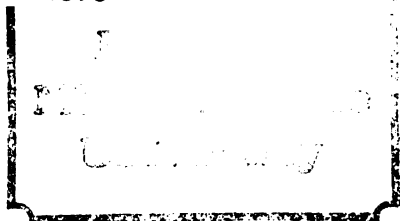




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BREEDING FOR SALT TOLERANCE IN  
BARLEY (HORDEUM VULGARE L.)

By

Azzildeen M. Al-Shamma

A DISSERTATION

Submitted to  
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## ABSTRACT

### BREEDING FOR SALT TOLERANCE IN BARLEY (HORDEUM VULGARE L.)

By

Azzildeen M. Al-Shamma

Selection was practiced in barley for low and high tolerance to salt during germination. The effect of selection on some agronomic characteristics was also investigated. An attempt was made to identify the mechanism of salt tolerance at the germination stage. The possibility of transferring tolerance to salt from spring barley to winter barley was also investigated.

Four F<sub>3</sub> populations of crosses involving five parental lines of spring barley were chosen for the selection study. Two cycles of selection preceded by crossing were employed. The total gains in germination percentage from selection were -12.7 and +17.4 for low and high tolerance, respectively. The results of this study indicated that selection can be used to improve tolerance to salt in barley.

There was little indication that tolerance to salt at the germination stage can be transferred from spring barley to winter barley, at least in a relatively short time. The F<sub>2</sub> seeds of crosses between the spring barley, California Mariout, and seven populations of winter barley showed a significant improvement in salt tolerance. However, when plants with winter habit were isolated from the

F<sub>2</sub> populations, tolerance declined in many lines and approached that in the original populations.

Seed weight, seed volume and seed density were not correlated with reaction to salt in the spring barley populations. They were, however, correlated in the winter barley populations. Positive correlations were obtained, suggesting a linkage between tolerance and seed weight and seed volume.

Number of tillers, type of spike, heading date, filling period and length of growing season were not correlated with reaction to salt at germination. Plant height was negatively correlated with reaction to salt.

Based on results from an imbibition test, conductivity test and an electron microprobe analysis, the sensitive line SS-1425 accumulated more sodium and chloride ions in the lemma and palea than did the tolerant line TT-1523. It was suggested that the mechanism of salt tolerance may involve (1) low permeability of the lemma and palea to sodium and chloride ions and (2) high concentrations of organic solutes in the embryonic and endospermic cells.

To my mother, sisters and brothers.

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## INTRODUCTION

Soil salinity is becoming a major threat to agriculture, especially in the arid and semi-arid regions of the world where rainfall is insufficient to leach the salt accumulated out of the root zone. The use of irrigation water with high salt content in these areas is a major contributing factor among others leading to salt accumulation. Soil factors such as soil structure in which a layer of clay lies at various depths beneath the surface can result in developing a poor drainage system in the soil. In some areas a high ground water table may provide poor drainage which in turn aids in salt accumulation. In addition to these conditions, climatic factors play their role in bringing the salt to the top layers of the soil. In a climate where high evaporation takes place, salts may be carried with water by capillary movement to the soil surface where they accumulate.

Soils severely affected can easily be recognized by a thin layer of white powdery material covering the soil surface. Chloride, sulfate, carbonate and bicarbonate salts of sodium, calcium, magnesium and potassium are generally found in these layers. The concentration of these salts in the soil may vary greatly from one region to another.

With the increase in world population and demand for food, agricultural land has become too valuable to lose.

Arid and semi-arid lands make up about one-third of the total land area. According to a recent FAO publication (51), this represents about  $4,464 \times 10^6$  HA. Half of this, i.e.  $2,232 \times 10^6$  HA, is salt affected. This makes up about 17% of the total land area. Some areas are so severely affected that farming has become non-profitable, which has led farmers to leave their land since reclamation and maintenance are very costly.

Awareness of this problem has led many farmers to grow crops that show considerable tolerance to this condition. Barley is one tolerant plant among other crops and the most tolerant of the other cereals. The importance of barley as an industrial and food crop makes it one of the major cereals produced world wide. In 1980, world barley production made up about 11% of the total cereals produced according to estimates made by FAO. Statistics indicate that barley is the fourth largest cereal produced annually, with wheat, rice and corn being the first, second and third respectively. Barley production in 1980 reached about  $162 \times 10^6$  metric tons on an area of approximately  $83 \times 10^6$  HA. For these reasons and the fact that barley possesses good tolerance to salt, it has been studied intensively with regard to its response to salinity. This has been indicated by a recent USDA publication which contains an indexed bibliography of literature from 1900 to 1977 on salt and boron effects on plants (119). It has also been indicated that only very few citations out of 2,357 were concerned

with the genetic and breeding aspects of this characteristic.

According to studies in this field, salt tolerance has been found to be a heritable characteristic in plants. Proper breeding programs then should be effective in developing or improving the tolerance of some important economic crops to salt. When breeding for this characteristic, all stages of growth should be considered since the reaction to salt may vary from one stage to another. More emphasis should be put on studying and understanding the mechanisms of salt tolerance of crop plants at various growth stages. Such information is definitely needed when breeding for salt tolerance. Recently, tissue culture techniques have been used in developing tolerant lines of in selected crops. The possibility of using the genetic engineering concept has been discussed in the literature and could be of greater importance in the near future. Tolerance to salt has been reported to exist among wild relatives of some crops which may be of value to plant breeders.

The present study involves breeding for tolerance to salt in barley during the germination stage. It has the following objectives: (1) selection for both extremes of salt tolerance, (2) the effect of selection on some important agronomic characteristics, (3) identification of the mechanism of salt tolerance during the germination stage, (4) an attempt to transfer salt tolerance from spring habit to winter habit barley.

## REVIEW OF LITERATURE

Soil salinity is common in the arid and semi-arid regions of the world where farming is dependent mainly on irrigation due to insufficient rainfall. The arid and semi-arid regions make up about one third of the total land area and half of that has highly saline soils (45). A saline soil is one with sufficient soluble salts to injure or reduce the growth of many plants. Although salts are naturally present in the soil, a considerable amount of additional salt may be added from irrigation waters, namely river and well waters. Depending on the mineral composition of the earth's crust through which streams and rivers pass, different waters have different salt contents. Allison (9) reports that most irrigation waters contain 0.1 to 5 tons of salt per acre-foot (70 to 3500 ppm). He classified irrigation water on the basis of electrical conductivity measurements into four classes: low salinity, medium salinity, high salinity and very high salinity, the dividing points between classes being 250, 750, and 2250  $\mu\text{mho}/\text{cm}$ . This range includes water that can be used for irrigation of most crops on most soils to water too harmful to use for irrigation under ordinary conditions. River water may contain as little as 85 ppm to as high as 5,620 ppm of salt

(9). Well water, on the other hand, may contain up to 3,775 ppm salt (9). The salt concentration of the Pacific Ocean was estimated to be about 35,000 ppm (46). A good quality irrigation water should not contain over 1,000 ppm salt, the marginal quality irrigation water being 1,000 to 3,000 ppm (46).

The salinity of the soil solution is almost always 2 or 3 times as great as the salinity of the irrigation water and may often be as great as 5 to 10 times. The degree of salinity increase is determined by three major factors. These are (1) the amounts of water applied in relation to water used by the crop and lost by evaporation, (2) drainage, and (3) height of water table. Saline soil with an electrical conductivity of 4 mmho/cm was set to be the lower limit for most plants (20). The growth of sensitive plants is affected at half of this salinity level, while the growth of tolerant ones is affected at about twice this salinity.

In saline soils, the cations calcium, magnesium, and sodium and the anions chloride, sulphate, bicarbonate and sometimes carbonates, are generally the predominant ions (9, 23). Carbonate may accumulate in considerable amounts in the soil if the pH rises to 9 or above (9). Potassium and nitrate may occur but to a lesser extent than other ions (21). Proportions and concentrations of these ions may vary considerably among saline soils. Addition of fertilizers may cause an increase in the variety of ionic species present in excess (23, 77).

The presence of these salts in the soil causes the osmotic pressure of the soil solution to rise high enough to create a water stress condition. The osmotic pressure within the root hairs of most plants is usually about 2 bars (81). In saline soils, the osmotic pressure may rise far beyond that value, and unless plants adjust to the osmotic pressure within their root hairs, they will not survive. Bernstein (19) provided evidence that the water absorption capacity of a given plant is relatively unaffected by salinity. He related the reduction in plant growth associated with osmotic stress to building up of the osmotic pressure of developing cells to meet the increasing osmotic pressure of the surrounding medium and still maintain turgor. He then defined salt tolerance accordingly as, "the degree to which osmotic adjustment can be made without sacrifice to growth." It was reported earlier, however, by Eaton (43) that as the salinity of the medium increases, the osmotic pressure of the leaves or above-ground parts of the plant increases. This results in the maintenance of essentially a constant gradient between medium and plant. Hayward and Spurr (66) measured the rate of entry of water into corn (Zea mays L.) roots under different osmotic pressures. A significant reduction in the rate of entry was found in both non-conditioned and pre-conditioned plants to a high osmotic pressure of the substrate. Seeds of alfalfa (Medicago sativa L.) showed a reduction in hydration as the concentration of the substrate increased with either sodium chloride

or mannitol (118).

The effect of salinity on plant growth has been given much attention by workers in this field during the last three decades. A single salt or more, or mix of salts, have been used and compared with regard to their effects on plant growth. Much emphasis has been placed on measuring the effect of salt and tolerance during germination and early growth stages (2, 13, 20, 35, 36, 37, 39, 69, 97, 114). Soil salinity seems to have a significant effect on plant growth in general. It decreased the percentage of germinating seeds, increased the time of germination, and delayed the emergence of seedlings. Barley (H. vulgare L.) seeds in general showed a 50% reduction in germination at a salt level of 16,000 ppm NaCl compared to those germinated in distilled water (10). Donovan and Day (39) found that barley seeds required an additional 3-5 days to germinate in a saline culture containing sodium chloride. Maliwal and Paliwal (84) also stated that the germination percentage of wheat (Triticum aestivum L.) and barley at different salt concentrations increased slowly with time. As the concentration of salt in the soil increased during germination, the number of germinating seeds decreased, followed by an increase in the percentage of plant mortality (2, 37). Barley seeds showed a 60-65% reduction in germination percentage at an NaCl level of 20,000 ppm compared to 50% reduction at 16,000 ppm of NaCl (10). Germination of rice (Oryza sativa L. var. Dular) seeds was greatly reduced when

the electrical conductivity of the medium rose to 8.9 mmho/cm and beyond, but failed to germinate at 59.5 mmho/cm. Removing the salt at any time during the germination period restored normal growth rates (53). Seeds of sugarbeet (Beta vulgaris L.), which failed to germinate but remained viable in a saline soil during an entire summer, germinated to produce normal seedlings the following fall when rains leached the salts from the vicinity of the seeds (24).

The reduction in germination under saline conditions could be due to a reduction in imbibition. Chapman et al (32) studied the gain in weight of barley seeds soaked in two solutions, distilled water and a 15 mmho/cm sodium chloride solution, for 36 hours. Barley seeds in the salt solution showed slower rates of imbibition and ultimately less imbibition than those in distilled water. Signs of germination were not detected until the end of the 36-hour period for the seeds in the salt solution, while germination was evident after a 24-hour period for those in distilled water. The delay in germination, therefore, could be, in part, due to the rate of imbibition and the final solution imbibed. Although salt accumulation was detected in the seeds at this stage, the retardation in germination was mainly due to reduced mobility of water in the salinized medium. The accumulation of salt would have more impact on seedling development than on imbibition (115). Salt accumulation in soybean (Glycine max L.) seeds seems to affect seedling development through a reduction in the metabolic

changes during germination (107). An increase in the dry weight of cotyledons, accompanied by a decrease in axis with increasing salt concentration, suggests reduced hydrolysis of reserve components in cotyledons and the mobilization to the axis. More specifically, the accumulation of salt ions imposes a significant effect on the synthesis of carbohydrate metabolism enzymes in germinating seeds (129). This has an inhibitory action on meristematic activity and thereby growth is retarded. Salts were not found to affect swelling of seeds, which occurred at a 12-hour period.

Germination can be delayed when low oxygen is associated with salinity. In semi-dwarf Mexican wheat, oxygen concentration below 5% delayed germination one day for each additional bar of osmotic potential applied (3). In addition, low oxygen and high salinity levels (-6 bars and below) depressed germination below economic levels.

Bernstein and Hayward (23) used the percentage of emergence as a criterion for germination and salinity level at a given time. In other words, if a number of crops are to be compared for salt tolerance, salinity levels are compared for each crop to produce a 50% reduction in emergence at a given period of time. The longer the period of time, the higher the salinity level must be to impose a 50% reduction in emergence. For example, barley seedlings required a salinity level of  $13.6 \text{ ECe} \times 10^3$  to produce a 50% reduction in emergence at a period of 7 days. At 15 days, however, the salinity level required was  $19.6 \text{ ECe} \times 10^3$ .

Salinity level, according to this criterion, would differ from one crop to another. It was found that seedlings of red kidney bean (Phaseolus vulgaris L.) sugarbeet, alfalfa (Medicago sativa L.) and field corn (Zea mays L.) required salinity levels of 3.7, 5.3, 8.7, and 9.4  $\text{ECe} \times 10^3$  for a 50% reduction in emergence at a 7-day period, respectively. The figures for salinities are higher at the 15-day period; they are 8.0, 6.0, 9.0, and 14.0  $\text{ECe} \times 10^3$ . George and Williams (57) used salinity levels measured in osmotic pressure required to obtain a 50% reduction in germination instead of emergence. Salinity levels required for a 50% reduction were 8.5, 4.3 and 3.3 atmospheres for barley, strawberry clover (Trifolium fragiferum L.), and Ladino clover (T. repens L.), respectively. Radicle emergence has been used by some authors (124) as a criterion of germination. However, Prisco and O'Leary (99) stated that this can be misleading because radicles may emerge but may not continue to grow. This was well demonstrated by their data in which radicle emergence occurred in 98% of the seeds at -12 bar water potential but none of them germinated. Their measurements of germination were based on a radicle length of 5 cm or more.

Most of the studies have been conducted under uniform salt concentrations in a controlled environment. Under field conditions, however, the concentration of salt varies significantly from one area to another and at various depths in the soil. In addition, environmental factors may play a

major role in maximizing or minimizing the effects of salinity on plant growth. This subject will be discussed in more detail later in this chapter as we look at the interaction of these factors with salinity and how they modify the effect of the latter on plant growth. Salt concentration is usually higher at the surface of the soil (0-2 cm) than at several centimeters deep (96). Pasternak et al (96) determined the electrical conductivity of saturation soil extract at several depths in the soil after 10 equal daily applications of 5 mm of brackish water. At depths of 0-2 cm, 2-5 cm, 5-10 cm, and 10-60 cm, the electrical conductivity values were 18.5, 4.6, 4.2, 3.2 mmho/cm, respectively. Seeds are usually sown somewhere within the first two depths where the concentration of salt(s) is much higher and most harmful to germinating seeds. Crops that have good tolerance to salt during the germination stage but develop a shallow root system, onions (Alium cepa L.), for example, may suffer more than those that have the capacity to produce a long tap root or a fast growing root system that will rapidly grow down to less saline soil layers (96).

Certain salts may depress germination more than others at equal concentrations. George and Williams (57) studied the effect of sodium chloride, calcium chloride, sodium sulfate and 1:1 isosmotic mixtures of sodium chloride with calcium chloride and sodium chloride with sodium sulfate on the germination of barley, strawberry clovers, and Ladino clovers. The results showed that the germination of both

clovers was less affected by calcium chloride than by the other individual salts, while barley was least affected by sodium chloride. However, barley and Ladino clover were more sensitive to sodium sulfate than to sodium chloride. This, as they concluded, was due to a higher concentration of sodium ions in sodium sulfate as it contained 40% more sodium than the sodium chloride solution. As for the salt mixtures, germination of all species was more affected in the mixture of the two sodium salts than in the mixture of sodium chloride and calcium chloride. They related this to the fact that accumulation of one salt might have been reduced by the presence of the other. The accumulation of certain cations and/or anions, therefore, may cause toxic effects in plant tissues in addition to the osmotic effects. Isosmotic pressures induced by mannitol and salts seem to have different effects on germination. Uhvits (118), using Arizona grown Chilean alfalfa seeds, observed that the germination percentage was reduced much more with sodium chloride than with mannitol at equal osmotic pressures. Similar results were also obtained for seeds of radish and sorghum (124). Sodium chloride and polyvinylpyrrolidone (PVP), a long chain polymer, showed greater effects on germination of radish and sorghum seeds and radicle growth than sucrose and glucose at the same osmotic pressures (124). Antibiotics such as aureomycin, tetracycline, and penicillin and a fungicide, Diathane D-14 up to 200 ppm, alone or in combination, or with a 2.5-atmosphere concentration of

sucrose, had no consistent effect on germination and radicle growth.

In an attempt to distinguish between the osmotic and the toxic effects of salt on the germination of red kidney bean seeds, Prisco and O'Leary (99) used sodium chloride and carbowax 1540 (polyethylene glycol), a non-permeating solute. The seeds absorbed the same amount of water during the first 6 hours irrespective of the type and the water potential of the substrate. From 12 hours on (to 96 hours), water uptake was higher in sodium chloride than in the carbowax 1540. This was, as they concluded, due to the uptake of sodium chloride which in turn increased the osmotic pressure in the seeds. As a result, the water potential gradient from ambient solution to seeds was maintained and more water was absorbed. It was also concluded that sodium chloride imposed a toxic effect at a water potential of -12 bars since more seeds germinated in carbowax at this osmotic level and no differences in germination were found at -8 bars in either solution. They then suggested that sodium chloride had an osmotic effect down to -8 bars and was toxic at low water potentials.

Most plants seem to be more sensitive to salt during the seedling stage than during other stages of growth. This may be because the tissues are tender and roots are shallow (81). Dumbroff and Cooper (42) showed that osmotic stress induced with salt was most deleterious to tomato plants (Lycopersicon esculentum Mill.) when applied during early

growth, especially during the succulent seedling stage. Rice varieties which were highly tolerant to salt during germination (salinity level of 20,000 ppm of sodium chloride and calcium chloride), showed high sensitivity to salt (3,000 ppm) during the seedling stage (5). Salinity, in general, reduced the dry weight of shoots and roots, although the latter were more affected at higher salinity levels. Seedling height and root length were also reduced by salinity. Pearson et al (97) found that reduction in seedling growth was greater when salinity was imposed at the early tillering stage than when imposed at the late tillering stage. The reduction in growth of seedlings could be due to a decrease in the moisture content and the osmotic potential of the plant sap (42). Seedlings of barley tolerated salt up to 9,000 ppm NaCl (83), however, barley seeds germinated at much higher concentrations of salt (20,000 ppm NaCl) (10). Mature plants of barley on the other hand, tolerated salt at concentrations comparable to those at the germination stage. This variation in tolerance throughout the plant life cycle seems insignificant in practice, especially under high levels of salinity when plants can survive through the germination stage but fail to pass the seedling stage (97). The importance of this stage is in determining whether or not a plant can reach maturity. Seedling development suffered both osmotic and toxic effects under saline conditions (99). Root growth was more affected by sodium chloride than by carbowax 1540, an osmotic agent,

at isosmotic levels. This was related to the toxic effects of sodium chloride accumulated during seedling development.

Excess salinity reduced both rates of growth and total plant size. Forage and seed yield were also usually reduced (2, 81). Increasing salinity levels in soybeans reduced green leaf color, leaflet size, dry stem production, plant height, seed yield and seed quality (2). Plant mortality, leaf necrosis, and accumulation of chloride in stems were increased under increasing salinity levels. Growth of most plants tested under saline conditions showed similar responses, except for leaf color which darkened under increasing salinity in the case of barley (14), alfalfa (29), and red kidney bean (55).

Many cereal crops have been studied extensively in terms of their response to water stress conditions and especially under saline environment. Among cereals, barley plants have been used in studies widely due to their known resistance to salt. They have been known to withstand higher concentrations of salt than other cereal crops. A concentration range of 20,000 ppm NaCl was found to affect the growth of six barley varieties (10). In spite of the degree of tolerance, salinity reduced fresh and dry weights of shoots, number of tillers, and height. The number of leaves and yield was also affected (71). Salinity, however, increased water content per gram of dry matter of barley varieties except for "California Mariout," a well-known tolerant variety which came originally from Egypt. This was probably

due to the fact that less tolerant varieties accumulated more salt in their tissue than the tolerant ones (10).

Salinity decreased grain yield and prolonged the filling period among barley plants (58). Seeds were shrivelled due to dessication stress brought about by the delay in maturity. Sugar content in developing seeds was reduced under saline conditions 15 days after anthesis. This was not, however, related to starch formation since the latter increased little or ceased after 15 days. This might have contributed to the reduction of grain weight and ultimately lowered yield. The decrease in yields of barley (var. CM-67), wheat (var. Siete Cerros), and sweet corn (var. Bonanza) was found to be due to the decrease in the number of tillers and thus a decrease in the number of heads or ears per plant (68). Bole and Wells (26) reported that the reduction in yield under saline conditions could be due to different factors when 6-row and 2-row barley are compared. The reduction in yield in the 6-row barley was due to a lower number of kernels per spike, while it was due to differential spike production in the 2-row type. The 6-row type showed more tolerance under saline conditions.

Rice plants showed a different response with regard to growth and yield when compared with other cereals (89). At a critical electrical conductivity (7.2 mmho/cm), the dry weight of panicles and grain yield declined sharply. The reduction in the yield was caused by an increase in the number of sterile florets per panicle and by a reduction in

the germination percentage of pollen grains. The dry weight of roots and shoots, on the other hand, did not decrease so markedly (89). The reduction in grain formation occurred when salinity was increased during the tillering stage and the period of initiation and differentiation of panicle primordium (76). Salinity seems to interrupt the normal cell growth and differentiation and ultimately influences flowering, fertilization, and development. In testing rice varieties from different regions of the world, Akbar et al (7) stated that salinization reduced the panicle weight, number of branches per panicle, panicle length, number of spikelets per panicle and seed setting percentage. All these factors contributed to the reduction of grain yield. Salinity also affected seed maturation by producing smaller seeds with darker color (7). Pollen viability, however, was not affected by salt treatment.

Despite the effect of sodium salts on the growth of plants, sodium was found to be an essential element in some higher plants. Sodium was found to be essential for some halophytes such as Atriplex species as their response to a sodium-free medium showed deficiency symptoms of this element (30). When sodium was supplied, plants increased in dry weight and deficiency symptoms disappeared. A salt-tolerant barley line also responded to sodium by increasing in dry weight. Yields of five varieties of barley increased at moderate levels of sodium (5 to 10 me./L). At higher levels (40 me./L), however, yields decreased slightly (106).

Other plants such as sugarbeet, tomato, cabbage, white clover, and lettuce showed no response to sodium. It was suggested that these plants either had no requirements for sodium or their requirements were so small that they obtained adequate sodium for normal growth and development already present in the medium (containing less than 0.07 ue/l Na) (30).

There are many reports in the literature concerning the effects of salinity on the metabolic processes in plants. Such effects seem to vary among plants and the type of salt utilized. Nieman (90) studied the effect of NaCl on photosynthesis in twelve crop plants from six different families. These were Chenopodiaceae, Compositae, Cruciferae, Leguminosae, Liliaceae and Solanaceae. The data showed that photosynthetic activity per unit leaf area was not affected by NaCl. He concluded that photosynthate is generally not a limiting factor in the growth of salt-stunted plants due to the pronounced tendency of various crops for carbohydrate accumulation. Petolino and Leone (98) noticed similar effects on bush bean (Phaseolus vulgaris L.) even after symptoms of chlorosis appeared, along with the accumulation of soluble sugars. This, as they stated, was an indication of carbohydrate utilization rather than photosynthetic activity being affected by salt. Ackerson and Younger (4) also showed that net photosynthesis in bermuda grass (Cynodon hybrid, Cultivar Santa Ana) was not significantly affected by increasing levels of NaCl, CaCl<sub>2</sub>, or K<sub>2</sub>SO<sub>4</sub>.

Top growth was decreased accompanied by an increase in root structural carbohydrate and by greater storage of nonstructural carbohydrates in crowns and leaf bases. These findings led the authors to the conclusion that shunting of photosynthate from top growth to root growth and storage may play an important role in bermuda grass survival and/or recovery under saline conditions. In wheat and barley, the activity of photosynthesis was decreased under NaCl salinization, but only initially (117). Later, however, the activity was restored and became similar to that observed in the control. This type of effect could be related to the specific activity of enzymes that are part of the photosynthetic apparatus under a salinized medium. The specific activity of such enzymes showed slower change in salt treated pea seedlings, but the final activity and the final concentration of each was the same as in the control plants (122).

Photosynthesis in cotton plants (Gossypium hirsutum L.) was affected by sodium chloride salinization (27). At an osmotic level of -8.5, photosynthesis was reduced 25% or more based on leaf area, fresh and dry weights of leaves, or chlorophyll content. Nieman and Poulson (92) found that salt affected light-dependent growth and chloroplast development. The development of chloroplasts was being delayed rather than inhibited, since carbohydrate utilization for nucleic acid and protein synthesis was reduced in growing cells.

Salts were also found to affect respiration in plants in a larger extent than photosynthesis. Based on oxygen uptake measurements, the rate of respiration tended to increase in the presence of sodium chloride in twelve crop plants tested (90). The increase was detected in both tolerant and sensitive species, however, the latter showed greater increase in the rate of respiration and occurred at lower levels of salt. Petolino and Leone (98) also reported an increase in the rate of respiration in bush bean plants (Phaseolus vulgaris L. 'Topcrop') under saline aerosol treatment with a synthetic seawater. Respiration rates in wheat and barley were also found to increase under salinization (117). Tolerant varieties of barley and wheat, however, showed higher rates of respiration than less tolerant ones under NaCl and Na<sub>2</sub>SO<sub>4</sub> salinization (116). Cotton plants showed different responses to salt as the respiration rate decreased to 25% or more at an osmotic pressure of -8.5 bars (27).

The respiration rate tended to increase more in the leaves and roots of some plants (90). Wheat and barley plants, however, showed the reverse, at least in the less tolerant varieties (116). The effect of salt on the respiration rate could be related to the high sensitivity of the mitochondria to the ionic composition of their environment (63). Also, cells of salt-affected plants may require higher energy for the accumulation of salts and the maintenance of higher osmotic pressures (90).

The mechanisms of salt tolerance in plants have been investigated by several workers. Most of these investigations, however, involved halophytic species which thrive in the presence of salts. The term "halophyte" literally means salt plant and is used to describe plants that can grow in the presence of high concentrations of sodium salts (79). Glycophyte (sweet plant) is a term used to describe plants that cannot grow in the presence of high concentrations of sodium salts (79). Most crop plants are glycophytes, examples being barley, potatoes (Solanum tuberosum L.), soybeans, and dry beans. Some glycophytes have been found to grow under saline conditions and possess similar cell structure to help in salt removal as those found in halophytes. It has been suggested that these plants have a common halophytic ancestor (80).

Since halophytes are adapted to grow under high concentrations of salts (soils with up to 20% salt), the mechanisms to salt tolerance are well established through various means. These mechanisms are generally based on keeping the levels of ions in the plant tissue below toxic levels. In addition, plants must have the ability to compensate for the lower water potentials associated with salinity by either increasing levels of organic solute or maintaining a constant concentration of ions in their tissues (103).

Some halophytes possess salt glands located in the leaves as small dimples (104). These were found in some species of the mangroves such as Aegialitis, Aegiceras and

Avicennia. The mechanism of salt tolerance in such species involves taking up a considerable amount of salts and excreting them through the salt glands. The concentration of sodium chloride in the xylem sap was measured to be about 0.2% to 0.5% (104). Such concentration exceeds that of non-secreting species by some 10 times, and that of ordinary land plants by about 100 times. The concentration of sodium chloride in the secreted liquid from salt glands of the Aegialitis species varied from 1.8 to 4.9%, the highest being secreted in the middle of the day. These concentrations exceeded that of the root medium. However, the water potential of the secretion is close to that in the leaf suggesting that secretion involves active transport of salt and passive movement of water by osmosis (11). The structure of salt glands may vary from a simple two-celled type to multicelled types which consist of five to nine or more cells (121). The two-celled gland, which has a hair-like shape, is found in Spartine townsendii. The multicelled glands are found in Avicenniaceae, in which the cells are arranged in a group of four or more located on top of one stalk and two-four collecting cells. Salt glands are found on almost every aerial part of these plants but are more abundant on the leaves (121). Salt gland cells have a higher number of mitochondria and other organelles than the surrounding epidermal or parenchymatous cells, but they lack a central vacuole (121). This suggests that salt gland cells do not serve primarily as accumulating organs

but rather as transit cells.

The salt tolerant species of the genus Atriplex do not possess salt glands but rather balloon-like, vesiculated hairs (trichomes) distributed in several layers on the surface of the leaves (25). The vesiculated hairs in A. halimus were found to play a significant role in removing excessive salts and preventing their accumulation in the parenchyma and vascular tissue (86). The concentration of salt in these hairs was found to increase as the salinity of the medium increased. The concentration of salt in the leaf sap, however, did not change appreciably as the salinity increased, indicating the efficiency of these plants in removing the salt and keeping it below toxic levels in their tissues (86). Salt concentration in the vesiculated hairs was about 60 times as great as that of the leaf sap. A vesiculated hair consists of two cells, a small stalk cell and a large bladder cell (121).

Shedding of salt saturated organs, especially the aging leaves, had been considered as an important salt regulation mechanism (8). Salt concentration in the leaves increases with increasing age until they turn yellow and die. While this continues to occur, essential elements (N, P, K) are probably withdrawn and translocated to the developing young leaves (8).

Salts may be excluded at the roots by the root membranes, leaving plant tissue almost free of salt (11, 104). These are the salt excluding mechanisms and are most likely the

main salt regulating factor in mangroves (8). Salt exclusion is also found in some grass species such as the tolerant clones of Festuca rubra (64). In Rhizophora mucronata and Bruguiera prob. exaristata, the salt concentration in the xylem sap was about 10 times less than that of the salt secreting species which was measured to be about 0.2 to 0.5% (104). The sap pressure in the salt excluding species was found to be below ambient and seldom became negative by a few atmospheres. Since the separation of fresh water from the seawater demands a permanent sap pressure of -20 atm or less, it was suggested that the separation could involve a case of active transport. This may be supported by the fact that the mangrove roots are well ventilated through pneumatic tissues, and an aerobic energy source is, therefore, available for active transport (104).

Dilution mechanisms are another means by which plants can cope with high salinities. This involves an increase in succulence, i.e., salt is diluted by increasing in water content during leaf maturation (8). Such a mechanism was found in mangroves (11) and many other halophytes (8).

Other means of salt removal may be found in the literature, for example through gutation fluid, salt leaching via a transpiration stream or salt retransportation back to the roots via the phloem and then to the surrounding media (121).

Osmotic adjustment in some halophytic species was also reported in the literature (52, 121). Such adjustment requires salt accumulation by plants grown under stress to

maintain a proper potential gradient between plant and external solution. The major portion of the total osmotic solutes in halophytes consists of sodium and chloride ions (67), while the major osmotic components in glycophytes are potassium salts of organic acids and sugars.

Carbohydrates, organic compounds and nitrogen compounds may be involved in the osmotic adjustment process (52). Glucose and inositol were found to accumulate at higher concentrations in the leaves of bean plants exposed to salt stress (125). There is evidence that sugars are withdrawn from the basal or older joints of sugarcane (Saccharum spp. L.) when grown under high osmotic pressure (113). As the osmotic stress was removed, the sugars returned to those joints from which they were withdrawn. Organic acids such as oxalic and malic acids accumulated in halophytes and other plants when exposed to high concentrations of salt or water stress (67). The accumulation of such compounds is due to the increase in cation uptake over anions under increasing levels of salinity (101).

Nitrogen compounds such as amino acids (101) and  $\alpha$ -amino nitrogen (73) were reported to increase under increasing salinities. Proline is one amino acid found to accumulate in halophytes (31, 101, 108, 112) and in other plants such as barley (33, 112, 127). Proline accumulation is not limited to plants growing in saline environments, but rather to plants subject to low water potentials in general (72). The amount of free proline accumulated is dependent on the

plant species (31) and the degree of osmotic stress (108). Depending on plant species, the amount and the significance of proline accumulation in the adaptation to a saline environment may vary. Cavalieri and Huang (31) studied the role of proline accumulation in the adaptation of eight major species of salt marsh halophytes to saline environments. Three patterns were identified. Limonium carolinianum (Walt.) Britt. and Juncus roemerianus Sheele accumulated proline at low salinity levels with maximum accumulation at higher salinities. C<sub>4</sub> species such as Spartina alterniflora Loisel., Spartina patens (Aiton) Muhly and Distichlis Spicata (L.) Greene accumulated proline at intermediate salinity levels. The succulents, Salicornia bigelovii Torr., Salicornia virginica L., and Borrchia frutescens (L.) DC., did not accumulate proline until very high salinities were reached. It was suggested, therefore, that the role of proline accumulation in the adaptation to a saline environment was of considerable importance in the first group, important to the C<sub>4</sub> grasses, and of little importance to the succulents.

Barley plants were among those which accumulated proline when exposed to osmotic stress (33, 127). However, proline accumulation in barley seemed to have an insignificant role in osmoregulation (33). This was due to the fact that the concentration of proline accumulated in the leaf exerted an osmotic potential of less than -0.5 bar, an amount too small to contribute significantly to the total

decrease of approximately -10 bar (33). Higher concentrations of proline may be attained in the plant tissue but only when growth is severely reduced (127). No varietal differences due to proline accumulation under a saline environment have been detected (65, 110). Salt tolerance was not found to correlate with basal proline levels among species (109). Current literature on this subject suggests that an adaptive role of proline is related to survival rather than to maintenance of growth (61).

Betaines and other methylated quaternary ammonium compounds are found to accumulate under saline conditions (109, 112, 127, 128). Glycinebetaine is one compound found to be produced in a number of higher plants, particularly in Gramineae and Chenopodiaceae (72). Salt-tolerant species contain more glycinebetaine than salt-sensitive species and it is found to increase in the former under high concentrations of sodium chloride (110). Other nitrogenous compounds such as betaine and choline tended to increase under stress conditions, suggesting their involvement in the osmotic adjustment (109).

Rains (101) suggested that nitrogenous compounds play an important role in the ability of plants to tolerate salinity. This was based on the dramatic changes in amino acid levels, such as proline, and the increased movement of reduced nitrogen via transamination of organic acids and synthesis of amines. There is evidence that abscisic acid may play a role in signaling a cellular requirement for

additional solute represented by ion accumulation and the biosynthesis of osmotically active compounds (41). This was supported by the fact that abscisic acid concentration increased before proline and other compounds and ions started to accumulate.

Many glycophytes, a group to which most agricultural crop plants belong, are not adapted to withstand and grow under high concentrations of salts. Unlike halophytes, glycophytes cannot tolerate and accumulate high levels of salt in their cells as a major part of the osmotic adjustment (60). Tolerance to salt in crop plants is based on ion exclusion (60, 79, 101). Salt-tolerant varieties of soybean showed minimal concentrations of chloride in their leaflets and petioles and, therefore, no necrosis was observed (1). The sensitive varieties, however, showed both high concentrations of chloride and necrosis. The former group was termed "chloride excluders" while the latter was termed "chloride includers." Similar results were obtained by Greenway (59) and Storey and Wyn Jones (111) on barley plants. The salt-tolerant barley cultivar "California mariout" excluded sodium in addition to chloride ions from the shoot better than the sensitive ones but only at lower salinity levels (111). Rice seedlings accumulated more sodium and calcium ions in their shoots and roots than wheat and barley seedlings (56). Wheat seedlings, on the other hand, accumulated more sodium but less calcium in the shoot than barley, whereas the roots had less sodium and more

calcium than barley seedlings. When compared to Agropyron elongatum (Host) Beauv., plants of the most tolerant barley variety accumulated more chloride and sodium ions in their tissues under saline conditions (62). Tolerant varieties may rely in part on organic solute accumulation for osmotic adjustment (126) in addition to the ions absorbed.

In order for osmotic regulation to be accomplished, it must involve a mechanism for ion transport into cells (101). Such a mechanism must have the characteristic of transporting specific ions required for physiological reaction aside from having the capacity of absorbing large amounts of ions. The dual mechanism has been suggested to explain this system (50, 100). Certain properties of the dual mechanism must be provided in order for plants to survive under saline environments (101). One of these properties is the selectivity of absorption of an essential ion such as potassium. The uptake of a specific ion occurs in the presence of high levels of chemically similar ions such as sodium, a predominant ion in saline soils. The accumulation of both ions would regulate the internal ionic content of the cells, and in the meantime the physiological processes would proceed in the presence of high salt concentrations. In barley, the rate of potassium absorption remains fairly constant at the highest sodium concentration and is equal to the maximal rate obtained by the mechanism (46). This and other evidence suggests that this mechanism is responsible for supplying the plant with adequate amounts of potassium even

at high concentrations of sodium by having a selective affinity for potassium.

The second property of this mechanism suggests that at increasing salt levels another mechanism becomes available which shows little selectivity to ions. As a result, the available ions accumulate at a higher rate which reduces the osmotic potential of the cell sap to ensure a minimum turgor. High rates of ion accumulation may impose some problems such as ion toxicity, especially in the salt-sensitive plants. Therefore, a synchronization of ion compartmentation by leaf cells along with a high rate of ions to the shoot is suggested (61). This may occur in the tolerant varieties on the assumption that they contain genes for efficient ion compartmentation (i.e. accumulation in the vacuole). Rice varieties may have been already selected for ion compartmentation unintentionally (61). Salt-tolerant varieties of rice have been found to contain salt-tolerant individual plants with high sodium in the leaves.

Calcium was found to play a role in the tolerance of plants both sensitive and tolerant to sodium salts. Bean plants tolerated high concentrations of sodium when considerable amounts of calcium ions were present in the medium (16, 78). Sodium uptake and translocation were competitively inhibited in the presence of calcium at a concentration range of 2.0 to 8.0 mmol/L (16). At lower concentrations, however, calcium markedly inhibited sodium translocation to the tops only. Similar results were found in barley (70). Tolerant

of plants to salt was found to improve as the ratio of calcium to sodium increased in the medium. It was suggested that calcium plays a role in sodium exclusion at the absorption site (78), and that a high sodium to calcium ratio (a value exceeding 17) (61) or low calcium per se increased membrane permeability (78). This in turn led to an increase in passive chloride and sodium ion transport and ultimately increased their concentrations in the tops (61). Such a role of calcium may be of greater importance in the salt-sensitive species such as beans which show a considerable tolerance in the presence of calcium.

Environmental factors can modify the tolerance of plants to salt. In other words, the effect of salts on plant growth and yield can be minimized or maximized by these factors as they interact with salinity. Soil fertility is an important factor. Salinity and fertility may impose different effects depending on the crop. When salinity is the limiting factor, as for wheat at -9 bars, carrots, onions, lettuce and cabbage at -4 bars, increasing fertility will be less effective than decreasing fertility (22). Similarly, when fertility is the limiting factor, as for broccoli and beets at both -2 and -4 bars, or for barley and wheat at -6 bars, the response will be greater with fertilization. The type of fertilization in this case becomes important. Higher levels of phosphorus seemed to increase salt injury and decrease salt tolerance in corn. Increasing levels of P did not appreciably affect dry matter or grain yield in

barley (120). Deficient levels of P and N, on the other hand, did not decrease salt tolerance of various crops.

The oxygen level around plants was found to interact with salinity to produce further effects. Tomato plants showed similar symptoms of stunting and growth reduction when exposed to low levels of oxygen or high salinities (12). Severe symptoms, however, were found under low oxygen and moderate to high salinities. Salt effect was more pronounced under adequate levels than under low or inadequate levels of oxygen.

Relative humidity is another factor which was found to affect salt tolerance in plants (68, 91). Salinity alone reduced plant height, number of heads per plant and grain weight in barley, wheat and sweet corn (68). Independent of salinity, increased relative humidity from 45% to 90% increased wheat grain yield by 24%, but decreased barley grain yield by 16% and had no significant effect on corn ear weight. The interaction of salinity with relative humidity, however, introduced different effects. Data on corn and barley plants showed that increased relative humidity from 45 to 90% decreased salinity levels (measured on relative basis) and ultimately increased the tolerance of these plants (68). Wheat plants, on the other hand, were not significantly affected by the interaction of both factors. The authors suggested that relative humidity should be considered when evaluating the salt tolerance of barley and corn but not for wheat. Relative humidity also reduced transpiration in bean

and cotton plants, and ultimately increased the water content of leaves under saline conditions (91). The effect of relative humidity had a greater effect in relieving the suppressive action of salinity in cotton plants than in bean plants.

Light and salinity were reported to have an interactive effect on the growth of bean plants (92). At low light intensities, salinity had an unexpected stimulating effect on the dry weight of bean seedlings. At high light intensities, however, salinity suppressed the growth of the plants but had no effect on their water content. The effect of salinity was more obvious on light-dependent growth as it had very little effect on heterotrophic growth in the dark. Chloroplasts were thought to be the target for the interactive effects of salt and light since their development, response to light and the light-dependent production of nucleic acid and protein were found to be affected by salts.

Temperature can modify the effect of salinity on plant growth. Germination of sugarbeet seeds was significantly affected by the interactive action of salt and temperature (54). At temperatures of 10°C and 15°C, salinity had little effect on germination but as the temperature increased from 25°C to 40°C, less seeds were germinating. The inhibitory action of salinity at all levels was maximum at 45°C.

Irrigation frequency is another factor that was reported to have an interactive effect with salinity on plant growth. Dry weight yield in barley decreased as the

frequency of irrigation decreased at a given salinity level (120). This action may have been due to the fact that frequent irrigation prevented salt buildup.

The degree of salt tolerance differs widely among plants. Barley was found to be a salt tolerant crop even at high salt concentrations. Ayers and Hayward (15) reported that barley germinated fairly well at high salt concentrations. In addition, they found that sugarbeets germinated poorly, and kidney beans did not germinate at all at moderate salt concentrations. Barley was also found to be tolerant to salt over strawberry clover, Ladino clover (57), wheat (84) and oats (17). In their review article, Mass and Hoffman (82) classified various crops according to their tolerance to salt based on yield decrease per unit increase in salinity beyond threshold (salinity at initial yield decline). Barley, cotton, date, sugarbeet, wheatgrass, wildrye and bermuda grass were found to be the most tolerant crops, while crops like almond, bean, carrot, grapefruit, onion, and strawberry were classified as sensitive. Other important crops such as alfalfa, corn, cucumber, grape, pepper, rice, sweet potato and tomato were considered to have moderate tolerance to salt.

In order for a plant breeder to improve plant tolerance to salt at any stage of growth, he or she must have populations with sufficient genetic variability. Genetic variability for salt tolerance is known to exist among cultivars of various crop plants at one or more stages of

growth when compared to others (10, 13, 39, 83, 93). Wheat varieties (T. aestivum L.) showed different responses at different levels of salinity during germination (18). A decrement of 50% in germination of sensitive varieties was found at a salinity level of -16 atm., while tolerant varieties showed the same decrement but at a salinity level of -20 atm. Similar results were also found among wheat varieties with regard to reaction to salt at the germination stage. Varietal differences for salt tolerance were also found to exist within wheatgrass species at various stages of growth. Varieties of crested wheatgrass (Agropyron desertorum) showed differences in genetic ability in terms of their reaction to salt at the germination stage (37). Genetic variability among varieties of intermediate wheatgrass (Agropyron intermedium (Host) Beauv) during seedling and mature-plant stages was also reported (69). Salt-tolerant and salt-sensitive varieties were also reported for tall wheatgrass (Agropyron elongatum (Host) Beauv) (105). Varietal differences were also found in grain sorghum (Sorghum bicolor (L.) Moench) when forty-eight varieties were screened for salt tolerance during germination and seedling stages (114). Rice varieties showed differences in reaction to salt during germination, seedling and mature stages (5, 7, 74, 75). Japanese upland rice varieties in general were more resistant than the Egyptian varieties and a Philippine variety, IR8 (44). Similar reports of this kind are found in the literature with regard to other species.

A salt tolerant species or variety does not necessarily tolerate salt throughout its life cycle just because it has a good tolerance during the germination period (13). Hunt (69) found little or no correlation between salt tolerance of intermediate wheatgrass during the seedling stage and later growth stages. Abel and Mackenzie (2) also noticed in soybeans that salt tolerance during germination and in the later stages was not apparently related. Barley cultivars showed different responses throughout their life cycle (10, 94). One cultivar coped with high salt stress at emergence but did not make the transition into the vegetative phase under high stress. Another cultivar, on the other hand, showed the opposite reaction (94). Similar findings led to the suggestion that two sets of genes might be involved (10).

The genetics of salt tolerance is not yet fully investigated. The few reports available indicate the complexity of the inheritance of this characteristic, at least in barley (10, 83). Based on coleoptile growth in barley, Maddur (83) stated that salt tolerance during the germination stage was carried out by partially dominant genes. However, when root growth was the basis, two genetic systems were proposed (10). At the seedling stage the tolerance to salt in barley was found to be controlled by partial to complete dominant genes (83). Simple inheritance of this character has been found in soybeans (1). In rice,  $F_1$  hybrids of two varieties showed high resistance to salinization when compared to their

parents for number of spikelets per panicle, panicle weight and grain yield per plant (6).

Several attempts were made to improve the tolerance of plants to adverse soil conditions through breeding. Through crossing and selection, improvement was made in the ability of alfalfa seeds to germinate under high osmotic pressures (using mannitol) (40). Selection of high yielding clones of crested wheatgrass under saline conditions was also successful in their tolerance to salt (36). However, selection was ineffective in producing high yielding lines when it was made on the basis of germinating seeds. This was probably due to the lack of correlation between tolerance in the germination stage and the mature stage. Epstein and colleagues have been working with crop improvement to tolerate salinity conditions using diluted and undiluted seawater (49). In a recent study they obtained 22 genotypes of barley (out of 7,200 entries) that could grow from seed to seed under salinity levels of 75-90‰ seawater (48). These were then planted in the field receiving water directly from the Pacific Ocean. One of these selections yielded 1,580 kg/ha compared to 2,070 kg/ha for the world average for barley (47). Wheat lines also showed promising results when grown under 50‰ seawater. An attempt was also made to transfer salt tolerance from wild species into the cultivated species. The salt-tolerant species of tomato (Lycopersicon cheesmanii) was used to introduce salt tolerance to the cultivated tomato (45). The progeny seeds underwent several cycles of

selection under salt treatment followed by backcrosses to the commercial line. The resultant crosses were able to survive, flower and set fruit about the size of large cherry tomatoes under treatment with 70% seawater, a concentration the cultivated parent could not survive (49).

There have been few reports on the possibility of using seawater for irrigation (48, 95). The practicality of this is questionable unless the soil is sandy enough to provide adequate leaching (95).

In recent years, tissue culture has been employed in breeding programs for screening and selection purposes (34, 38, 87, 88). Croughan et al (34) were able to obtain salt-tolerant lines of alfalfa cells through selection. The cells were taken from the cotyledons of germinating seeds and were exposed to a medium containing 1.0% NaCl. The selected line was reported to perform better under a saline than under a non-saline environment. The authors suggested that such tolerance was the consequence of a shift towards a true halophytic nature since the selected line was found to behave like a halophyte. Similar behavior was found with rice cells where the selected line grew better in the presence of 0.5% NaCl and withstood a concentration of 1.5% NaCl. In another study, Nabors et al (88) obtained salt-tolerant tobacco plants (Nicotiana tabacum L.) regenerated from cultured cells that tolerated a NaCl level of 8.8 g/L. The regenerated plants also withstood a concentration of 33.4 g/L NaCl. Selection from cell cultures containing

sodium chloride was also used in other species. A number of cell lines of Nicotiana sylvestris and Capsicum annum have been obtained which are able to grow in liquid media containing 1.0% and 2.0% NaCl.

The use of the genetic engineering concept and methodology in the improvement of salt tolerance of crops will probably be of greater importance in the near future. Mielenz et al (85) discussed the possibility of using this concept in transferring genes from salt tolerant microorganisms, such as bacteria, to cultivated crops. They also emphasized the importance of some beneficial bacteria, such as some strains of Rhizobium spp., and the possibility of their use in this field due to their possession of certain degrees of tolerance to salt and to their economic value.

## MATERIALS AND METHODS

This work consisted of two main studies. The first dealt with selection for salt tolerance at the germination stage in spring barley. The second study was an attempt to transfer salt tolerance during germination from spring barley to heterogeneous populations of winter barley (Schoolers barley).

In both studies, the same screening test at the germination stage was employed. The screening method similar to the one developed by Whitmore and Sparrow (123) for laboratory malting was applied with some modifications to fit the purpose of this study.

The seeds were germinated in test tubes using sodium chloride solutions of various concentrations. The concentrations were chosen according to preliminary tests to determine the critical level of sodium chloride at which varietal differences could be detected. The salt solutions were prepared by dissolving an equivalent amount of table salt (Iodine free) in a proper volume of distilled water.

Twenty-five seeds from each line tested were placed in a 15 x 1.7 cm test tube. A volume of 15 ml of solution was poured into the test tubes. The test tubes were then placed in a growth chamber at 12°C for 48 hours. The

solutions were changed every 12 hours.

At the end of 48 hours, the solutions were filtered off and the kernels blotted to remove excess moisture. They were then placed back in their test tubes and stoppered with porous foam rubber. The test tubes were then covered with aluminum foil and placed in the growth chamber at 17°C for 6 days. On alternate days the germinating seeds were carefully removed from the test tubes to prevent rootlets from tangling together. Germination measurements were based on root growth. Seeds were considered germinated when the first seminal root appeared.

#### I. Selection Study:

This study involved two cycles of selection for both extremes, i.e., selecting for high and low tolerance to salt, respectively, at the germination stage. The selection cycles were preceded by crossing the chosen lines. The idea was to increase genetic variability in addition to forming populations with various combinations of genes for salt tolerance (previously found to be polygenic in nature, at least in barley, 10, 83).

According to their performance during germination at 20,000 ppm NaCl, four crosses were chosen to be used in this study. These crosses were part of a diallel cross set (involving six parental lines) made earlier

for a previous study (10). Five of the six parents were involved in the four crosses selected. Their names, identification and source are given in Table 1. The performances of the five parents and their intercrosses during germination at 20,000 ppm NaCl are given in Table 2.

Table 1. Barley varieties (parental lines) that were used in this study.

| Variety name           | Identification | Source  |
|------------------------|----------------|---------|
| 1. Lajbjey Drosihezy A | Not available  | Denmark |
| 2. Ingrid              | CI 10083       | Sweden  |
| 3. Coho                | CI 13852       | USA     |
| 4. Orge Saida 183      | Not available  | Algeria |
| 5. California Mariout  | CI 1455        | Egypt   |

Table 2. Average germination percentage of parents and their intercrosses (F<sub>3</sub>) during germination at 20,000 ppm NaCl.

| Parental Number | 1     | 2     | 3     | 4     | 5     |
|-----------------|-------|-------|-------|-------|-------|
| 1               | 22.67 |       |       |       |       |
| 2               | 33.33 | 45.33 |       |       |       |
| 3               | 33.33 | 45.33 | 33.33 |       |       |
| 4               | 17.33 | 14.67 | 17.33 | 10.0  |       |
| 5               | 37.33 | 26.67 | 24.0  | 16.67 | 65.33 |

Crosses 1 x 4 and 2 x 5 were chosen for selection for low tolerance, while crosses 1 x 5 and 2 x 3 were chosen

for selection for high tolerance. These crosses were in the  $F_3$  generation. The identification of the crosses are designated by the numbers given to the parental lines in Table 1.

The first selection cycle was started with these crosses at 20,000 ppm NaCl. When selecting for low tolerance, the non-germinating seeds from the first two crosses were isolated and rinsed with distilled water to wash off the salt that may have accumulated on the seed coat. The seeds were then planted in 22-centimeter pots. Selection was concentrated on those seedlings that emerged later (4 to 5 days). This was done to eliminate the possibility of selecting those (emerged after 2 or 3 days) that were in the process of germination while in the test tubes. The emerging seedlings which were selected (usually at the one-leaf stage) were then transplanted into separate 11-centimeter pots, i.e., one seedling per pot.

Selection for high tolerance involved saving 9% of the total number of seeds that were used in the test. This percentage included seeds with the most vigorous growth which were identified visually by having a root length ranging from 10 to 20 mm with 1 to 3 seminal roots. These selected germinating seeds were transplanted into separate 11-centimeter pots.

The selected populations were allowed to grow in the greenhouse to be used as parents for crosses to

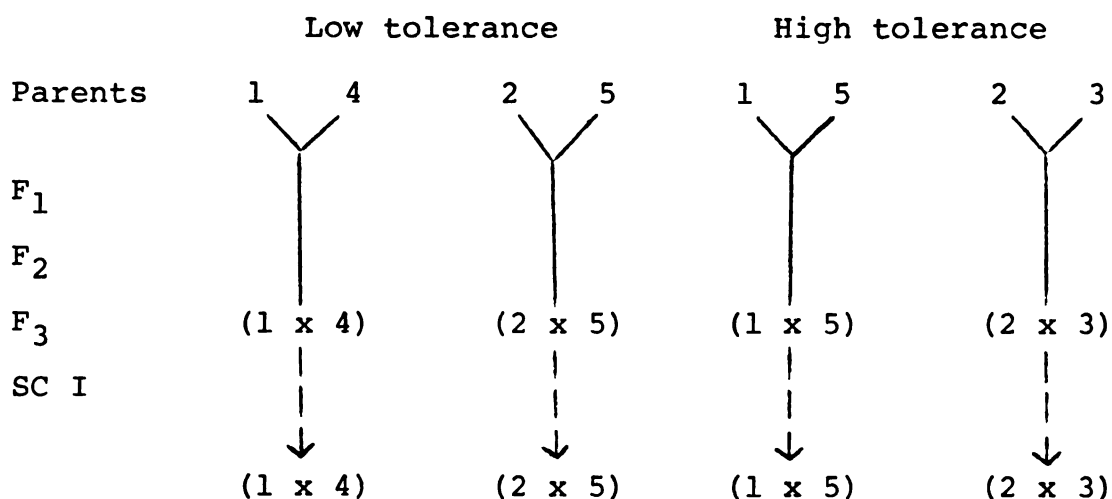
form new populations. The selected populations were intercrossed to form two new populations. These two populations were subsequently used for further selections for low and high tolerance, respectively.

The  $F_1$  seeds of these crossings were planted and grown in the greenhouse and  $F_2$  seeds were obtained. The second cycle of selection was made on the  $F_2$  seeds. Similar methodology to the one used in the first cycle was applied in the second cycle, except for sodium chloride concentrations. NaCl concentrations of 16,000 ppm and 24,000 ppm were used to select for low-tolerance and high-tolerance lines, respectively. The selected seeds were treated and planted in the same manner as discussed earlier. Plants were allowed to reach maturity and the progeny seeds were obtained. The selection study was terminated by obtaining seeds which represent low-tolerance and high-tolerance lines. Progress from selection was evaluated in terms of germination percentage at a salt level of 20,000 ppm according to the criterion discussed earlier. The entire experiment is summarized in Figure 1 in which a scheme is presented to explain the steps followed in this study.

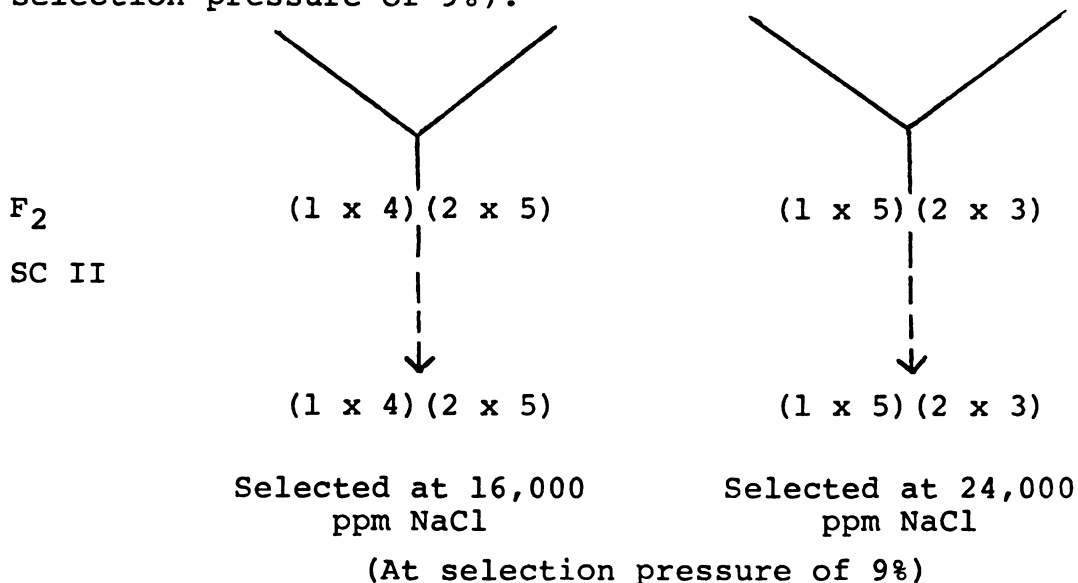
To study the effect of selection for salt tolerance at the germination stage on certain characteristics, another experiment was set up. This was to provide information on whether there was any association between salt tolerance and other agronomic characters. To do

this, all five groups of populations (Figure 1) for both low and high tolerance, including the parents, were included in the experiment. Five seeds from each line were planted in 11-centimeter pots (i.e., one seed per pot) in the greenhouse. These were repeated three times (3 replications). Randomization was carried out

Figure 1. A scheme representing the steps that were followed in the selection study.



These are parents selected for low and high tolerance to salt at 20,000 ppm NaCl during the germination stage (at selection pressure of 9%).



within each replication. The plants were given adequate irrigation and fertilization throughout the growing period. Each line was harvested at the time the plants reached physiological maturity.

The following are the characteristics measured and their criteria of measurement:

1. Seed weight:

This was measured by weighing 3 grams of seeds and dividing by number of seeds in the sample.

Four random samples were used.

2. Seed volume:

A random sample of 100 seeds was placed in a 50-ml graduated cylinder. Fifteen ml of distilled water was pipeted into the cylinder. Before reading the final volume, the cylinder was shaken to remove air bubbles which usually accumulated around the seeds. Seed volume was measured by subtracting 15 from the final volume divided by 100. Measurements were made on four random samples.

3. Heading date (days to flowering):

This was based on the time period from planting until the appearance of the awns from the boot.

4. Filling period (in days):

This was measured from the time of heading until the time of physiological maturity (physiological maturity in barley is indicated by the yellowish color of the kernels).

5. Number of tillers:

This was measured after plants had reached physiological maturity. It included fertile and non-fertile tillers.

6. Plant height:

Measured from the soil surface to the top of the spike.

7. Type (2-row and 6-row):

The parents used in this study were of different types in terms of kernel arrangement on the spike. Parents 1, 2 and 3 were 2-row, while parents 4 and 5 were 6-row barley.

Mechanism of Salt Tolerance:

To assist in identifying the mechanism by which barley plants tolerate salts during the germination stage, three tests were conducted. These were (1) imbibition test, (2) conductivity test, and (3) electron microprobe analysis of the seed. These tests were applied to the sensitive and tolerant lines produced from the final selection cycle.

1. Imbibition test:

This was conducted to see whether sensitive and tolerant lines differ in their ability to imbibe water under salt treatment. Twenty-five seeds from each line were randomly selected, weighed and placed in a 15 x 1.7 cm test tube. Two treatment solutions

were used, 0 ppm NaCl (distilled water) and 20,000 ppm NaCl. Fifteen ml of the solution was poured in the test tubes. The tubes were then placed in the growth chamber and kept for 48 hours at a temperature of 12°C. At the end of the 48 hour period, the test tubes were removed from the growth chamber. The seeds were carefully removed from the test tubes, gently blotted with paper towels and weighed immediately. This test was replicated three times. The total amount of moisture imbibed by the twenty-five seeds from each treatment was calculated by subtracting the weights before the treatment from that after the treatment.

## 2. Conductivity test:

This was conducted to determine the change in the concentrations of the salt solution before and after the treatment and to determine whether this change was related to the ability of seeds to germinate under salt treatments. Salt solutions before and after soaking the seeds of both the sensitive and the tolerant lines were obtained from the imbibition test. The solutions were diluted to 100th to have approximately 0.02% of salt. Six standard salt solutions with concentrations of 0.001, 0.005, 0.01, 0.02, 0.03 and 0.05 percent sodium chloride were used for concentration prediction purposes. A conductivity meter was used to measure the resistance

of the solutions at a room temperature of 26°C. The conductivity of the solutions was measured by taking the reciprocals of the resistance. The concentration of salt in the unknown (in this case the treatment solutions) was determined from a figure in which the conductivity of the standard solutions was plotted against their salt concentrations (standard curve).

### 3. Microprobe analysis:

The purpose of this test was to detect differences, if any existed, in the accumulation and localization of sodium and chloride ions in the seeds of the sensitive and tolerant lines. The same procedure and materials used in the imbibition test were repeated. At the end of the 48 hour period the solutions were filtered off and the seeds were gently removed and blotted without being touched to reduce contamination. They were then transferred to 25 ml beakers and placed over dry ice for about one hour. After freezing, the beakers were placed in a freeze drying apparatus evacuated to 100 millitorr<sup>1</sup> and held at -80°C for four days. At this point, the seeds were ready to be sectioned for the microprobe analysis. The sectioning of the

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<sup>1</sup> 1 atmosphere = 760,000 millitorr

seeds was made at three sites on the seed and slices of tissue (approximately 1/2 mm in thickness) were obtained. The sectioning included cross sections at the embryo and the center of the seed and a longitudinal section along the axis as indicated by the diagram in Figure 2.

The slices from each treatment were mounted on carbon discs and placed in a dessicator to prevent uptake of moisture from the atmosphere until the time of the analysis. They were then coated with approximately 300 Å of carbon in a Varian VE-10 vacuum evaporator.

The microprobe analysis of tissues is based on x-rays emitted from the tissue after being exposed to a beam of accelerated electrons. The wavelengths of the emitted x-rays are characteristic of a given element. A wavelength is selected by a spectrometer with a differential crystal, and the intensity of the emission is measured with a conventional x-ray detector (Applied Research Laboratories model EMX-SM microprobe operated at 15KV and 20 nA.).

On each slice, two sites were chosen to measure the accumulation of sodium and chloride ions. The first site was at the outer surface of the seed (lemma or palea) and the second was beyond the first site moving toward the center of the tissue. This was done to determine the site at which the

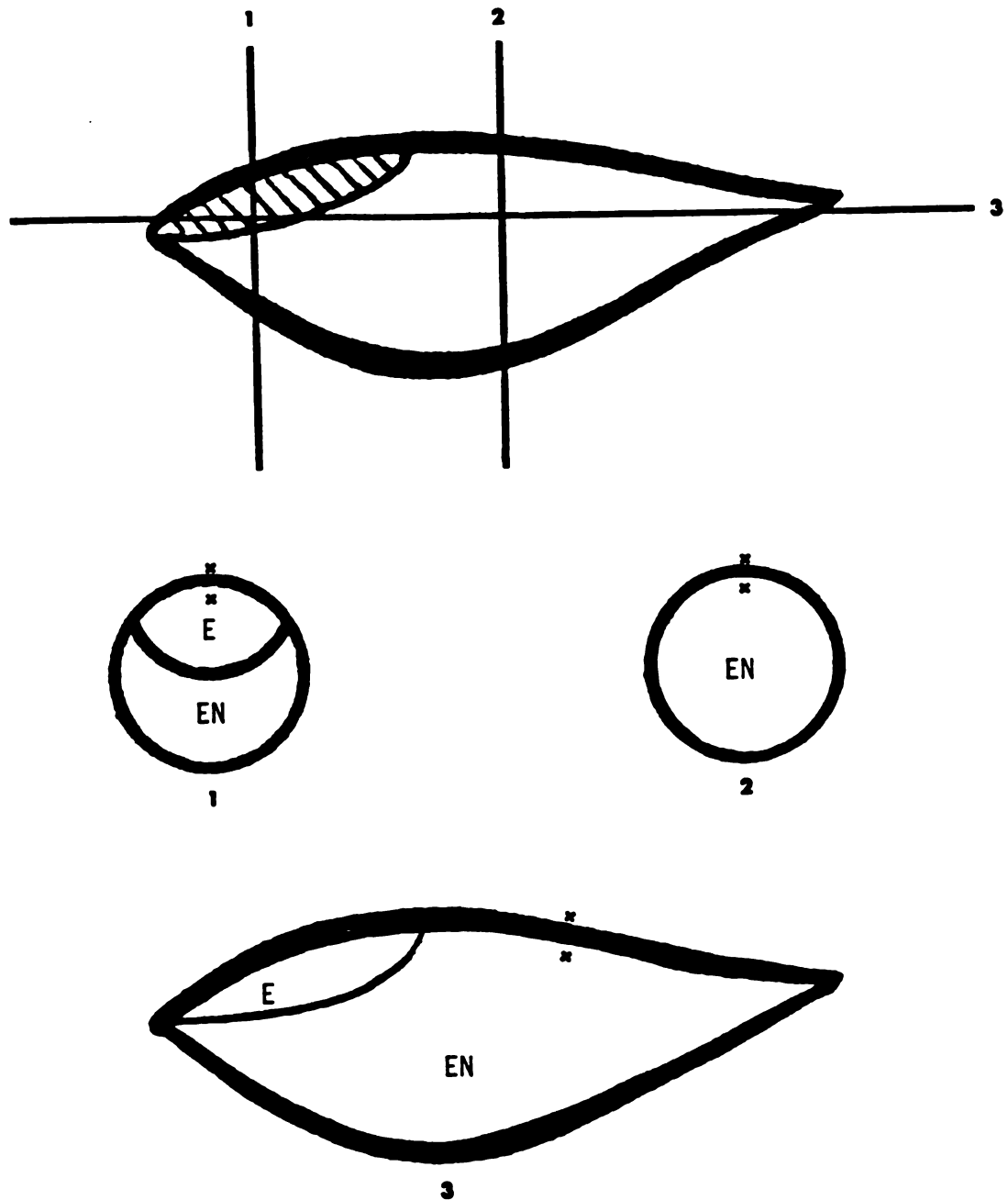


Figure 2. Sectioning of barley seed for the electron microprobe analysis. Numbers 1 and 2 are cross sections, while number 3 is a longitudinal section through the axis. The letter x designates the site around which the scanning was done. E and EN are embryo and endosperm, respectively.

accumulation of both ions was greatest and to see how far in the tissue they penetrated. To obtain a clearer picture about such information, a graph was produced to illustrate the accumulation and the penetration of ions starting with the outer surface of the seed while moving toward the center of it (approximately 600  $\mu\text{m}$ ). This was done as the tissue was being scanned.

## II. Salt Tolerance Transfer Attempt From Spring to Winter Barley:

The well-known salt tolerant variety "California Mariout" was used as the spring barley. The winter barley was represented by five heterogeneous lines screened earlier among others for winter hardiness and provided by Dr. Arnold B. Schooler of North Dakota State University in 1976. A preliminary test for salt tolerance at germination was made to evaluate 46 of these lines. The five lines involved in this study were selected according to their high tolerance to salt. However, their ability to germinate under saline conditions was inferior to California Mariout.

The selected lines were 4711-75-7, 4712-75-3, 4712-75-12, 4712-75-13, and 4732-75-1\*. In this study they were designated by the names Schooler 7, 10, 17, 21, and 43, respectively. In the spring of 1978, crosses were made among these lines in the field. F<sub>1</sub> seeds were grown in the growth chamber after being vernalized for 30 days to obtain F<sub>2</sub> seeds. Similarly, F<sub>2</sub> plants were grown in the growth chamber to obtain F<sub>3</sub> seeds. Crosses were made between these lines and California Mariout. Not all the crosses with California Mariout were successful, limiting the number to 7 lines. F<sub>1</sub> seeds of these crosses were obtained and planted in separate 11-centimeter pots and the plants were grown in the greenhouse. Since spring habit is dominant over winter habit, vernalization was not imposed.

The next step was to save all winter habit plants that were to segregate in the next generation. To do this, F<sub>2</sub> seeds were planted in 22-centimeter pots, three seeds per pot, and placed in the growth chamber. Segregation for winter and spring habit was evident 6-8 weeks after planting. The spring type plants grew

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\*These are segregating populations at early generations of crosses involving more than three parents. 4711-75-7 is a cross between Dicktoo (2X), (H. brachyantherum x H. bogdanii) (6X) and H. vulgare (4X Traill). 4712-75-3, 4712-75-12, and 4712-75-13 are crosses between Dicktoo and 4703-74-1 (Dicktoo X H. brachyantherum X H. bogdanii X Traill). 4732-75-1 is a cross between 47-75-8 (similar to 4711-75-7) and H. vulgare (2-row) X Elymus.

rapidly and headed in a relatively short time. The early heading time expressed by these types resembled that of California Mariout. The winter types, however, were short and bushy. At this stage, after 6-8 weeks in the growth chamber, pots containing the winter types were transferred to a cold room for vernalization after removing the spring types. After a period of 30 days, plants were taken back to the growth chamber. Seeds obtained from these plants were assumed to carry genes for salt tolerance. They were then tested for salt tolerance at the germination stage using the screening method discussed earlier. Other measurements were taken on these seeds such as seed weight and seed volume. These measurements were used to calculate seed density.

## RESULTS

Crosses between California Mariout and lines from the selection study and the salt tolerance transfer study exhibited three types of gene action. Figure 3 shows that crosses between this line and lines 7-21, 17-21, 7-43 and 10-21 exhibited a partial to complete dominance for genes expressing salt tolerance, whereas partial to complete dominance for sensitivity was shown with lines 1, 4, 7-10, 7-17 and 10-17. Crosses with lines 2 and 3, on the other hand, showed transgressive effects for sensitivity. The values given in this figure are germination percentages taken as percentage of California Mariout at 20,000 ppm NaCl.

### I. Selection Study:

The data from the germination test including those of the parental lines, crosses and selections were analyzed together. The results from the analysis of variance are summarized in Table 3.

The results from the germination test of the parents, crosses and selections are given in Table 4 for both the control and 20,000 ppm NaCl. These figures represent averages of three replications.

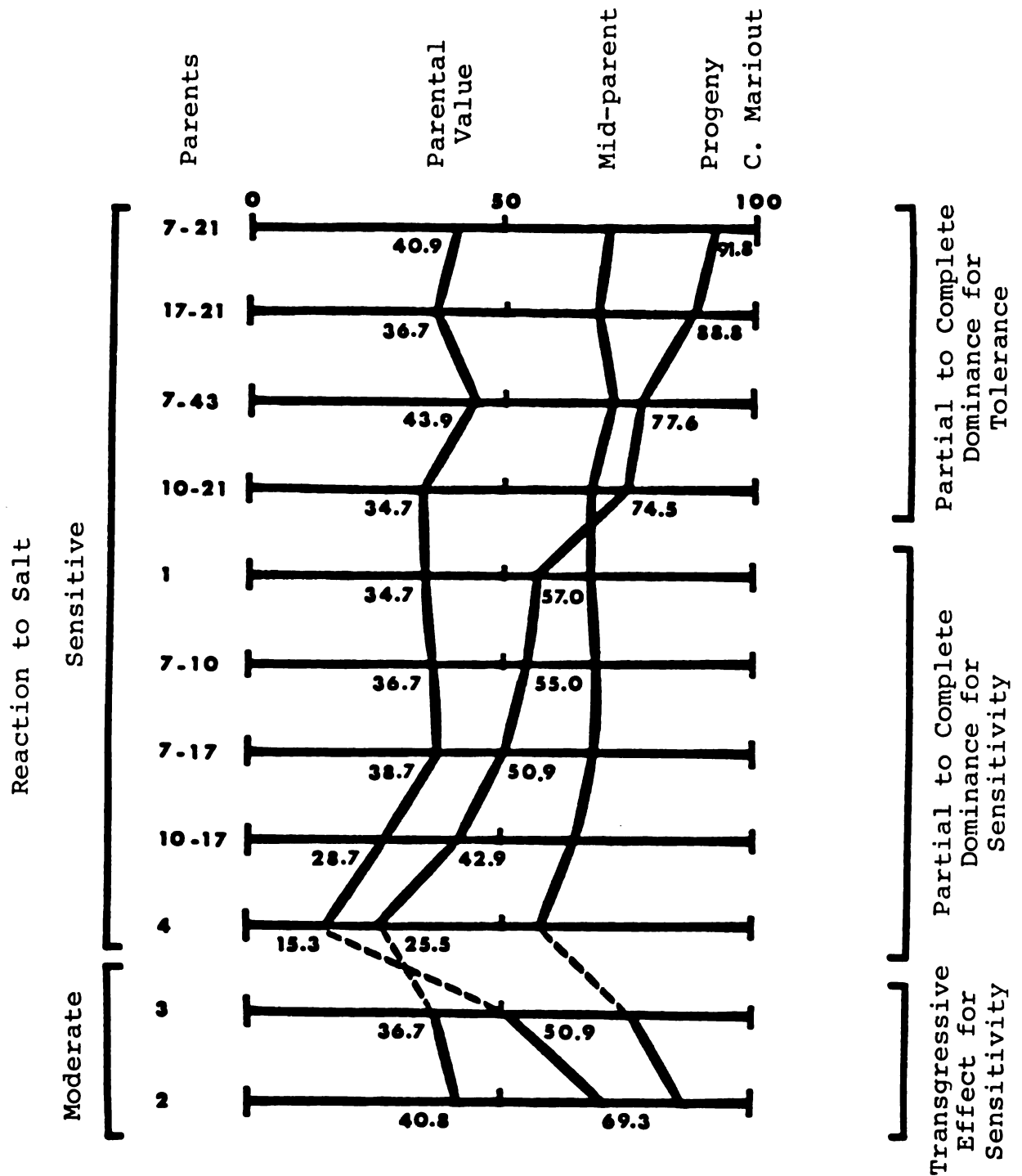


Figure 3. Mid-parent and progeny performance of crosses between California Mariout and eleven genotypes.

Table 3. Analysis of variance of salt tolerance as represented by germination percentages of seventeen lines of barley.

| Source       | Degrees<br>of Freedom | Mean<br>Squares | F         |
|--------------|-----------------------|-----------------|-----------|
| Replications | 2                     | 1.30            | n.s       |
| Entries      | 16                    | 671.91          | 30.24**   |
| Error (a)    | 32                    | 22.22           |           |
| Treatments   | 1                     | 83477.69        | 5252.79** |
| Interaction  | 16                    | 470.19          | 29.59**   |
| Error (b)    | 34                    | 15.89           |           |

\*\*P < .01

The single digit numbers designate the parental lines (namely 1, 2, 3, 4 and 5). Lines with two or four digit numbers are crosses among parental lines. Each of these numbers represents a parental line, for example, line number 14 is a cross between the parental lines 1 and 4. The letters S and T represent selections from the cross whose number is placed next to the letter. The letters S and T also stand for whether selection was for low tolerance (S) or high tolerance (T) to salt.

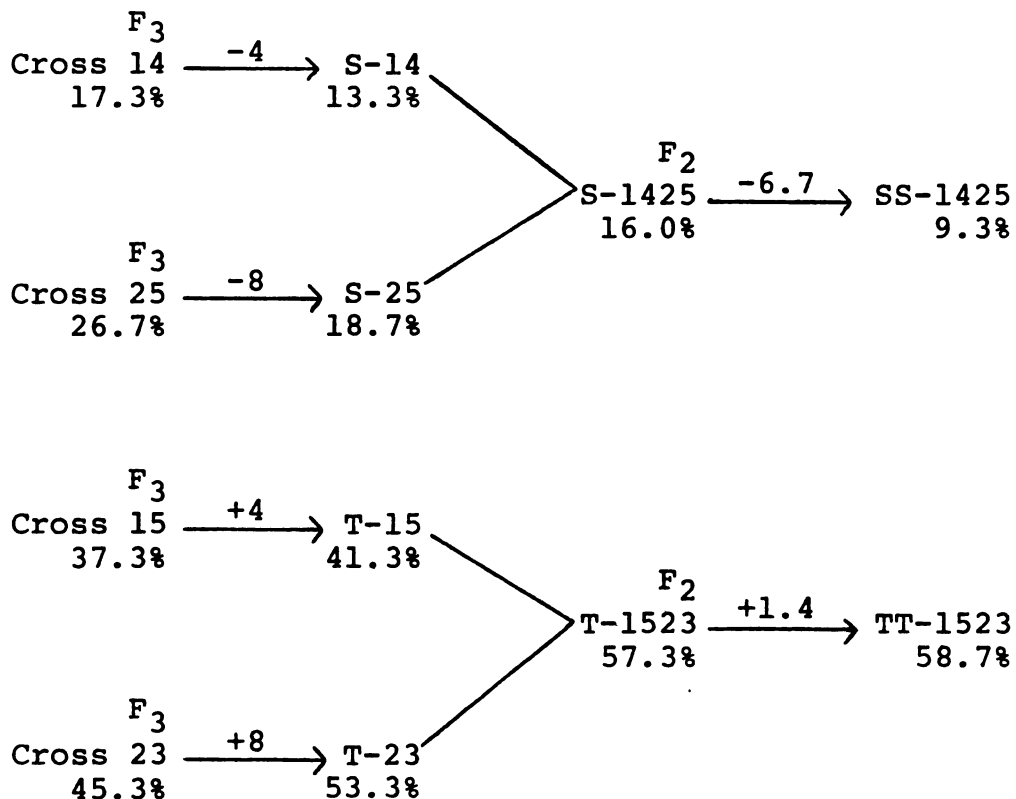
In the first selection cycle (selection from single crosses), the average gains were -4% and -8% when selecting for low tolerance from lines 14 and 25, respectively. When selecting for high tolerance from lines 15 and 23, the average gains were +4% and +8%, respectively.

Table 4. Average<sup>1</sup> germination percentages of parents, crosses and selections at 0 and 20,000 ppm NaCl.

| Line                          | <u>Germination Percentage</u> |            |
|-------------------------------|-------------------------------|------------|
|                               | 0 ppm                         | 20,000 ppm |
| Parents:                      |                               |            |
| 1                             | 85.3                          | 22.7       |
| 2                             | 90.7                          | 45.3       |
| 3                             | 100.0                         | 33.3       |
| 4                             | 96.0                          | 10.0       |
| 5                             | 92.0                          | 65.3       |
| Selection for low tolerance:  |                               |            |
| 14                            | 96.0                          | 17.3       |
| 25                            | 86.7                          | 26.7       |
| S-14                          | 85.3                          | 13.3       |
| S-25                          | 90.7                          | 18.7       |
| S-1425                        | 97.3                          | 16.0       |
| SS-1425                       | 73.3                          | 9.3        |
| Selection for high tolerance: |                               |            |
| 15                            | 90.7                          | 37.3       |
| 23                            | 90.7                          | 45.3       |
| T-15                          | 86.7                          | 41.3       |
| T-23                          | 93.3                          | 53.3       |
| T-1523                        | 97.3                          | 57.3       |
| TT-1523                       | 92.0                          | 58.7       |

<sup>1</sup>Average of three replications (25 seeds/replication)

In the second cycle of selection, i.e., selections from double crosses, the average gain was greater when selecting for low than for high tolerance. The gains (changes) in germination percentage were -6.7 and +1.4 when selecting for low tolerance and for high tolerance, respectively. However, when selections from the first selection cycle were combined, the tolerant line (T-1523) exceeded the better parent by 4%; while the sensitive line (S-1425) showed a mid-parental value. The following diagram summarizes the progress that was made in the germination percentage through crossing and selection. The gain figures are placed on the arrows. The numbers below are average germination percentages.



The total gain from selection for low tolerance to salt in the two cycles of selection was estimated to be -12.7%. The total gain from selection for high tolerance was estimated to be +17.4%. Note that 10% was gained from crossing the two selections from the first cycle and that only 7.4% was the result of the two selection cycles.

The impact of selection for low tolerance and high tolerance to salt on seed weight, seed volume and seed density is given in Tables 5 and 6. Table 5 shows the average values of these characteristics of the parents, crosses and selections, while Table 6 shows the association of these and other characteristics with low tolerance.

Selection for low tolerance did not seem to affect seed weight as indicated by its stability throughout the two cycles of selection (Table 5) and by the lack of association between this characteristic and sensitivity to salt (Table 6). The effect on seed volume, however, was more pronounced although a non-significant correlation was found between this characteristic and sensitivity to salt. The gradual decrease in seed volume and the stability of seed weight throughout the crossing and selection have caused an increase in seed density. This in turn created a stronger negative yet non-significant association between density and sensitivity to salt.

Table 5. Average seed weight, seed volume and seed density of parents, crosses and selections.

| Line                             |         | Average<br>Seed<br>Weight<br>(gm) | Average<br>Seed<br>Volume<br>(cm <sup>3</sup> ) | Average<br>Seed<br>Density<br>(gm/cm <sup>3</sup> ) |
|----------------------------------|---------|-----------------------------------|-------------------------------------------------|-----------------------------------------------------|
| Parents:                         | 1       | .0381                             | .0253                                           | 1.51                                                |
|                                  | 2       | .0371                             | .0255                                           | 1.45                                                |
|                                  | 3       | .0529                             | .0290                                           | 1.82                                                |
|                                  | 4       | .0482                             | .0345                                           | 1.40                                                |
|                                  | 5       | .0410                             | .0295                                           | 1.39                                                |
| Selection for<br>low tolerance:  | 14      | .0478                             | .0360                                           | 1.33                                                |
|                                  | 25      | .0485                             | .0320                                           | 1.52                                                |
|                                  | S-14    | .0445                             | .0248                                           | 1.79                                                |
|                                  | S-25    | .0430                             | .0210                                           | 2.05                                                |
|                                  | S-1425  | .0445                             | .0185                                           | 2.41                                                |
|                                  | SS-1425 | .0491                             | .0173                                           | 2.84                                                |
| Selection for<br>high tolerance: | 15      | .0489                             | .0360                                           | 1.36                                                |
|                                  | 23      | .0429                             | .0260                                           | 1.65                                                |
|                                  | T-15    | .0482                             | .0275                                           | 1.75                                                |
|                                  | T-23    | .0393                             | .0158                                           | 2.49                                                |
|                                  | T-1523  | .0428                             | .0165                                           | 2.60                                                |
|                                  | TT-1523 | .0459                             | .0175                                           | 2.62                                                |

Table 6. Correlations between reaction to salt at the germination stage and some agronomic characteristics.

| Characteristics          | Degrees of Freedom | Low Tolerance r | High Tolerance r |
|--------------------------|--------------------|-----------------|------------------|
| Seed weight              | 8                  | -0.06           | -0.19            |
| Seed volume              | 8                  | +0.18           | -0.45            |
| Seed density             | 8                  | -0.48           | +0.46            |
| Numbers of tillers       | 8                  | +0.58           | -0.04            |
| Plant height             | 8                  | -0.70*          | -0.15            |
| Days to flowering        | 8                  | -0.23           | -0.36            |
| Filling period           | 8                  | +0.54           | +0.37            |
| Length of growing season | 8                  | -0.04           | -0.28            |

\*P < .05

Seed weight had some tendency to decrease under selection for high tolerance as indicated by the figures in Table 5 and the negative correlation in Table 6. A stronger negative, yet not significant, association between seed volume and tolerance has been found. Accordingly, seed density increased as tolerance to salt increased. This has been verified by the positive correlation, although not significant, between the two characteristics.

The effect of selection on the type of spike (2-row and 6-row) is shown in Table 7. The values given are frequencies of the two types which occurred

in a random sample of fifteen plants of each line. It should be noted that parents 1, 2 and 3 are of 2-row type, and parents 4 and 5 are of 6-row type. The 2-row type appeared to increase in frequency as crossing and selection progressed. In the second cycle of selection, only one out of fifteen plants of the sensitive line was of 6-row type. The frequency of the 6-row type in the tolerant line was two out of fifteen.

Table 7. Frequency of 2-row and 6-row in barley crosses and selections in a total of fifteen plants per line.

| Line          | Type of Spike |       |
|---------------|---------------|-------|
|               | 2-row         | 6-row |
| Sensitive: 14 | 6             | 9     |
| 25            | 6             | 9     |
| S-14          | 7             | 8     |
| S-25          | 15            | -     |
| S-1425        | 14            | 1     |
| SS-1425       | 14            | 1     |
| Tolerant: 15  | 9             | 6     |
| 23            | 15            | -     |
| T-15          | 10            | 5     |
| T-23          | 15            | -     |
| T-1523        | 13            | 2     |
| TT-1523       | 13            | 2     |

Note: Parents 1, 2 and 3 are 2-row.  
Parents 4 and 5 are 6-row.

The impact of selection for low tolerance on other characteristics, namely, number of tillers, plant height, days to flowering, filling period and length of growing season, is summarized in Table 8. The overall effect of selection on the number of tillers per plant is indicated by the high association between sensitivity to salt and this characteristic (Table 6). Number of tillers per plant decreased drastically in the second cycle of selection as it went down from an average of 10.3 to an average of 7.7.

Plant height showed significant negative association with sensitivity to salt. Generally, height tended to increase as selection for low tolerance progressed.

A negative but low association between the number of days to flowering and salt sensitivity has been found. The number of days increased with the decrease in salt tolerance. As for the filling period, a high positive association with low tolerance was indicated. Filling period tended to decrease with selection for low tolerance to salt. Selection for low tolerance on the other hand, had no apparent effect on the length of growing season as no association between the two characteristics has been found. Generally, the second cycle of selection produced a line (SS-1425) that resembled in all characteristics but plant height parent number 4, the most sensitive to salt.

Table 8. Average<sup>1</sup> values of some agronomic characteristics of barley lines involved in the selection for low tolerance to salt.

| Line    | No. of Tillers | Height<br>(cm) | Days to<br>Flowering | Filling<br>Period<br>(days) | Length of<br>Growing Season<br>(days) |
|---------|----------------|----------------|----------------------|-----------------------------|---------------------------------------|
| 1       | 14             | 73.0           | 89.5                 | 37.3                        | 126.8                                 |
| 2       | 11.8           | 76.8           | 90.7                 | 38.2                        | 128.9                                 |
| 4       | 6.5            | 94.5           | 81.5                 | 33.9                        | 115.4                                 |
| 5       | 12.2           | 67.5           | 59.1                 | 42.7                        | 101.8                                 |
| 14      | 8.8            | 81.7           | 63.6                 | 40.1                        | 103.7                                 |
| 25      | 10.3           | 77.7           | 67.3                 | 44.3                        | 111.6                                 |
| S-14    | 9.5            | 83.5           | 71.3                 | 38.3                        | 109.6                                 |
| S-25    | 11.7           | 71.9           | 70.0                 | 40.8                        | 110.8                                 |
| S-1425  | 10.3           | 85.7           | 73.7                 | 38.5                        | 112.2                                 |
| SS-1425 | 7.7            | 83.2           | 81.3                 | 31.3                        | 112.6                                 |

<sup>1</sup>Average of measurements on fifteen plants.

The effect of selection for high tolerance on the above characteristics is summarized in Table 9.

There was no association found between high tolerance to salt and number of tillers per plant (Table 6). However, a transgressive effect was shown when the two selections (T-15 and T-23) from the first cycle of selection were combined. The increase was about 5 tillers per plant more than the mid-parental value. This increase was diminished in the second cycle of selection to produce plants with an average of 10.3 tillers per plant.

A negative yet non-significant association was found between tolerance to salt and plant height. A transgressive effect was also shown here as the two selections from the first cycle were combined.

The number of days to flowering tended to decrease but not significantly throughout the crossing and selection for high tolerance. This was indicated by the negative correlation between the two characteristics. The effect of selection for high tolerance on the filling period was a positive one as it increased, yet not significantly, as crossing and selection progressed. As for the length of growing season, it showed a small negative association with tolerance to salt.

Table 9. Average<sup>1</sup> values of some agronomic characteristics of barley lines involved in the selection for high tolerance to salt.

| Line    | No. of Tillers | Height<br>(cm) | Days to<br>Flowering | Filling<br>Period<br>(days) | Length of<br>Growing Season<br>(days) |
|---------|----------------|----------------|----------------------|-----------------------------|---------------------------------------|
| 1       | 14.0           | 73.0           | 89.5                 | 37.3                        | 126.8                                 |
| 2       | 11.8           | 76.8           | 90.7                 | 38.2                        | 128.9                                 |
| 3       | 15.1           | 75.9           | 81.4                 | 34.4                        | 115.8                                 |
| 5       | 12.2           | 67.5           | 59.1                 | 42.7                        | 101.8                                 |
| 15      | 12.0           | 76.4           | 63.8                 | 43.8                        | 107.6                                 |
| 23      | 15.0           | 76.2           | 89.5                 | 32.4                        | 121.9                                 |
| T-15    | 11.4           | 76.0           | 76.2                 | 37.4                        | 113.6                                 |
| T-23    | 15.4           | 72.4           | 85.5                 | 41.6                        | 127.1                                 |
| T-1523  | 18.0           | 80.7           | 79.1                 | 37.3                        | 116.4                                 |
| TT-1523 | 10.3           | 76.6           | 81.7                 | 40.4                        | 122.1                                 |

<sup>1</sup>Average of measurements on fifteen plants.

The mechanism of salt tolerance:

Results from the imbibition test showed that both the sensitive and the tolerant lines imbibed similar amounts of water in the control treatment with a difference of about .015 gms per 25 seeds (Table 10). Under salt treatment, however, the seeds of both lines absorbed less water. The sensitive line, however, tended to absorb more water than the tolerant line, with a difference of about .060 gms per 25 seeds.

The conductivity test showed that solutions obtained from test tubes containing seeds of the sensitive line had lower conductivity values than those obtained from test tubes containing seeds of the tolerant line. The concentrations of these solutions were predicted from the standard curve (Figure 4) and are given along with resistance and conductivity values in Table 11.

It is apparent that the concentration of the solutions obtained from tolerant line seed treatment were higher than those from the sensitive line seed treatment. Solutions from the sensitive line seed treatment, however, had a lower concentration than the initial one.

The results from the microprobe analysis of the seeds for sodium and chloride at two sites (the endosperm and the embryo) are summarized in Table 12. Under salt treatment, the accumulation of the ions is concentrated at the lemma or palea. Generally, there seems

Table 10. Average<sup>1</sup> amount of water absorbed by twenty-five seeds of barley after being soaked in treatment solutions for 48 hours.

| Line                   | 0 ppm NaCl                 |         |                             | 20,000 ppm NaCl            |         |                             |
|------------------------|----------------------------|---------|-----------------------------|----------------------------|---------|-----------------------------|
|                        | Weight of 25 Seeds<br>(gm) |         | gms of<br>Water<br>Absorbed | Weight of 25 Seeds<br>(gm) |         | gms of<br>Water<br>Absorbed |
|                        | Before                     | After   |                             | Before                     | After   |                             |
| Sensitive<br>(SS-1425) | 1.12433                    | 1.87233 | 0.748<br>+ <u>.0291</u>     | 1.16200                    | 1.79667 | 0.635<br>+ <u>.0135</u>     |
| Tolerant<br>(TT-1523)  | 1.15967                    | 1.89233 | 0.733<br>+ <u>.0058</u>     | 1.15900                    | 1.73367 | 0.575<br>+ <u>.0035</u>     |

<sup>1</sup>Average of three replications.

to be no significant penetration beyond this point, at least under the conditions of the treatment where the seeds had been exposed to salt for a period of 48 hours. A large difference in the ion accumulation between the sensitive and the tolerant lines was observed. The sensitive line accumulated approximately three times more sodium and two times more chloride at the lemma or palea than the tolerant line.

Table 11. Resistance and conductivity values<sup>1</sup> of the treatment solutions and their predicted concentrations.

| Treatment/line | Resistance<br>(R) | Conductivity<br>(1/R) | Predicted<br>Conc.<br>(ppm NaCl) |
|----------------|-------------------|-----------------------|----------------------------------|
| Initial Conc.  | 1775              | 0.00056               | 21,500                           |
| Sensitive      | 1833              | 0.00055               | 21,000                           |
| Tolerant       | 1617              | 0.00062               | 23,000                           |

<sup>1</sup>Average of three replications (2 readings/replication)

Figure 5 shows the pattern of sodium accumulation at the lemma or palea (embryo site) and beyond. In the tolerant line, the accumulation of sodium ions appeared to concentrate at the outer portion of the lemma or palea (within the first 50 microns), at least at the embryo site. The accumulation of sodium ions in the sensitive line, on the other hand, seems to be across the lemma or palea (a width of approximately 200 microns). A similar pattern was noticed for

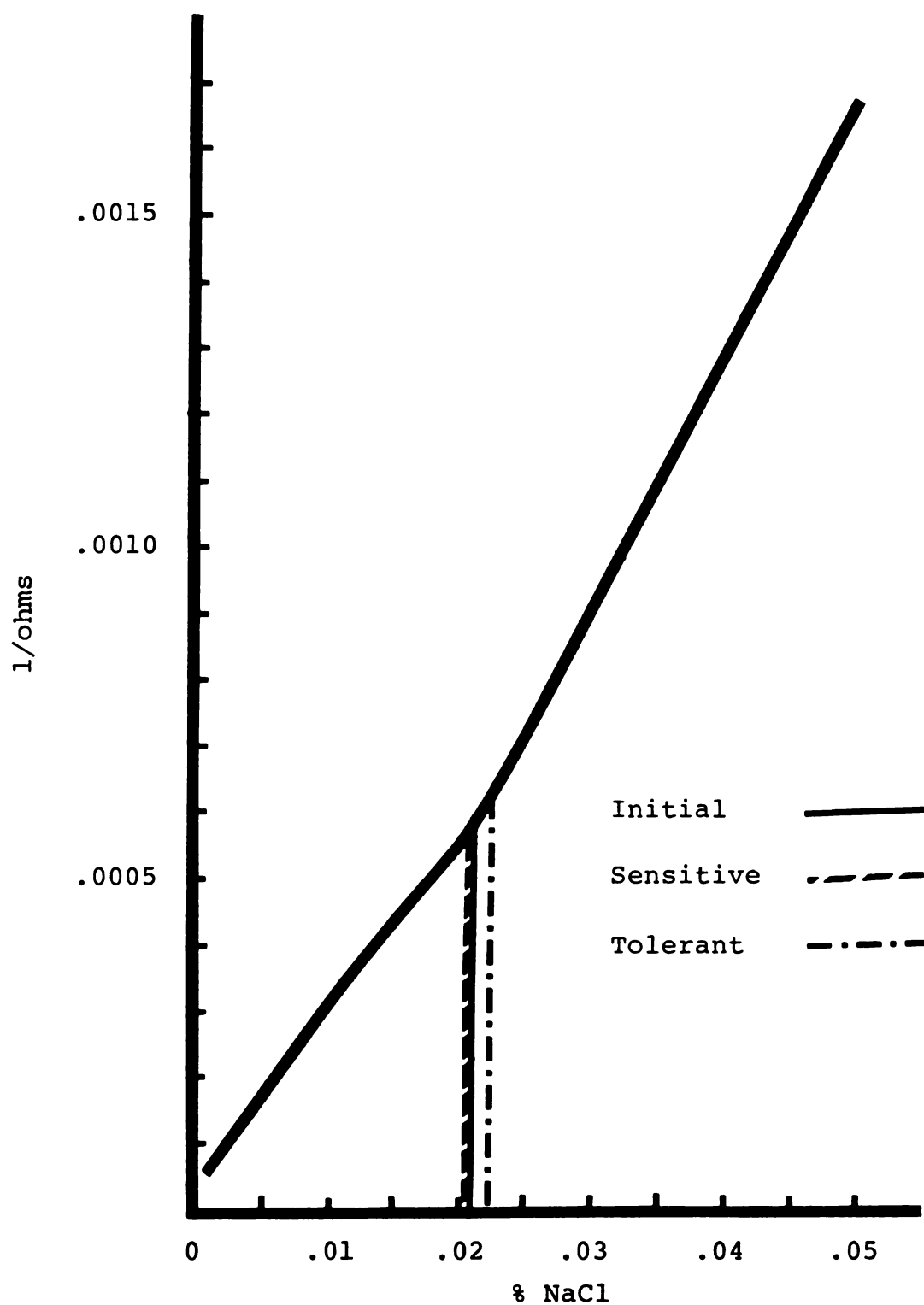


Figure 4. A standard curve of the reciprocal of resistance (1/ohms) against % NaCl.

Table 12. Average<sup>1</sup> counts/10 seconds of sodium and chloride ions in barley seeds under 0 and 20,000 ppm NaCl treatments.

| Line                   | Site      | Treat-<br>ment<br>ppm NaCl | Na <sup>+</sup>    |                   | Cl <sup>-</sup>    |                   |
|------------------------|-----------|----------------------------|--------------------|-------------------|--------------------|-------------------|
|                        |           |                            | Internal<br>Tissue | Lemma or<br>Palea | Internal<br>Tissue | Lemma or<br>Palea |
| Sensitive<br>(SS-1452) | Embryo    | - C.S. <sup>2</sup>        | 49.90              | 48.73             | 66.97              | 184.79            |
|                        | Endosperm | - C.S.                     | 33.80              | 45.75             | 65.90              | 200.18            |
|                        |           | - L.S. <sup>3</sup>        | 33.40              | 39.40             | 79.63              | 269.50            |
|                        | Embryo    | 20,000                     | 58.44              | 1613.81           | 103.15             | 891.35            |
|                        | Endosperm | 20,000                     | 62.74              | 1692.51           | 126.76             | 643.45            |
|                        |           | - L.S.                     | 63.20              | 2005.77           | 89.45              | 607.96            |
| Tolerant<br>(TT-1523)  | Embryo    | - C.S.                     | 54.0               | 66.48             | 64.39              | 125.87            |
|                        | Endosperm | - C.S.                     | 46.98              | 48.42             | 73.35              | 104.80            |
|                        |           | - L.S.                     | 38.24              | 58.56             | 108.53             | 158.05            |
|                        | Embryo    | 20,000                     | 97.0               | 525.58            | 162.68             | 349.12            |
|                        | Endosperm | 20,000                     | 59.22              | 630.51            | 110.39             | 410.88            |
|                        |           | - L.S.                     | 53.22              | 762.18            | 99.76              | 406.10            |

<sup>1</sup>Average of three replications (three readings/replication)

<sup>2</sup>C.S.: cross section.

<sup>3</sup>L.S.: longitudinal section.

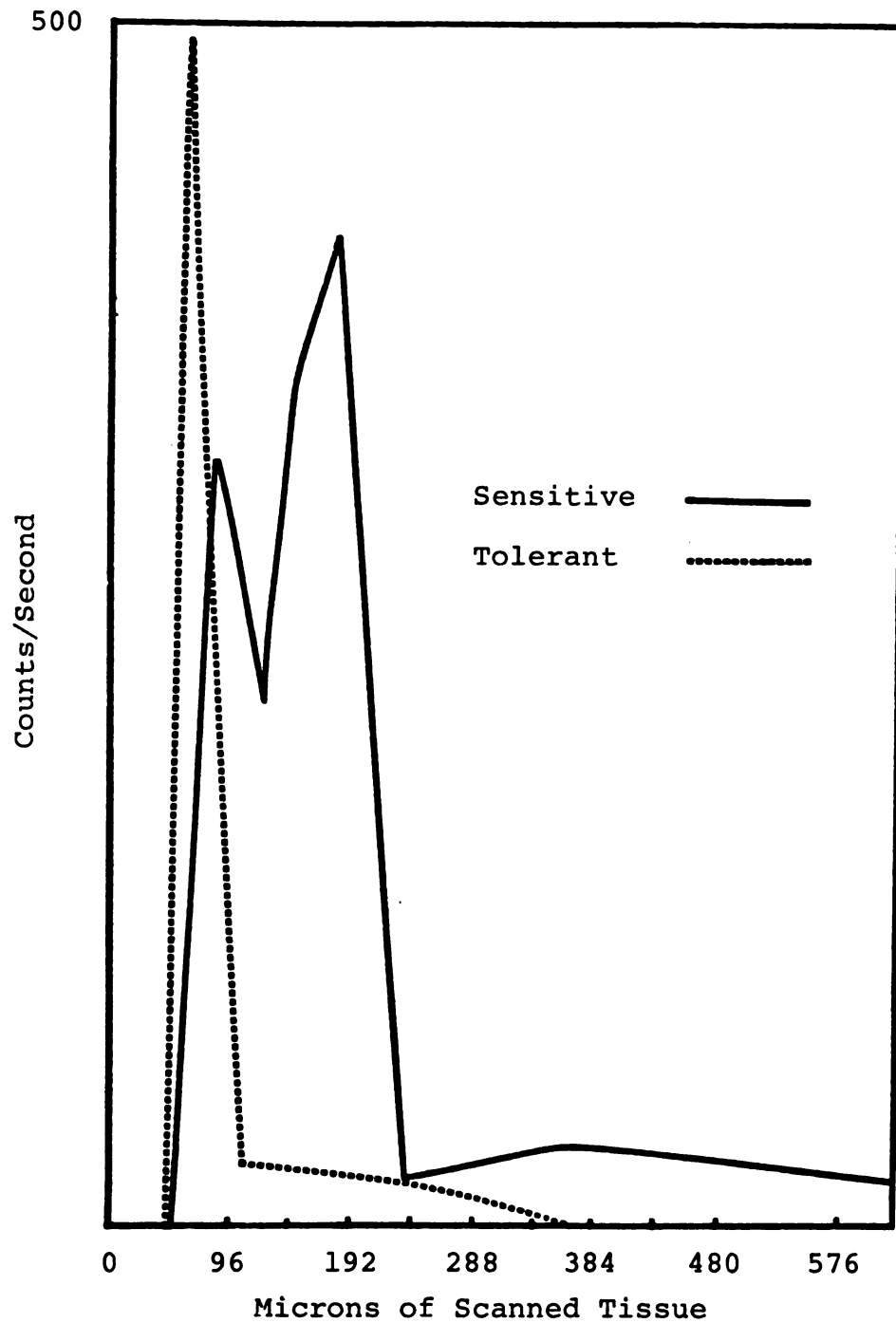


Figure 5. Pattern of sodium ion accumulation at the embryo site in the seeds of the sensitive and the tolerant lines after soaking in 20,000 ppm NaCl for 48 hours.

chloride ions (Figure 6) except that this ion may have penetrated a little beyond the lemma or palea. The photograph in Figure 7 gives a clear picture of the accumulation of these ions in both the sensitive and the tolerant lines at the embryo site.

The accumulation of sodium ions at the endosperm site exhibits a pattern similar to that found at the embryo site, except that in the first the ions showed a little penetration across the lemma or palea of the tolerant line (Figures 8, 10). This was also true for chloride ion accumulation at this site (Figure 9). The amount of ions accumulated by the sensitive line, however, was still larger than that accumulated by the tolerant line.

## II. Salt tolerance transfer attempt from spring to winter barley:

The data on the germination percentage of all entries were statistically analyzed together, and the results are given in Table 13.

Significant differences were found among lines, treatments, and line x treatment interaction. For the purpose of comparison, the average values of germination percentages from three replications (25 seeds per replication) are presented in Table 14. In this table, the first group represents five parental lines of winter

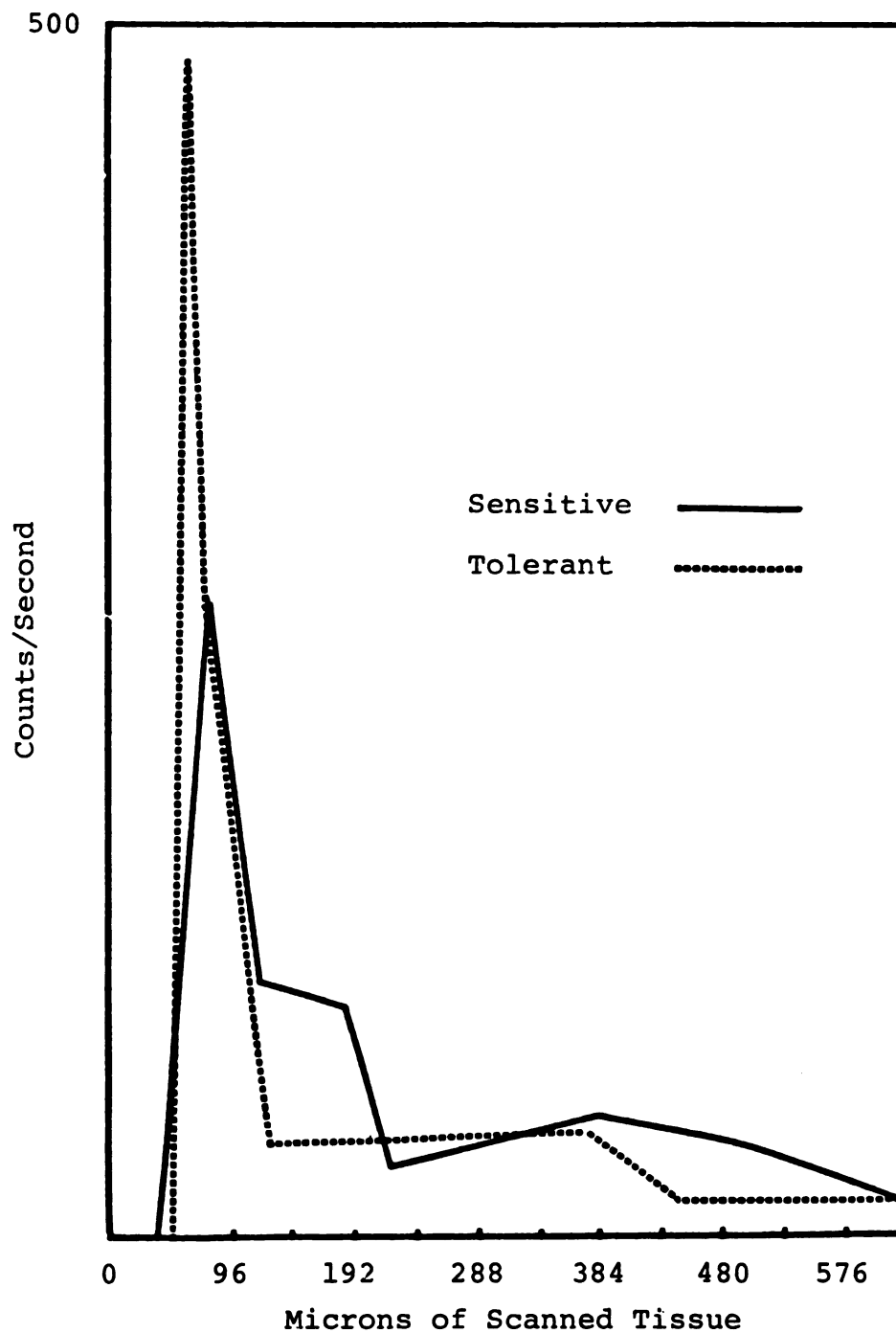


Figure 6. Pattern of chloride ion accumulation at the embryo site in the seeds of the sensitive and the tolerant lines after soaking in 20,000 ppm NaCl for 48 hours.

Figure 7. Microprobe photographs, 500X, of the lemma at the embryo site of the sensitive and tolerant lines (A and D), electrical charges for sodium ions (B and E) and chloride ions (C and F).

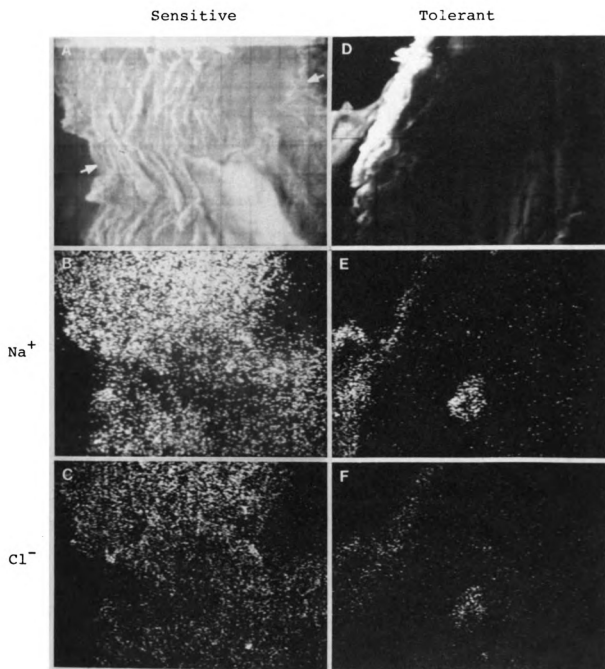


Figure 7.

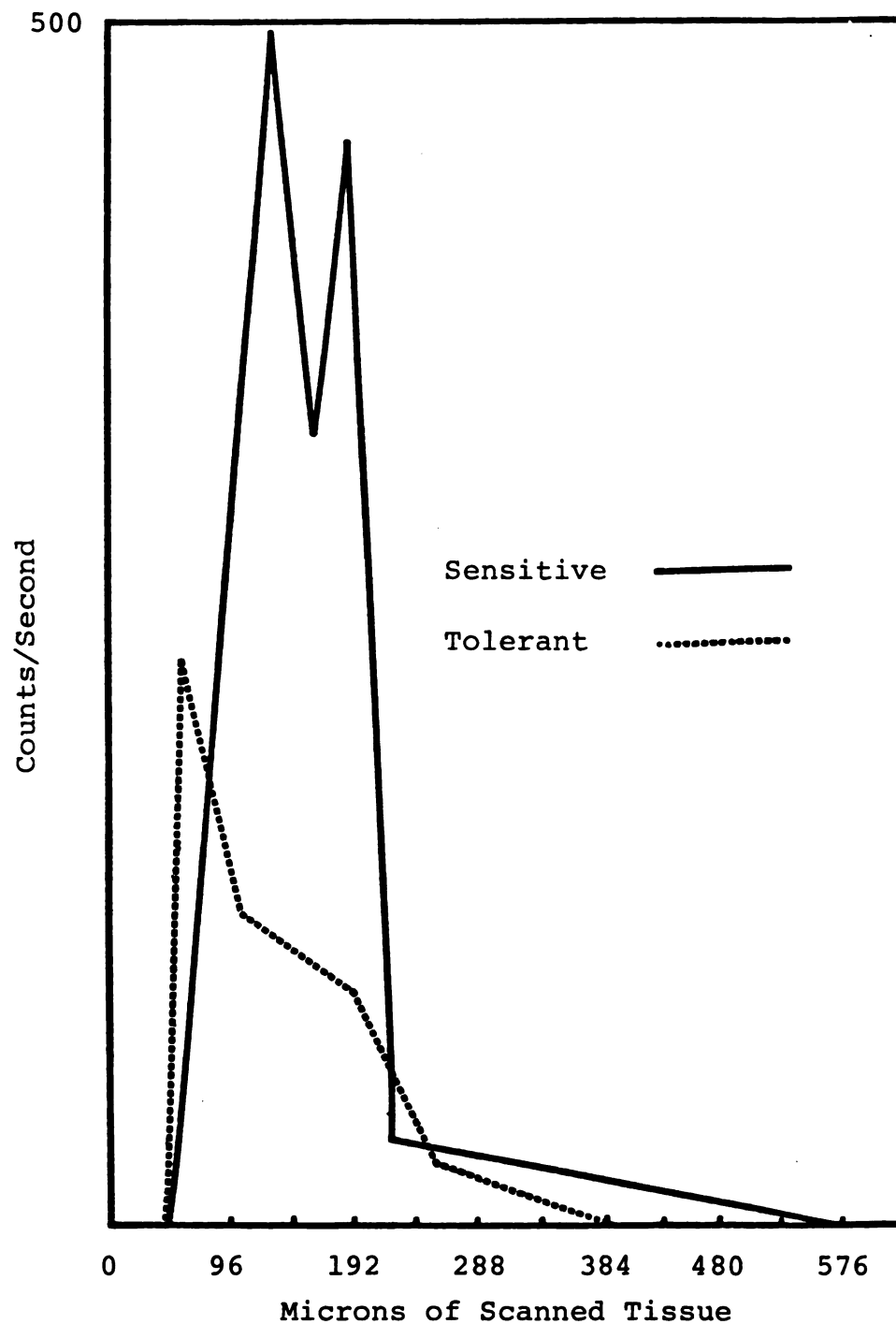


Figure 8. Pattern of sodium ion accumulation at the endosperm site in the seeds of the sensitive and the tolerant lines after soaking in 20,000 ppm NaCl for 48 hours.

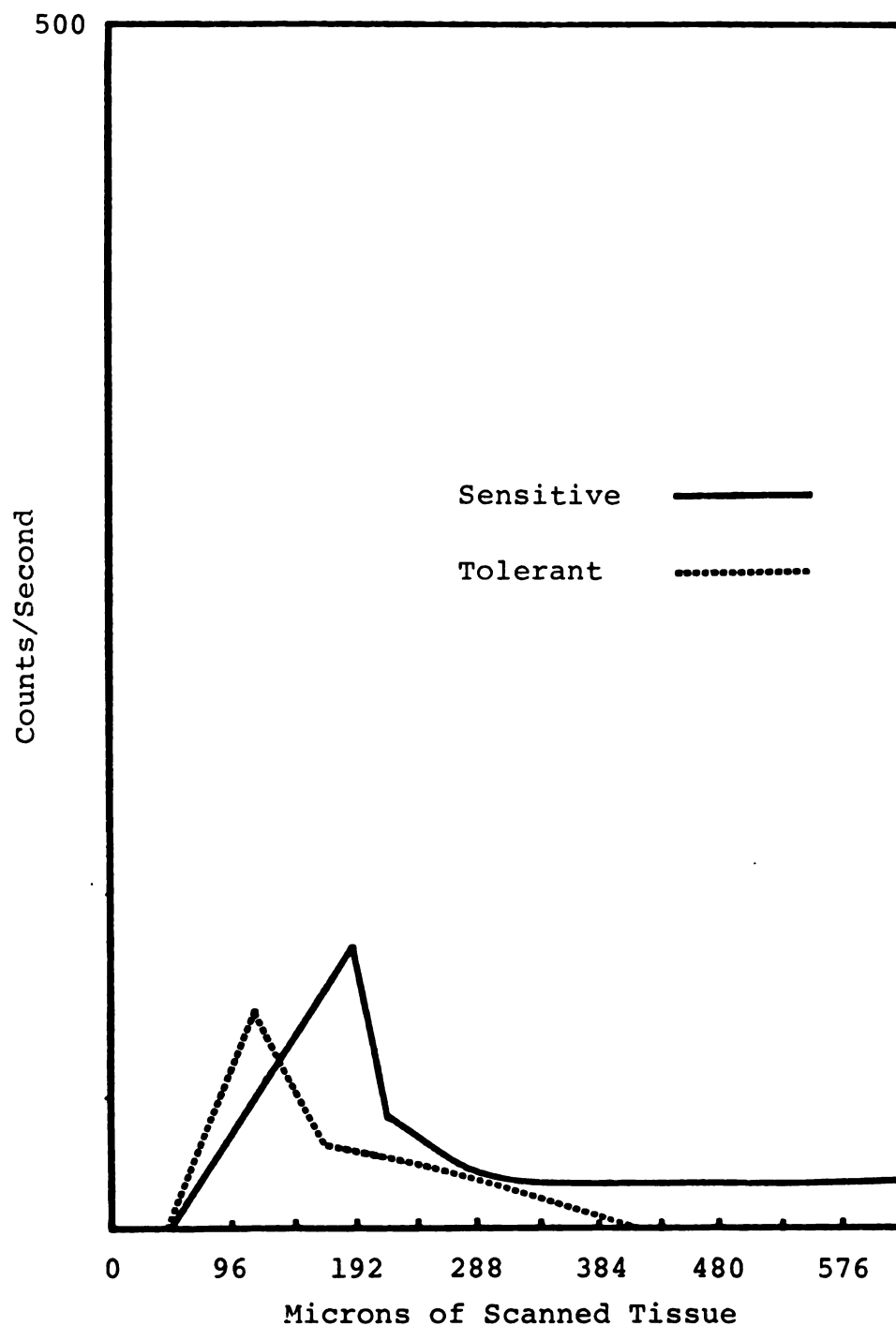


Figure 9. Pattern of chloride ion accumulation at the endosperm site in the seeds of the sensitive and the tolerant lines after soaking in 20,000 ppm NaCl for 48 hours.

Figure 10. Microprobe photographs, 500X, of the lemma or palea at the endosperm site of the sensitive and tolerant lines (A and D), electrical charges for sodium ions (B and E) and chloride ions (C and F).

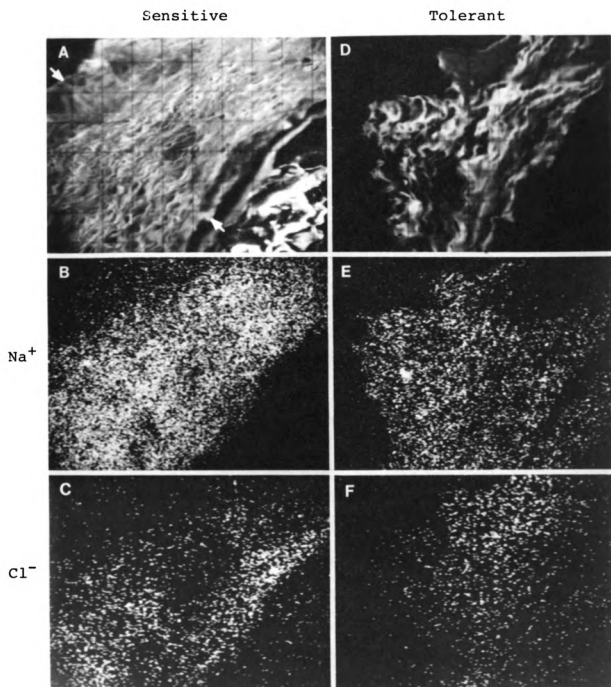


Figure 10.

habit (7, 10, 17, 21 and 43) and a spring habit barley, California Mariout.

Table 13. Analysis of variance of salt tolerance as represented by germination percentages of twenty-seven lines of barley.

| Source       | Degrees<br>of Freedom | Mean<br>Squares | F         |
|--------------|-----------------------|-----------------|-----------|
| Replications | 2                     | 36.07           | 1.30      |
| Entries      | 26                    | 354.10          | 12.78**   |
| Error (a)    | 52                    | 27.72           |           |
| Treatments   | 1                     | 134767.14       | 8488.47** |
| Interaction  | 26                    | 591.14          | 37.23**   |
| Error (b)    | 54                    | 15.88           |           |

\*\*p < .01

Group 2 represents a third generation (F<sub>3</sub>) of crosses among parental lines. Group 3 are the second generation of those in group 2 crossed with "California Mariout," which is designated by number 5. The last group represents lines with winter habit selected from the previous group. All parental lines of winter habit had similar performance under salt treatment although parents number 7, 21 and 43 showed a little advantage over the rest. Parent number 7 showed a dominant effect when crossed with parents 10, 17, 21 and 43. Crosses 10-17, 10-21 and 17-21 showed values within the parental range.

Table 14. Average germination percentages of twenty-seven lines of barley in 0 and 20,000 ppm NaCl.

| Line           |                  | Germination % |            |
|----------------|------------------|---------------|------------|
|                |                  | 0 ppm         | 20,000 ppm |
| G <sub>1</sub> | 7                | 96.0          | 26.7       |
|                | 10               | 94.7          | 18.7       |
|                | 17               | 94.7          | 21.3       |
|                | 21               | 97.3          | 24.0       |
|                | 43               | 97.3          | 26.7       |
|                | Cal. Mariout (5) | 92.0          | 65.3       |
| G <sub>2</sub> | 7-10             | 88.0          | 24.0       |
| F <sub>3</sub> | 7-17             | 89.3          | 25.3       |
|                | 7-21             | 90.7          | 26.7       |
|                | 7-43             | 97.3          | 28.7       |
|                | 10-17            | 89.3          | 18.7       |
|                | 10-21            | 98.7          | 22.7       |
|                | 17-21            | 96.0          | 24.0       |
| G <sub>3</sub> | 7-10-5           | 93.3          | 36.0       |
| F <sub>2</sub> | 7-17-5           | 97.3          | 33.3       |
|                | 7-21-5           | 97.3          | 60.0       |
|                | 7-43-5           | 100.0         | 50.7       |
|                | 10-17-5          | 96.0          | 28.0       |
|                | 10-21-5          | 97.3          | 48.7       |
|                | 17-21-5          | 97.3          | 58.7       |
| G <sub>4</sub> | 7-10-5W          | 90.7          | 26.7       |
| F <sub>3</sub> | 7-17-5W          | 74.7          | 29.3       |
|                | 7-21-5W          | 89.3          | 29.3       |
|                | 7-43-5W          | 94.7          | 32.0       |
|                | 10-17-5W         | 78.7          | 25.3       |
|                | 10-21-5W         | 89.3          | 38.7       |
|                | 17-21-5W         | 89.3          | 37.3       |

Group 3 lines exhibited various responses to salt treatment. Note that these lines were the second generation of crosses between California Mariout and each of group 2 lines, and should contain both spring and winter types. All lines showed better performance than those in group 2. The best performances were exhibited by the lines 7-21-5, 7-43-5, 10-21-5 and 17-21-5 as their average germination percentages under salt treatment were 60.0, 50.7, 48.7, and 58.7, respectively. Lines 7-21-5 and 17-21-5 showed performances similar to California Mariout, while the rest showed values within the range of the parents.

Group 4, which represents lines with winter habit selected from group 3, showed a setback type of reaction to salt as compared to group 3 lines. The best performance was shown by lines 10-21-5W and 17-21-5W with average germination percentages of 38.7 and 37.3, respectively. It should be noted, however, that the average germination percentages in the control of some lines were below 90%. This was probably due to post-harvest dormancy. For a clearer picture of the progress made in this study, see Table 15.

The fate of seed weight, seed volume and seed density in this study was investigated. The average seed weight, seed volume and seed density of twenty-seven lines are given in Table 16. Seed weight did not seem to change significantly in the first three groups.

Table 15. Average germination percentages of barley lines at 20,000 ppm NaCl.

| F <sub>3</sub> Populations<br>(G <sub>2</sub> ) | F <sub>2</sub> (F <sub>3</sub> x Cal. Mariout)<br>(G <sub>3</sub> ) | Winter Habit<br>(G <sub>4</sub> ) |
|-------------------------------------------------|---------------------------------------------------------------------|-----------------------------------|
| 24.0%<br>7-10                                   | 36.0%<br>7-10-5                                                     | 26.7%<br>7-10-5W                  |
| 25.3%<br>7-17                                   | 33.3%<br>7-17-5                                                     | 29.3%<br>7-17-5W                  |
| 26.7%<br>7-21                                   | 60.0%<br>7-21-5                                                     | 29.3%<br>7-21-5W                  |
| 28.7%<br>7-43                                   | 50.7%<br>7-43-5                                                     | 32.0%<br>7-43-5W                  |
| 18.7%<br>10-17                                  | 28.0%<br>10-17-5                                                    | 25.3%<br>10-17-5W                 |
| 22.7%<br>10-21                                  | 48.7%<br>10-21-5                                                    | 38.7%<br>10-21-5W                 |
| 24.0%<br>17-21                                  | 58.7%<br>17-21-5                                                    | 37.3%<br>17-21-5W                 |

Note: The average germination percentages of parents 7, 10, 17, 21, 43 and Cal. Mariout are 26.7, 18.7, 21.3, 24.0, 26.7 and 65.3, respectively.

Table 16. Average seed weight, seed volume and seed density of twenty-seven lines of barley.

|                | Line             | Average<br>Seed<br>Weight<br>(gm) | Average<br>Seed<br>Volume<br>(cm <sup>3</sup> ) | Average<br>Seed<br>Density<br>(gm/cm <sup>3</sup> ) |
|----------------|------------------|-----------------------------------|-------------------------------------------------|-----------------------------------------------------|
| G <sub>1</sub> | 7                | .0336                             | .0235                                           | 1.43                                                |
|                | 10               | .0262                             | .0160                                           | 1.64                                                |
|                | 17               | .0404                             | .0255                                           | 1.58                                                |
|                | 21               | .0497                             | .0315                                           | 1.58                                                |
|                | 43               | .0450                             | .0305                                           | 1.48                                                |
|                | Cal. Mariout (5) | .0409                             | .0295                                           | 1.39                                                |
| G <sub>2</sub> | 7-10             | .0325                             | .0135                                           | 2.41                                                |
| F <sub>3</sub> | 7-17             | .0395                             | .0173                                           | 2.28                                                |
|                | 7-21             | .0418                             | .0195                                           | 2.14                                                |
|                | 7-43             | .0423                             | .0180                                           | 2.35                                                |
|                | 10-17            | .0309                             | .0108                                           | 2.86                                                |
|                | 10-21            | .0406                             | .0155                                           | 2.62                                                |
|                | 17-21            | .0427                             | .0168                                           | 2.54                                                |
| G <sub>3</sub> | 7-10-5           | .0374                             | .0255                                           | 1.47                                                |
| F <sub>2</sub> | 7-17-5           | .0358                             | .0225                                           | 1.59                                                |
|                | 7-21-5           | .0462                             | .0310                                           | 1.49                                                |
|                | 7-43-5           | .0417                             | .0295                                           | 1.41                                                |
|                | 10-17-5          | .0294                             | .0200                                           | 1.47                                                |
|                | 10-21-5          | .0428                             | .0285                                           | 1.50                                                |
|                | 17-21-5          | .0472                             | .0325                                           | 1.45                                                |
| G <sub>4</sub> | 7-10-5W          | .0256                             | .0165                                           | 1.55                                                |
| F <sub>3</sub> | 7-17-5W          | .0240                             | .0155                                           | 1.55                                                |
|                | 7-21-5W          | .0374                             | .0230                                           | 1.63                                                |
|                | 7-43-5W          | .0368                             | .0230                                           | 1.60                                                |
|                | 10-17-5W         | .0250                             | .0165                                           | 1.52                                                |
|                | 10-21-5W         | .0391                             | .0230                                           | 1.70                                                |
|                | 17-21-5W         | .0450                             | .0275                                           | 1.64                                                |

However, it decreased in group 4 lines which represent those selected according to their winter habit. Seed volume showed instability throughout the study. All  $F_3$  populations of crosses among parental lines (group 2) showed seed volume less than the lower parent. However, when California Mariout was incorporated into these genotypes, seed volume increased to match that in group 1. On the other hand, when winter types were selected from group 3, seed volume was once again decreased. It would be more meaningful to look at seed density as a function of both seed weight and volume than looking at each factor independently. The change in seed density was dramatic in the  $F_3$  populations of group 2 as their densities have risen significantly from a range of 1.39 - 1.64 in group 1 to 2.14 - 2.86 in group 2. This change was mainly due to a decrease in the seed volume. Seed density of lines in groups 3 and 4 was decreased and remained at a close range. Correlations between reaction to salt and seed volume, seed weight and seed density were found statistically significant at  $P < .05$ . Tolerance to salt was positively correlated with seed weight and seed volume, while it was negatively correlated with seed density (Table 17).

Table 17. Correlations between tolerance to salt at the germination stage and seed weight, seed volume and seed density.

| Characteristic | Degrees of Freedom | r     |
|----------------|--------------------|-------|
| Seed weight    | 25                 | +.44* |
| Seed volume    | 25                 | +.69* |
| Seed density   | 25                 | -.48* |

\*P < .05



## DISCUSSION

Before getting into the discussion of the major points of this work, certain aspects about the genetics of salt tolerance should be clarified. It was suggested earlier (10) that salt tolerance in the most tolerant line, i.e., California Mariout, was regulated by recessive genes as indicated by the diallel cross analysis. Tolerance to salt in the rest of the parents in the set, on the other hand, was suggested to be controlled by dominant genes, with parent number 3 (Coho) possessing most of the dominant genes. This analysis was based on the performance of parents and progenies germinated in 16,000 ppm NaCl. However, similar behavior was detected when they were tested at 20,000 ppm NaCl. In this test, F<sub>3</sub> progenies from crosses with California Mariout showed two distinct patterns. Progenies from crosses with the sensitive lines, i.e., parents 1 and 4, showed values within the range of the parents yet below the value of the mid-parent. More specifically, they showed partial to complete dominance for sensitivity to salt. Progenies from crosses with the lines with intermediate tolerance, i.e., parents 2 and 3, showed values below the lower parent or transgressive effects for sensitivity to salt.

When California Mariout was crossed with  $F_3$  progenies of crosses among the winter heterogeneous barley lines, the  $F_2$  progenies showed similar behavior in 20,000 ppm NaCl. Partial to complete dominance for sensitivity to salt was found with lines 7-10, 7-17 and 10-17, and partial to complete dominance for tolerance with the rest, i.e., 7-21, 7-43, 10-21 and 17-21. Note that crosses among the parental lines of winter barley, i.e., parents 7, 10, 17, 21 and 43, showed a partial to complete dominance type of gene action.

According to the performances of  $F_3$  populations of crosses between California Mariout and other lines, three types of gene action were observed. These are as follows: (1) transgressive effects for sensitivity with lines 2 and 3, (2) partial dominance for sensitivity with lines 1, 4, 7-10, 7-17 and 10-17, and (3) partial dominance for tolerance with lines 7-21, 7-43, 10-21 and 17-21.

The transgressive effect for sensitivity which was shown in the crosses between California Mariout and parents 2 and 3 suggests that certain interactions among genes may have occurred when two genotypes were combined. Such interactions, which were brought about by combining two gene complexes, resulted in types more sensitive to salt. As for those genotypes which exhibited partial to complete dominance for sensitivity when crossed with California Mariout, it is assumed their genes showed some degree of dominance over genes of California Mariout. In other words, the action of genes of California Mariout was overridden by

the action of genes of those genotypes. In effect, progenies with values below the mid-parent were produced. In the last group the case is reversed, i.e., genes of California Mariout showed a partial dominance type of action over the genes of the genotypes of this group.

#### I. Selection Study:

In this study, it was possible to decrease and increase salt tolerance at the germination stage through crossing and selection. In a program like this, the amount of gain from selection depends on the heritability of the characteristic as well as on the selection differential. The importance of the heritability value lies in expressing the proportion of additive genetic variance that exists in a population. Crossing among lines which differ widely in a given characteristic before selection is one way to increase the additive genetic variance. This was the intention behind using this approach in this study. The type of gene action is also important in a successful selection program. Improvement of populations is best attained when an additive type of gene action prevails. Progress from selection is said to diminish once the additive genetic variance is exhausted.

Although different types of gene action have been inferred in the population studied here, some progress from selection was still possible, indicating the

presence of a certain amount of additive genetic variance. In the first cycle of selection, the gain in the germination percentage from selection for low tolerance from cross 14 was -4. The gain in this case was expected due to the inference that this cross showed additivity in as much as its mean was close to that of the mid-parent. In the case of cross 25, the gain in the germination percentage from selection was -8. It should be noted that this cross had a mean value lower than that of the lower parent. Apparently, the selection was in favor of those genotypes with certain gene complexes that favored sensitivity to salt.

When the two selections from the first cycle, i.e., S-14 and S-25, were combined, the second generation (S-1425) showed complete additivity. In the second cycle of selection, which involved selecting for low tolerance from line S-1425, a gain of -6.7 in the germination percentage was obtained. This has resulted in producing a line (SS-1425) with a germination percentage of 9.3% which is similar to that of parent number 4, the most sensitive to salt. Note that in this cycle, the screening test was done at a concentration of 16,000 ppm instead of 20,000 ppm NaCl, while maintaining the selection pressure at 9%. This step helped in narrowing down the number of germinating seeds at the higher concentration of salt. In other words, the superior genotypes have been reduced by this step. Accordingly,

further gain from selection may be attained from this line by lowering the concentration of salt beyond 16,000 ppm.

When selection was practiced for high tolerance to salt from cross 15, the gain in germination percentage was +4. A gain of +8 was obtained, on the other hand, from cross 23. However, when the two selections, i.e., T-15 and T-23, were combined, the mean of the  $F_2$  population (T-1523) showed transgressive effects for tolerance. It exceeded the mean value of the higher parent T-23 by 4% and the mid-parent by 10%. In the second cycle of selection, the gain was only 1.4%, probably because in this cycle the concentration of salt was raised to 24,000 ppm in contrast with 20,000 ppm in the first cycle, with selection pressure being maintained at 9%. Another reason for not obtaining a higher gain from selection in the second cycle could be the fact the genetic variance in the  $F_2$  population included a substantial fraction of non-additive genetic variance. It might, then, be surmised that a large portion of the increase in the germination percentage resulted from crossing per se rather than from selection. This was due mainly to the transgressive effects which were displayed by crossing the two selections from the first cycle.

The association between sensitivity or tolerance to salt at the germination stage and some seed characteristics may be useful in providing some information to

the plant breeder. Such information may be of use in the explanation or even identification of the mechanism(s) of salt tolerance in addition to a possible use in breeding programs. The value of such information depends, of course, on the magnitude or the degree of association in question.

Seed weight and seed volume were the seed characteristics examined in this study. The effect of selection on these two characteristics separately may not appear so meaningful, especially in the case of selection for low tolerance. However, when they were considered jointly, i.e., as seed density, the effect was more pronounced. Seed density tended to increase with selection independent of which extreme it was for. Seed volume seemed to be the main factor that caused this increase to occur due to its gradual decrease with selection. This was accompanied by a stability of seed weight throughout this study. Although seed density has risen during selection for both extremes, it continued to increase in the sensitive lines through the second cycle of selection, while no change was found in the density of the tolerant lines after the second crossing. Since the seeds were produced in the greenhouse it is assumed that this could have contributed to producing smaller seeds. The amount of contribution of this factor is not known. However, the effect of crossing and selection may have made the larger contribution due to the

gradual increase followed by each of these steps. If the greenhouse had a large effect, one would expect a significant change in both seed weight and volume. This, however, was not the case since only seed volume changed. This argument does not suggest a strong association between these characteristics and the reaction to salt since the correlations were statistically not significant. It was merely considered because of their importance as agronomic characteristics, although these correlations would be statistically significant if there were more degrees of freedom than 8.

It has been found that tolerance to salt at the germination stage was not correlated with that at other stages of growth (10, 94). Associations may exist, however, between tolerance to salt at the germination stage and other characteristics of mature stages of growth. In the case of the type of spike, it may seem that most selections, whether they were for low or high tolerance, favored the 2-row type (Table 7). A possible association may exist between low tolerance to salt and the type of spike. This is supported by the fact that all selections from line 25 produced plants with 2-row type. Note that this line is a cross between a 2-row which is parent number 2 (Ingrid) that possesses an intermediate tolerance to salt, and a 6-row which is parent number 5 (California Mariout), the most tolerant line. As for cross 14, selections from it produced both

types at about equal frequencies. Although parent number 1 is a 2-row type and parent number 4 is a 6-row type, both are sensitive to salt. In this case, both types (2-row and 6-row) were expected to appear in the selections.

The 2-row type was also predominant in the final selections of the tolerant lines. Although this was the case, it is not likely that the 2-row type is associated with salt tolerance for the following reasons: (1) selections from line 15 which is a 2-row x 6-row produced both types at about equal frequencies found in the original cross, (2) the 2-row type is more abundant in these populations since three out of four parents were of 2-row type, (3) systematic bias may have played a role in increasing the 2-row/6-row ratio since plants were randomly chosen for the second crossing to produce the double cross T-1523, and (4) most important, is that the most tolerant parent, i.e., California Mariout, is a 6-row barley.

All other characteristics except height did not seem to correlate with reaction to salt as the  $r$  values were not statistically significant. Even height was not significantly correlated with tolerance to salt. However, it tended to correlate with sensitivity to salt as the  $r$  value was found to be significant at  $P < .05$ . The possible explanation for this association could be due to the fact that selection was in favor of selecting

types that are similar to parent number 4, the most sensitive to salt. This is also true for other characteristics since the product of the final selection resembled this parent in most of the characteristics measured.

The mechanism of salt tolerance at the germination stage:

The fact that the presence of salts in a medium reduces the amount of water imbibed by barley seeds has been mentioned in the literature (32). Seeds of both tolerant and sensitive lines imbibed less water under salt treatment. However, the sensitive line SS-1425 tended to absorb more water than the tolerant line TT-1523.

The results of the conductivity test and the microprobe analysis are in line with results of the imbibition test. These tests showed that the sensitive line tended to absorb more salt than the tolerant line. As the salt builds up in the seed, more water is absorbed due to the fact that salt ions (sodium and chloride in this case) have a tendency to attract water molecules.

The tolerant line appeared to have the advantage of accumulating less salt ions in its tissue. In other words, this line excluded salt more effectively than the sensitive line. This must involve a mechanism that is operating to ensure initiation and progression of germination under high osmotic pressure. A salt-exclusion

mechanism has been suggested in the literature as one of the means by which plants escape salt injury. This mechanism has been found to operate in stages beyond germination, and was found in some halophytes (97) as well as in some glycophytes (60, 79, 101, 111). California Mariout, the salt tolerant cultivar, has been reported to possess such a mechanism by which sodium and chloride ions are excluded from the shoot, whereas the sensitive lines did not do so effeciently (111). Since California Mariout was found to be tolerant throughout its life cycle (10, 83), it is postulated that the tolerant line TT-1523 had inherited the exclusion mechanism from its California Mariout parent. This is based on the assumption that California Mariout possesses the exclusion mechanism at the germination stage. Specific inferences cannot be made about how this mechanism operates due to the lack of supportive data. Based on the results provided by this study, however, the following model is proposed to explain the mechanism of salt tolerance on a general basis.

#### Model

The fact that salt imposed osmotic effects rather than salt toxicity was indicated by the failure of ions to penetrate the testa in the seeds of both the sensitive and the tolerant lines although very little penetration was found in the sensitive seeds. The testa, which is

a thin layer located next to the pericarp, was found to be impermeable to many ions and unless it was damaged mechanically or by other means salt ions are excluded (28). The main reason for such property could be the presence of the waxy substance that coats the testa. The osmotic rather than toxic effect of salt was also indicated by the fact that germination was restored after the treated seeds were rinsed with distilled water and planted in the soil. This was an indication that germination under the conditions of the treatment was delayed rather than inhibited. However, under field conditions further delay in germination may cause seeds to dessicate.

The accumulation of ions in both lines was concentrated in the lemma and palea of the seed. Seeds of the sensitive line accumulated approximately three times more sodium and two times more chloride ions than seeds of the tolerant line. This may indicate that constituents of the lemma and palea of the tolerant line show low permeability to sodium and chloride ions. The large accumulation of ions in the seeds of the sensitive line caused the osmotic potential to rise and consequently more water was taken up so that water potentials between the seed and the medium came to an equilibrium. As for the seeds of the tolerant line, the accumulation of ions was far less, yet they were able to take up moisture and the process of germination proceeded. This ability to

absorb water in spite of high osmotic pressure in the lemma and palea may indicate the presence of high concentrations of organic solute in the cells.

The above discussion was based on measurements made on the seeds immediately after 48 hours of soaking in salt solutions. Measurements beyond this period were not made except that the tolerant line exhibited much greater ability to germinate under high salt concentration than the sensitive line. Any discussion of what happens beyond the 48-hour period would be mainly speculation.

During the 6-day period, i.e., after the seeds were blotted and allowed to germinate, the water potentials in the seeds were assumed to be in equilibrium. During this process water movements will be by osmosis. The testa in the seeds of both the sensitive and the tolerant lines acts as a barrier to ions. Water mobility across the testa would be determined according to concentration gradient. The fact that the seeds of the tolerant line showed signs of germination during the 6-day period is an evidence of water mobility across the testa and the capacity of the embryonic and endospermal cells to swell and maintain turgidity. To do so, these cells must contain high concentrations of organic solute. In other words, seeds of the tolerant line rely mainly on organic solutes for osmotic adjustment. Accordingly, the failure of seeds of sensitive lines to germinate may

have been due to the low organic solute concentration in the cells. In effect, water mobility was limited and cells failed to expand and become turgid (see Figures 11 and 12).

In conclusion, the mechanism of salt tolerance at the germination stage may involve the following factors: (1) low permeability of the lemma or palea to sodium and chloride ions, and (2) the presence of high concentrations of organic solutes in the embryonic and endospermal cells. Organic compounds may have been deposited during the filling period.

If this were the case, selection for high tolerance was probably in favor of those seeds with lemma and palea that show low permeability to ions and cells with high concentrations of organic solutes.

## II. Salt tolerance transfer attempt from spring to winter barley:

There is some indication that tolerance to salt during the germination stage can be transferred from spring barley to winter barley, at least in a short period of time. However, certain genotypic combinations have resulted in an increase in tolerance but not large enough to be compared with California Mariout's performance. The only promising combinations were those in lines 10-21-5W, 17-21-5W and 10-17-5W. These lines showed average increases in germination percentage of

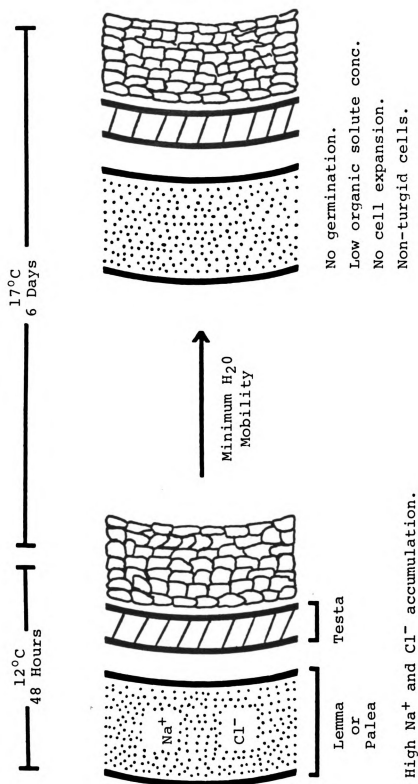


Figure 11. Diagrams showing ionic state in a seed of the sensitive line after 48 hours of soaking in salt solution, and 6 days later, respectively.

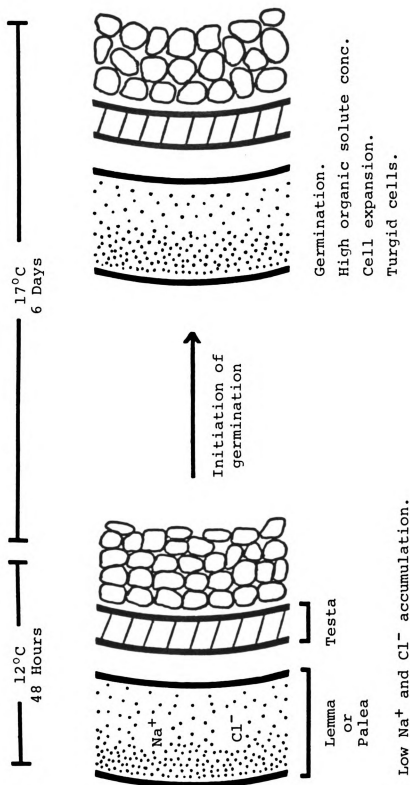


Figure 12. Diagrams showing ionic state and cell expansion in a seed of the tolerant line after 48 hours of soaking in salt solution, and 6 days later, respectively.

16.0, 13.3 and 6.6, respectively, from the original  $F_3$  populations (i.e., 10-21, 17-21 and 10-17). Note that the parents involved in these crosses, i.e., parents number 10, 17 and 21, have the same genetic background since they came from one multiway cross. Other lines, although some showed high performance when crossed with California Mariout in the  $F_2$  generation, tended to regress to their parental values after being selected according to winter habit.

Generally, all isolated winter habit populations (G4) showed dramatic reduction in performance compared to the populations from which they were derived. This may be an indication of a weak linkage between sensitivity to salt and winter habit. It is important, however, to mention that part of this setback may have been due to the fact that the salt test was done on the  $F_3$  populations. In such populations the frequency of the heterozygotes under selfing has been reduced to 25% in contrast to 100% in the  $F_1$  populations. Thus, dominance effects are expected to decrease in the  $F_3$  populations.

This study showed that tolerance of a given population to salt stress at the germination stage is related to a certain combination of genotypes and their genetic contribution. For instance, all intercrosses between lines 10, 17, 21 and California Mariout showed certain amounts of increase in tolerance to salt. Although these lines, i.e., 10, 17 and 21, share most of their genes in

common, they contain an extra dose of genes from Dicktoo in contrast with line 7. When Dicktoo was substituted with another H. vulgare and Elymus (as in line number 43), the outcome did not show any improvement in the final group, although the mean was high in G<sub>3</sub>. Therefore, the best combinations of genes for tolerance to salt were obtained from crosses involving lines 10, 17 and 21 with California Mariout. However, the maximum improvement was obtained from a cross between lines 10, 21 and California Mariout.

In this study, reaction to salt was found to be correlated significantly with seed weight, seed volume and seed density. Both seed weight and seed volume were positively correlated with salt tolerance. Seed density, on the other hand, was negatively correlated with salt tolerance. The significant correlation with seed density may be attributed, in part, to the dramatic increase in this factor in group 2 plants. It is obvious that this increase in density was due mainly to a decrease in seed volume as indicated by values given in Table 16. This variation in seed volume may be attributed, in part, to the fact that these populations were in the third generation of selfing where segregation was still taking place for parental types which contained wild germplasm. Part of this variation may be attributed to the greenhouse effect since all seeds of all groups except the parents were produced in the greenhouse. This effect

seems very small, if any, since such large variation in seed volume was not consistent in the following groups. In addition, seed weight in group 2 did not seem to vary as the values of this characteristic were within the parental range.

When California Mariout was incorporated in group 2 genotypes,  $F_2$  seeds showed an increase in volume along with an increase in tolerance. However, when plants with winter habit were selected from group 3, seed volume tended to decrease once again. This and the other facts mentioned above may suggest a linkage between reaction to salt and seed volume. Similar behavior was observed for seed weight in groups 3 and 4. This may have contributed to the strong positive association between the two characteristics.

## SUMMARY AND CONCLUSIONS

This study acquired information on the possibility of breeding for salt tolerance in barley during the germination stage. Selection for low and high tolerance to salt was practiced for two cycles preceded by crossings among genotypes. It also revealed information about a possible association between this characteristic and other agronomic characteristics. Information on the mechanism of salt tolerance at that stage was provided. The possibility of transferring salt tolerance during germination from spring barley to a heterogeneous population of winter barley was also discussed. Further knowledge about the inheritance of this characteristic in these populations was acquired.

Evaluation of the populations under investigation was based on a screening test at the germination stage using a concentration of 20,000 ppm NaCl. The test was carried out in a growth chamber using 15 x 1.7 cm test tubes and 25 seeds of each genotype. The criterion of germination was based on root growth.

According to previous work, salt tolerance in the most tolerant barley variety, California Mariout, appeared to be controlled by recessive genes. Further knowledge about the genetics of salt tolerance was provided when California

Mariout was crossed with  $F_3$  populations of intercrosses among a number of heterogeneous barley lines. These lines were previously obtained from Dr. A.B. Schooler of North Dakota State University in 1976. Each of these lines were in an early generation of segregation from crosses involving more than three genotypes. According to crosses between California Mariout and these lines and other spring barleys, two main types of gene action were inferred. A transgressive effect for sensitivity type of gene action was exhibited when it was crossed with the moderately tolerant lines 2 and 3. When it was crossed with the sensitive lines, the type of gene action varied from partial dominance for sensitivity to partial dominance for tolerance with various degrees of dominance within that range. Lines which showed partial dominance for sensitivity with California Mariout were 1, 4, 7-10, 7-17 and 10-17, while those that showed partial dominance for tolerance were 7-21, 7-43, 10-21 and 17-21. The possible explanation for the transgressive effects for sensitivity is that certain genetic interactions may have occurred when two genotypes or more were combined which produced types that favored sensitivity to salt. As for the partial dominance for sensitivity, it seemed that the action of California Mariout's genes were overridden by the action of genes of the genotypes in question. The reverse is, then, true for the case of partial dominance for tolerance.

Selection was based upon selecting 9% of the population at a salt concentration of 20,000 ppm NaCl in the first cycle of selection. For the selection for high tolerance, the 9% represented seeds with superior root growth, while in the selections for low tolerance it represented non-germinating seeds. The non-germinated seeds were, later on, rinsed with distilled water and planted in soil to germinate and grow to produce seeds. In the second cycle of selection, the same criteria were used except that the salt concentration was increased to 24,000 ppm NaCl for selections for high tolerance, whereas it was lowered to 16,000 ppm NaCl for selection for low tolerance. Crossing among populations used in this study preceded each selection cycle. Four F<sub>3</sub> populations of crosses involving five parents were chosen for the study. Two of these populations were used in the selection for low tolerance and the other two were used in the selection for high tolerance.

It was possible to decrease and increase tolerance to salt at the germination stage through crossing and selection. The overall gain in the germination percentage from selection for low tolerance was -12.7%. The overall gain in higher tolerance, however, was +17.4%. Note that only 7.4% out of this figure was gained from selection and that 10% was obtained by crossing the two selections from the first cycle of selection.

In these populations of spring barley, there was no association found between tolerance or sensitivity to salt

and seed weight, seed volume and seed density. This was indicated by the non-significance of the correlation coefficients which were based on 8 degrees of freedom.

Associations between tolerance or sensitivity to salt with some agronomic characteristics at the mature stage were also studied. These characteristics were type of spike (2-row or 6-row), number of tillers per plant, plant height, days to flowering, filling period and length of growing season. There seemed to exist a strong association between a 2-row type of spike and both sensitivity and tolerance to salt. However, such associations were suspect for the following reasons: (1) the abundance of this head type in the population studied, (2) the 2-row is dominant over the 6-row type, (3) systematic bias may have played a role in increasing the 2-row type during the crossing since plants were randomly chosen for this step, and (4) and most important, is that the most tolerant line (California Mariout) is of 6-row type and that this type is also a property of line 4 (Orge Saida 183), the most sensitive to salt.

Among other characteristics, only plant height was found to correlate with sensitivity to salt. This was indicated by the significant value of the correlation coefficient ( $r$ ) at  $P < .05$ . The possible explanation for this association could have been the fact that selection for low tolerance was in favor of choosing types that are similar to parent number 4 (Orge Saida 183), the most

sensitive to salt. This was also true for most of the other characteristics since the product of the final selection cycle resembled this parent in many ways.

An attempt was made to identify the mechanism of salt tolerance at the germination stage. Three tests were conducted; these were an imbibition test, a conductivity test and the microprobe analysis. The results from these tests provided evidence that the sensitive line accumulated more salts in the lemma or palea than the tolerant line.

The effect of salts on seed germination appeared to be mainly osmotic rather than toxic. This was indicated by the fact that ions failed to penetrate the testa in both the sensitive and the tolerant lines. It was also indicated by the evidence that germination was restored after rinsing the salt-treated seeds with distilled water and planting them in the soil. It is postulated then that sodium chloride delays rather than inhibits seed germination. Under field conditions, however, further delay may cause seeds to dessicate.

A model to explain the mechanism of salt tolerance during germination was presented. In this model, it was suggested that the lemma or palea (due to their constituents) of the tolerant line showed low permeability to sodium and chloride ions, a property that did not exist in the sensitive line. The fact that seeds of the tolerant line germinated under high salt concentration is one evidence of sufficient moisture uptake for the cells to expand and

maintain turgidity during the germination process. In order for this to occur, the cells must contain a high concentration of organic solute. Accordingly, the failure of the sensitive line to germinate under salt treatment could be due to low organic solute concentration in addition to higher permeability of the lemma or palea of the seeds to sodium and chloride ions. It is concluded, then, that tolerance to salt during germination may involve (1) low permeability of the lemma or palea to sodium and chloride ions and (2) the presence of high concentrations of organic solutes in the embryonic and endospermal cells.

There was little indication that salt tolerance at the germination stage could be transferred from spring barley to winter barley at least in a relatively short time. The  $F_2$  seeds of crosses between the spring barley, California Mariout, and seven heterogeneous populations of winter barley showed a considerable amount of improvement in salt tolerance. However, when plants with winter habit were selected from the  $F_2$  populations, tolerance declined in many lines and approached that in the original populations. Some improvement in tolerance was observed in certain combinations. The only promising combinations were those that involved lines 10, 17, 21 and California Mariout. However, the maximum improvement was obtained from a cross involving lines 10, 21 and California Mariout with an increase of 16.0% in germination percentage under salt treatment. Note that these parents, i.e., 10, 17 and 21, had the same genetic

background since they came from one multiway cross. This argument had led to the speculation that a weak association may exist between sensitivity to salt and winter habit.

In these populations of winter barley, reaction to salt was found to correlate significantly with seed weight, seed volume and seed density. Both seed weight and seed volume were positively correlated with salt tolerance. Seed density, on the other hand, was negatively correlated with salt tolerance. Seed volume was found to show the least consistency in all populations and especially in group 2 which represented  $F_3$  populations of crosses among heterogeneous winter barleys. These were still segregating for parental types which contained wild germplasm. This may have contributed to the significant correlation between the two characteristics. The inconsistency of seed volume throughout this study, with a little change in seed weight, has led seed density to vary accordingly.

The present work suggests a linkage between salt tolerance and seed weight and seed volume at least in the winter barley populations and under the environment in which the work was done, although no correlation was found between these characteristics and reaction to salt in the spring barley.

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