shows the total morphology of *A. hortensis*-GOP seedlings germinated and grown in Hoagland & Arnon solution (control), and in the same solution with 100 mM NaCl or with 10 mM KNO₃. The nitrate led to a healthy growth rate and plant height, while NaCl severely inhibited plant growth. The general appearance of the plants grown with NaCl is very poor. Plant growth in the control medium also looks poor. The plants in Figure 14 were grown without molybdate.

Salinity can affect plants in three ways. Initially, salt makes it more difficult for plants to draw water from the soil, even if the soil appears quite moist. In effect, the plant suffers from a form of drought which can result in retarded growth and reduced yield. Secondly, some salts, such as Na and Cl can be directly toxic to plants. Plants take up salts with the water that they use, and often these salts can damage the plant internally, affecting the plant's physiological processes and often resulting in reduced growth, leaf burn and even plant death. Thirdly, high amounts of ions such as Na and Cl may affect the availability of other ions, e.g., K, Mg, N or P, which are extremely important for plant growth.

Figure 15 compares *A. hortensis*-GOP seedlings germinated and grown in nutrient solution containing NO₃ + 0.3 μM Mo (Ho) with those that were grown in NaCl + NO₃ + 0.3 μM Mo (Ho). Growth was better when the treatment lacked NaCl. The presence of NaCl inhibited plant growth, germination and root/shoot biomass (data not shown). Both plants were grown in the presence of 0.3 μM molybdate.

+Mo (Ho) 0.3 μ M+Mo (Ho) 0.3 μ M

Fig. 15 Effect of NaCl, nitrate and molybdate on growth of *A. hortensis*-GOP

Molybdenum is essential for many plant functions. It functions in converting nitrates (NO_3) into amino acids within the plant. Deficiency of molybdenum caused chlorosis of leaf margins, or more general chlorosis in some cases whiptail of leaves. The most common visible symptom is a pale yellowing resembling nitrogen deficiency. Other symptoms include whiptail of leaves and distorted curding in cauliflower and destruction of embryonic tissue in some legumes (Anderson, 1956; Chatterjee and Nautiyal, 2001).

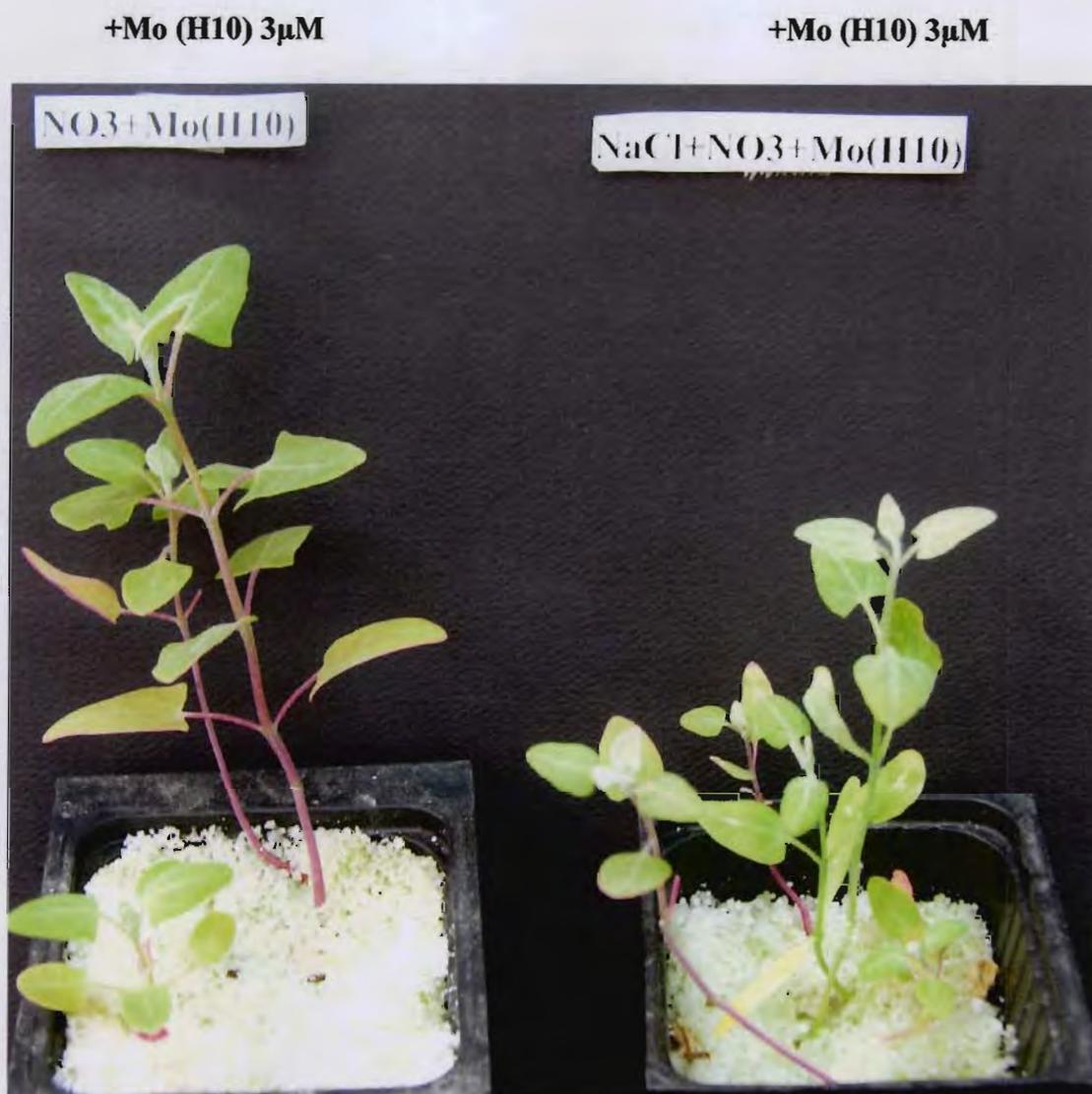


Fig. 16 Effect of NaCl with nitrate and molybdate on growth of *A. hortensis*-GOP

Figure 16 compares *A. hortensis*-GOP seedlings treated with NO₃ + 3.0 µM Mo with those that were treated with NaCl + NO₃ + 3.0 µM Mo. Seedlings grown without salinity look better than plants grown with salinity. Comparing the plants in Figures 15 and 16, it is clear that the higher concentration of molybdate positively influenced the plant growth and height of the seedlings.

Nitrogen in one form or another account for about 80% of the total mineral nutrients absorbed by plants (Marschner, 1995). Moreover, inadequate nitrogen is often the growth-limiting nutritional stress in field soils. Consequently, addition of N usually improves plant growth and yield regardless of whether the crop is salt-stressed or not. In many field studies, agronomists set out to test the hypothesis that N-fertilizer additions alleviate, at least to some extent, the deleterious effect of salinity on plant.

Salinity is an environmental factor that critically influences halophyte seed germination and plant establishment. Salinity affects imbibitions, germination and root elongation.

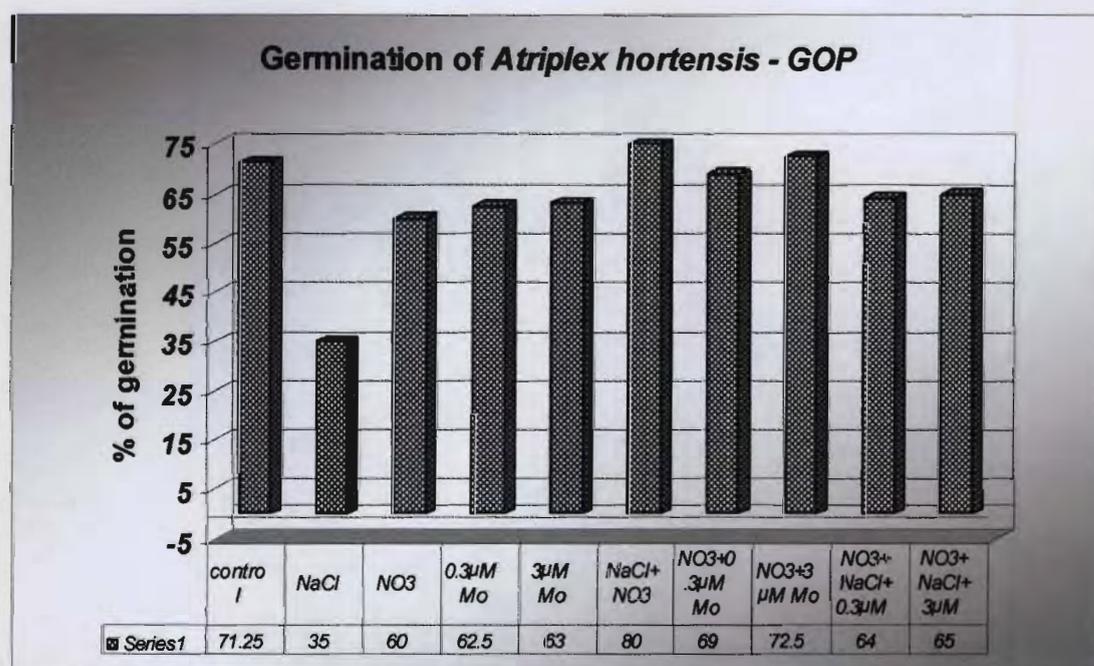


Fig.17 Effect of salinity, nitrate, and molybdate to percentage of germination of *A. hortensis*-GOP

Figure 17 shows that NaCl negatively affected germination of *A. hortensis*-GOP seeds, but nitrates and traces of molybdate improved seed germination under conditions of salinity. The seed germination percentage in the nutrient solution

(control) was 71.3%. Adding NaCl, nitrate or molybdate individually, however, led to a reduction in the germination percentage below this rate. Adding 100 mM NaCl reduced the germination percentage by half to 35%, adding nitrate reduced it by one-sixth to 60% and adding 0.3 μM or 3.0 μM molybdate reduced it by about one-eighth to 62.5% and 63%, respectively. Combining NaCl and nitrate, however, improved the germination percentage to 80%, beyond that obtained when each was added separately and beyond that obtained with no additives at all (control). Combining nitrate with molybdate also improved the germination percentages (to 69% with nitrate + 0.3 μM and 72.5% with nitrate + 3.0 μM molybdate) beyond that obtained by adding each of these, separately. The nitrate-molybdate combination with the stronger molybdate concentration even obtained a germination percentage that slightly exceeded that of the control treatment. Adding NaCl to both nitrate-molybdate combinations led to results that were lower than when NaCl was not present (64% germination with NaCl + nitrate + 0.3 μM molybdate and 65% germination with NaCl + nitrate + 3.0 μM molybdate). By the same token, adding nitrate and molybdate improved the germination percentage of the NaCl treatment, but not as effectively as adding nitrate without molybdate. In every instance, the stronger molybdate concentration obtained the better the results.

We measured the total height of *A. hortensis*-GOP plants germinated and grown under the aforementioned treatments. Salinity not only inhibited seed germination it also inhibited the total height of the seedlings.

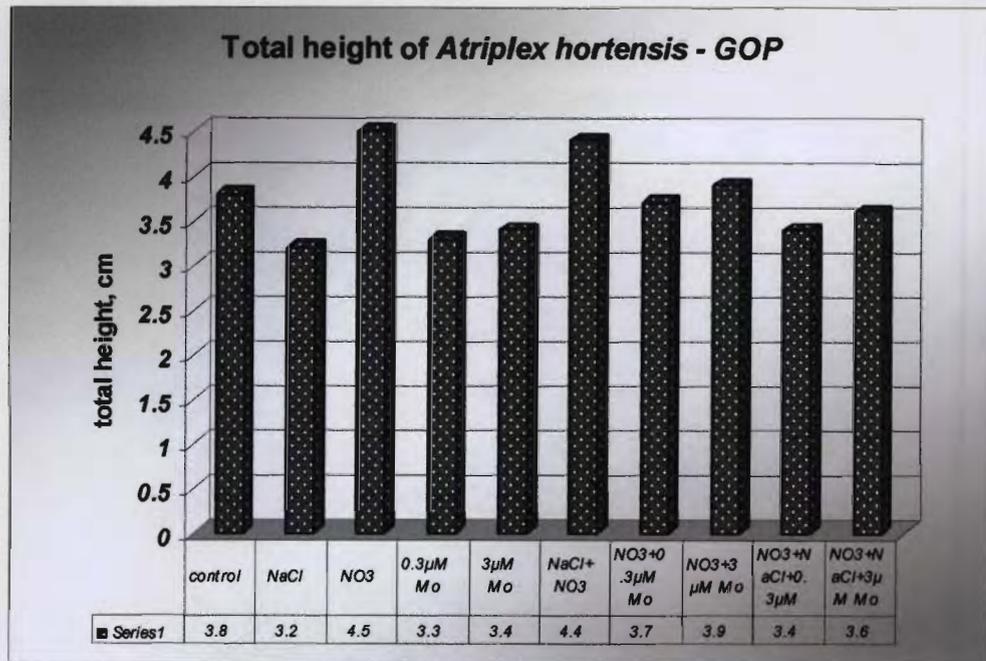


Fig. 18 Effect of salinity, nitrate and molybdate on total height of *A. hortensis*-GOP.

Salinity not only inhibited seed germination, it also inhibited the total height the seedlings obtained after 30 days of growth. We measured the total height of *A. hortensis*-GOP plants germinated and grown under the same treatment conditions as above. Figure 18 shows that the total height of plants grown without additives (control) was 3.8 cm. Adding 100 mM NaCl reduced the total height by one-sixth to 3.2 cm. Adding 0.3 µM and 3.0 µM molybdate reduced the total height by one-eighth and one-ninth, respectively, to 3.3 cm and 3.4 cm. However, adding 10 mM nitrate increased the total height by one-fifth to 4.5 cm. Combining NaCl + nitrate resulted in a total height (4.4 cm) nearly identical to that obtained with nitrate alone. Combining nitrate and molybdate obtained results similar to those obtained with no additives (control), however nitrate + 0.3 µM molybdate resulted in marginally shorter (3.7 cm) and nitrate + 3.0 µM molybdate resulted in marginally taller (3.9 cm) total heights. Adding NaCl to the two nitrate-molybdate combinations (NaCl + nitrate + 0.3 µM

molybdate and NaCl + nitrate + 3.0 μM molybdate) reduced their effectiveness by about one-twelfth and one-thirteenth, to 3.4 cm and 3.6 cm, respectively. These values are intermediate between the results obtained with the NaCl treatment and the control treatment, and although they represent an improvement over NaCl alone, this improvement was much less effective than when only nitrate was combined with NaCl. Results in Figure 18 show that, as with Figure 17, the greater the concentration of molybdate the taller the seedlings, in every case.

Effects of seed priming and germination under saline condition.

Interacting effects of KNO_3 , NH_4Cl and Na_2MoO_4 (molybdate) on germination was promoted by presown seed priming. Germination was increased by increasing either the nitrate concentration or molybdate in the priming solutions. There was significant interaction between these three factors, the germination response to the KNO_3 supply increasing with its concentrations during seed priming and decreasing with NaCl and NH_4Cl concentrations (Table 1).

When dormant intact seeds received a 30 h priming with 100 mM KNO_3 and after a further 6 days of incubation in water, their germination response to the nitrate pre-treatment was significantly increased by the application of water to the seeds. The interaction between the nitrate and water application was significant at 6 and 12 days after treatment.

When intact seeds were supplied with a 100 mM KNO_3 plus-minus 50 mM Na_2MoO_4 solution for 12 days, the nitrate content of the seed increased to a maximum of 0.145 $\mu\text{g}/\text{seed}$ after 6 days and then declined to 0.065 $\mu\text{g}/\text{seed}$ during the last 3 days of incubation, but in the presence of molybdate the nitrate content significantly decreased (Table 2). These changes were associated with a gradual increase in amino-nitrogen which became significantly higher than in the seeds in the 12 day sample.

The water content of the seed in the nitrate treatment was lower than that of the controls after both 3 and 6 days but increased to the same values as those of the controls during the last 6 days of incubation. The same reduction in seed water content occurred after 3 and 6 days when intact seeds were supplied with a 100 mM KNO_3 solution but did not occur if they had been pre-treated (data not shown).

Table 1. Effects of KNO_3 , NH_4Cl and Na_2MoO_4 on seed germination of *A. litoralis*.

Seed treatments (priming)	Germination in different NaCl concentrations (%)				
	0	50 mM	100 mM	200 mM	300 mM
Without priming (dry seeds)	65	50	25	15	3-5
Priming in H_2O	67	65	48	27	6-8
Priming in H_2O + 50 mM Na_2MoO_4	68	65	50	26	8-10
Priming in 25 mM KNO_3	72	72	62	28	10-13
Priming in 50 mM KNO_3	78	75	72	35	15
Priming in 75 mM KNO_3	82	80	78	42	15-20

Priming in 100 mM KNO ₃	87	87	80	45	18-20
Priming in 25 mM KNO ₃ + 50 mM Na ₂ MoO ₄	75	76	66	30	10-13
Priming in 50 mM KNO ₃ + 50 mM Na ₂ MoO ₄	80	78	75	38	17-20
Priming in 75 mM KNO ₃ + 50 mM Na ₂ MoO ₄	85	80	78	40	15-20
Priming in 100 mM KNO ₃ + 50 mM Na ₂ MoO ₄	89	87	82	47	18-20
Priming in 25 mM NH ₄ Cl	67	63	45	25	4-6
Priming in 50 mM NH ₄ Cl	65	60	45	25	4-6
Priming in 75 mM NH ₄ Cl	65	58	40	20	3-5
Priming in 100 mM NH ₄ Cl	63	50	35	15	2-3
Priming in 25 mM NH ₄ Cl + 50 mM Na ₂ MoO ₄	68	65	45	20	2-5
Priming in 50 mM NH ₄ Cl + 50 mM Na ₂ MoO ₄	65	60	40	18	2-5
Priming in 75 mM NH ₄ Cl + 50 mM Na ₂ MoO ₄	65	58	35	15	1-3
Priming in 100 mM NH ₄ Cl	63	50	28	8	1-2

+ 50 mM Na ₂ MoO ₄					
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A second set of seeds that received the same nitrate treatment as those used for seed analysis was used to determine effects on germination. Pre-sown priming of the seeds in nitrate and molybdate induced germination, which was initiated 2–3 days after priming and reached approximately 90% after 12–15 days (**Table 1**). Although the rate of germination of the nitrate pre-treated seeds was initially more rapid than the water pre-treated controls, this difference was statistically significant at the 8–10% level. Germination of both treatments of the intact seeds reached a maximum of 10% during the 15-day incubation period.

Table 2. Effect of 100 mM KNO₃ on the nitrate and amino-nitrogen content of

A. littoralis seeds (+ radicles)

Priming treatments	Time course changes of nitrate content in the seeds (µg/seed)			
	3 d	6 d	9 d	12 d
Priming in H ₂ O	0.005	0.000	0.000	0.000
Priming in 100 mM KNO ₃	0.115 ± 0.025	0.145 ± 0.040	0.090 ± 0.015	0.065 ± 0.015
Priming in 100 mM KNO ₃ + 50 mM Na ₂ MoO ₄	0.110 ± 0.020	0.125 ± 0.025	0.070 ± 0.015	0.035 ± 0.008
Time course changes of amino-nitrogen content (µg/seed)				

Priming in 100 mM KNO ₃	2.5 ± 0.25	2.6 ± 0.40	2.7 ± 0.75	2.9 ± 0.45
Priming in 100 mM KNO ₃ + 50 mM Na ₂ MoO ₄	2.7 ± 0.50	3.2 ± 0.65	3.5 ± 0.55	3.5 ± 0.60

The next investigation was the effect of 100 mM KNO₃ on the nitrate and amino-nitrogen content of the seeds in the nitrate and control treatments. The exogenous nitrate supply increased the nitrate content of the seed after 3 days, but the increase was less significant in the 6-day sample (**Table 2**). When the seeds were treated in the absence of the exogenous KNO₃, the nitrate content of the seed was not greater than that of the control seeds after 3 days, but was considerably reduced between 3 and 6 days after treatment. The highest seed nitrate levels occurred when the nitrate and water treatments were combined, increasing to 0.115 µg/seed after 3 days and 0.145 µg/seed after 6 days. The interaction between pre-treatment and nitrate was significant in the 6-day sample. The amino-nitrogen content of the seeds was not significantly affected by the nitrate treatment but increased after 3 and 6 days when nitrate and molybdenum were combined. The apparent increase in the amino-nitrogen content of the seeds in the molybdenum and control treatment after 6 days may account for the associated reduction in the nitrate level; this increase was not significant at the 5% level.

Germination of seeds primed in the presence of molybdenum plus nitrate increased rapidly from 45% after 3 days to a maximum of 89% after 15 days (**Table 1**). Germination was also induced by molybdenum treatment of the seeds, while the initial rate of germination was slightly slower than when molybdenum and nitrate treatments were combined—there was only a 2-5% difference in the final

percent germination in these two treatments after 15 days. Germination of the seeds supplied with KNO_3 reached 12% after 30 days, but was significantly higher than the controls, all of which remained dormant. The treatments had the same relative effects on both seed nitrogen content and germination when the experiment was repeated but the absolute values were lower than in the first experiment (data not shown). This difference can probably be attributed to the higher degree of dormancy of the seeds used in the repeated experiment, which were stored for only 4 months prior to treatment.

Effects of nutritional nitrate and molybdate solutions on seed germination

According to the hypothesis of Esashi and coworkers (1979), nitrate promotes germination of plant seeds. We studied the effects of combinations of nutritional nitrate and molybdate experimentally by comparing the effect of 100 mM and 50 mM solutions of KNO_3 and Na_2MoO_4 (respectively) on seed germination under saline conditions. Modi and Cairns (1994) showed that molybdenum may play an important role in seed germination of cereals. The nitrate, and—to a lesser extent—the molybdate solutions in growth medium both produced significant and similar increases in seed germination at 100 mM NaCl. The germination rates of the seeds in both these treatments were higher than for the water-treated controls at each sampling time, but these differences were statistically significant for nitrate treatment only after 6 days and for the molybdate treatment only in the 12-day sample. When the experiment was repeated, samples were taken after only 6 and 12 days (**Table 3**). Similar results were obtained in that seed germination in both the KNO_3 and Na_2MoO_4 treatments were significantly higher than those of the water treated controls.

A second set of seeds received the same treatments and was used to compare the effect of these treatments on germination. The germination induced by the nitrate plus molybdate treatments was the same, while the rate of germination of the nitrate-treated seeds was intermediate between the rates of these two treatments. The difference between the rates of germination of seeds in these three treatments became clearly established between 4 and 8 days after the start of germination, although the difference in percent germination between the nitrate and nitrate plus molybdate treatments did not become statistically significant until 4 days later.

Table 3. Effect of 100 mM KNO₃ and 50 mM Na₂MoO₄ in the growth medium on germination of dry unprimed seeds of *A. littoralis* under saline conditions (100 mM NaCl).

Treatments	Days of germination						
	0	4	8	12	16	20	24
	Germination frequency (%)						
In H ₂ O	0	10	20	25	25	25	25
In 100 mM KNO ₃	0	15	25	30	40	45	45
In 50 mM Na ₂ MoO ₄	0	10	20	30	35	35	35
In 100 mM KNO ₃	0	15	30	35	45	45	45

	Content of molybdenum (ppm)						
In H ₂ O	0.150	0.150	0.150	0.150	0.150	0.150	0.150
In 100 mM KNO ₃	0.150	0.150	0.150	0.150	0.150	0.150	0.150
In 100 mM NH ₄ Cl	0.150	0.150	0.150	0.150	0.150	0.150	0.150
In 50 mM Na ₂ MoO ₄	0.150	0.160	0.190	0.210	0.210	0.210	0.210
In 100 mM KNO ₃ + 50 mM Na ₂ MoO ₄	0.150	0.200	0.300	0.340	0.340	0.340	0.340
In 100 mM NH ₄ Cl + 50 mM Na ₂ MoO ₄	0.150	0.160	0.200	0.200	0.200	0.200	0.200
In 100 mM NH ₄ NO ₃	0.150	0.150	0.150	0.150	0.150	0.150	0.150
In 100 mM NH ₄ Cl + 50 mM Na ₂ MoO ₄	0.150	0.190	0.280	0.320	0.320	0.320	0.320

Although germination showed the same statistically significant treatment effects in both experiments and slow germination of the untreated control seeds, the rate of germination induced by each treatment was somewhat higher. The differences between these experiments with respect to nitrogen and molybdenum content and the

rate of germination were related to the age of the seed, which had been stored for 1 and 2 years prior to treatment in these experiments. In further experiments conducted to determine the effects of different pretreatments on the germination response to 100 mM NH_4Cl , the untreated and NH_4Cl treatments showed the same rate of germination, but NH_4Cl failed to induce germination of any of the seeds during the 30-day period of incubation, as did KNO_3 .

Although numerous studies have been conducted on the effect of various nitrogenous compounds on seed germination, very little attention has been given to the effect of such treatments on halophyte seed germination. An exception is the study by Hilhorst (1990), who showed that the embryos of *Sisymbrium officinale* seeds collected in the field had a higher nitrate content and that the nitrate content of intact seeds from different habitats was linearly related to their rate of germination. He also showed that most of the nitrate could be rapidly leached from the seed, and that the loss of nitrate by leaching was closely correlated with the reduction of germination. It was therefore concluded that germination was limited by the nitrate content of the seed. This conclusion is consistent with the results of our investigation, which showed that supplying the seeds of *A. littoralis* with KNO_3 , increased the nitrate content of the seed prior to germination.

However, the intact seed coat was a major limiting factor in nitrate uptake by the embryo. This was evident from the large increase in seed nitrate content induced by priming the seeds and may account for the significant interaction between the nitrate-induced germination (Table 1). We concluded that the nitrate absorbed by the intact seed was not only being held in the outer tissues, but also infiltrated into the endosperm and embryo of the seed. It is also likely that priming the seeds resulted in the accumulation of KNO_3 accumulated into the seed. It is further suggested that these

changes may also increase the uptake of nitrate or other solutes that have been shown to stimulate germination. In support of this hypothesis, the apparent similarity and interaction between the effects of after-ripening and seed coat permeability on nitrate-induced germination could be attributed to their similar capacity to promote water and nitrate uptake by the embryo. Our hypothesis that nitrate promotes germination in *A. litoralis* by accumulating in the seed is consistent with evidence in the literature that nitrate plays an important role in the regulation of plant growth and development. However, Hilhorst and Karssen (1989) showed that the endogenous nitrate in seeds of *Sisymbrium officinale* could induce germination without being metabolically reduced. It is quite possible, however, that the increase in amino-N induced by the KNO_3 treatment in the presence of molybdenum also had a significant effect showing nitrate metabolism by halophyte seeds. Sharma and Gupta (1986) showed that the high concentration of amino-nitrogen that accumulated in the apical region of the radicle could generate the turgor pressure and growth potential required for germination. In experiments on nitrate-induced germination in *A. litoralis* and several other species, Hilton and Thomas (1986) reported that water uptake by the seeds during their priming was the same whether the seeds were imbibed on water or on nitrate solution. Our results clearly suggest that the lack of induction of germination by NH_4Cl was due, at least in some cases, to the failure of the NH_4Cl to generate energy in sufficient amounts to induce germination. This postulated mechanism is basically the same as that which we have proposed to account for the promotion of germination by an exogenous nitrate and molybdenum supply.

ABA content in seeds of *Aeluropus* under control and saline conditions.

The response of plants to salt stress depends on many factors, but phytohormones are thought to be among the most important endogenous substances involved in the mechanisms of tolerance or susceptibility of various plant species. Under salinity, the quantity of abscisic acid (ABA) increases and that of cytokinins decreases (Omarov et al., 1999). Some investigators think that ABA plays a central role in regulating osmotic adaptation of plants, as well as in the synthesis of proline (Hayashi et al., 1997). A relationship has been established between the ability of some plants to survive under salinization and the concentration of proline in plants tissues (Hayashi et al., 1997). Exogenous ABA induces accumulation of proline (Delauney and Verma, 1993). It was established that exogenous ABA facilitates the adaptation of isolated cells to increased sodium chloride concentration. Treatment of plants grown under high salinity, with ABA in proper concentrations results in stimulation of growth. ABA may regulate the level of some inorganic cations, such as K^+ and Na^+ and of some osmotically active substances, such as sucrose, proline and betaine. The increased content of ABA under stress is probably related to the hormonal control of the adaptive response of the plants (Walton and Li, 1995).

Therefore the objective of our study was to investigate the possible role of ABA as a modulator of response of halophyte plants to salt stress, and as a regulator of plant tolerance and adaptation to increased concentrations of sodium chloride.

The radicles of control seeds were emerged at 4–5 days in the water. Sodium chloride at a concentration of 100 mM had an inhibitory effect on *A. litoralis* seed germination. We found that treatment of the seeds with 100 mM NaCl resulted in an increase of endogenous ABA content. Since salt stress inhibits seed germination of halophytes, we studied the content of ABA in the seeds exposed to water and 100 mM

NaCl. In our experiments, *A. littoralis* seeds were salt-stressed for four days and then transferred to distilled water. The ABA content of these seeds was determined during the next 16 days (**Table 5**).

We suggest that ABA modulates the response of seeds to salt stress or alleviates the toxic effect of NaCl, and that ABA—a phytohormone that is known to inhibit plant growth—also inhibits seed germination under salinity.

Table 5. Relative content (%) of ABA and proline in salt-stressed and non-stressed seeds of *A. littoralis* (the data are averages from three separate experiments).

Treatment	ABA or proline	Days of germination					
		0	4	8	12	16	20
		Content ABA and proline in %					
Seeds in H ₂ O	ABA	100	65	40	35	30	30
	proline	100	100	85	80	75	75
Seeds in 100 mM NaCl	ABA	100	125	165	180	210	210
	proline	100	95	95	100	95	95
Seeds were incubated in H ₂ O for 4 days and then	ABA	100	70	125	160	190	200

transferred to 100 mM NaCl	proline	100	100	110	105	110	110
Seeds were incubated in 100 mM NaCl for 4 days and then transferred to H ₂ O	ABA	100	130	90	40	35	35
	proline	100	110	95	90	90	90

It is well known that under stress conditions, such as salinity, drought and cold, the synthesis of ABA in plant cells increases dramatically. Therefore, this phytohormone is usually thought to be a mediator of adaptation processes to stresses in higher plants. At the same time, this phytohormone inhibits seed germination in a wide range of plant species. Therefore we conclude that the inhibition of seed germination by 100 mM NaCl is due to increased endogenous ABA. Thus, in our investigation the inhibition of seed germination correlated with the increased level of endogenous ABA under saline conditions.

It is known that gibberellins, phytohormones antagonist of ABA, induce seed germination (Hilhorst and Karssen, 1992). However, supplying *A. littoralis* seeds with gibberellins (GA3) for 3 days did not influence their germination. Exogenous GA3 in a concentration as high as 10^{-5} M had no significant effect on germination of salt-stressed seeds, while GA3 accelerated the induction of germination in non-stressed control seeds. The lack of a direct effect of high exogenous concentration of gibberellins on seed germination of halophyte plants under saline condition remains unclear.

It is widely accepted that ABA induces accumulation of proline in the plants and that proline may regulate the osmotic balance of the cell, thus relieving the negative effect of NaCl. We studied the content of proline in salt-stressed and non-

stressed halophyte seeds. However, the proline content was almost the same in both these seeds in 20 days of incubation (**Table 5**).

D. Desalinizing ability of *A. litoralis*

A. litoralis is a perennial grass and one of the most salt tolerant plants occurring in the coastal and inland areas of the Aral Sea areas of Kazakhstan. *A. litoralis* has an advantage among the halophytes: it grows quickly and produces large clumps of rhizomes. Rhizomes can form adventitious roots and establish new plant clones. Once established, this species tends to form large, continuous, clonal root masses, extending sometimes through several square meters. Its horizontal root system spreads under the soil surface (Kurochkina, 1966). In view of the high evaporation rates of the arid areas, which increase salt concentration at the soil surface, the root system of *A. litoralis* seems to be ideally suited to take up these surface salts more effectively, accumulating them in its aerial parts.

A. litoralis is a halophyte which can absorb salts from saline soils and transport and accumulate them on leaf surface as soil glands. *A. litoralis* grows normally in highly saline soils and the accumulation of salt on leaf surface may significantly decrease the salt content of the soils.

Pot experiment. Table 6 shows that dry weight and Na content of the *A. litoralis* plants increased during the 120-day experimental period. Table 2 shows that the Na content of the soil in pots without *A. litoralis* plants increased during the experiment by an amount approximately equal to that added as NaCl in the irrigation water. In contrast, the Na-ion content of soil in pots with *A. litoralis* decreased, despite the addition due to irrigation with the NaCl solution.

Table 6. Changes in dry weight and Na content of *A. litoralis* plants in pots (mean of five replicates)

Initial dry weight (g/pot)	Initial Na content (g/pot)	Final dry weight (g/pot)	Final Na content (g/pot)
0.42 ± 0.01	0.14 ± 0.03	32.3 ± 0.8	12.4 ± 0.3

Table 7 shows, that there was a mean net loss of 10.6 g by the *A. litoralis* plant. This suggestion is confirmed by the data in Table 1, which shows that the Na content of the *A. litoralis* plants increased by 10.94 g pot⁻¹ during the experiment. *A. litoralis* is clearly capable of taking up salt from the soil. In the pot experiment, on average, 10.9 g of Na ions were absorbed by plants with a total final dry weight of 42.33 g.

Table 7. Changes in Na-ion content of soil in pots with and without *A. littoralis* (mean of 5 replicates)

Treatment	Initial Na content (g/pot)	Na-ion added by irrigation (g/pot)	Final Na content (g/pot)	Change in Na content (g/pot)
<i>+A. littoralis</i>	12.6 ± 0.4	8.0	10.0 ± 0.6	-10.6 ± 0.4
<i>-A. littoralis</i>	12.8 ± 0.4	8.0	21.0 ± 0.8	-0.2 ± 0.6

Field experiment. Table.8 shows Na content of soil in which *Sueda salsa* was grown decreased and that this decrease was greater with a higher plant density. The reduction in soil Na content by *A. litoralis* was greatest in the 20–30 cm soil horizon. Another halophyte plant, *Anabasis aphylla* (endemic to the Aral Sea region), also reduced the Na content of the soil, albeit less effectively that did *A. litoralis*. The Na content of soil with no plant cover increased, especially in the 0–10 cm horizon, presumably due to evaporation of saline groundwater, which can lead to a long-term accumulation of salts at the soil surface.

A. litoralis growing in saline soil can adsorb and accumulate considerable amounts of salt within one growing season, thereby decreasing the salt content of the soil.

Table 8. Changes in Na content at different depth of soil in plots sown with *A. litoralis* or *Anabasis aphylla*, or left unsown (the means of four replicates).

Halophyte plant species	Initial Na content (g/kg DW)			Final Na content (g/kg DW)			Change in Na content (% of initial)		
	Depth (cm)			Depth (cm)			Depth (cm)		
	0-10	20-20	50-60	0-10	20-30	50-60	0-20	20-30	50-60
	4.30	4.00	3.54	4.42	4.08	3.81	-1.8	-1.0	-0.5

Anabasis aphylla 15 plants m ⁻²									
A. litoralis 15 plants m ⁻²	4.55	4.20	3.90	4.49	4.02	3.90	-2.4	-4.5	-2.5
A. litoralis 30 plants m ⁻²	4.65	4.20	3.82	4.52	3.88	3.78	-3.8	-6.7	-3.6
Unsown	4.45	4.10	3.73	4.97	4.38	4.02	+9.0	+3.8	+3.9

The dry weights of individual *A. litoralis* plants in the field typically reach 40 to 60 g during the growing season. On this basis it would be theoretically possible for *A. litoralis*, at 15 plants m⁻², to remove 2990–3040 kg of Na from 1 ha of the field soil. From the field trial data in **Table 8**, it can be calculated that the average Na-ion content of the 0–60 cm soil horizon decreased by 950 g kg⁻¹, with 15 *A. litoralis* plants m⁻², and by 0.17 g kg⁻¹ with 30 plants m⁻². If it is assumed that this soil has a bulk density of 1320 kg m⁻³, then the decrease in Na content observed in the field represents a reduction of 1125 and 1640 kg Na-ions ha⁻¹ with 2000 plants m⁻², respectively.

If *A. litoralis* were harvested at the end of the growing season, a significant reduction in soil salinity might be achieved. It is possible that many other halophytes, including species of the genera *Atriplex*, *Sueda*, *Tamarix*, *Salicornia* and *Aster* could

also be used in soil improvement. Thus, a reduction in the salt content of soil would increase the area of land available for cultivation and would raise the yield of crops grown on marginal saline soils.

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C. Scientific Impact of Collaboration

The data obtained will be an important factor in increasing the interactions between the research groups active in the area. At December 2004 Malika Myrzabayeva started her training in physiology and Biochemistry of halophytes under salinity and different nutritional conditions. The joint data analysis that will be carried out together with the training will contribute to the Israeli and Kazakhstan researchers.

D. Description of Project Impact

The data collected in this project is now on the way of scaling up and field experiments are to be conducted. As more data will be obtained we expect to start the application in the farmers plots.

E. Strengthening of developing country institutions

Malika Myrzabayeva a Msc graduate student arrived to my lab ca 15 months ago and making her training and studying *Salicornia*, *Aster tripolium* and *Atriplex* as well as

seeds of other halophyte species from Kazakhstan. Moshe Sagi and Zerekby Alikulov are in tied email and telephone connection about the project development.

F. Future work

The research in the field experiments and in the labs both is Israel and in Kazakhstan will continue as planned. **The following experiments are in progress:**

-Research with 2 ecotypes of *Aster tripolium*, 2 ecotypes of *Salicornia herbacea*, 3 ecotypes of *Atriplex hortensis* as Sea Vegetables for export to the EU market.

- Determination of physical and chemical qualities of halophytes as Sea Vegetables for export to the EU market.

- We shall investigate the activity of nitrate reductase to seek ways to reduce nitrate accumulation in commercial plant parts of the halophytes.

Section II

A. Management issues

No special management issues

B. Special concerns

No special concerns.

C. Collaboration, Travel, training and publications.

Msc graduat student Malika Myrzabayeva arrived to Israel to make her training and study on *Salicornia*, *Aster tripolium*, *Atriplex* and on seeds of other halophyte species exist in Kazakhstan. Moshe Sagi and Zerekby Alikulov are in tied email connection about the project development.

D. Summary of requests for CDR program actions

No special requests