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SALT TOLERANCE OF ATRIPLEX NUMMULARIA

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ABSTRACT

UCHIYAMA, Y. 1987 Salt tolerance of *Atriplex nummularia*

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With a view to introducing *Atriplex nummularia* in the arid lands of developing countries, the author studied the growth habit of the plant under high salinity conditions along with the physiological characteristics of the plant. Although the salt tolerance of *Atriplex nummularia* at the germination and seedling establishment stages was not particularly high compared with that of other crops, the seeds remained dormant under the high saline environment and sprouted after the high saline conditions were removed. Adult *Atriplex nummularia* plants exhibited so high salt tolerance that they were able to survive in a saline medium containing 5% NaCl. The vesiculated hairs excreting excess NaCl from the lamina were considered to play an important role in the salt tolerance of *Atriplex nummularia*. The plants grown at a daily mean temperature of 25°C showed the optimum growth and the plants grown at the temperature of 25°C with 1% of NaCl showed a growth pattern and yield similar to those of the plants grown at high and low temperatures without NaCl addition in the culture solution. Dry weight of leaves and stems increased almost in parallel to the increase of the concentration of N, P and K in the culture solution. Growth and yield of the plants growing at higher levels of nutrient along with the 1% NaCl treatment were significantly higher than those of the plants growing at lower levels of nutrient without NaCl treatment. The seeds produced in the plants growing in a higher salinity culture medium showed a higher salt tolerance at the germination stage than the seeds produced in a low salinity culture medium. Feeding value of *Atriplex nummularia* which had been growing under suitable environmental conditions, was equal or superior to that of alfalfa at the flowering stage. On the basis of the results obtained, the mechanism of the high salt tolerance of *Atriplex nummularia*, as well as the methods for improving the growth of the plant on high salinity soils was discussed.

Index words: atriplex, bladder cell, saltbush, salt tolerance, vesiculated hairs

CONTENTS

I. Introduction

The genus *Atriplex* comprises more than 200 species worldwide. Most of the *Atriplex* species are found in arid and saline habitats. They are highly salt-tolerant, and many species are perennial shrubs that remain green all the year round. They make useful forage in arid zones of the world, for example, in Australia and the United States of America, and they are being introduced to the developing countries in arid zones (NATIONAL ACADEMY OF SCIENCES, 1975). *Atriplex nummularia,* one species of salt-bushes, has a high adaptability to the soil conditions and grows well in deep soils with only 150—250 mm annual rainfall.

Atriplex nummularia is one of the most palatable among the *Atriplex* and it has a high protein content. Many studies on the production and the agricultural use of *Atriplex nummularia* have been carried out in Australia.

The environmental conditions such as drought, soil salinity, higher temperature, in the Middle East and North Africa, etc. where agricultural development is a prerequisite are more severe than in Australia.

It is necessary to analyse the physiological and ecological characteristics of *Atriplex nummularia,* especially the salt tolerance and drought resistance of the plant under severe environmental conditions, for the introduction of this plant into the undeveloped arid zones of the Middle East and North Africa, etc.

The author studied the mechanism of salt tolerance of *Atriplex nummularia* and the growth behavior of the plant under a saline environment, in order to develop a technology for introducing the plant into undeveloped arid zones worldwide.

The saline soils in the arid zone generally contain a high concentration of Na, Mg, Ca, Cl and SO_4, etc. : Na content of soil often amounts to 300 me/l, and Na often accounts for more than 50% of the total cation contents of soil (TADANO, 1983) where most of the crops in the glycophyte group suffer considerable damage.

In this paper, salt tolerance refers to the tolerance to NaCl. The main reasons for using NaCl for the salt tolerance studies are as follows: NaCl problems play a major role in saline soils, some *Atriplex* species have a special attraction to Na and Cl ions (see IV-2), and Na is the essential micronutrient element for *Atriplex* (BROWNELL, 1957, 1968; BROWNELL and CROSSLAND, 1972; OSMOND, 1970).

It is hoped that the results obtained in this study will contribute to the agricultural development of the arid zone in enabling the cultivation of *Atriplex* as a forage crop on saline soil. These results may also pave the way for the development of new salt-tolerant crops in future.

The author carried out these studies mainly at the Tropical Agriculture Research Center (TARC) in Tsukuba, Japan from 1981 to 1984 and some experiments were carried out in the laboratories of the National Institute of Agricultural Sciences, which was reorganized as the National Institute of Agrobiological Resources and the National Institute of Agro-Environmental Sciences in December 1983, with the necessary facilities and instruments to complete the studies.

II. Synopsis on the known characteristics of *Atriplex nummularia*

1. Systematic and geographical status

Atriplex nummularia Lindl. is one of the species of the genus *Atriplex*.

Atriplex is a large and widely distributed genus of the Chenopodiaceae family. The genus is said to comprise about 200 species (KELLEY et al., 1982) or about 400 species (SCHIRMER and BRECKLE, 1982). OSMOND et al. (1980) listed 247 species of *Atriplex* after analysing many publications. They stated also that the members of the genus *Atriplex* were established in all the continents and many islands, and that their distribution ranged from 70° N to 46° S, with the major regions including South America (73 species), North America (59 species) and Australia (59 species). The coastal weeds, *Atriplex subcordata* Kitagawa and *Atriplex gmelinii* C. A. Mey, are known to occur in Japan (OHWI, 1973).

The genus *Atriplex* comprises salt tolerant species which are called "saltbushes". The chenopod shrublands, including saltbushes and bluebushes (*Maireana,* Syn. *Kochia*) which cover about 500 thousands km^2 and account for 6.5% of Australia's land area, are used for rangelands (GRAETZ and WILSON, 1984).

Above all *Atriplex nummularia* has played a major role as a forage plant with *Atriplex vesicaria* in Australia (LEIGH, 1972). *Atriplex nummularia* is called "Oldman saltbush" or "Giant saltbush" in English.

2. Morphological characteristics

Atriplex nummularia is a perennial shrub that remains green all the year round and grows to a height and diameter of about 3m with very brittle woody rootstocks and stems. The alternate bluish-grey leaves covered with a scaly-white layer, measure up to 5~6cm across and are roughly oval in shape; their margins are irregular serrate or entire. The flowering season of the plant is about May to June in Tsukuba, Japan. LEIGH (1972) mentioned that the plants were usually unisexual. However, in the experimental materials which originated from the seeds introduced from CSIRO, Australia, diclinism was observed and female flowers were found on the basal part of the male inflorescence. The pistil bifurcates and the stigma protrudes out of the perianth. The male flower has five stamens (Figs. 1-4 and 1-5). The fruiting body consists of two flat paper-like bracteoles, hemispherical in outline, pressed closely together but united only at the base, where the seed is enclosed, the whole structure being 5~8mm across (Fig. 1-3). The seed is very small spheroidal, about 1.5mm in diameter. Seeds with an air-dry weight of more than 1mg retain their viability for 5 years after harvesting if they are preserved in a dry environment, but seeds less than 0.5mg in air-dry weight are hardly viable (UCHIYAMA, 1981).

Fig. 1 − 1

Fig. 1 − 2

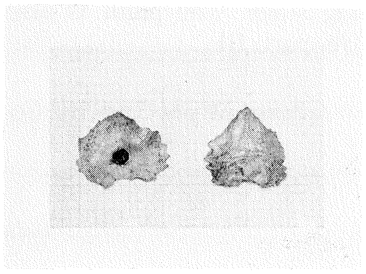

Fig. 1 − 3

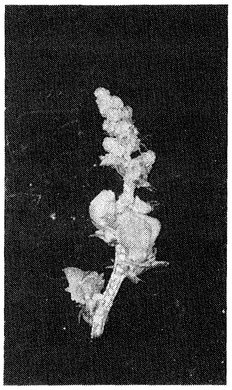

Fig. 1 − 4

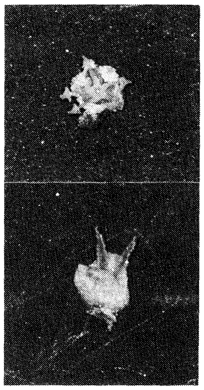

Fig. 1 − 5

Fig. 1. Morphology of *Atriplex nummularia*.
Fig. 1-1. Growing plant in the field.
Fig. 1-2. Fruits on the plant.
Fig. 1-3. Seed with bracteole.
Fig. 1-4. Branch bearing male and female flowers.
Fig. 1-5. Male flower with five stamens (above) and female flower with bifurcated stigma of pistil (below).

3. Ecophysiological characteristics

The seeds of *Atriplex nummularia* are generally sown with the bracteoles because it is not easy to remove the bracteole from the seed. Germination percentage of the seed enclosed in the bracteole is generally low. When the seeds with the bracteoles are leached with water the germination percentage rises because water soluble salts inhibiting seed germination are removed (JONES, 1968 ; UCHIYAMA, 1981 ; WILLIAMS, 1963a). Germination percentage of naked seeds more than 1mg in air-dry weight which is usually 90~100% at temperatures ranging from 4 to 22.5°C, decreases remarkably with the increase of the temperature above 25°C (UCHIYAMA, 1984), and most of the germinated seeds die a few days after germination at temperatures above 30°C (SHARMA, 1956; UCHIYAMA, 1981). Beadle (1952) reported that the optimum temperature for *Atriplex nummularia* ranged from 20 to 25°C. However it is considered that the optimum temperature actually is ranges from 10 to 20°C, and that a temperature of about 15°C is best based on the authors results (UCHIYAMA, 1981, 1984).

It has been reported that the *Atriplex* can tolerate temperatures as low as -10° to -12°C (NATIONAL ACADEMY of SCIENCES, 1975). About 100 *Atriplex nummularia* plants grew in the field of TARC in Tsukuba which is situated at 140° 02′ E and 36° 04′ N and they survived the winter of 1982 and 1983 but all of them died in the winter of 1984. Average minimum temperatures of January and February and minimum temperature of each year were -2.0°C, -2.1°C and -8.0°C in 1982, -2.9°C, -2.1°C and -9.3°C in 1983 and -5.1°C, -4.5°C and -12.3°C in 1984, respectively.

Although *Atriplex nummularia* prefers alkaline soils of moderate to heavy texture, it frequently occurs on sand dunes and, if planted, will grow vigorously on any soil type—alkaline, acid, sand, or clay (LEIGH and MULHAM, 1965). *Atriplex nummularia* is extremely resistant to drought, even at the seedling stage. The plant is also salt-tolerant. The level of salt tolerance is described in part III.

III. Salt tolerance level of *Atriplex nummularia*

There are many agronomic studies on the growth of *Atriplex nummularia* on saline soils. However the author did not find any reports which stated explicitly the level of salt tolerance of the plant. On the other hand it is considered that the salt tolerance of a plant varies with the growing stages.

Then the author evaluated the level of salt tolerance of *Atriplex nummularia* at the seed germination stage, seedling establishment stage and adult growing stage, respectively.

1. Effect of sodium chloride (NaCl) concentration on seed germination and seedling establishment

The level of salt tolerance at the seed germination and seedling establishment stages are the limiting factors for the introduction of a plant to saline soil because it is considered that the tolerance level at the seed germination stage is generally lower than that at the adult growing stage and that the seedling establishment stage is the most susceptible to environmental conditions.

Although there are many reports on salt tolerance at the seed germination stage and at the seedling establishment stage of halophytes including *Atriplex* species as reviewed by UNGAR (1978, 1982), few data are available for *Atriplex nummularia*. The effect of the NaCl concentration on the seed germination and seedling establishment was analysed in this section.

Materials and methods

The seeds were produced in New South Wales, Australia in 1977 and were introduced to TARC in Tsukuba, Japan in May 1979. The seeds were stored under dry conditions at 5°C and they were used for the experiments. Germination percentage of the seeds at temperatures ranging from 15°C to 20°C did not decrease at all during the 2 years' storage. Naked seeds (UCHIYAMA, 1981) more than 0.8mg in air-dry weight were used in the experiments. Germination was tested in petri dishes 9cm in diameter containing two sheets of Toyo No.2 filter paper and moistened with 3.5ml of the test solution. Four replications of each treatment were carried out with 20 seeds per dish.

Germination was considered to have taken place when the radicle had penetrated through the seed coat. The germination beds including petri dishes, filter papers and the test solution were changed every day. Na, K, Mg and Ca were extracted with 1*N* HCl from the bracteoles and their concentrations were measured with the atomic absorption spectrophotometer, Shimazu AA-650. Cl was extracted with water and its concentration was measured with the ionmeter, Orion 901.

1) Effect of NaCl concentration on seed germination

In the first experiment, the germination beds were kept in the incubator at 15°C. NaCl concentrations of the test solutions were 0.25, 0.5, 0.75, 1.0, 1.5 and 2.0%, respectively. The experiment was continued for 21 days.

In the second experiment, NaCl concentrations of the test solutions were 0.5, 1.0 and 1.5%, respectively and temperatures were 10, 20 and 30°C, respectively for each

NaCl concentration. The experiment was continued for 21 days.

In the third experiment, non-germinated seeds subjected to the NaCl treatment (first treatment) were transferred to a germination bed with pure water in the incubator at 15°C (second treatment) and their viability was determined. NaCl concentrations were 1.0, 2.0 and 3.0% and temperatures were 10, 15 and 20°C, respectively in the first treatment. The viability was expressed as follows;

$$\frac{\text{number of germinated seeds in the second treatment}}{\text{number of non-germinated seeds in the first treatment}} \times 100$$

2) Effect of NaCl concentration on seedling establishment

The germination beds were kept in the incubator at 10, 15, 20°C. NaCl concentrations of the test solutions were 1.0, 2.0 and 3.0%, respectively. After the germination percentage was measured for 21 days, the seedlings were maintained in the germination beds for the same treatment for 14 days and the number of seedlings which survived was determined.

Results

1) Effect of NaCl concentration on seed germination

Effect of the NaCl concentration on the germination percentage and the mean number of days to germinate at 15°C is presented in Table 1. The 0.25% NaCl treatment did not affect the germination. There was no significant difference in the

Table 1. Effect of NaCl concentration on the seed* germination of *Atriplex nummularia* at 15°C.

Articles	NaCl concentration of medium, %						
	0	0.25	0.5	0.75	1.0	1.5	2.0
Germination percentage**	100	100	93 ± 7	93 ± 5	78 ± 15	63 ± 8	25 ± 15
Mean no. of days to germinate	4.9	4.1	6.0	5.9	5.6	7.4	8.2

* Naked seeds showing normal appearance with a weight heavier than 0.8mg each were used.
** Measured on 21st day after the beginning of seed soaking.

germination percentage between the pure water and 0.75% NaCl treatments although the mean number of days to germinate of the latter was slightly longer than that of the former. When the NaCl concentration exceeded 1.0% the germination percentage decreased gradually to a value of about 25%.

The effect of the NaCl concentration and temperature on the germination percentage is presented in Table 2. At temperatures ranging from 10°C to 30°C, the germination percentage decreased more remarkably at higher temperatures when the concentration of NaCl in the solution increased.

Table 2. Effect of NaCl concentration on the seed* germination of *Atriplex nummularia* at different temperatures.

Articles	Temperature °C	NaCl concentration of medium, % 0	0.5	1.0	1.5
Germination percentage**	10	93 ± 5	93 ± 5	80 ± 0	73 ± 15
	20	100 ± 0	85 ± 13	70 ± 16	28 ± 15
	30	25 ± 13	13 ± 15	13 ± 5	5 ± 6
Index***	10	100	100	86	78
	20	100	85	70	28
	30	100	52	52	20

* & ** See notes of Table 1.
*** Index to 0% NaCl at each temperature.

The percentage of the seeds which did not germinate with the NaCl treatment (first treatment) and the percentage of germination shown by these seeds after their transfer from the NaCl solution to pure water (second treatment) are presented in Table 3.

Table 3. Percentage of *Atriplex nummularia* seeds* which did not germinate with NaCl treatment and percentage of germination shown by these seeds after NaCl solution was changed to pure water.

Temp. treatment °C	NaCl treatment %	Percentage of non-germinated seeds**	Germination percentage in pure water***
10	1.0	26± 7	17±14
	2.0	74±10	74±13
	3.0	95± 4	71± 9
15	1.0	20± 9	26±21
	2.0	72± 8	82± 8
	3.0	84± 5	84±11
20	1.0	25± 9	33±20
	2.0	79±12	86±12
	3.0	92± 3	79± 6

* & ** See notes of Table 1.
*** Measured on 10th day after the change to pure water bed.

Approximately 70% of the non-germinated seeds placed in the 2.0% and 3.0% NaCl solution in the first treatment germinated at 10°C in the second treatment. A percentage of 80—85% of the non-germinated seeds placed in the 2.0% and 3.0% NaCl solution in the first treatment germinated at 15°C and 20°C, respectively in the second treatment. Only 20—30% of the non-germinated seeds placed in the 1.0% NaCl solution in the first treatment germinated at temperatures ranging from 10°C to 20°C in the second treatment.

Contents of bases and chlorine in the bracteole are presented in Table 4. The bracteole contained 4.90% of Na and 2.30% of K on a dry weight basis and about 90% of these minerals were water soluble. The content of Mg and Ca was low and that of water soluble Mg and Ca was less than half and less than 5% of the total, respectively. The bracteole contained also 9.91% of water soluble Cl.

Table 4. Contents of bases and chlorine in bracteoles of *Atriplex nummularia* seeds.

Method of samples extraction	Content (% on dry weight basis) of					
	Na	K	Mg	Ca	Total	Cl
1*N* HCl extraction	4.90	2.30	0.50	0.48	8.18	
Water extraction	4.42	2.05	0.22	0.02	6.71	9.91

2) Effect of NaCl concentration on seedling establishment

Effect of NaCl concentration on the seed germination and seedling establishment at different temperatures is presented in Table 5. All the seeds which had germinated became seedlings with the pure water treatment at temperatures ranging from 10°C to 20°C. About 35% of the germinated seeds at 15°C and about 75% of the germinated seeds at 10°C became seedlings, respectively when the concentration of NaCl was 1% and 2%. However all the germinated seeds died a few days after germination when the concentration of the NaCl solution exceeded 1% at 20°C and when the concentration of the NaCl solution was 3% at 15°C and 10°C.

Table 5. Effect of NaCl concentration on the seed* germination and seedling establishment of *Atriplex nummularia* at different temperatures.

Articles	Temperature °C	NaCl concentration of medium, % 0	1.0	2.0	3.0
Germination	10	96±5	74± 9	26±10	5±4
percentage**	15	96±3	80±11	28±10	16±6
(A)	20	98±5	75±11	21±14	8±3
Establishment***	10	96±5	56±10	19± 3	0
(B)	15	96±3	30±10	10± 4	0
	20	98±5	0	0	0
Index	10	100	76	73	0
$\frac{(B)}{(A)} \times 100$	15	100	38	36	0
	20	100	0	0	0

* & ** See notes of Table 1.

*** Measured on 35th day after the beginning of seed soaking.

Discussion

The germination percentage of the seeds of *Atriplex nummularia* was only 25—30% when they were placed in 2% NaCl solution at the temperature of 15°C which is considered to be the optimum temperature for their germination. Although in many instances rice and wheat seeds scarcely germinated in the 2.0% NaCl solution (IWAKI and OTA, 1952; NODA and HAYASHI, 1961; OTA and OGO, 1953), in a few reports the germination percentage of paddy rice was 56% in a 2.18% NaCl solution at 25°C (MASUBUCHI, 1927) or that the germination percentage of brown rice was 72% in a 3.0% NaCl solution at 30°C (NODA and HAYASHI, 1961).

The wheat cultivar "Shirasagi komugi" showed a germination percentage of 98% in a 2.0% of NaCl solution at 20°C (UCHIYAMA, unpublished). Thus it is difficult to consider that the salt tolerance of *Atriplex nummularia* at the germination stage was higher than that of other crops like rice and wheat judging from these results.

Although the salt tolerance of *Atriplex nummularia* at the seedling establishment stage was lower than that at the germination stage, about 35% and 75% of the germinated seeds continued to grow in 2.0% NaCl solution at temperatures of 15°C and 10°C, respectively. However the brown rice mentioned above which germinated in 3.0% NaCl solution died soon after and the wheat cultivar showing a germination percentage of 98% in 2.0% NaCl solution also died at the seedling establishment stage. Thus it can be concluded that the salt tolerance of *Atriplex nummularia* at the seedling establishment stage is higher than that of other crops.

Although *Atriplex halimus* and some halophyte seeds did not germinate in the NaCl solution at a high concentration, they did not lose their viability and they germinated when they were transferred from the NaCl solution to pure water (BREEN et al., 1977; OKUSANYA, 1977; UNGAR, 1977; Zid and BOURKHRIS, 1977). *Atriplex nummularia* in this experiment showed the same tendency.

The seeds of some halophytes remain dormant in the soil at high salt concentration and they germinate when rainfall reduces the salt concentration of the surface soils (UNGAR, 1962, 1974, 1982; WARD, 1967; WILLIAMS and UNGAR, 1972). UNGAR (1982) mentioned that the period of seedling establishment is probably one of the most sensitive periods in the life cycle of halophytes. He also stated that the delay in the time of germination until the stress associated soil salinity decreased, prevented the seedlings from dying immediately and provided them with some chance of surviving to maturity.

It was reported that adult plants were more salt tolerant than seeds (MILINGTON et al., 1951; MAYER and POLJAKOFF-MAYBER, 1963). UNGAR (1982) reported that an extremely high salt tolerance in terms of germination of seeds could become deleterious at later stages of development, and seeds may have been selected for germination at reduced salinity to allow for the survival of the developing seedlings. Salt tolerance of *Atriplex nummularia* at the germination stage was lower than that of the adult plant, enabling the plant to adapt to a saline environment.

The bracteole contained 4.42% of water-soluble Na, 2.30% of water-soluble K and 9.91% of water-soluble Cl on a dry weight basis.

If it is assumed that all the water-soluble Na contained in the bracteole is combined with Cl, the bracteole may contain 11.23% of NaCl. The critical concentration of NaCl for the survival of adult *Atriplex nummularia* will be about 5% (Refer Part III-2). The content of water soluble NaCl of the bracteole is undoubtedly higher than the critical concentration of NaCl and is enough to inhibit the germination. When rain leaches salt in the surface soils, water soluble salts in the bracteole containing the seed in the soil will also be leached. On the other hand when the NaCl concentration of the bracteole decreases enough to make the seed germinate by rain, the soil salinity also will have thoroughly decreased. A high content of salt in the bracteole will not be a disadvantage for the propagation of the species, but will be one of the important mechanisms of the species to adapt to a saline environment.

Although the mechanism of the interaction of salinity and temperature on germination is not clearly understood, various hypotheses have been put forward; the increase of the temperature increases the activity of ions resulting in a high degree of dissociation of the salt (AHI and POWER, 1938); high temperature promotes a rapid ion uptake and this way causes an apparent increase in specific ion toxicity or osmotic inhibition of the enzymatic processes (UNGAR, 1982).

Many authors have reported that high temperatures reduce seed germination (FRANÇOIS and GOODIN, 1972; MAHMOUD and HILL, 1980; TADMOR et al., 1969; UNGAR, 1978 and 1982). On the other hand some reports have shown that there is an optimum temperature peculiar to the species and that the germination rate is higher at the optimum temperature than at higher and lower temperatures regardless of the osmotic concentrations (KHAN and UNGAR, 1984; RIVERS and WEBER, 1971; SPRINGFIELD, 1966; TADMOR et al., 1969; UNGAR, 1978 and 1982; UNGAR and HOGAN, 1970).

It was observed in this experiment that the higher the temperature of the saline germination bed, the lower the germination percentage of *Atriplex nummularia*. However in further studies the interaction between temperature and salinity for seed germination of the species should be considered.

The present results show that 50 to 30% of the seeds sown will become established under 1.0% concentration of the NaCl of soil solution and at the daily mean temperature ranging from 10 to 15°C.

Summary

The purpose of this study was to evaluate the salt tolerance of *Atriplex nummularia* at the stages of seed germination and seedling establishment under different NaCl concentrations and at various temperatures. The results obtained are as follows:

1. Germination percentage of seeds placed in 2% and 3% NaCl solutions was only about 25% and 16%, respectively at 15°C, although almost no difference in the germination percentage was observed between the 0.75% NaCl and pure water treatment at 15°C. At temperatures ranging from 10°C to 30°C, the germination percentage decreased more remarkably at higher temperatures when the concentration of NaCl in the solution increased.

2. Salt tolerance at the seedling establishment stage was lower than that at the germination stage; all the seeds which had germinated died a few days after germination when the concentration of the NaCl solution exceeded 1% at the temperature of 20°C and when the concentration of the NaCl solution was 3% at 15°C and 10°C. However about 35% of the germinated seeds at 15°C and about 75% of the germinated seeds at 10°C became seedlings, respectively when the concentration of NaCl was 1% and 2%.

3. Non-germinated seeds were maintained in 2% and 3% NaCl solution for 21 days. Approximately 70% of these seeds germinated at 10°C and 80—85% of these seeds germinated at 15°C and 20°C, respectively, after being transferred into pure water.

4. The bracteole contained 4.90% of Na and 2.30% of K on a dry weight basis and about 90% of these minerals were water soluble. The bracteole contained also 9.91% of water soluble Cl.

On the basis of the results obtained, the adjustment mechanism of *Atriplex nummularia* to saline soil was discussed.

2. Effect of sodium chloride (NaCl) concentration on growth and yield

There are numerous reports on the physiological relationship between some plants of the genus *Atriplex* and NaCl as reviewed by KELLEY et al. (1982).

It is generally accepted that the growth of plant is stimulated by a small quantity of NaCl and inhibited by a large quantity of NaCl (BLUMENTHAL-GOLDSCHMIDT and POLJAKOFF-MAYBER, 1968; BROWNELL, 1965 and 1968; BROWNELL and CROSSLAND, 1972).

However reports on the salt tolerance of *Atriplex nummularia* are limited and basic information on introduction and establishment of the plant into virgin soil in the arid zone is insufficient.

Therefore the author analysed the growth and yield of *Atriplex nummularia* exposed to a high concentration of sodium chloride.

Materials and methods

The experimental plants were derived from a single plant growing in the greenhouse by using cuttings with the purpose of preparing uniform plants; the cutting which was about 5cm long and bare about 5 nodes was planted on a vermiculite medium without nutrient supply. After one month, the cutting was transplanted to a pot 15cm in diameter filled with vermiculite. The pots were placed in a shallow container containing culture solution at a depth of 2cm. The culture solution was absorbed through the bottom hole of the pot. The culture solution, a mixture of Otsuka House Compound No.1 and No.2 diluted by city water contained 100ppm nitrogen, 48ppm phosphate, 138 ppm potassium, 30ppm magnesium, 90ppm calcium and a few micronutrients. The city water contained naturally 25ppm of Na. Three weeks later the salinization treatment was initiated. Sodium chloride was added to the culture solution to reach NaCl a concentration of 1, 2, 3, 4, and 5%, respectively. Six plants were used in each treatment. Immediately before the NaCl treatment, the top of all the plants was cut off at 4cm above the surface of the culture medium and the regenerated plants were used for measurements 40 days after the beginning of the treatment.

The pots were leached weekly with the corresponding NaCl treatment solution respectively to prevent salt accumulation in the medium.

The experiment was carried out in a growth chamber equipped with six 400W "BOC lamps" (Mitsubishi MLBOC 400F-U, reflected type) as the light source. Photosynthetically active photon fluence rate at the upper level of the plants was adjusted to about 900 $\mu E\, m^{-2} s^{-1}$ (400—700nm) with LI-COR Quantum Sensor, LI-190S. Daylength was 12h, day and night temperatures were 28±1°C and 21±1°C, and the relative humidity was approximately 45—50%.

The photosynthetic rate was measured by the oxygen electrode method (SAKA and CHISAKA, 1981) using 15 pieces of disk like leaf sections 5mm in diameter for one experimental sample. The transpiration rate was measured by the LI-COR superporometer, LI-1600. Na, K, Ca and Mg were extracted with 1*N* HCl from the leaves and stems and were measured with the atomic absorption spectrophotometer, Hitachi 180-70. Cl was extracted with water and was measured with the ion-chromatography apparatus, Dionex 14.

Additional experiment: solution culture of *Atriplex nummularia*. The seedlings were transplanted into Wagner pots with a 500 cm^2 surface area which contained a nutrient solution and were kept continuously aerated. Two plants were placed in each pot. Nutrient solution was the same as that used in the above-mentioned experiment and the NaCl concentrations of the solution were 1, 2, 3, and 4% respectively. The pots were placed in a greenhouse. Temperature was kept at a minimum higher than 12°C in winter, but the upper limit could not be controlled in the summer when the temperature rose occasionally to more than 40°C. The water culture was continued for 2 years.

Results

Effect of NaCl concentration on the growth of *Atriplex nummularia* is presented in Fig. 2. There were significant differences between the plants treated with the lower NaCl concentration (0~1% NaCl) and those with the higher NaCl concentration (2~5%) 15 days after the beginning of the treatment. The difference between the treatment with NaCl concentrations of 0% and 1% was not observed until the 30th day. The differences among each treatment were clearly observed on the 40th day, and the higher the concentration of NaCl, the less satisfactory the growth of the plants. The dry weight of the leaves and stems 40 days after the beginning of the

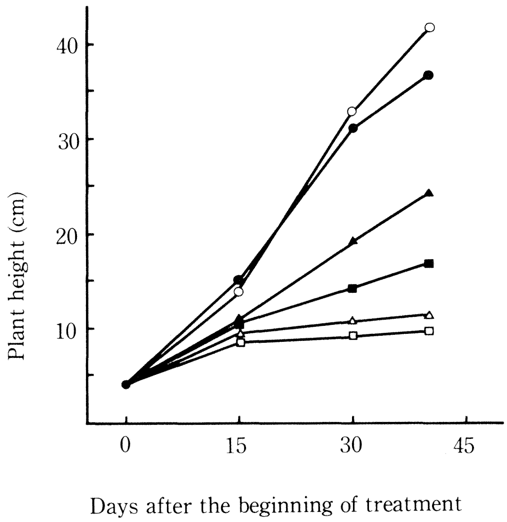

Fig. 2. Plant height of *Atriplex nummularia* grown under different NaCl concentrations.
Applicated NaCl concentration of culture solution;
○——○ 0%, ●——● 1%, ▲——▲ 2%,
■——■ 3%, △——△ 4%, □——□ 5%

treatment is presented in Table 6. The higher the concentration of NaCl, the lower the dry weight of the leaves and stems; the dry weight of the plants treated with NaCl 4% and 5% was about 20% and 10%, respectively that of the plant which were not subjected to the NaCl treatment. However the dry weight of the plants treated with NaCl 1% remained at a high level, about 80% of that of the untreated plants. Leaf area per plant and per leaf showed a similar tendency to the dry weight of leaves and stems.

Table 6. Growth and yield of *Atriplex nummularia* grown at different NaCl concentrations.

Treatment	Plant length cm	Dry weight of top g/plant	Dry matter content of top %	Leaf area dm²/plant	Leaf area cm²/leaf	Areal weight of leaf mg/cm²	Water cont. of leaf g/g DW
Control	4.18 [a]	22.8 [a]	19.4 [ab]	21.4 [a]	2.93 [a]	7.18 [e]	4.85 [ab]
NaCl 1%	31.1 [b]	18.5 [b]	17.7 [b]	16.3 [b]	2.79 [a]	7.88 [d]	5.51 [a]
NaCl 2%	19.1 [c]	12.7 [c]	19.9 [a]	8.9 [c]	2.40 [b]	10.99 [c]	4.48 [b]
NaCl 3%	14.3 [d]	8.8 [d]	21.2 [a]	5.3 [d]	1.78 [c]	12.91 [b]	4.08 [bc]
NaCl 4%	10.7 [e]	4.2 [e]	20.2 [a]	2.5 [e]	1.58 [cd]	13.78 [ad]	4.24 [bc]
NaCl 5%	9.2 [e]	1.9 [f]	21.0 [a]	1.1 [f]	1.25 [d]	14.35 [a]	3.98 [c]

Notes: Immediately before NaCl treatment, top of all plants was cut off at 4cm above the surface of culture media, and regenerated plants were used for measurements 40 days after the beginning of the treatment. Each figure indicates an average of 6 plants. Figures not bearing the same superscript within a column are significantly different at the 5% level.

The NaCl 4% and 5% treatments were continued for 1 year in the greenhouse after the examination. Although the growth was very slow, the stems and leaves were able to regenerate and all the plants treated with NaCl 4% survived more than 1 year. However 4 plants out of 6 plants treated with NaCl 5% died in the summer of 1984 when the maximum temperature in the greenhouse rose ranging from 40 to 45°C every day for 2 months.

Mineral contents of harvested leaf and stem are presented in Table 7. K content was high in the untreated plants and the Na content was remarkably high in the NaCl

Table 7. Mineral contents of harvested *Atriplex nummularia* (on dry weight basis %).

Treatment	Leaf Na	Leaf K	Leaf Ca	Leaf Mg	Leaf Cl	Leaf SO_4	Stem Na	Stem K	Stem Ca	Stem Mg	Stem Cl	Stem SO_4
Control	3.19	5.28	1.89	1.23	3.63	0.62	0.73	4.80	2.16	0.24	1.94	0.83
NaCl 1%	12.78	1.79	0.38	0.33	11.38	0.40	6.48	1.79	0.73	0.15	7.38	0.22
NaCl 2%	15.85	1.34	0.35	0.27	13.63	0.24	8.68	1.68	0.46	0.14	9.50	0.07
NaCl 3%	15.85	1.29	0.31	0.21	16.75	0.07	9.53	1.46	0.44	0.13	10.38	0.07
NaCl 4%	15.75	1.04	0.26	0.17	19.63	0.02	9.93	1.21	0.42	0.11	11.42	0.01
NaCl 5%	18.10	1.56	0.28	0.17	21.13		11.04	2.17	0.77	0.10	12.51	

treated plants. Mg and Ca contents were lower than the K and Na contents, particularly in the NaCl treated plants. Na and Mg contents of leaf were higher than those of stem, while the Ca content of leaf was lower than that of stem, and the K content was about the same in both organs.

Photosynthetic rate 31 to 33 days after the beginning of the treatment is presented in Fig. 3. The rate decreased in inverse proportion to the concentration of NaCl: the tendency was similar to that of the dry weight of the leaves and stems.

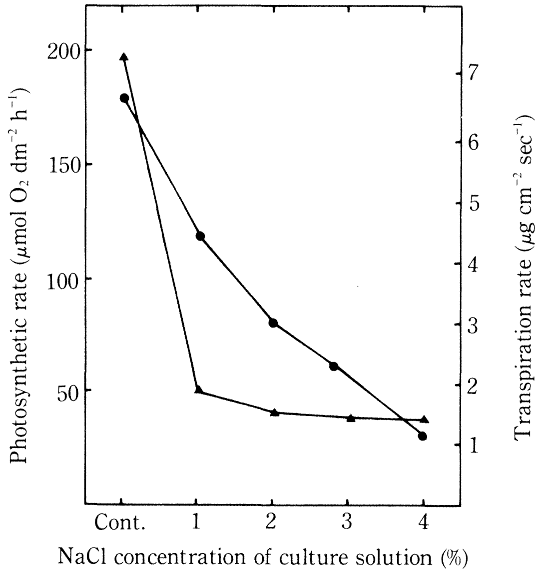

Fig. 3. Photosynthetic rate (●——●) and transpiration rate (▲——▲) of *Atriplex nummularia* growing at different NaCl concentrations. The measurements were carried out from 30 to 33 days after the beginning of the treatment.

Transpiration rate 30 days after the beginning of the treatment is presented in Fig. 3. The rate was affected by the NaCl treatment: the rate in the plants treated with 1% NaCl decreased to a quarter of that of the untreated plants and there was hardly any difference between the rate in the plants treated with NaCl 1% and that in the plants subjected to higher concentrations of NaCl.

In the additional experiment using a solution culture, a similar tendency to that of the vermiculite culture was observed. However the damage to the plants was more severe in the case of the solution culture than in the vermiculite culture: the plants treated with NaCl 4% wilted completely 5 days after the beginning of the treatment and they died on the 10th day.

The plants treated with NaCl 3% continued to grow for more than 2 years and survived extremely high levels of temperature when the daily maximum temperature in the greenhouse ranged from 40 to 45°C for 2 months.

Discussion

Species of the genus *Atriplex* with the C_4 pathway require Na as an essential micronutrient (BROWNELL, 1957, 1965, 1968; BROWNELL and CROSSLAND, 1972; OSMOND, 1970). *Atriplex nummularia* grown in a sodium-free culture solution showed distinct symptoms of leaf chlorosis and the dry weight decreased remarkably. However the addition of 0.1me/l of Na_2SO_4 into the culture solution induced a five fold increase in the dry weight (BROWNELL, 1968; BROWNELL and CROSSLAND, 1972). GREENWAY (1968) reported that the dry weight of *Atriplex nummularia* grown in a culture solution containing 100m*M* NaCl and 300m*M* NaCl was 1.4 times and 1.1 times, respectively that of the plants grown in a solution ranging from 1 to 10m*M* NaCl.

It is considered that the Na concentration of the soil solution generally ranges from 9 to 30ppm (BOWEN, 1966). OSMOND et al. (1980) stated that it is improbable that Na deficiency would ever be encountered under natural conditions because it is unlikely that any natural habitat would be sufficiently free of Na for deficiency symptoms to be expressed. Therefore it is reasonable to assume that a control plant is a plant grown in a culture solution containing enough Na as essential micronutrient when the salt tolerance of *Atriplex nummularia* is examined.

Growth of the control plant in this experiment was considered to be normal because the culture solution contained about 25 ppm of Na originating from the city water. Therefore it was recognized that the growth of *Atriplex nummularia* was inhibited by a culture solution containing NaCl at a concentration higher than 1%.

Regarding the morphology of salt-tolerant plants grown in a high salinity culture medium TADANO (1983) stated that plants under a saline condition show the following morphological changes: (1) decrease of leaf number and leaf area per plant, (2) decrease of stomata number per leaf area, (3) increase of leaf thickness and (4) accumulation of wax on leaf epidermis.

These morphological changes promote the inhibition of the transpiration of the plant which may be interpreted as a defense mechanism to compensate for the decrease of water uptake by the roots caused by the increase in the osmotic pressure of the culture medium. In the author's experiments (1) and (2) of the phenomena mentioned above were obviously observed and the transpiration rate of the plants treated with a concentration of NaCl higher than 1% decreased remarkably. However this defense mechanism results in a decrease of growth and of the photosynthetic rate. In addition *Atriplex nummularia* has a low transpiration rate: transpiration rates of sweet potato, groundnut and *Atriplex nummularia* under the same environmental conditions were 28.8±1.8, 19.7±2.2 and 4.0±0.7 $\mu g\ cm^{-2}\ sec^{-1}$, respectively.

BERNSTEIN (1961) suggested that growth inhibited by a high salinity medium was not caused by the prevention of water intake but by the increase in the salt content of the plant. Based on studies on the relationship between the concentration of the cell sap of cotton and the culture solution, he observed that the difference of osmotic pressure between the cell sap and culture solution was constant regardless of the salinity level of the culture solution and that water absorption was not prevented by the increase of the salinity level of the culture solution. The adjustment of the osmotic pressure may operate as a defense mechanism for the increase of the water absorption ability of the plant. *Atriplex nummularia,* in the author's experiment, also showed a considerable increase in the Na and Cl contents of leaf and stem by the NaCl treatment in the culture solution.

However excessive absorption of salt for the adjustment of the osmotic pressure of sap results in the accumulation of salt in the intercellular space and affects the structure of the cell. As a result the growth of the plant is inhibited by cell destruction, inhibition of the assimilation of certain essential nutrients, inhibition of the translocation of photosynthetic products, disturbance of hormonal balance of the plant, etc. (KELLY et al., 1982; TADANO, 1983). BLUMENTHAL-GOLDSCHMIDT and POLJAKOFF-MAYBER (1968) reported the occurrence of changes in the fine structure of leaf cells, viz. swelling of chloroplasts and mitochondria, appearance of numerous large lipid droplets, swelling of the nuclear membrane, vacuolization, and distortion of the tonoplasts, etc. in *Atriplex halimus,* for a salt concentration of 9—14 atm.

SHIMOSE (1963) pointed out that the remarkable increase of Na absorption in rice caused the antagonistic inhibition of absorption of K, Ca, Mg, etc. and that the deficiency in these elements resulted in physiological disorders in rice. In *Atriplex nummularia* the mechanisms mentioned above for the maintenance of life under high salinity conditions were operating but these functions inhibited growth. Therefore the growth rate decreased in inverse proportion to the increase of the NaCl concentration of the culture solution.

IWAKI and OTA (1953) reported that rice seedlings (variety Asahi) died in a culture solution containing 1.0% NaCl for 13 days. In addition, SHIMOSE (1965) reported that rice occasionally died even for a 50me/l (about 0.2%) NaCl treatment. Salt tolerance of *Atriplex nummularia,* which was able to grow for more than one year under the 5% NaCl treatment, is markedly high in comparison with rice. NaCl concentration halving the dry weight of some crops in the solution culture was as follows: garden beet about 1.4% (HOFFMAN and RAWLINS, 1971), cotton about 0.7 to 1.4% (HOFFMAN et al., 1971; BOYER, 1965), creeping bentgrass about 0.7 to 1.0% (YOUNGNER et al., 1967), barley about 0.6% (GAUCH and EATON, 1942), rice about 0.5% (SHIMOSE, 1963). Therefore the salt tolerance of *Atriplex nummularia* appears to be very high in comparison with that of these crops.

Summary

Salt tolerance of *Atriplex nummularia* was investigated in plants derived from a single plant by using cuttings with the purpose of preparing uniform plants as the experimental materials. The plants were grown in a culture solution with high NaCl concentrations in both vermiculite culture and solution culture. The results obtained are as follows:

1. The plants in the vermiculite culture survived in the culture solution containing 5% NaCl although their growth rate and yield were very low. Dry matter production of the plants grown in the 1% NaCl solution was 80% of that of the control plants at a daily mean temperature of 24.5°C. The plants grown in a culture solution containing a NaCl concentration of 3% at daily maximum temperatures of 40 to 45°C (mean temperature ranged from 30 to 33°C) were able to survive for 2 months but could not survive in a culture solution containing 4% NaCl.

2. NaCl-treated plants which showed a low growth rate, regardless of the NaCl concentration, exhibited a remarkable decrease of the leaf area and of the transpiration rate.

3. The Na content of the plants increased markedly whereas the K content decreased and the total mineral content of the plants increased markedly with the rise of the NaCl concentration in the culture solution.

IV. Morphological features of leaf and stem, and salt-excreting function of vesiculated hairs of *Atriplex nummularia*

As mentioned in parts III-1 and 2, the adult plant of *Atriplex nummularia* shows an extremely high salt tolerance although the seedlings do not show remarkably high salt tolerance in comparison with rice and wheat.

Halophytes growing on high salinity soils survive either by salt exclusion or by salt accumulation (SHARMA, 1982). The characteristics of salt accumulator plant include a rapid salt uptake, regulation of internal salt concentration and tolerance of the metabolic machinery to a high salt concentration (SHARMA, 1982). GREENWAY and OSMOND (1970) suggested that *Atriplex* species fall into the category of salt accumulator plants. The mechanisms of salt tolerance in *Atriplex* have been investigated fairly extensively, and the presence of vesiculated hairs on the upper and lower epidermis of the leaves has been recognized. The vesiculated hairs are thought to be the main structure for the salt tolerance of the *Atriplex* species, though their role in salt tolerance is not yet clearly understood.

The morhological features of vesiculated hairs of some species have been observed under optical and electron microscopy; *Atriplex buchananii* (TROUGHTON and CARD, 1974), *A. halimus* (MOZAFAR and GOODIN, 1970; OSMOND et al., 1980), *A. hastata* and *A. hortensis* (SCHIRMER, 1982), *A. spongiosa* (OSMOND et al., 1969), etc. The morphology of these structures which vary with the species have not been thoroughly studied in *Atriplex nummularia.* The author observed the morphological features of the vesiculated hairs of *Atriplex nummularia* growing in a saline culture medium and examined their physiologial role on the basis of the results of studies on the distribution of Na, K and Cl ions in the lamina and the vesiculated hairs.

In addition the presence of vesiculated hairs on the cotyledon was examined.

1. Morphological structure of the surface of the leaf and stem

Materials and methods

1) Methods of culture and treatments of the materials

The experimental plants were derived from a single plant by using cuttings with purpose of preparing uniform plants. The methods of culture of the materials and NaCl treatments were the same as those described in part III-2. NaCl concentration of the culture solution was 2%. Expanded 5th or 6th leaves from the top were observed.

2) Fixation and embedding of specimens for optical microscopic examination

Lamina of *Atriplex nummularia* was fixed by soaking into 5% glutaraldehyde solution containing 0.1*M* cacodylic acid buffer solution (pH 6.8) for one day.

After being washed with the cacodylic acid buffer solution, the materials were dehydrated and embedded according to the method of SENOO (1978). Since the dehydration in graded series of ethanol brought about changes in the surface structure of the lamina, dehydration was performed by soaking into A-solution (mixed solution of glycol methacrylate and butoxyethanol) from JB-4 solution Kit, made by Polyscience Company, containing C-powder (benzoyl peroxide) from the kit. Soaking was repeated four times each for 20 minutes and the materials were allowed to stand for one night.

Mounted blocks of specimens were prepared by using the JB-4 Kit which is composed of a resin solution consisting of a mixture of A-solution, C-powder and B-solution (mixed solution of polyethylene glycol and N, N-dimethylaniline) for one night at 4°C. Sections about 15μm thick were cut from the resin block with a microtome and the sections were stained with a toluidine blue solution.

3) Preparation of specimens for scanning electron microscopic examination

Small pieces of the lamina were quickly frozen by liquid nitrogen, and after sublimation of the ice on the surface in the cryo air lock chamber at -130°C, evaporation coating with gold was carried out. Scanning electron microscope, Japan Electron JSM-35CF equipped with an X-ray micro-probe was used for the examination.

Results and discussion

1) Morphological structure of the surface of leaf

(1) Observation by optical microscope

The surface of the leaf was covered all over with spherical cells (Fig. 4-1) which were observed not only on the leaves of the NaCl treated plants but also on those of the untreated plants. However the density of these spherical cells was higher in the former than in the latter. The spherical cells covered closely the epidermis of the upper and lower surfaces of leaf (Fig. 4-2). These cells were considered to be the bladder cells already observed in *Atriplex buchananii* (TROUGHTON and CARD, 1974), *A. halimus* (MOZAFAR and GOODIN, 1970), *A. hastata* and *A. hortensis* (SCHIRMER and BRECKLE, 1982), *A. spongiosa* (OSMOND et al., 1980; STOREY et al., 1983) and *A. vesicaria* (BLACK, 1954) etc. It is assumed that the vesiculated hair consists of bladder cell and stalk cell connected with the epidermis. However stalk cell was not observed in the present experiment.

OSMOND et al. (1980) reported the existence of C_3 plants and C_4 plants in the genus *Atriplex,* while *Atriplex nummularia* was designated as a C_4 plant, as confirmed by the observation of a "Kranz" arrangement (Fig. 4-2).

(2) Observation by scanning electron microscope

On the basis of the results obtained by optical microscopic examination, more detailed studies were carried out by using a scanning electron microscope.

Critical point drying method was adopted first after double fixation of the lamina with a glutaraldehyde solution and osmic acid solution. However the bladder cells showed considerable changes because they were exserted out of the surface of the leaf. Therefore the cryo-scanning electron microscopic method was adopted.

The results are shown on the photographs in Fig. 4-3 to Fig. 4-7 and Fig. 4-9. All the samples except those illustrated in Fig. 4-9 were obtained from the plants treated with 2% NaCl.

Bladder cells densely grew and covered the epidermis in forming 2-3 layers (Fig. 4-3) and the surface of the bladder cells was covered with waxy blooms (Fig. 4-4). Diameter of the bladder cell was about 100 to 200 μm.

Residues of collapsed bladder cells containing some crystalline material were observed on the intact bladder cells (arrows in Fig. 4-3). This crystalline material was examined by X-ray micro-probe. Fig. 4-6 shows the chlorine mapping of the crystalline material in the collapsed bladder cell shown in Fig. 4-5 (White grains indicate the presence of chlorine). Chlorines were found in the crystalline material.

Fig. 4-7 shows representative X-ray energy dispersive spectra from the crystalline material shown in Fig. 4-5 (①) and from sodium chloride (②). As a result of the examination, the crystalline material was identified as sodium chloride. In addition NaCl solution frozen in the same way as the lamina in the cryo air lock chamber was examined by scanning electron microscopy. However crystallization of NaCl did not occur. As a result of these examinations, it was considered that the bladder cells specifically accumulated Na and Cl ions. When excessive amounts of Na and Cl ions accumulated, the bladder cells collapsed, and the crystallized NaCl remained in adhering to the collapsed cell. Similar crystalline material which was observed on the surface of the leaf of *Atriplex Hymenelytra* was assumed to be NaCl (OSMOND et al. 1980). Stalk cells were not observed in the leaves of the NaCl-treated plants, but were observed in the leaves of the un-treated plants. Length of the stalk cell of *Atriplex nummularia* in this experiment seemed to be shorter than that of *Atriplex hortensis* (SCHIRMER and BRECKLE, 1982) and of *Atriplex spongiosa* (STOREY et al., 1983).

2) Morphological structure of the surface of the stem

Although there are some reports on the presence of vesiculated hairs on the surface of the leaf of *Atriplex* plants, the author did not find reports on the presence of vesiculated hairs on the surface of the stem. The author confirmed the existence of vesiculated hairs on the surface of the stem by examination by both optical and scanning electron microscopy (Fig. 4-8 and 4-10). However a large amount of residues of collapsed bladder cells was observed on the surface of the stem in contrast with the small number of intact bladder cells.

Summary

The morphological structure of the surface of the leaf and stem of *Atriplex nummularia,* grown in a culture solution with or without 2% NaCl, was observed by optical as well as cryo-scanning electron microscopy. The results obtained are as follows:

1. Both upper and lower surfaces of the leaf were covered with 2—3 layers of densely grown vesiculated hairs. The vesiculated hair consisted of bladder cell (100 to 200 μm in diameter) and stalk cell connected with the epidermis. The bladder cell was covered with waxy blooms. The stem surface was also covered with vesiculated hairs.
2. Residues of collapsed bladder cells were often observed on the intact bladder cells. Crystals of NaCl were often found in the collapsed bladder cells.

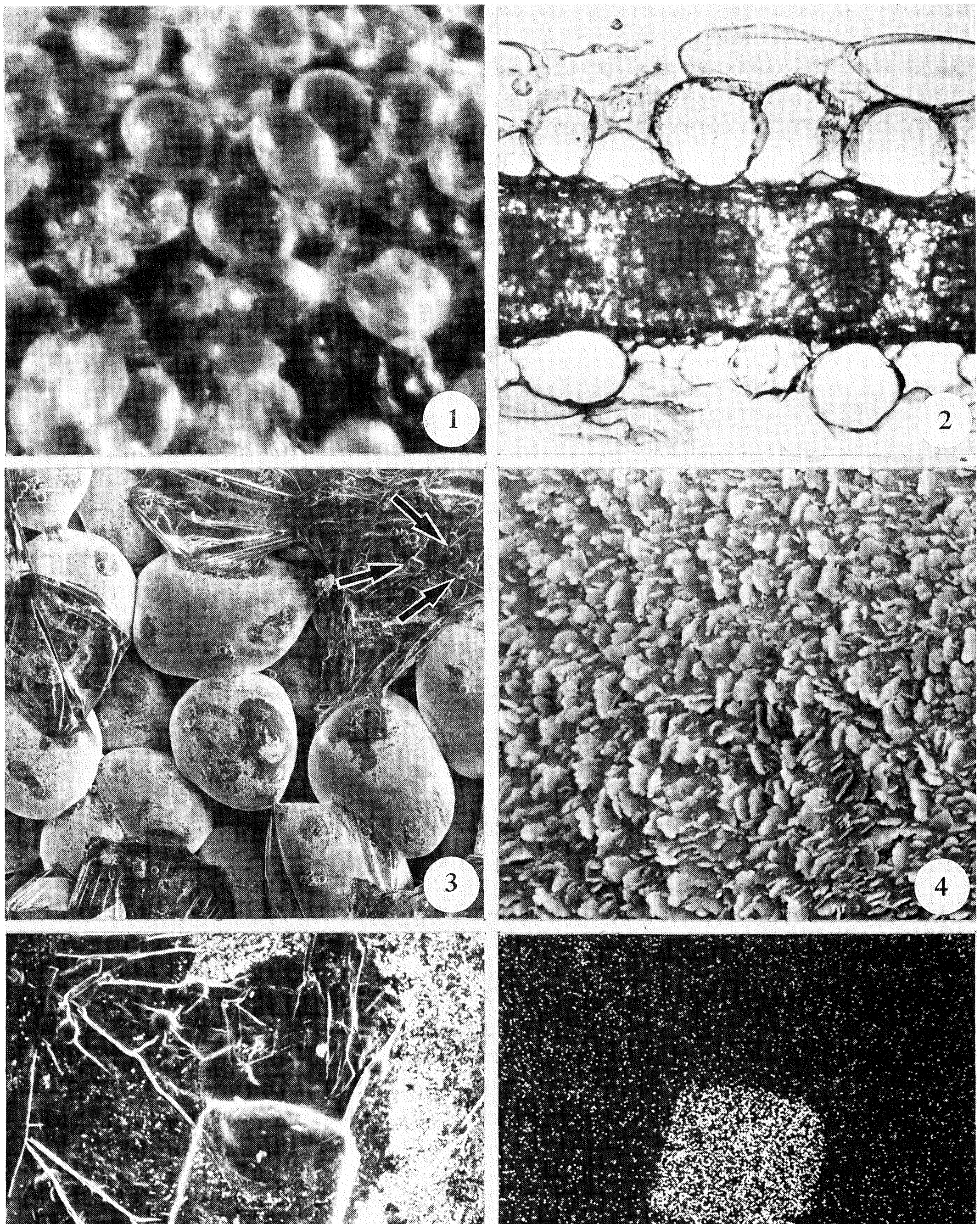

Fig. 4. Micrographs of vesiculated hairs on *Atriplex nummularia* leaf and stem (1—10).

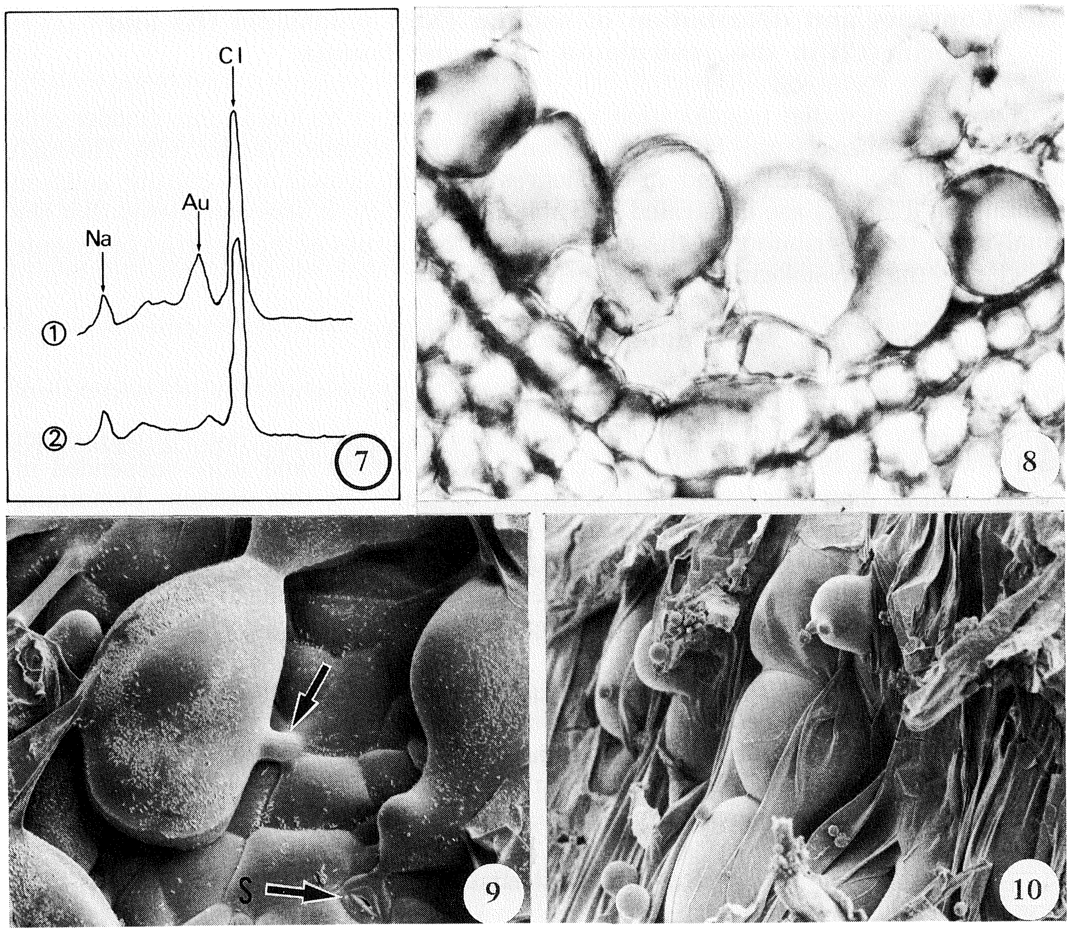

Fig. 4-1. Surface of ***Atriplex nummularia*** leaf, showing bladder cells of vesiculated hairs. (× 80)
Fig. 4-2. Transverse section of *A. nummularia* leaf, showing bladder cells closely covering the epidermis of upper and lower surfaces of leaf and "kranz" type structure of lamina. (× 100)
Fig. 4-3. Cryo-scanning electron micrograph (SEM) of the surface of *A. nummularia* leaf, showing densely grown bladder cells and residues of collapsed bladder cells containing some crystalline materials (arrows). (× 125)
Fig. 4-4. SEM of the surface of a bladder cell showing waxy blooms (× 3500)
Fig. 4-5. Crystalline material in collapsed bladder cell on the surface of leaf. (× 520)
Fig. 4-6. The chlorine mapping of same specimen as shown in Fig. 4-5 by X-ray microprobe. White grain density shows concentration of chlorine. (× 520)
Fig. 4-7. Representative X-ray energy dispersive spectra from the crystalline material shown in Fig. 4-5 (①) and from sodium chloride (②). See Fig. 5-2. for Au peak.
Fig. 4-8. Transverse section of *A. nummularia* stem, showing bladder cells and stalk cells. (× 200)
Fig. 4-9. SEM of the surface of *A. nummularia* leaf obtained from control plant, showing a bladder cell connected with a stalk cell (arrow). S: Stoma. (× 300)
Fig. 4-10. SEM of the surface of *A. nummularia* stem, showing intact and collapsed bladder cells. (× 250)
All samples excepting Fig. 4-9 were obtained from the plants treated with 2% sodium chloride.

2. Contents and distribution of sodium (Na), potassium (K) and chlorine(Cl) in the vesiculated hairs and lamina

It was assumed that translocation and accumulation of Na and Cl into bladder cells took place because crystals of NaCl were found in the collapsed bladder cells. The high contents of Na and Cl found in the leaves of plant grown in a culture solution containing 2% NaCl are illustrated in Table 7 (part III-2). It was considered that the analysis of the distribution of these elements in the tissues of the leaf may contribute to clarify the physiological role of vesiculated hair.

Materials and methods

1) Separation of vesiculated hairs and lamina

Using some of the materials from experiment IV-1, vesiculated hairs of both upper and lower surfaces of the leaf were chipped with a razor and were suspended in water. These materials are called "Fraction of blad." in this section. Remaining tissues were ground with a homogenizer, and these are called "Fraction of lam." in this section. Na and K contents in the extracted solutions from both fractions were measured with the atomic absorption spectrophotometer, Shimazu AA-650 and the contents were expressed in unit leaf area.

2) Examination of the distribution of Na, K and Cl in bladder cell and in various cells of the lamina

A part of the frozen leaf used in experiment IV-1 was fractured in the cryo air lock chamber. Distribution of Na, K and Cl on the surface of the fractured sections was examined with X-ray micro-probe.

Results and discussion

1) Contents of Na and K in vesiculated hairs (Fraction of blad.) and Lamina (Fraction of lam.)

Content of Na+K in the Fraction of lam. showed little difference between the NaCl-treated plant and un-treated plant. On the other hand the content of Na+K in the Fraction of blad. from the NaCl-treated plant was three times that of the un-treated plant. This increase in the content of Na+K was attributed mainly to the increase of the Na content (Table 8). It is considered that the content of Na+K in the lamina remains constant regardless of NaCl treatment, whereas the function of the vesiculated hairs is to increase the Na content by the NaCl treatment. Similar results have been obtained in *Atriplex halimus* (MOZAFAR, 1969, requoted from SCHIRMER and BRECKLE, 1982).

Table 8. Contents of sodium and potassium in vesiculated hairs and lamina of *Atriplex nummularia* plants grown in a culture solution containing 2% sodium chloride (treated) or not containing it (control).

Plant organ	Element species	Content ($mg/10cm^2$ leaf area)	
		Control	Treated
Vesiculated hair	Na + K	1.64±0.22	4.15±0.33
	Na	0.65±0.07	3.63±0.28
	K	0.99±0.24	0.52±0.08
Lamina	Na + K	3.44±0.34	3.22±0.23
	Na	0.68±0.08	2.17±0.18
	K	2.76±0.30	1.05±0.08

Notes: Figures indicate mean ± standard deviation. The experiment was conducted with five replications.

2) Distribution of Na, K and Cl in bladder cell and in various cells of the lamina

The results of the examination of the distribution of Na, K and Cl in the bladder cell, epidermal cell, mesophyll cell and bundle sheath cell by X-ray micro-probe under a scanning electron microscope are shown in Figs. 5-1 and 5-2. Crystals of NaCl were found in the internal part of collapsed bladder cell (Fig. 5-1 ①) suggesting the crystals of NaCl were not extraneous. The peaks corresponding to Na and Cl were observed on the surface (Figs. 5-1 ② and 5-2 ②), in the cytoplasm (Figs. 5-1 ③ and 5-2 ③) and in the vacuole (Figs. 5-1 ④ and 5-2 ④) of the bladder cell, while K was scarcely detected in the same parts. On the other hand the small peaks corresponding to K and Cl were observed in the epidermal cell, (Figs. 5-1 ⑤ and 5-2 ⑤), mesophyll cell (Figs. 5-1 ⑥ and 5-2 ⑥) and bundle sheath cell (Figs. 5-1 ⑦ and 5-2 ⑦).

In *Atriplex hortensis* grown in a culture solution containing Na and K, a larger amount of Na accumulated in the vesiculated hairs than in the lamina (JESCHKE and STELTER, 1983). The Na/K ratio in the vacuole of the bladder cell of *Atriplex spongiosa* was higher than that observed in various cells of the lamina (STOREY et al., 1983). It was considered in this experiment that the transport and accumulation of K into the bladder cells was restricted and that Na was transported and accumulated preferentially into the bladder cells.

The peaks corresponding to Na and Cl in the cytoplasm of the bladder cell were obviously higher than those in the vacuole of the same bladder cell (Fig. 5-2 ③ and ④). This finding supports the results obtained by OSMOND et al. (1969) who reported that radioactive Cl was concentrated in a thin circular of the cytoplasm of the bladder cell.

It is considered that NaCl absorbed by the roots was transported actively to the bladder cells through the conducting tissue, mesophyll cells and epidermal cells. It is also considered that NaCl is transported to the cytoplasm first and thereafter moves to vacuole in the bladder cell. Using radioactive Cl, OSMOND et al. (1969) observed a light-stimulated increase in the Cl content of the bladder cells which took place against an electrochemical gradient and they suggested that uptake of Cl into the bladder cell was an active process. Subsequently, LÜTTGE and OSMOND (1970) and

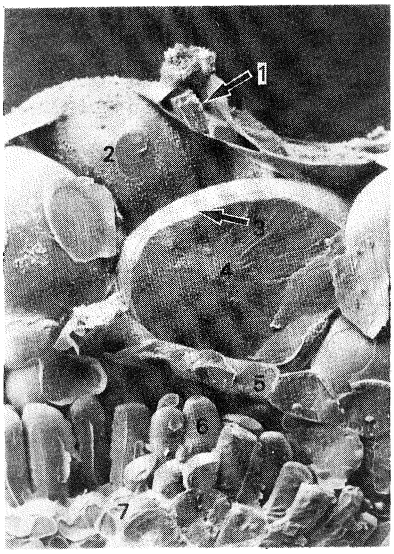

Fig. 5-1

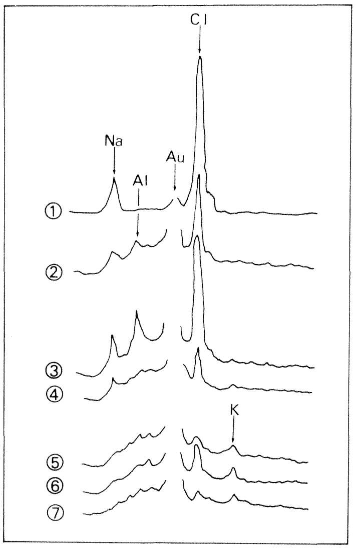

Fig. 5-2

Fig. 5-1. SEM of a fracture of frozen leaf of *A. nummularia* grown in a culture solution containing 2% sodium chloride. ① Crystalline material in collapsed bladder cell. ② Surface of bladder cell. ③ Cytoplasm of bladder cell. ④ Large vacuole of bladder cell. ⑤ Epidermal cell. ⑥ Mesophyll cell. ⑦ Bundle sheath cell. (× 300)

Fig. 5-2. Representative X-ray energy dispersive spectra from cellular parts numbered in Fig. 5-1. Au peak appeared after the sample had been in contact with the gold used for evaporation coating. Al peak appeared after contact with the sample-holder made of aluminium.

LÜTTGE and PALLAGHY (1969) reported that the light-stimulated active Cl transport was inhibited by the photosystem II inhibitor 3-(3,4-dichlorophenyl)-1, 1-dimethylurea [DCMU] and that light-dependent CO_2 fixation was inhibited by DCMU in both the leaf and the bladder cells. They suggested that active transport of Cl into the bladder cell was directly linked to photosynthesis.

The bladder cells of *Atriplex nummularia* are considered to be associated with the salt tolerance of the plant. Based on these observations it is suggested that *Atriplex nummularia*, grown under high salinity conditions, is able to excrete the excess of sodium chloride from the lamina of plants which absorbed the ions through roots and conducting tissues by the repetition of the following cycle; ① transport of Na and Cl from the lamina to the bladder cells and accumulation of Na and Cl in these cells, ② collapse of the bladder cells containing crystals of NaCl and ③ liberation of the crystals from the collapsed bladder cells. It is postulated that sodium chloride can be excreted from the surface of the stem by the same mechanism since similar vesiculated hairs were observed on the surface of the stem (Figs. 4-8 and 4-10).

Summary

Distribution of Na, K and Cl in bladder cell and in various cells of the lamina was investigated.

The results obtained are as follows:

1. The vesiculated hairs and the lamina were separated, and Na and K contents of each fraction were analysed. When NaCl was added to the culture solution, Na content markedly increased in the vesiculated hairs unlike in the lamina.

2. Ion contents of the bladder cell and of various cells of the lamina were determined by using X-ray micro-probe under scanning electron microscope. The peaks corresponding to Na and Cl were observed on the surface, in the cytoplasm, and in the vacuole of the bladder cell. On the other hand the small peaks corresponding to K and Cl were observed in the epidermal cell, mesophyll cell and bundle sheath cell. Crystals of NaCl could be seen in the internal part of the collapsed bladder cell.

On the basis of the results obtained, the salt-excreting function of vesiculated hairs of *Atriplex nummularia* is discussed.

3. Morphological structure of the surface of cotyledon

Low salt tolerance of *Atriplex nummularia* at the seedling stage was pointed out in part III-1. The experiment to determine the existence of vesiculated hairs was carried out because one of the factors for the low salt tolerance may be ascribed to the lack of vesiculated hairs on the cotyledon.

Materials and methods

The seeds of *Atriplex nummularia* were germinated in distilled water and in 1.5% NaCl solution, respectively. The cotyledons of the seedlings on the 5th and 12th day after germination were examined.

The sections were made by a hand microtome and these were mounted in glycerin and observed by optical microscopy.

Results and discussion

Transverse sections of the cotyledons are shown in Figs. 6-1, 6-2 and 6-3. Fig. 6-1 shows specimen from the cotyledon of a seedling on the 5th day after germination in 1.5% NaCl solution and Figs. 6-2 and 6-3 show that of a seedling on the 12th day after germination in the same solution. Vesiculated hairs were not found even on the 12th day after germination in 1.5% NaCl solution, nor in the specimen from seedlings which germinated in distilled water. It is considered that the lack of growth of the vesiculated hairs at this stage is one of the reasons for the low salt tolerance of the seedlings of *Atriplex nummularia*.

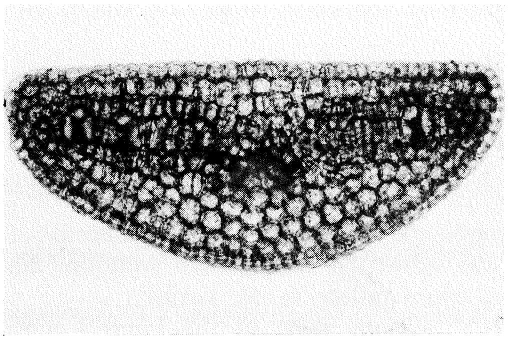

Fig. 6 − 1

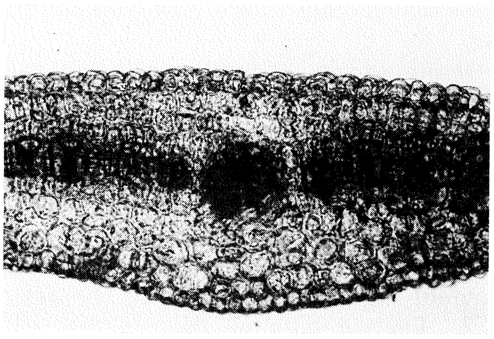

Fig. 6 − 2

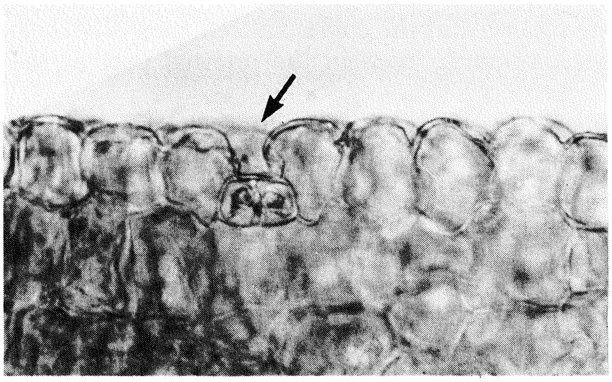

Fig. 6 − 3

Fig. 6. Transverse sections of cotyledon of *Atriplex nummularia*.
Fig. 6-1. On the 5th day after the germination. (× 100)
Fig. 6-2. On the 12th day after the germination. (× 100)
Fig. 6-3. On the 12th day after the germination, showing epidermal cells and stoma (arrow). (× 850)

Summary

Vesiculated hairs could not be detected on the cotyledons of the seedlings within 12 days after germination in culture in both distilled water and 1.5% NaCl solution.

V. Changes in the salt tolerance of *Atriplex nummularia* with the environmental conditions

High salt tolerance of the adult plant of *Atriplex nummularia* was mentioned in part III-2. However the salt tolerance of the plant was found to vary with the environmental conditions.

It is very important to understand the relationship between the salt tolerance of *Atriplex nummularia* and environmental conditions in order to introduce the plant to a new area and achieve high yields. The effect of thermal and nutritional conditions on the salt tolerance of *Atriplex nummularia* was investigated, and the effect of the salinity conditions of the seed production field on the salt tolerance at the germination stage of the plant was also investigated.

1. Salt tolerance of *Atriplex nummularia* growing at different temperatures

The experiment described in part III-2 was carried out at a mean air temperature of 24.5°C. However since the salt tolerance of the plant varies with the temperature, the effect of the air temperature on the salt tolerance of *Atriplex nummularia* was investigated.

Materials and methods

The experimental materials were derived from a single plant using cuttings in order to prepare uniform plants. The methods for obtaining the cuttings, culture and NaCl treatments were identical with those described in part III-2. NaCl concentrations of the culture solution containing 100ppm N, 48ppm P and 148ppm K were 0.5% and 1%. The control culture solution contained only 25ppm Na originating from city water. Six plants were used for each treatment. Immediately before the NaCl treatment, the top of all the plants was cut off at 4cm above the surface of the culture media and the regenerated plants were used for the measurements performed 48 days after the beginning of the treatment. The pots were leached weekly with the corresponding NaCl treatment solution, respectively to prevent salt accumulation in the medium.

The conditions of temperature in each treatment were as follows:

Treatment	Temp. at day time (12h.)	Temp. at night time (12h.)	Daily mean temp.
	°C	°C	°C
Low	20.5	13.5	17.0
Medium	28.5	21.5	25.0
High	36.5	29.5	33.0

The experiment was carried out in a growth chamber under the same light conditions as those described in part III-2. Relative humidity was approximately 35—45%.

Photosynthetic rate, transpiration rate and base contents were measured by the same methods as those described in part III-2. Chlorophyl was extracted with 80% acetone from the leaf, using 4 leaf fractions of 0.5cm^2 per sample, and its content was measured with a spectrophotometer, double-beam, Hitachi 100-60.

Results

Growth and yield of *Atriplex nummularia* 48 days after the beginning of the treatment are presented in Table 9 and Fig. 7. The plants cultivated under the

Table 9. Growth and yield of *Atriplex nummularia* grown at different NaCl levels combined with different temperatures*.

Temp.**	NaCl %	Plant length cm	Dry weight of top g/plant	Dry matter content of top %	Leaf area dm²/plant	Leaf area cm²/leaf	Areal weight of leaf mg/cm²	Water cont. of leaf g/g DW
L	0	25.0 [c]	13.1 [c]	21.1 [a]	9.2 [c]	2.96 [a]	10.22 [a]	4.11 [d]
	0.5	25.6 [c]	15.2 [bc]	17.2 [cd]	11.5 [cd]	3.13 [a]	9.73 [a]	5.42 [ab]
	1	23.1 [c]	15.5 [bc]	18.0 [c]	10.8 [d]	2.99 [a]	10.48 [a]	5.15 [b]
M	0	40.0 [a]	23.3 [a]	20.3 [ab]	20.4 [a]	2.54 [b]	7.71 [b]	4.60 [c]
	0.5	39.6 [a]	25.0 [a]	19.6 [b]	17.9 [b]	2.39 [b]	9.84 [a]	4.84 [c]
	1	32.6 [b]	17.4 [b]	19.2 [b]	12.4 [cd]	2.35 [b]	10.21 [a]	4.81 [c]
H	0	35.5 [ab]	18.3 [b]	18.5 [bcd]	17.7 [b]	1.64 [d]	6.79 [b]	5.18 [ab]
	0.5	30.3 [bc]	14.5 [c]	16.0 [d]	12.9 [c]	2.01 [c]	8.15 [b]	5.93 [a]
	1	24.5 [c]	9.3 [d]	16.9 [d]	6.9 [f]	2.06 [c]	9.97 [a]	5.53 [a]

* Immediately before temperature and NaCl treatment, top of all plants was cut off at 4cm above the surface of culture media, and regenerated plants were used for measurement 48 days after the beginning of the treatment. Each figure indicates on average of 6 plants. Figures not bearing the same superscript within a column are significantly different at the 5% level.

** Mean daily temperature, L is 17°C(20.5°C × 12h, 13.5°C × 12h), M is 25°C(28.5°C × 12h, 21.5°C × 12h) and H is 33°C(36.5°C × 12h, 29.5°C × 12h).

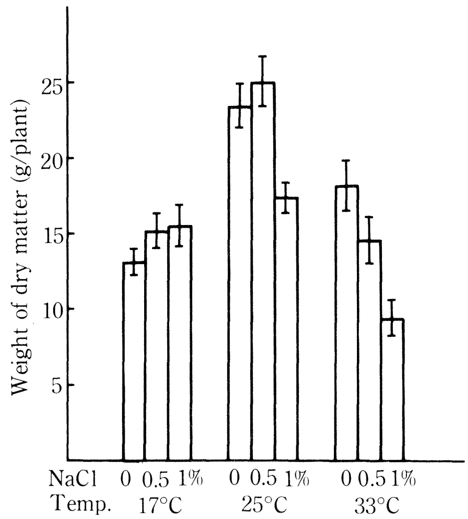

Fig. 7. Yield of *Atriplex nummularia* grown at different NaCl levels combined with different temperatures.

medium temperature (daily mean 25°C) conditions showed the optimum growth among the 3 temperature-plots at each level of NaCl concentration in the culture solution. No decrease in dry weight occurred for concentrations of 0.5 and 1% in the culture solution at a low temperature (daily mean 17°C). No decrease in dry weight was observed at a 0.5% NaCl concentration in the culture solution but the dry weight decreased significantly by 1% NaCl under the medium temperature condition. At a high temperature (daily mean 33°C) the decrease was remarkable for both the 0.5% and 1% NaCl concentrations in the culture solution. Leaf area per plant showed a similar tendency to that of the dry weight of the leaves and stems. The leaf area per leaf decreased with the rise of temperature. However the leaf area per leaf was not affected by the differences in the NaCl treatment.

Na and K contents of harvested leaves are presented in Fig. 8. The Na content of the leaves increased and the K content decreased with the increase of the NaCl concentration in the culture solution. The contents of both Na and K increased with the increase of temperature regardless of the levels of NaCl. This trend was more pronounced in the Na content.

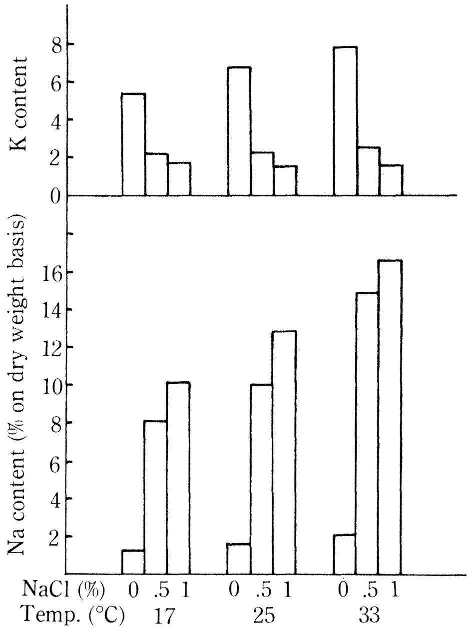

Fig. 8. Na and K contents of *Atriplex nummularia* leaf grown at different NaCl levels combined with different temperatures.

Photosynthetic rate 38 to 40 days after the beginning of the treatment is presented in Fig. 9-A. The rate decreased remarkably by the NaCl treatment at medium and high temperatures but scarcely at a low temperature. The chlorophyl content in the leaves 43 days after the beginning of the treatment showed a similar tendency to that of the photosynthetic rate, as shown in Fig. 9-B.

Transpiration rate 31 to 32 days after the beginning of the treatment is presented

Fig. 9-C. The rate decreased remarkably by the NaCl treatment at all the temperature levels.

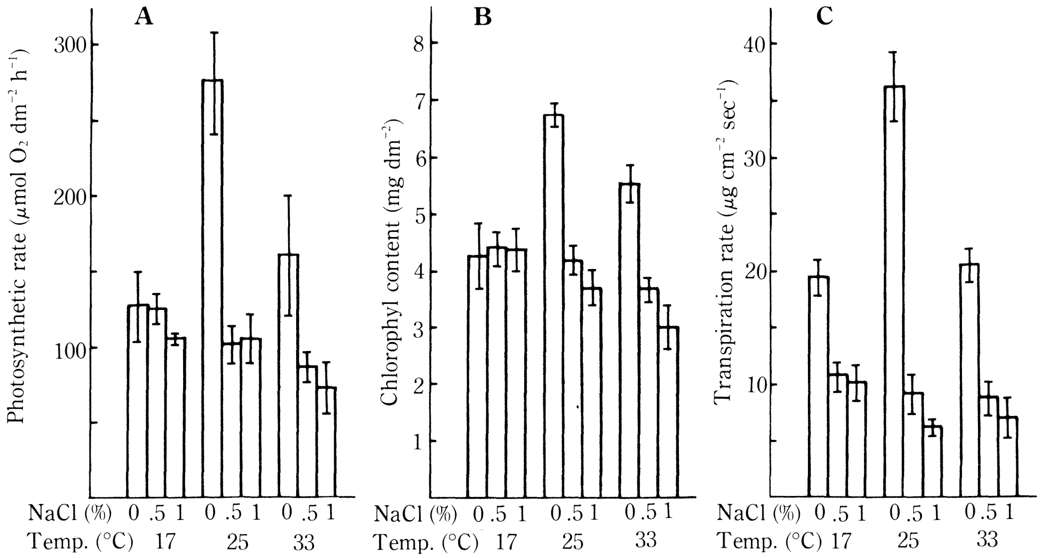

Fig. 9. Photosynthetic rate, chlorophyl content of leaf and transpiration rate of *Atriplex nummularia* grown at different NaCl levels combined with different temperatures.

Discussion

Rise in temperature increases the activity of ions in promoting the dissociation of the salt which affects plant growth. Reports on the effect of salinity and temperature on plant growth indicate that a higher temperature is likely to damage the plants (AHI and POWER, 1938; BERNSTEIN and AYERS, 1951, 1953; EHLIG, 1960; WALL and HARTMAN, 1942).

Although salt injury and high or low temperature injury may have acted concurrently, in this experiment, it was not possible to discriminate between both injuries. However, assuming that the same level of temperature injury affects each NaCl treated plant, the effect of the temperature on the salt tolerance of the plant can be deduced by estimating the differences in the decrease of growth of the NaCl treated plants compared with the control plants at each temperature.

The indices of dry weight of the plants grown at different NaCl levels at each temperature are presented in Fig. 10. Injury caused by the NaCl treatment was conspicuous at high temperatures. Injury which was not observed at NaCl concentrations lower than 0.5% was evident at NaCl concentration of 1% under medium temperature conditions. However injury by the NaCl treatment was not observed at low temperatures. Although the level of salt tolerance varies *Atriplex nummularia* is also likely to experience injury by salt at higher temperatures like other crops (AHI and POWER, 1938; BERNSTEIN and AYERS, 1951, 1953; EHLIG, 1960; WALL and HARTMAN 1942).

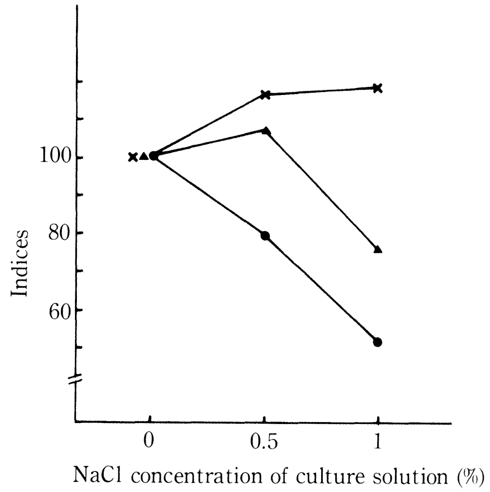

Fig. 10. Indices of dry weight of *Atriplex nummularia* grown at different NaCl levels at each temperature. Daily mean temperature; ×——× 17°C, ▲——▲ 25°C, ●——● 33°C.

The increase of the Na content and the decrease of the K content in the plant with the increase of the NaCl concentration of the culture solution, may be one of the factors inhibiting growth. In this case, the remarkable increase of the Na content with the rise of temperature may account for the severity of the injury at higher temperatures. The dry weight of the leaves and stems did not decrease by the 0.5% NaCl treatment at medium temperatures in spite of the decrease of the photosynthetic rate, chlorophyl content and transpiration rate for unknown reasons. However excessive absorption of Na may affect the distribution of the photosynthates because the leaf area per plant decreased remarkably by the 0.5% NaCl treatment.

The plants cultivated at a medium temperature (daily mean 25°C) showed the highest level of growth among the 3 temperature-plots for each level of NaCl in the culture solution, and the plants grown at a medium temperature with 1% NaCl showed growth patterns and yield similar to those of the plants grown at high and low temperatures without NaCl addition in the culture solution.

In view of the results so far obtained it is considered that for a region to be suitable for the growth of *Atriplex nummularia* many days with a daily mean temperature of 25°C are required. The yield may be very high when the plant is grown on low salinity soil. However the yield may hardly decrease in the case of saline soil containing 0.5% NaCl and even in the case of a high salinity soil containing 1% NaCl, the yield may be maintained at a level of about 75% of that of plants growing on nonsaline soil.

In regions where the mean temperature is 17°C, the yield may decrease to about 60% of that of the plants growing at a medium temperature on soils with a very low salinity. However the yield will be maintained at the same level in plants grown on saline soil containing 1% NaCl. Therefore a region, with numerous days with a daily mean temperature of less than 20°C, will not generally be suitable for the growth of *Atriplex nummularia*. However, in the case of a soil with a very high salinity where other crops are unable to grow, *Atriplex nummularia* can be planted.

On the other hand high temperature (daily mean 33°C) decreases the dry matter production of the plant, with a concomitant decrease in yield. Furthermore the rise of the soil salinity inhibits remarkably plant growth and the yield of plants grown on saline soil containing 1% NaCl will be half to that of the plants grown at a medium temperature. However *Atriplex nummularia* is able to survive under environmental conditions with a very high temperature (daily mean temperature more than 30°C) and a soil with a very high salinity (containing more than 1% NaCl). As mentioned in part III-2, *Atriplex nummularia* survived in the culture solution containing 4% NaCl at daily mean temperatures ranging from 33 to 35°C and at daily maximum temperatures ranging from 40 to 45°C, during more than 2 months. Though such climate and soil conditions may prevail for 2 months each year, if in the other months the daily mean temperature is less than 30°C and the soil salinity is less than 1% NaCl, the growth of *Atriplex nummularia* will not be seriously impaired.

Summary

Salt tolerance of *Atriplex nummularia* growing at different temperatures was investigated. The plants were grown in vermiculite using a culture solution containing NaCl at a concentration of less than 1%. The results obtained are as follows:

1. No decrease in the growth of *Atriplex nummularia* occurred at NaCl concentrations of 0.5 and 1% in the culture solution at a low temperature (daily mean 17°C), although the growth decreased significantly at a concentration of 1% NaCl at medium temperature (mean 25°C). At high temperature (mean 33°C) the decrease was more remarkable. A similar trend was observed for the leaf area.
2. The Na content of the plant increased and the K content decreased with the increase of the NaCl concentration of the culture solution. The content of both Na and K increased with the increase of the temperature regardless of the level of NaCl. This trend was more pronounced in the Na content. These observations support the assumption that growth inhibition caused by excessive absorption of Na is promoted by high temperatures.
3. The plants cultivated at the medium temperature of 25°C, showed the optimum growth among the 3 temperature-plots at each level of NaCl in the culture solution. The plants grown at the temperature of 25°C with 1% of NaCl showed a growth pattern and yield similar to those of the plants grown at high and low temperatures without NaCl addition in the culture solution.

On the basis of the results obtained, the climate and soil conditions of the region most suitable for plant *Atriplex nummularia* are discussed.

2. Salt tolerance of *Atriplex nummularia* growing at different nutrient levels in the culture solution

Tolerance of plants to environmental conditions varies with the nutrient conditions. Since the salt tolerance of *Atriplex nummularia* also will change with the nutrient conditions, the effect of nutrient levels on the salt tolerance of *Atriplex nummularia* was investigated.

Materials and methods

The preparation methods of the experimental materials and the method for preventing salt accumulation in the culture medium were identical with those described in part V-1.

The experiment was carried out in a growth chamber under same the light and temperature conditions as those described for the medium-temperature-plot in part V-1. Relative humidity was approximately 50–55% and 55–65% in experiments 1) and 2), respectively. Photosynthetic rate, transpiration rate, chlorophyl content of leaves and base contents of leaves and stems were measured by the same methods as those outlined in part V-1. Nitrogen content of leaves and stems was measured with a Technicon auto-analyzer, after decomposition by the Kjeldahl method.

1) Effect of the levels of applied nitrogen

The culture solution was prepared in adding 96.0mg KH_2PO_4, 176.2mg KCl, 253.2mg $Ca(NO_3)_2 \cdot 4H_2O$, 245.0mg $MgSO_4 \cdot 7H_2O$, 5mg $FeCl_3 \cdot 6H_2O$, 3.7mg $MnCl_2 \cdot 4H_2O$, 5.8mg H_3BO_3, 0.9mg $ZnSO_4 \cdot 7H_2O$, 50mg NaCl per 1 liter solution and additional 85.8mg and 257.4mg NH_4NO_3 for the medium nitrogen level and high nitrogen level, respectively. Nitrogen levels comprised three levels, i.e. low (30ppm), medium (60ppm) and high (120ppm). NaCl concentrations of the culture solution were 0.5% and 1%.

For the control, the culture solution contained 30–35ppm Na originating from the city water. Six plants were used for each treatment.

2) Effect of the levels of application of the three elements

The culture solution which was a mixture of Otsuka House Compounds No.1 and No.2, diluted by city water, contained 25ppm nitrogen, 12ppm phosphate, 34.5ppm potassium, 7.5ppm magnesium, 22.5ppm calcium and some micronutrients for the "low nutrient plot", a twofold nutrient concentration for the "medium nutrient plot" and a fourfold nutrient concentration for the "high nutrient plot". NaCl concentrations of the culture solution were 0.5% and 1%. The culture solution of the control contained 30–35ppm Na originating from the city water. Six plants were used for each treatment.

Additional experiment: in order to determine the marginal response to fertilizer of *Atriplex nummularia,* plots with 1.2 fold (N-120 plot) and twofold (N-200 plot) concentrations of the "high nutrient plot (N-100 plot)" were prepared.

Results

1) Effect of the levels of applied nitrogen

The plants of the high N plot grew most actively at first in each NaCl treatment plot, but they showed symptoms of nitrogen excess such as drooping hanging about one month after the beginning of the treatment (Fig. 11). These symptoms were remarkable in the plots of the control and 0.5% NaCl.

Growth and yield of the plants 47 days after the beginning of the treatment are presented in Table 10. The dry weight of the leaves and stems increased with the increase of the nitrogen concentration in the culture solution: in the medium N plot the dry weight was 1.7–1.9 times that of the low N plot, and in the high N plot the dry weight was 1.1–1.5 times that of the medium N plot. No decrease in the dry weight of the leaves and stems occurred by the NaCl treatment in the low N and medium N

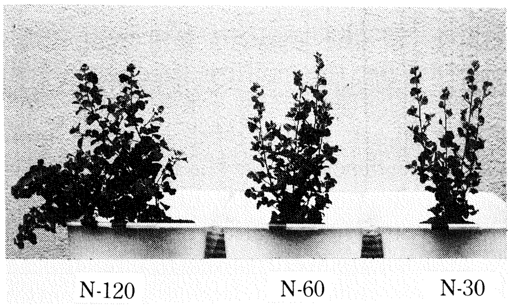

Fig. 11. Growth of *Atriplex nummularia* grown at different nitrogen concentrations without NaCl in culture solution.

Table 10. Growth and yield of *Atriplex nummularia* grown at different NaCl levels combined with different nitrogen concentrations.

Treatment Nitrogen ppm	NaCl %	Plant length cm^2	Dry weight of top g/plant	Dry matter content of top %	Leaf area dm^2/plant	Leaf area cm^2/leaf	Areal weight of leaf mg/cm^2	Water cont. of leaf g/g DW
N-30	0	34.3^{b}	6.5^{d}	19.5^{ab}	4.3^{c}	1.70^{d}	8.62^{b}	5.42^{b}
	0.5	34.0^{bcd}	7.1^{d}	20.1^{a}	4.2^{c}	1.85^{d}	10.23^{a}	5.05^{b}
	1	29.7^{d}	6.2^{d}	19.0^{b}	3.6^{c}	1.68^{d}	10.19^{ab}	5.38^{b}
N-60	0	38.4^{a}	11.3^{c}	18.7^{ab}	8.9^{b}	2.14^{c}	7.81^{bc}	5.50^{b}
	0.5	38.9^{ab}	13.2^{b}	19.5^{ab}	8.8^{b}	2.49^{b}	9.70^{ab}	5.06^{b}
	1	33.6^{c}	11.9^{bc}	19.2^{abc}	7.8^{b}	2.51^{b}	10.06^{a}	5.07^{b}
N-120	0	38.8^{a}	17.1^{a}	15.2^{d}	18.9^{a}	2.73^{b}	6.12^{c}	6.76^{a}
	0.5	41.2^{ab}	17.1^{a}	16.0^{cd}	17.0^{a}	3.73^{a}	7.03^{c}	6.15^{ab}
	1	37.3^{c}	13.0^{b}	17.4^{c}	10.3^{b}	2.93^{b}	8.66^{b}	5.60^{b}

Notes: Immediately before NaCl and nitrogen treatment, top of all plants was cut off at 4cm above the surface of culture media, and regenerated plants were used for measurements 47 days after the beginning of the treatment. Each figure indicates an average of 6 plants. Figures not bearing the same superscript within a column are significantly different at the 5% level.

plots but the dry weight decreased in the high N plot.

The leaf area per plant showed a similar tendency to that of the dry weight of the leaves and stems. On the other hand the areal weight of the leaves decreased with the increase of the nitrogen concentration in the culture solution but increased with the NaCl treatment.

Na and K contents of harvested leaves are presented in Fig. 12. The Na content increased and the K content decreased with the increase of the NaCl concentration in the culture solution. The Na content of the leaves grown at a higher nitrogen concentration was slightly higher than that of leaves grown at lower nitrogen concentrations regardless of the NaCl level of the culture solution, while the K content showed a reverse pattern.

The chlorophyl content in the leaves 40 days after the beginning of the treatment increased with the increase of the nitrogen concentration in the culture solution and

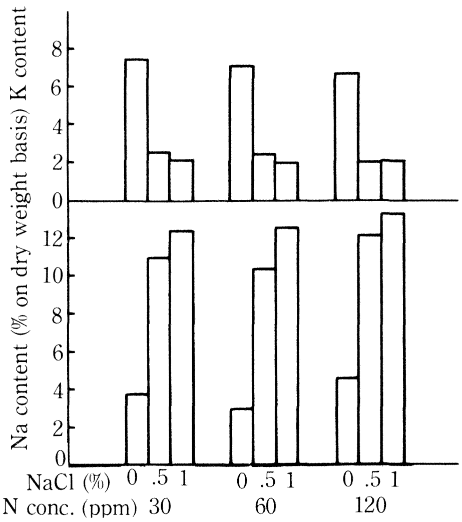

Fig. 12. Na and K contents of *Atriplex nummularia* leaf grown at different NaCl levels combined with different nitrogen concentrations.

decreased with the increase of the NaCl concentration in the culture solution (Fig. 13).

Total nitrogen content of leaf increased remarkably with the increase of the

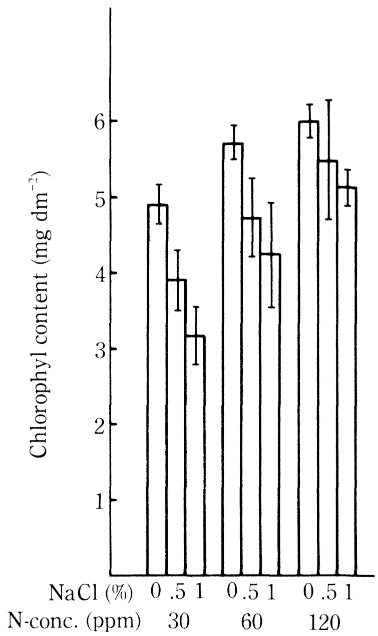

Fig. 13. Chlorophyl content of leaf of *Atriplex nummularia* grown at different NaCl levels combined with different nitrogen concentrations.

nitrogen concentration in the culture solution: the content in the high N plot was twofold that of the low N plot. Total nitrogen content of the leaves of the plants grown without NaCl treatment was significantly higher than that of the plants treated with NaCl, regardless of the nitrogen level of the culture solution, though there was no significant difference between the plants treated with 0.5% NaCl and those treated with 1% NaCl (Table 11).

Table 11. Total nitrogen content in *Atriplex nummularia* leaf grown at different NaCl levels combined with different nitrogen concentrations (% on dry weight basis).

NaCl treatment	Nitrogen concentration treatment		
	N-30	N-60	N-120
%	%	%	%
0	2.31	2.65	4.38
0.5	1.50	2.13	3.04
1	1.57	2.06	2.68

2) Effect of the levels of application of the three elements

Growth and yield of *Atriplex nummularia* 60 days after the beginning of the treatment are presented in Table 12 and Fig. 14. The dry weight of the leaves and stems increased almost in parallel to the increase of the concentration of the nutrients, N, P, K and some micronutrients, in the culture solution.

There were no significant differences in the dry weight of the leaves and stems between the plants treated with 0.5% NaCl and those in which the NaCl treatment was omitted regardless of the nutrient level of the culture solution.

Table 12. Growth and yield of *Atriplex nummularia* grown at different NaCl levels combined with different nutrient concentrations.*

Treatment: Nutrient conc.**	Treatment: NaCl %	Plant length cm	Dry weight of top g/plant	Dry matter content of top %	Leaf area dm²/plant	Leaf area cm²/leaf	Areal weight of leaf mg/cm²	Water cont. of leaf g/g DW
Low	0	34.0 [c]	6.6 [d]	26.7 [a]	3.1 [e]	1.32 [e]	11.0 [d]	3.74 [bc]
	0.5	36.9 [bc]	6.6 [d]	26.5 [a]	2.6 [ef]	1.29 [de]	13.0 [bc]	3.80 [bc]
	1	28.8 [d]	5.2 [d]	26.0 [a]	2.2 [f]	1.26 [e]	14.0 [b]	3.63 [bc]
Medium (twofold)	0	42.9 [b]	13.9 [c]	25.5 [a]	7.6 [c]	1.65 [c]	10.1 [e]	3.83 [b]
	0.5	40.3 [b]	13.1 [c]	25.4 [ab]	5.4 [d]	1.44 [d]	14.1 [b]	3.71 [bc]
	1	31.1 [cd]	9.5 [cd]	26.6 [a]	3.7 [def]	1.30 [de]	16.1 [a]	3.42 [c]
High (fourfold)	0	52.8 [a]	30.3 [a]	21.3 [b]	24.9 [a]	2.35 [a]	7.9 [f]	4.42 [a]
	0.5	48.6 [ab]	28.5 [a]	23.3 [b]	15.2 [b]	1.74 [bc]	12.9 [bc]	3.95 [b]
	1	42.4 [b]	22.6 [b]	23.2 [b]	11.8 [b]	1.86 [b]	13.0 [c]	3.94 [b]

* Immediately before nutrient and NaCl treatment, top of all plants was cut off at 4cm above the surface of culture media, and regenerated plants were used for measurement 60 days after the beginning of the treatment. Each figure indicates an average of 6 plants. Figures not bearing the same superscript within a column are significantly different at the 5% level.

** Concentration of N, P and K in the low nutrient culture solution was 25, 12 and 34.5ppm respectively.

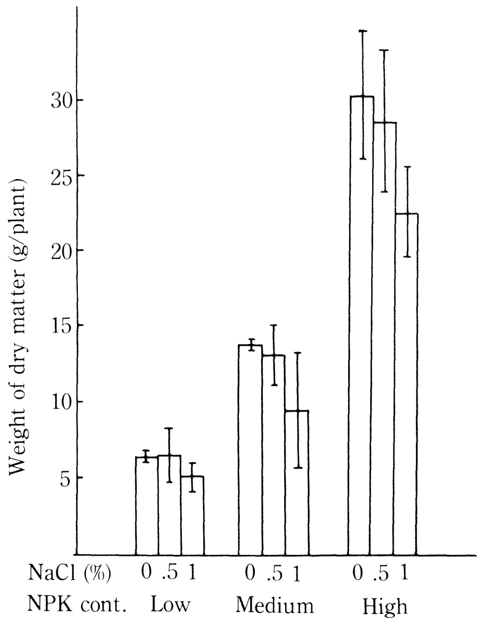

Fig. 14. Yield of *Atriplex nummularia* grown at different NaCl levels combined with different nutrient concentrations.

However the dry weight of the leaves and stems of the plants treated with 1% NaCl was lower than that of the plants treated with NaCl at other concentrations regardless of the nutrient level. The dry weight of the leaves and stems of the plants treated with 1% NaCl in the high nutrient plot was about 1.5 times and 3.5 times that of the plants grown without NaCl treatment in the medium nutrient plot and low nutrient plot, respectively. Leaf area per plant showed similar tendency to that of the dry weight of the leaves and stems, while the areal weight of the leaves showed an opposite tendency.

Na and K contents of harvested leaves are presented in Fig. 15. The Na content of the leaves increased and the K content decreased by the NaCl treatment, but there were no differences among the nutrient treatment plots for the same NaCl level in the culture solution. On the other hand the Na content of the leaves decreased and the K content increased with the increase of the nutrient concentration in the solution in the plots without NaCl treatment. Na and K contents of harvested stems showed a similar tendency to that of the leaves though the Na and K contents of the stems were lower than those of the leaves.

Total nitrogen content of the leaves increased with the increase of the nutrient concentration in the culture solution and it decreased also by the NaCl treatment in the culture solution. However there were no appreciable differences in the nitrogen content between the NaCl 0.5% and 1.0% treatments (Table 13).

The photosynthetic rate and transpiration rate from the 24 to 26th day and in the 28th day after the beginning of the treatment, respectively decreased remarkably with the increase of the NaCl concentration in the culture solution, while there were no variations in the rates with the changes in the nutrient concentration in the culture

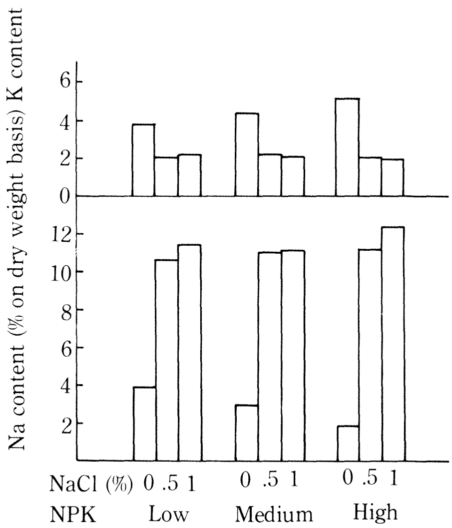

Fig. 15. Na and K contents of *Atriplex nummularia* leaf grown at different NaCl levels combined with different nutrient concentrations.

Table 13. Total nitrogen content in *Atriplex nummularia* leaf grown at different NaCl levels combined with different nutrient concentrations (% on dry weight basis).

NaCl treatment	Nutrient concentration treatment		
	Low*	Medium (twofold)	High (fourfold)
%	%	%	%
0	1.63	2.22	3.68
0.5	1.33	1.55	2.11
1	1.28	1.62	2.56

* Concentration of N, P and K in the low nutrient culture solution was 25, 12 and 34.5 ppm respectively.

solution (Figs. 16-A and C).

The chlorophyl content of the leaves 55 days after the beginning of the treatment increased with the increase of the nutrient concentration in the culture solution and decreased remarkably by the NaCl treatment though differences in the chlorophyl content between the NaCl 0.5% and 1.0% treatments were not clear (Fig. 16-B).

Additional experiment: As the plants of the N-120 and N-200 plots exhibited lodging, as shown in Fig. 17, due to the softening of the stems, they were harvested 50 days after the beginning of the treatment although they grew vigorously at first. The dry weight of the leaves and stems per plant in the N-100, N-120 and N-200 plots was 24.7±3.9g, 24.5±2.3g and 24.9±5.5g, respectively.

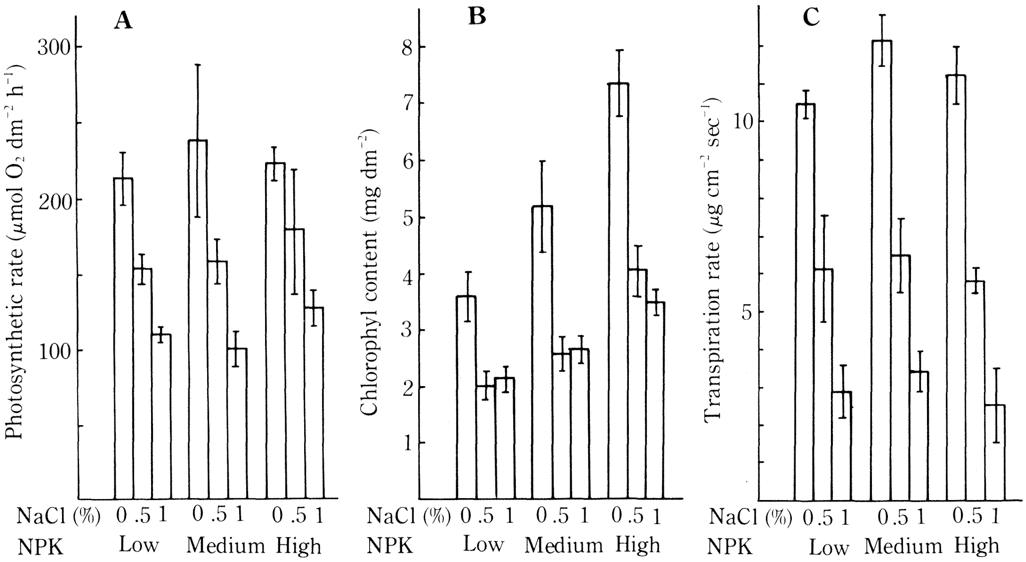

Fig. 16. Photosynthetic rate, chlorophyl content of leaf and transpiration rate of *Atriplex nummularia* grown at different NaCl levels combined with different nutrient concentrations.

N-100 N-120 N-200

Fig. 17. Lodging of *Atriplex nummularia* by nitrogen excess.

Discussion

It was observed that the increase of the nutrient concentration in the culture solution promoted significantly the growth of *Atriplex nummularia.* The growth and yield of the plants treated with a higher level of nutrients in the 1% NaCl treatment were higher than those of the plants treated with lower levels of nutrients without the NaCl treatment. A similar tendency was observed when only the nitrogen concentration was increased: the growth and yield of *Atriplex nummularia* receiving higher levels of nitrogen in the 1% NaCl treatment were higher than those of the plants growing under lower levels of nitrogen without the NaCl treatment. Rice plants grown in the culture solution containing NaCl and 40ppm nitrogen suffered from salt injury, while rice grown in the culture solution containing NaCl at the same concentration and more than 80ppm nitrogen did not experience any salt injury and the growth was promoted (SHIMOSE, 1965). On the basis of the results of experiments using *Spergularia* spp., OKUSANYA and UNGAR (1984) reported that the addition of certain nutrients to seedlings growing under high salinity conditions could help overcome the problems associated with high salinity. These reports support the results obtained in the current studies.

Many *Atriplex* spp. are described at nitrophilous plants, particularly the weedy species, and the leaf nitrogen content of these plants is among the highest ever found in wild plants (LEXANDER et al., 1970; OSMOND et al., 1980). WILLIAMS (1963 b) also reported that *Atriplex* spp. showed a strong response to nitrogenous fertilizer: the dry matter production of *Atriplex nummularia* plants receiving urea was threefold that of the control plants (without fertilizer application). The dry matter production of the plants receiving urea and superphosphate was about 4.5 times that of the control plants, while there were no differences between the superphosphate treatment and the control.

It is suggested that the ability of *Atriplex* to grow in arid region soils or coastal dune sands which are unusually low in PO_4 implies that the plants are able to absorb more effectively this nutrient anion (JONES et al., 1970; OSMOND et al., 1980). Therefore the application of nitrogenous fertilizer is one of the most important measures for promoting the growth of *Atriplex nummularia.*

In experiment 2), the dry weight of the leaves and stems increased almost in parallel to the increase of the concentration of nutrients, N, P, K and some micronutrients, while in experiment 1), the increase of only the N concentration did not affect significantly the dry weight of the plants: for example, the dry weight of the plants in the N 120ppm treatment increased only 1.1~1.5 times compared with that of the plants in the N 60ppm treatment. Nitrogen efficiency for dry matter production of *Atriplex nummularia,* which was expressed as g dry matter increment/ppm N applied/day, is presented in Table 14. In experiment 2), the nitrogen efficiency

Table 14. Nitrogen efficiency for dry matter production* of *Atriplex nummularia* grown at different NaCl levels combined with different nutrient concentrations.

NaCl treatment	N conc. (ppm) balanced with P and K**			N conc. (ppm) at constant P and K***		
%	25	50	100	30	60	120
0	4.40	4.87	5.47	4.82	3.56	2.16
0.5	4.40	4.33	5.13	5.27	4.51	1.44
1	3.47	2.87	4.37	4.58	4.22	0.40

* Nitrogen efficiency for dry matter production was expressed as g dry matter increment/ppm N applied/day.

** Concentrations of phosphate and potassium were varied at the same rate as the change of nitrogen concentration. Data were quoted from table 12.

*** Concentrations of phosphate (50ppm) and potassium (140ppm) were kept constant. Data were quoted from table 10.

increased slightly with the increase of the N, P, K concentration, while in experiment 1), the nitrogen efficiency decreased with the increase of the N concentration, and the decrease in the N 120 ppm treated plants was remarkable in comparison with that in the N 60ppm treated plants.

The decrease of the nitrogen efficiency may not be due to the increase of the N concentration at constant P and K concentrations, but may be ascribed to the fact that the marginal response to nitrogenous fertilizer of *Atriplex nummularia* is about 100ppm N. The fact that the remarkable increase of the N concentration along with the P and K concentration did not result in the increase of the dry weight of the leaves and stems suggests that the marginal response to nitrogenous fertilizer of *Atriplex nummularia* is in the range of 100 and 120ppm N.

Summary

Salt tolerance of *Atriplex nummularia* was investigated at different NaCl levels combined with different nutrient concentrations. The plants were grown in vermiculite using a culture solution containing various levels of NaCl and nutrients. The results obtained are as follows:

1. Dry weight of leaves and stems increased almost in parallel to the increase of the concentration of N, P and K in the culture solution when the values of N, P and K were below 100, 48 and 138ppm, respectively. There were no significant differences in the dry weight of the leaves and stems between the plants treated with 0.5% NaCl and those in which the NaCl treatment was omitted regardless of the nutrient level of the culture solution. However the dry weight of the leaves and stems of the plants treated with 1% NaCl was lower than that of the plants treated with other NaCl concentrations regardless of the nutrient level.

When the N concentration increased and the P and K concentrations remained constant, a similar tendency was observed but the degree of the increase of the dry weight of the leaves and stems was not remarkable.

2. Growth and yield of plants growing at higher levels of nutrients with 1% NaCl treatment were considerably higher than those of the plants growing at lower levels of nutrients without NaCl treatment. When the levels of N were higher but the levels of P and K remained constant, a similar tendency was observed.

3. The photosynthetic rate and transpiration rate decreased with the increase of the NaCl concentration in the culture solution. However the increase of the nutrient level of the culture solution did not affect these physiological responses.

4. Symptoms of nitrogen excess were observed in plants grown in the culture solution containing 120ppm nitrogen, although the plants grew vigorously at first. Subsequently the stems of the plants became soft and prone to lodging and their nitrogen efficiency decreased remarkably.

3. Salt tolerance at the germination stage of seeds produced under different saline environments

It was reported in part III-1 that the germination percentage of seeds of *Atriplex nummularia* placed in 2% NaCl solution was about 25%. However it has been reported that the germination percentage of halophyte seeds produced in soils with a higher salinity is higher than that of the seeds produced in soil with a lower salinity (Bazzaz, 1973; Clarke and West, 1969; Keren and Evenari, 1974). In order to analyse the relationship between the salt tolerance at the germination stage of *Atriplex nummularia* seeds and the saline environment of the production sites of the seeds, germination tests of seeds produced in different salinity environments were carried out using a saline solution.

Materials and methods

1) Salt tolerance at the germination stage of the seeds collected from three sites with different saline environments

The collected seeds were as follows: Western Australia seeds (WA), supplied by the Ministry of Agriculture, Western Australia State, Australia, September 1979; Beer-Sheva seeds (BE), supplied by the Ben-Gurion University in the Negev, Israel, December 1979; New South Wales seeds (NS), supplied by the Division of Plant Industry, CSIRO, Australia, June, 1981. The seeds were stored under dry conditions at 5°C. The germination percentage of the seeds at temperatures ranging from 15°C to 20°C did not decrease at all in during the storage. The naked seeds (UCHIYAMA, 1981) more than 0.8mg in air-dry weight were used in the experiments. Germination was tested in petri dishes 9cm in diameter containing two sheets of Toyo No. 2 filter paper, moistended with 3.5ml of test solution, and the germination beds including petri dishes, filter papers and the test solution were changed every day as described in part III-1. The germination beds were kept in the incubator at 15°C. NaCl concentrations of the test solutions were 0.25, 0.5, 0.75, 1.0, 1.5 and 2.0%, respectively. The experiment was continued for 21 days. Four replications of each treatment were carried out with 20 seeds per dish. Bases were extracted with 1*N* HCl from the bracteoles and were measured with the atomic absorption spectrophotometer, Shimazu AA-650.

2) Salt tolerance at the germination stage of the NS seeds and their progeny seeds (TK) produced in Tsukuba, Japan

The NS seeds were introduced from CSIRO, Australia, in May 1979. A part of the NS seeds was sown in the field of the Tropical Agriculture Research Center, Tsukuba, Japan and their progeny seeds (TK) were harvested in August 1981. The NS and TK seeds were examined by the same methods as those described above except for the NaCl concentrations of the test solutions which were 0.5, 1.0, 1.5 and 2.0%. The measurement of the bases contained in the bracteoles was carried out the same methods as those described above.

3) Salt tolerance at the germination stage of the seeds produced in plants grown at different NaCl levels in the culture medium

Homogeneous plants were obtained from a single plant by using cuttings: the methods for obtaining the cuttings and culture were identical with those outlined in part III-2. These plants were transplanted to Wagner pots 200cm^2 in surface area. The pots with the plants were placed in a shallow container containing the culture solution at a 4cm depth. The culture solution was absorbed through the bottom hole of the pot. The culture solution had the same composition as that described in part III-2 and contained 0.5, 1.0 and 2.0% NaCl, respectively. Control culture solution contained 25—35ppm Na originating from the city water. The seeds were harvested about 1 year after the beginning of the NaCl treatment. The germination percentages of the seeds harvested from each NaCl treatment pot were tested in NaCl solutions. The methods applied for the germination test were the same as those outlined above, except for the NaCl concentrations of the solutions which were 1.0, 1.5 and 2.0%. Four replications of each treatment were carried out with 40 seeds per dish.

The measurement of the bases contained in the bracteoles was carried out by the same methods as those described above.

Results

1) Salt tolerance at the germination stage of the seeds collected from three sites with different saline environments

The differences in the salt tolerance at the germination stage of the seeds collected from three sites with different saline environments are presented Table 15. The germination percentage of the WA seeds was lower than that of the NS and BE seeds when the concentration of the solution exceeded 0.75% NaCl and the germination percentage of the NS seeds was slightly lower than that of the BE seeds when the concentration of the solution exceeded 1.0% NaCl.

Table 15. Effect of NaCl concentration on germination percentage of *Atriplex nummularia* seeds collected from different places.

Place of seed production	NaCl concentration (%) of medium						
	0	0.25	0.5	0.75	1.0	1.5	2.0
WA	100	100	88±10	75±17	58±17	28±15	
NS	100	100	93±13	93± 5	78±15	63± 8	25±15
BE	98±5	95±6	93± 5	93±10	93±10	70±17	40±12

Notes: WA, Western Australia State, Australia; NS, New South Wales State, Australia; BE, Beer-Sheva, Israel. Naked seeds showing normal appearance with the weight heavier than 0.8mg each were used. Experiment was carried out at 15°C and measurement was made on the 21st day after the beginning of seed soaking.

Contents of Na and K in the bracteoles of the seeds which are presented in Table 16, were higher in the seeds with a higher germination percentage at a concentration of more than 1.0% NaCl in the solution.

Table 16. Contents of Na and K in bracteoles of *Atriplex nummularia* seeds collected from different places.

Place of seed production*	Content** (% on dry weight basis) of	
	Na	K
WA	4.13	0.90
NS	4.95	2.77
BE	5.43	4.18

* See notes of Table 15.
** Bases were extracted with 1*N* HCl.

2) Salt tolerance at the germination stage of the NS seeds and their progeny seeds (TK) produced in Tsukuba, Japan

The NS seeds showed a higher germination percentage than the progeny seeds (TK) produced in Tsukuba, Japan at a concentration of more than 1.5% NaCl as shown in Table 17. Contents of bases in the bracteoles of these seeds are listed in Table 18.

Table 17. Effect of NaCl concentration on germination percentage of *Atriplex nummularia* seeds produced in Australia and their progeny seeds produced in Japan.

Place of seed production	NaCl concentration (%) of medium				
	0	0.5	1.0	1.5	2.0
NS	98±5	95±5	80±11	65± 8	33±17
TK	97±5	95±6	80± 4	42±13	9± 9

Notes: NS, New South Wales State, Australia; TK, Tsukuba, Ibaraki-ken, Japan. See notes of Table 15.

Table 18. Contents of bases* in bracteoles of *Atriplex nummularia* seeds produced in Australia (NS) and their progeny seeds produced in Japan (TK).

Place of seed production	Content (% on dry weight basis) of				
	Na	K	Mg	Ca	Total
NS	4.90	2.30	0.50	0.48	8.18
TK	0.91	1.67	0.55	0.91	4.04

* Bases were extracted with 1*N* HCl.

Contents of Na and K in the bracteoles of the TK seeds were about 20% and 70% for those of the NS seeds, respectively. Content of Ca in the bracteoles of the TK seeds was about twofold that of the NS seeds whereas the content of Mg was not different between both groups of seeds. Total content of the bases of the TK seeds was about half of those of the NS seeds.

3) Salt tolerance at the germination stage of the seeds produced in plants grown at different NaCl levels in the culture medium

Germination percentage of the seeds produced in the plants grown at different NaCl levels in the culture medium is presented in Table 19. The seeds produced in the plants which grew in the culture solution containing more than 0.5% NaCl showed a remarkably higher germination percentage than the seeds produced in the plants grown in the culture solution without NaCl treatment in the germination beds with 1.5% and 2.0% NaCl solutions. Especially the seeds produced in the plants grown in the culture solution containing 2.0% NaCl showed clearly a higher germination percentage than the other seeds, even in the germination beds with 1.0% NaCl solution.

The content of Na in the bracteoles of the seeds produced in the plants which grew in the culture solution containing more than 0.5% NaCl was remarkably higher than

Table 19. Effect of NaCl concentration on germination percentage of *Atriplex nummularia* seeds produced on the plants grown at different NaCl levels of culture solution.

NaCl concentration of culture solution used for seed production %	NaCl concentration (%) of medium			
	0	1.0	1.5	2.0
0	99±1	84±6	32±14	4±4
0.5	99±1	84±8	64± 7	23±5
1.0	99±1	86±3	53± 6	25±9
2.0	99±3	96±2	78± 5	41±9

See notes of Table 15 except place of seed production.

that of the seeds produced in the plants grown in the culture solution without NaCl treatment and the total contents of the bases of the former were also higher. However there was no difference in the Na and base contents between the seeds produced in the plants grown in the culture solution containing 1.0% NaCl and those in the 2% NaCl solution (Table 20).

Table 20. Contents of bases* in bracteoles of *Atriplex nummularia* seeds produced on the plants grown at different NaCl levels of culture solution.

NaCl concentration of culture solution used for seed production	Content (% on dry weight basis) of				
	Na	K	Mg	Ca	Total
0	1.79	6.13	1.34	1.58	10.84
0.5	9.48	2.14	0.32	0.40	12.34
1.0	11.85	2.01	0.38	0.43	14.67
2.0	11.98	1.65	0.31	0.51	14.45

*Bases were extracted with 1*N* HCl.

Discussion

Contents of bases in the soil of New South Wales and Tsukuba where the seeds were produced respectively, are presented in Table 21. The soil of the site which produced the NS seeds, with a higher salt tolerance at the germination stage and higher base content in the bracteole, showed a higher contents of exchangeable bases at depths of 10-40cm, with values 2-3 times those of the TK soil, especially the Na content of the former was remarkably high. It is difficult to determine whether there is a relationship between the salinity level of the soil which produced the seeds and the salt tolerance of the seeds at the germination stage, on the basis of the results of only experiments 1) and 2), because the viability of the seeds varied with the storage conditions, storage period and climatic conditions of the production site.

However some reports state that the halophyte seeds produced on a soil with a

Table 21. Contents of bases in the soils growing *Atriplex nummularia* which produced the seeds.

NS*						TK*					
Depth of soil sampling cm	Contents of bases me/100g					Depth of soil sampling cm	Contents of bases me/100g				
	Ca	Mg	K	Na	Total		Ca	Mg	K	Na	Total
3	4.5	4.6	0.8	0.8	10.7	0	11.27	4.31	0.92	0.75	17.25
10	7.5	11.7	1.0	3.2	22.7	10	7.59	2.87	0.75	0.39	11.60
30	7.7	12.0	0.9	7.6	28.2	30	5.90	1.97	0.87	0.29	9.03
40	6.3	11.0	0.8	7.2	25.3	42	5.25	1.60	0.41	0.29	7.55

* Place of seed production
NS: New South Wales State, Australia (see references, Stace et al., 1968).
TK: Tsukuba, Ibaraki, Japan (see references, National Institute of Agro-Environmental Sciences, 1984).

higher salinity show a higher salt tolerance at the germination stage than the seeds of the same species produced on a soil with a lower salinity (BAZZAZ, 1973; CLARKE and WEST, 1969; KEREN and EVENARI, 1974; UNGAR, 1982) and these reports are in agreement with the results of the present experiments. In addition, rice which is glycophyte shows also a similar tendency (FUJII, 1962).

UNGAR (1982) suggested that the variations in the salt tolerance of the seeds at the germination stage are caused by differences in populations or ecotypes within a plant species. He suggested also that there is a possibility of genetic selection having taken place for increased salt tolerance in the evolution of some taxa. However experiment 3) suggests that the salinity of the culture medium affects the salt tolerance of the seeds of the next generation of *Atriplex nummularia* at the germination stage, because the seeds produced from homogeneous plants showed variations in the salt tolerance at the germination stage depending on the changes in the salinity of the culture medium in the previous generation. Then it is considered that the salinity of the soil which produced the seeds affects the physiological activity of the seeds, in addition to the theory of ecotype proposed by UNGAR (1982).

FUJII (1962) reported that the germination percentage of the rice seeds (Variety: Sanin 55) which were produced from plants growing in a culture solution containing 0.5% NaCl was higher than that of the seeds produced from the plants growing in a culture solution without NaCl, and that there were differences in the paper-electrophoretic patterns of protein of brown rice between both groups of seeds. He suggested that the NaCl treatment in the previous generation may have affected enzyme systems of the seeds. Therefore when *Atriplex nummularia* is planted on saline soil, the salinity conditions of the soil which produced the seeds to be planted must be considered.

The contents of the bases in the bracteole which contains the seed with a higher salt tolerance at the germination stage are higher than those in the bracteole which contains the seed with a lower salt tolerance. On the other hand the salt tolerance of the seeds produced on a soil with a higher salinity is higher than that of the seeds produced on a soil with a lower salinity, and the contents of the bases in the bracteole of the former are higher than those of the latter. However care should be exercised when adapting the contents of the bases in the bracteole as an index of the salt tolerance level of the seed because the contents of bases in the bracteole do not increase above a certain level.

Summary

The purpose of this study was to clarify the relationship between salt tolerance at the germination stage of seeds and the saline environment of the production sites of the seeds. The results obtained are as follows:

1. There were differences in salt tolerance at the germination stage of the seeds collected from three sites with different saline environments. The seeds from Beer-Sheva (BE), Israel, showed the highest salt tolerance, those from the New South Wales State (NS), Australia, showed a medium salt tolerance, and the seeds from the Western Australia State (WA), Australia, showed the lowest salt tolerance. This order was in agreement with the order of the total contents of Na and K in the bracteoles.
2. The seeds from NS showed a higher salt tolerance than their progeny seeds produced in Tsukuba, Japan, without soil salinity. Contents of bases in the bracteoles of the seeds from NS were remarkably higher than those of the seeds from Tsukuba.
3. The seeds produced in plants grown at different NaCl levels in the culture medium showed that the higher the NaCl level, the higher the salt tolerance at their germination stage, and the higher the contents of bases in their bracteoles.

On the basis of the results obtained, the relationship between the salinity of the soil used for seed production and the salt tolerance of the seeds was discussed.

Note : Among the three types of *Atriplex* seeds used in the experiment (WA, NS, BE) the WA seeds exhibited the lowest tolerance to salt. However there is a wide range of salt tolerance among the *Atriplex* seeds produced in WA and some of them display a definit tolerance to salt.

VI Effect of sodium chloride on the chemical composition of feed made of *Atriplex nummularia*

It was demonstrated in this study that *Atriplex nummularia* possesses the very high salt tolerance: for example, the plant was able to survive in a saline medium containing 4% NaCl at daily maximum temperatures of more than 40°C (daily mean temperature more than 33°C); plants which grew on the saline medium with 1% NaCl at a daily mean temperature of 25°C with an adequate supply of nutrients could produce 75–80% of the dry matter produced by plants grown under normal conditions. When *Atriplex nummularia* is cultivated so as to use it as an animal feed, the feeding value of the forage must be considered. Although the evaluation of the feed value should be carried out in considering various aspects, the chemical analysis of the general components of the feed and the relationship between the NaCl concentration of the culture solution and feed production were invesgigated here.

Furthermore it must be emphasized that *Atriplex nummularia* showed a strong absorption ability for Na and Cl, and that the contents of NaCl in the leaves and stems increased remarkably with the increase of the NaCl concentration of the culture solution. The major part of this NaCl is transported into the bladder cells as mentioned previously (part IV-2), and does not penetrate into the tissues but remains in a free state. This free NaCl affects considerably the physiology of the animals fed on *Atriplex nummularia.* Therefore the relationship between the NaCl concentration of the culture solution and animal feeding using *Atriplex nummularia* was also investigated.

Materials and methods

1) Chemical analysis of the general components of feed

Materials were collected from the plants used in the experiments described in part V-2-2): the culture solution contained 25ppm nitrogen, 12ppm phosphate, 34.5ppm potassium, 7.5ppm magnesium, 22.5ppm calcium and some micronutrients for the "low nutrient plot", a twofold nutrient concentration for the "medium nutrient plot" and fourfold nutrient concentration for the "high nutrient plot".

The NaCl concentrations of the culture solution were 0.5% and 1.0%. The culture solution of the control contained 30–35ppm Na originating from the city water. Daily mean temperature was 25°C. Chemical analysis was carried out for six components of feed in using the Japan Feed Component Chemical Analysis Standard.

2) Estimation of the content of NaCl in the leaves and stems

The materials were collected from the plants used in the experiments described in part V-2-2) and V-1. The latter plants grew in the culture solution containing the same nutrient elements as those of the "high nutrient plot" referred to in part V-2-2) and at a daily mean temperature of 17°C for the "low temperature plot", 25°C for the "medium temp. plot" and 33°C for the "high temp. plot", respectively. Na was extracted with 1*N* HCl from the dried shoots (leaves and stems) of the plant and was measured with the atomic absorption spectrophotometer, Shimazu AA-650. NaCl content was calculated from the Na content in assuming that all Na was in the NaCl form.

Results and discussion

1) Effect of NaCl concentration and nutrient concentration of the culture solution on feed production

The chemical composition of *Atriplex nummularia* grown at three levels of NaCl concentration combined with three levels of nutrient concentration is presented in Table 22. The data for the plants which grew in the field of TARC, Tsukuba, Japan, for two years and were harvested on 11th, June, 1983 are also presented in the Table for reference.

Table 22. Chemical composition of *Atriplex nummularia* grown at different NaCl levels combined with different nutrient concentrations*.

Treatment			Chemical composition (% on dry matter)				
Nutrient conc.	NaCl %	Dry matter %	Crude protein	C. fat	C. ash	C. fiber	N.F.E.***
Low	0	26.7	7.9	1.8	17.7	22.4	50.2
	0.5	26.5	6.8	2.0	25.2	19.2	47.9
	1.0	26.0	7.3	1.9	28.5	15.3	46.9
Medium (twofold)	0	25.5	11.5	1.8	17.2	19.1	50.5
	0.5	25.4	8.9	2.0	27.0	15.7	46.5
	1.0	26.6	9.8	2.0	28.7	12.5	46.8
High (fourfold)	0	21.3	19.5	1.8	19.4	13.9	45.4
	0.5	23.3	12.0	2.0	28.7	13.1	44.5
	1.0	23.2	15.0	1.9	31.7	9.9	41.5
Field culture**		21.2	22.7	2.4	21.2	13.4	40.4

* Concentration of N, P and K in the Low culture solution was 25, 12 and 34.5ppm, respectively. Mean daily temperature was 25°C. Measurement was carried out 60 days after the beginning of the treatment.
** The field of Tropical Agriculture Research Center, Tsukuba.
*** Nitrogen free extract

In the *Atriplex nummularia* plants which had been growing at a daily mean temperature of about 25°C in the culture solution containing an adequate amount of nutrients but lacking NaCl, the content of crude protein, nitrogen free extract and crude fiber amounted to about 20%, 45%, 14% on a dry weight basis respecively. The plants grown in the field of Tsukuba contained 22.7% of crude protein which is a value comparable to the value of 20% reported in Australia and South Africa (FAO, 1981; Wilson, 1966 a). The content of crude protein, nitrogen free extract, crude fiber and crude ash in alfalfa, shown in the Standard Tables of Feed Composition in Japan (1980), are 27%, 35-39%, 20-23% and 12% before the flowering stage, respectively, and 18-21%, 39%, 27-30% and 9-10% at the flowering stage, respectively. Therefore *Atriplex nummularia* appears to be equal or superior to alfalfa at the flowering stage with regard to the feed composition except for the content in crude ash. The high content of crude ash is one of the constraints on its use as forage. In this experiment, crude ash contained 4.7% K, 1.4% Na and 1.3% Ca in the high nutrient plot without NaCl. The effect of NaCl and nutrient concentration of the culture solution on the leaf-stem ratio on a dry weight basis is presented in Table 23. The leaf-stem ratio increased with the increase of the NaCl concentration and increased remarkably with the increase of nutrient concentration.

The chemical composition of the leaves and stems is presented in Tables 24-(1) and

Table 23. Leaf-stem (dry weight) ratio of *Atriplex nummularia* grown at different NaCl levels combined with different nutrient concentrations*.

NaCl concentration %	Nutrient concentration Low	Medium	High
0	1.08	1.25	1.83
0.5	1.07	1.42	1.94
1.0	1.39	1.62	2.12

* See notes (*) of Table 22.

24-(2), respectively. The feeding value of the leaves was higher than that of the stems except for the crude ash content because the former contained a larger amount of crude protein, crude fat and nitrogen free extract and less crude fiber. However the very high content of crude ash is one of the drawbacks for the utilization of the leaves

Table 24. Chemical composition of leaf and stem of *Atriplex nummularia* grown at different NaCl levels combined with different nutrient concentrations*.

(1) Leaf

Treatment			Chemical composition (% on dry matter)				
Nutrient conc.	NaCl %	Dry matter %	Crude protein	C. fat	C. ash	C. fiber	N.F.E.
Low	0	21.1	10.8	2.2	23.6	7.0	56.4
	0.5	20.6	8.7	2.3	34.2	6.4	48.3
	1.0	21.6	8.4	2.3	36.8	5.6	46.8
Medium	0	20.8	14.6	2.2	22.3	6.3	54.6
	0.5	21.2	10.1	2.4	35.0	5.0	47.6
	1.0	22.3	10.6	2.3	34.9	4.8	47.4
High	0	18.5	23.7	2.0	22.1	6.0	46.2
	0.5	20.0	13.6	2.3	35.4	4.9	43.9
	1.0	20.3	16.6	2.1	37.6	4.0	39.7

(2) Stem

Treatment			Chemical composition (% on dry matter)				
Nutrient conc.	NaCl %	Dry matter %	Crude protein	C. fat	C. ash	C. fiber	N.F.E.
Low	0	37.5	4.9	1.3	11.3	39.0	43.6
	0.5	36.9	4.6	1.6	15.5	33.0	45.3
	1.0	36.2	5.4	1.6	17.1	28.8	47.1
Medium	0	35.8	7.5	1.3	10.8	35.1	45.4
	0.5	36.8	7.2	1.5	15.5	31.0	44.9
	1.0	36.8	8.6	1.6	18.7	25.2	45.9
High	0	30.5	11.7	1.5	14.6	28.4	43.9
	0.5	34.0	8.9	1.5	15.7	29.0	45.6
	1.0	33.2	11.6	1.7	19.3	22.4	45.1

* See notes (*) of Table 22.

as feed.

The remarkable decrease of the crude protein content, remarkable increase of the crude ash content and decrease of the crude fiber and nitrogen free extract contents in the leaves were associated with the increase of the NaCl concentration of the culture solution. In the stems, the increase of the crude ash content and decrease of the crude fiber content were associated with the increase of the NaCl concentration of the culture solution. The increase of the NaCl concentration of the culture solution brought about a definite decrease of the crude protein content, a decrease of the crude fiber content and a remarkable increase of the crude ash content on a whole plant basis.

The remarkable increase of the crude protein content and definite decrease of the nitrogen free extract in the leaves, and remarkable increase of the crude protein content and distinct decrease of the crude fiber content in the stems were associated with the increase of the nutrient concentration of the culture solution. Also the increase of the nutrient concentration of the culture solution which brought about a remarkable increase of the crude protein content and a distinct decrease of the crude fiber content contributed significantly to the increase of the feeding value of *Atriplex nummularia*.

The increase of the NaCl concentration of the culture solution resulted in brought the increase of the leaf-stem ratio. However it contributed more to the remarkable decrease of the crude protein content and accumulation of NaCl in the leaves than to the increase of the number of leaves which containing a larger amount of crude protein and less crude fiber, hence the decrease in the quality of the feed. On the other hand the increase of the nutrient concentration of the culture solution resulted in the increase of production of feed due to the remarkable increase of the crude protein content as well as the increase of the leaf-stem ratio.

2) Effect of NaCl concentration of culture solution combined with different nutrients and different temperatures on the NaCl content of the plant

NaCl content of *Atriplex nummularia* grown at three levels of temperature and three levels of nutrient combined with three levels of NaCl concentration in the culture solution is presented in Table 25. The control plants for the NaCl treatment

Table 25. NaCl content of *Atriplex nummularia* grown at different temperatures and nutrients combined with different NaCl concentrations of culture solution (% on dry matter).

NaCl concentration of culture solution %	Temperature treatment*			Nutrient treatment**		
	Low	Medium	High	Low	Medium (twofold)	High (fourfold)
0	2.66	3.08	4.09	6.53	4.76	3.53
0.5	17.91	21.27	33.01	19.01	20.58	22.12
1.0	22.78	28.60	37.32	21.78	21.84	25.70

* Mean daily temperature, Low, Medium and High was 17°C, 25°C and 33°C, respectively. Concentration of N, P and K in the culture solution was 100, 48 and 138ppm, respectively. Measurement was carried out 48 days after the beginning of the treatment.

** Concentration of N, P and K in the low nutrient culture solution was 25, 12 and 34.5ppm, respectively. Mean daily temperature was 25°C. Measurement was carried out 60 days after the beginning of the treatment.

contained 2–4% NaCl, except for the plants with a poor growth due to the lack of nutrients, as very small quantities of Na were supplied to the plants from the city water which contained 30–35ppm Na. When the NaCl concentration of the culture solution exceeded 0.5%, the NaCl content of the plant increased remarkably, and reached values of more than 20% on a dry weight basis in most of the treatments. However no specific relation between the nutrient concentration of the culture solution and the NaCl content of the plant was detected. MEYER et al. (1955) reported that fattening steers were not adversely affected by feed to which 9.33% NaCl had been added for 84 days. MEYER and WEIR (1954) reported also that female sheep were not adversely affected by feed to which 13.1% NaCl had been added during the growing and fattening period but that weight loss was observed during the lactation period. WILSON (1966 b) reported that Merino sheep showed a reduction in weight gain when 10% NaCl was added to feed. The COMMITTEE ON ANIMAL NUTRITION, NATIONAL RESEARCH COUNCIL, U.S.A. (NATIONAL ACADEMY OF SCIENCES, 1980) defined the "maximum tolerable levels of dietary salt in animals" as follows: a level of 4% was set for lactating cows, since this level was the maximum level tested by DEMOTT et al. (1968); a level of 9.0% was set for other cattle and sheep based on the studies of MEYER et al. (1955) and MEYER and WEIR (1954), respectively. Therefore single feeding of *Atriplex nummularia* which was grown on saline soil containing more than 0.5% NaCl, is undesirable. However the maximum tolerable level may increase in areas where animals can obtain water easily, because the saline disorder can be alleviated if the animals drink large amount of water (NATIONAL ACADEMY OF SCIENCES, 1980; WILSON, 1966). Rangelands with *Atriplex nummularia* require a larger number of water places than other grasslands.

HASSAN (1979) reported that the addition of barley grains to fresh leaves and stems of *Atriplex nummularia* produced good results including the increase of feed intake, the decrease of water intake and the increase of body weight gain, as compared with a single feeding of *Atriplex nummularia*.

The development of a feeding method combining the use of *Atriplex nummularia* with other grasses which contain low amounts of NaCl, should be promoted in order to prevent salt disorders of livestock.

Content of Ca on a dry weight basis was 1.1–1.3% in the plants which were grown in the culture solution without NaCl treatment. Such a value is too low compared with that for alfalfa. Furthermore the increase of the NaCl concentration of the culture solution decreases remarkably the Ca content.

Summary

By using *Atriplex nummularia* plants which had been grown in a culture solution containing NaCl, the relationship between the chemical composition of feed and NaCl concentration in the medium was investigated. The results obtained are as follows:

1. In *Atriplex nummularia* plants which had been growing at a daily mean temperature of about 25°C in a culture solution containing an adequate amount of nutrients but lacking NaCl, the content of crude protein, nitrogen free extract and crude fiber amounted to about 20%, 45% and 14%, on a dry matter basis respectively.

2. Content of crude protein decreased remarkably whereas the content of crude fiber clearly increased with the lowering of the nutrient level of the culture solution. The content of crude protein decreased significantly whereas the content of crude ash increased remarkably with the rise of the NaCl concentration in the culture solution.

3. NaCl content of plants increased markedly with the rise of the NaCl concentration in the culture solution: for example the NaCl content of the plants which had been grown in the culture solution containing 0.5% NaCl at a daily mean temperature of 25°C exceeded 20%.

On the basis of the results obtained, the suitability of *Atriplex nummularia* as feed for livestock is discussed.

VII. General Discussion

1. Salt tolerance level of *Atriplex nummularia*

SATO (1983) defined the criteria for the salt tolerance level of a plant as follows: (1) the extreme margin of salt concentration which allows the plant to survive; (2) the growth rate of the plant grown at a certain concentration of salt to the growth increment of the plant grown under no saline environment; (3) absolute amount of production of the plant grown at a certain concentration of salt.

With regard to criterion (1), the salt tolerance level of *Atriplex nummularia* was remarkably higher than that of rice and wheat. *Atriplex nummularia* in the vermiculite culture survived when the culture solution applied contained 5% NaCl at a temperature of 25°C while the plants in the solution culture survived when the NaCl concentration of the solution was 3% at a daily mean temperature of 30 - 33°C or at a daily maximum temperature of 40 - 45°C. It is evident that the salt tolerance of *Atriplex nummularia* is very high in comparison with that of rice which died in the culture solution containing 1.5% NaCl for a few days (IWAKI and OTA, 1953).

As for criterion (2), it may be considered that the salt tolerance level of *Atriplex nummularia* was remarkably higher than that of other crops. NaCl concentration halving the dry weight of some crops in the solution culture was as follows: garden beet about 1.4% (HOFFMAN and RAWLINS, 1971), cotton about 0.7 - 1.4% (HOFFMAN et al., 1971; BOYER, 1965), creeping bentgrass about 0.7 - 1.0% (YOUNGNER et al., 1967), barley about 0.6% (GAUCH and EATON, 1942) and rice about 0.5% (SHIMOSE, 1963). In the present experiment, the dry matter production of *Atriplex nummularia* which was grown in the culture solutions containing 1% and 2% NaCl was about 80% and 55%, respectively of that of the plants grown in a culture solution to which NaCl was omitted.

Regarding criterion (3), the lack of data precludes any judgment.

It is considered that *Atriplex nummularia* could be introduced into regions with a high salinity soil because the salt tolerance level of the plant is remarkably higher than that of many species of crops, on the basis of the criteria discussed above.

2. Mechanism of salt tolerance of *Atriplex nummularia*

TADANO (1983) outlined the main causes of salt injury of crops as follows: (1) limitation of water intake by increase of the osmotic pressure of the culture medium; (2) physiological disorder or cytoclasis due to the excessive intake of salt; (3) antagonistic inhibition of absorption of essential elements by special elements contained in large amounts in the culture medium; (4) peculiar growth injury by special elements composing the salt.

It is considered that causes (1) and (2) are closely related to the mechanism of salt tolerance of *Atriplex nummularia* in the following aspects:

Ability to overcome the limitation of water intake
  Prevention of transpiration: Transformation of the leaf morphology
  Prevention of the decrease of water intake: Adjustment of the osmotic pressure of the cell sap by intake of salt from the culture medium
Ability to overcome the high salt concentration within the plant

Increase of the osmotic pressure of the cell sap: Peculiar tolerance of the plant to a high osmotic pressure
Excretion of sodium chloride: Function of vesiculated hairs
Adaptation to a high saline enviornment
Avoidance of high saline environment: Lack of germination when the soil salinity is high

1) Prevention of transpiration

When plants grow on a saline soil, their transpiration rate may decrease by transformation of the leaf morphology as follows: (1) decrease of leaf number and leaf area per plant, (2) decrease of stomata number per unit leaf area, (3) increase of leaf thickness and (4) accumulation of wax on leaf epidermis (MAAS and NIEMAN, 1978).

In the author's experiments (1) and (3) mentioned above were obviously observed in *Atriplex nummularia,* and the transpiration rate per unit leaf area of the plant grown in the culture solution containing 0.5% NaCl decreased remarkably.

Such a transformation of the leaf morphology is a form of protection against the decrease in water intake caused by the high salinity of the culture medium.

2) Prevention of the decrease of water intake

BERNSTEIN (1961) suggested that the cotton plant is able to increase the osmotic pressure of its cell sap by absorbing large amounts of salt ions from the culture medium and to keep a constant difference of osmotic pressure between the roots and culture medium in order to continue to absorb water.

The author measured the osmotic pressure of the cell sap of the leaves devoid of vesiculated hairs of *Atriplex nummularia* grown in the culture solution containing 0%, 0.5% and 1.0% of NaCl, respectively and of the each culture solution by using the vapor pressure osmometers, Wescor 5100. The difference in the osmotic pressure between the cell sap (higher) and corresponding culture solution (lower) were 17.9 atm., 28.8 atm. and 28.6 atm. respectively in the 0%, 0.5% and 1.0% NaCl treatment plots.

The findings recorded in the present study such as the increase of the total base contents of leaf and stem with the increase of the NaCl concentration of the culture solution, and the absence of difference in the water content of leaf per unit area among the plants grown in the culture solution at different NaCl levels, support Bernstein's theory.

The leaves of *Atriplex nummularia* contain generally 5 - 6% oxalate on a dry weight basis (WILSON, 1966a). The accumulation of oxalate in plant may also contribute to the increase of the osmotic pressure of the cell sap.

3) Increase of the osmotic pressure of the cell sap

REPP et al. (1959) studied the NaCl concentration of the solution in which 50% of the cells of the stem tissues of several species soaked in the solution for 24 hours were killed and observed that salt tolerance varied with the species.

The osmotic pressure of the cell sap of *Atriplex confertifolia* growing on a salt rich soil in Utah State, U.S.A., reached 202 atm. (HARRIS, 1934).

The osmotic pressure of the cell sap of the lamina of *Atriplex nummularia* grown in a culture solution containing 1% NaCl was about 45 atm. *Atriplex nummularia* grown in a solution containing 1% NaCl produced about 80% of the dry matter of the plants grown in a solution lacking NaCl (Part III-2). On the other hand Repp et al. reported that 50% of the cells of the stem tissue of beet (*Beta vulgaris*) died when soaked in a solution with an osmotic presure of 29 atm. for 24 hours.

Salt tolerance of the cells of *Atriplex nummularia* is remarkably high.

4) Excretion of sodium chloride

Vesiculated hair is the most important and characteristic structure of *Atriplex nummularia* in relation to the salt tolerance mechanism. *Atriplex nummularia* absorbs a large quantity of salt from the culture medium with a high salt concentration in order to be able to absorb water. The cells of the plant are tolerant to a high osmotic pressure originating from the salt. However regardless of the level of tolerance the cells will be destroyed if the plant absorbs continuously salt in order to absorb water.

The vesiculated hair of *Atriplex nummularia* consists of one bladder cell and several stalk cells. The bladder cell which accumulates NaCl collapses and excretes NaCl when the concentration of NaCl in the cell increases. The structure and mechanism of action of the vesiculated hair is definitely different from those of the salt gland of Mangroves, Tamarix and other plants (LIPHSCHITZ and WAISEL, 1982; SCHIRMER and BRECKLE, 1982; THOMSON, 1975). MOZAFAR and GOODIN (1970) observed a 60-fold accumulation of salt concentration in the bladder cells over the mesophyll in *Atriplex halimus*. SCHIRMER and BRECKLE (1982) estimated the amount of Na within the biomass of *Atriplex confertifolia* at about 460 kg per ha and calculated that its annual cycling amounted to at least 150 kg per ha.

The fact that vesiculated hairs are rarely found on the cotyledon of *Atriplex nummularia* may account for the low salt tolerance of *Atriplex nummularia* at the stage of seedling establishment.

5) Avoidance of high saline environment

Most of the seeds which did not germinate in a high NaCl solution (more than 2%) did not lose their viability and germinated after being transferred into pure water. It has been reported that the seeds of some halophytes remain dormant in the soil under high salinity conditions and germinate when rainfall reduces the salinity of the surface soil (UNGAR, 1962, 1974, 1977, 1978, 1982; WARD, 1967; WILLIAMS and UNGAR, 1972).

Seedling establishment is the most sensitive and vulnerable stage in the life cycle of halophytes (SCHIRMER and BRECKLE, 1982; UNGAR, 1982). Delay in the time of germination until the stress associated with soil salinity decreased, prevented the seedlings from dying immediately and provided them with some chance of surviving to maturity (UNGAR, 1982). This mechanism is very important for the preservation of the species. UNGAR (1982) reported that the seeds which do not germinate until the level of soil salinity is reduced might have been naturally selected after a long period of time.

Salt tolerance of adult *Atriplex nummularia* plants is higher than that of the plants at the seed germination and seedling establishment stages. The critical concentration of NaCl for the survival of adult *Atriplex nummularia* plants is about 5%, based on the data recorded in part III-2. The bracteroles, harvested from *Atriplex nummularia* plants growing on a high salinity soil, contained such high concentrations of NaCl that the plant could not survive. The NaCl content of the bracteole is obviously higher than that of the soil where the plant grew. When rain leaches salt in the surface soil, water-soluble salt present in the bracteole of the seed placed in the soil will be leached at the same time. When the NaCl concentration of the bracteole decreases enough to enable the seed to germinate due to rain, the soil salinity also will have throughly decreased so as to allow for the seed to germinate and for the seedling to become established. A high content of salt in the bracteole will not be a disadvantageous factor for the propagation of the species, but will be one of the important mechanisms of the adaptation of *Atriplex nummularia* to saline environments.

3. Factors controlling the growth increase of *Atriplex nummularia* in saline soil

GOODIN and MCKELL (1970) estimated at 16,000kg per hectare per year the potential yearly yields of fresh forage of *Atriplex lentiformis* on arid rangelands. However the actual yield levels are lower than this value for example the fresh forage yield per hectare per year ranges from 700kg to 1230kg in the rangeland of *Atriplex vesicaria* at Broken Hill, Australia (GRAETZ and WILSON, 1984). It is considered that the same figures apply to *Atriplex nummularia*. Therefore the development of a technology to promote the growth of the plant which is essential, is discussed as follows:

1) Relationship between growth and fertilizer application

Fertilizer response, especially nitrogen response, of *Atriplex nummularia* is very high. Concentrations of N, P, and K of the medium nutrient plot in the experiment V-2-2) were about the same as those of the "Kasugai solution for field crops" (KASUGAI, 1939). Dry weight of leaves and stems per plant in the high nutrient plot (about twice the concentrations of the Kasugai solution) was about twice that of the medium nutrient plot.

It was observed that the growth and yield of the plants cultured at a higher level of nutrients which experienced injury by 1% NaCl were markedly higher than those of the plants growing at a lower level of nutrients without salt injury.

Therefore the increase of the amount of fertilizer applied not only contribute to the increase of the growth and yield of *Atriplex nummularia* but also enables to grow the plant on a high salinity soil. The increase of the amount of nitrogen applied results in the increase of the content of crude protein in the leaves and stems and contributes to the increase of production of useful feed components.

Although it is difficult to apply a large dose of fertilizer in vast rangelands, fertilizer application for *Atriplex nummularia* is essential for the increase of the productivity of land and for the efficient utilization of vacant lands.

However excessive application of fertilizer results in the decrease of the efficiency of fertilizer use. Especially, excessive application of nitrogen inhibits the growth of *Atriplex nummularia* due to the softening and lodging of the plant stem. It was considered that the marginal response to nitrogenous fertilizer of *Atriplex nummularia* would be about 100ppm N under the conditions of the present experiment.

2) Relationship between growth and temperature

(1) Adult plant

Optimum temperature for adult *Atriplex nummularia* is considered to be 25°C (daily mean temperature). Therefore for a region to be suitable for the growth of *Atriplex nummularia,* numerous days with a daily mean temperature of 25°C are required. It can be anticipated that in such a region the yield of the plant may be very high on a soil with a low salinity, and that the yield of the plants grown on saline soil containing 1% NaCl is about the same as that of the plants grown on non-saline soils at daily mean temperatures of 33°C and 17°C, respectively.

In a region where the daily mean temperature is less than 20°C although the growth rate of the plant is rather low, the yield will not decrease on a saline soil containing 1% NaCl compared with the plants grown on non-saline soil. Therefore when it is difficult to grow other crops due to the high salinity of soil, the introduction of *Atriplex nummularia* can contribute to the efficient use of vacant lands.

In a region where the daily mean temperature is more than 30°C a certain amount of yield can be expected in the case of a low salinity soil containing less than 0.5% NaCl. On the other hand although during 2 months in a year the daily maximum temperature ranges between 40 and 45°C, if during the other months the mean temperature ranges between 20 and 30°C, the growth of *Atriplex nummularia* will not be significantly impaired. However since the content of NaCl in the leaves and stems increases with the temperature, the feeding value of such a plant will decrease.

(2) Germination stage and seedling establishment stage

Although *Atriplex nummularia* germinates very well at temperatures ranging from 4°C to 22.5°C (UCHIYAMA, 1984), the optimum temperature for the germination stage is about 15°C based on the observations of the germination percentage and the number of average days to germinate under salt stress. However the percentage of the seedlings which survived at lower temperatures was higher than that at higher temperatures; for example, at concentrations of more than 1% NaCl, the seedlings hardly survived at 20°C while some of the seedlings survived at 15°C and about twice as many seedlings survived at 10°C. Therefore it is necessary to consider carefully the effects of soil salinity and temperature on the germination of the seeds of *Atriplex nummularia* at the time of seeding.

3) Salt tolerant seeds

When *Atriplex nummularia* is introduced into a region with soils characterized by a high salinity, seeds with a high salt tolerance should be sown. It goes without saying that to select or to breed highly salt-tolerant varieties is very important. On the other hand the germination percentage of seeds harvested from plants growing on a high salinity soil is higher than that of seeds harvested from plants growing on a non-saline soil, as mentioned in part V-3. UNGAR (1982) attributed this phenomenon to differences in the populations or ecotypes within a plant species. However the results of the current experiments V-3-3) show that the physiological characteristics of the seeds may vary with the soil salinity in addition to the variations in populations or ecotypes. Therefore when *Atriplex nummularia* is planted on saline soil the salinity condition of the soil which produced the seeds to be planted must be considered. In this regard the contents of bases in the bracteole may enable to evaluate the salt tolerance of the seed to some extent.

4. Contribution of and problems in the use of *Atriplex nummularia* for agriculture

1) Use of Atriplex nummularia as feed resource

Atriplex nummularia is a very important source of feed in arid lands with saline soil because it grows better on saline soil than other crops. In terms of the contents of crude protein, nitrogen free extract and crude fiber, *Atriplex nummularia* is equal or superior to alfalfa at the flowering stage. However the high content of crude ash is a constraint on the utilization of *Atriplex nummularia* as feed.

The present experiment showed that the higher the nutrient concentration of the culture solution, the higher the feed production of the plant so far as the N concentration of the culture solution is less than 100ppm. Therefore the increase of the amount of fertilizer applied should promote plant growth and feed production.

The increase of the NaCl concentration of the culture medium resulted in the decrease of the production of the chemical components of feed and in a considerable increase of the NaCl content of leaf and stem which adversely affected livestock. Therefore the development of a method combining the use of *Atriplex nummularia* with other feeds with a low content in salt and the increase of the number of water places, etc. are very important.

However in certain seasons of the year when the soil salinity increases significantly due to high temperature and pronounced aridity, the plant should not be used as feed but as a cover crop for soil conservation and prevention of soil erosion.

2) *Other uses of Atriplex nummularia*

It has been suggested that salty soils could be reclaimed by growing and harvesting *Atriplex* on those soils (GOODIN and MOZAFAR, 1972; SHARMA, 1982). Although WAISEL (1972) concluded that desalinization in *Atriplex* is ineffective, it is necessary to reexamine this problem.

MCKELL (1975) stated that *Atriplex* could be used for soil stabilization and prevention of soil erosion, particularly in arid lands.

Recently perennial *Atriplex* species have been considered as a possible biomass crop plant for energy production. Among the plants which require minimal inputs of water, fertilizers, machineries, etc. for cultivation and those which can be effectively planted on vacant lands, *Atriplex* would be most suitable. Indeed this C_4 plant is highly tolerant to salinity and drought, can be produced at a low cost and is characterized by a large biomass productivity (KELLY et al., 1982).

On the other hand the recent remarkable development of biotechnology suggests the possibility of improving higher plants as food resources. In view of the increasing interest in the transfer of salt tolerance to crops, plants harboring genes with an extremely high tolerance to salt will be one of the most important genetic resources.

VIII. Summary

It is necessary to develop the arid lands of the Middle East and North Africa, etc. for agricultural use. However such a development is prevented by the lack of suitable crops.

With a view to introducing in these regions *Atriplex nummularia,* a plant with a high tolerance to salt and drought which can be used as feed, the author studied the growth habit of the plant under high salinity conditions along with the physiological characteristics of the plants. The results obtained are as follows:

1. Germination percentage of the seeds placed in a 2% NaCl solution at 15°C was only about 25%. Therefore the salt tolerance of *Atriplex nummularia* at the germination stage is not particularly high compared with that of other crops. However almost no difference in the germination percentage was observed between treatments with 0.75% NaCl and pure water at 15°C. At temperatures ranging from 20°C to 30°C, the germination percentage decreased more remarkably at higher temperatures when the concentration of NaCl in the solution increased.

2. Salt tolerance at the seedling establishment stage was lower than that at the germination stage: all the seeds which had germinated died a few days after germination when the concentration of the NaCl solution exceeded 1% at the temperature of 20°C. However about 35% of the germinated seeds at 15°C and about 75% of the germinated seeds at 10°C became seedlings, respectively when the concentration of NaCl was 1% and 2%.

3. Non-germinated seeds were maintained in 2% and 3% NaCl solution for 21 days. Approximately 70% of these seeds germinated at 10°C and 80 - 85% germinated at 15 and 20°C, respectively, after being transferred into pure water. The bracteole contained large amounts of salts which play an important role in the adjustment of the germination of the seed. This phenomenon is one of the important mechanisms of adaptation of *Atriplex nummularia* to saline environments.

4. Adult *Atriplex nummularia* plants exhibited a very high salt tolerance, and survived in a culture solution containing 5% NaCl in vermiculite culture. However the dry matter production of the plant grown in a 1% NaCl solution was 75 - 80% of that of the control plants at a daily mean air temperature of 24.5°C.

5. Na content of the plants increased markedly with the increase of the NaCl concentration in the culture solution. This phenomenon accounts for the adjustment of the osmotic pressure which acts as a defense mechanism for the increase of the water intake ability of the plant.

Leaf area per plant and transpiration rate decreased with the increase of the NaCl concentration in the culture solution. This phenomenon operates as a defense mechanism of the plant for the decrease of water intake by the increase of the osmotic pressure of the culture medium. However as a result plant growth eventually decreased.

6. In adult plants of *Atriplex nummularia,* the vesiculated hairs grow thickly on the

surface of the leaf and stem. The vesiculated hairs are considered to paly the important role in the salt tolerance of the plant. Therefore *Atriplex nummularia,* grown under high salinity conditions, can excrete excess sodium chloride from the lamina of plants which absorbed the ions through the roots and conducting tissues, by repetition of the following cycle; (1) transport of Na and Cl from the lamina to the bladder cells and accumulation of Na and Cl in these cells, (2) collapse of the bladder cells containing crystals of NaCl and (3) liberation of the crystals from the collapsed cells. It is postulated that sodium chloride can be excreted from the surface of stem by the same mechanism since similar vesiculated hairs were observed on the surface of stem.

However vesiculated hairs were not found at all on the cotyledons of seedlings within 12 days after germination which may account for the low salt tolerance of the seedlings.

7. The plants were cultivated at a daily mean temperature of 17°C (low temperature plot), 25°C (medium temperature plot) and 33°C (high temperature plot), respectively. The plants of the medium temperature plot showed the optimum growth among the 3 temperature-plots at each level of NaCl in the culture solution. The plants grown at the temperature of 25°C with 1% of NaCl showed a growth pattern and yield similar to those of the plants grown at high and low temperatures without NaCl addition in the culture solution.

No decrease in the growth of the plant occurred at NaCl concentrations of 0.5 and 1.0% in the culture solution in the low temperature plot, although growth decreased significantly by 1% NaCl in the medium temperature plot. In the high temperature plot the decrease of growth by NaCl at the concentrations of 0.5% and 1.0% was more remarkable.

8. The Na content of the plant increased and the K content decreased with the increase of the NaCl concentration of the culture solution. The contents of both Na and K increased with the increase of the temperature regardless of the levels of NaCl. This trend was more pronounced in the Na content. These observations support the assumption that growth inhibition caused by excessive absorption of Na is promoted by high temperatures.

9. Dry weight of leaves and stems increased almost in parallel to the increase of the concentration of N, P and K in the culture solution up to values of 100 ppm, 48 ppm and 138 ppm for N, P and K, respectively. There were no significant differences in the dry weight of the leaves and stems between plants treated with 0.5% NaCl and those without the NaCl treatment regardless of the nutrient level of the culture solution at the daily mean temperature of 25°C. However the dry weight of the leaves and stems of the plants treated with 1% NaCl was lower than that of the plants treated with 0.5% NaCl or untreated with NaCl regardless of the nutrient level.

Growth and yield of the plants growing at higher levels of nutrient along with the 1% NaCl treatment were significantly higher than those of the plants growing at lower levels of nutrient without NaCl treatment. In the case of the higher levels of N and identical levels of P and K, a similar tendency was observed.

Symptoms of nitrogen excess were observed in plants grown in the culture solution containing 120 ppm nitrogen, and the nitrogen efficiency for dry matter production was markedly decreased.

10. The seeds produced in the plants growing in a higher salinity culture medium showed a higher salt tolerance at the germination stage than the seeds produced in the plants growing in a lower salinity culture medium. It is considered that the salinity of the soil which produced the seeds affects the physiological activity of seeds, in addition to differences of salt tolerance associated with differences in the ecotypes as suggested by UNGAR.

11. Feeding value of *Atriplex nummularia* which had been growing at a daily mean temperature of about 25°C in a culture solution containing an adequate amount of nutrients but lacking NaCl, was equal or superior to that of alfalfa of the flowering stage. However when the NaCl concentration of the culture solution was high the feed quality decreased.

12. On the basis of the results obtained, the mechanism of the high salt tolerance of *Atriplex nummularia* as well as the methods for improving the growth of the plant on high salinity soils was discussed.

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