〔Article〕

Evaluation of Salinity Tolerance in Coastal and Inland Populations of *Setaria viridis* by Growth and Non-Growth Analyses

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Abstract

A total of 14 coastal and inland plants of *Setaria viridis* (L.) P. Beauv. were examined for evaluation of soil salinity tolerance and salt spray tolerance by growth and non-growth analyses. Soil salinity tolerance was measured by rooting technique (gauging root elongation in water culture) and by growth analysis (weighing plant growth in soil culture). For the salt spray tolerance, growth inhibition of plant which was sprayed with NaCl solution at various concentrations and percentage of leaf necrosis of plant sprayed sequentially with NaCl were observed. There was no significant difference in soil salinity tolerance between the coastal and inland plants examining in both water and soil cultures treated with NaCl. All plants of which roots were treated with NaCl solution were seriously damaged in root elongation and top biomass at 125mM/l of NaCl which was approximately equivalent to a quarter of seawater in net weight of NaCl. On the other hand, significant difference between the coastal and inland plants was found in salt spray tolerance. The inland plants sprayed with NaCl at 0.25 mM/l exhibited leaf necrosis right after the spray, and

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then got damaged seriously. The coastal plants showed no leaf necrosis and grew well even at 4000 mM/l of NaCl solution. They showed no damage even when they had the sequential spray at a total of 83.5 g/㎡ of NaCl. The extremely high salt spray tolerance of the coastal plants of *S. viridis* observed here may enable them to grow in the severe coastal habitats on which seawater splashes repeatedly under stormy conditions such as typhoon. Both the growth inhibition from NaCl solution and the date when necrosis occurred readily represented the salt spray tolerance. The measurement of growth inhibition is surely applicable for determining the rate of NaCl of salt spray tolerance while the scoring leaf necrosis is a convenient way to find the plant order in salt spray tolerance.

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Keywords：salinity tolerance, salt spray tolerance, *Setaria viridis*

Ⅰ. Introduction

Weeds growing in field boundaries such as hedge, grass strip, paddy levee, riverbank and seashore often play important roles in agro-ecosystem and are enhancing biodiversity by providing insects and birds with refuges and foods (Cordeau et al., 2012, Marshall, 1989, Marshall et al., 2003 and Petit et al., 2003). Some of the weeds and their relative species often comprise a part of coastal plant communities on seashore and rock cliff on which tourists and anglers frequently trespass. The coastal plants including these weeds are often in danger in Japan because natural and semi-natural environments of waterfronts have been lost by bank protection with concrete covers in last several decades. *Setaria viridis (L.) P.* Beauv. is a wide spread annual grass species in the world, growing not only in crop fields but also in field boundaries (Dekker, 2003a and Wan et al., 1995). Coastal plants of the *S. viridis* are usually assigned to a maritime variant, *Setaria viridis* var. pachystachys (Franch et Savat.) Makino et Nemoto, which is sometimes listed on Red Data Books by local organizations in Japan. Thus, research works on the coastal populations of *S. viridis*are crucial for conservation of seaside environments.

Ecological races with compact stature as the result of adaptation to coastal environments have been widely perceived in a variety of species growing in exposed bluffs (Boyce, 1954; Clausen, 1951). *S. viridis* var. *pachystachys* is one of those plants growing in coastal habitats by changing its form to be short stature with decumbent branches while an inland relative, *S. viridis* is usually tall and erect (Osada, 1989). Itoh et al. (2014a) reported that the coastal individuals of *S. viridis* consisted of three ecotypes differing in plant form; upright/dwarf type found in rock cliff and pebbly seashore, prostrate/dwarf type seen on rock cliff only and upright/normal type in sandy seashore. These two dwarf types appeared to coincide in form with the maritime variant, *S. viridis var. pachystachys*, whereas the other taller type in sandy shore resembled an inland relative, *S. viridis*. Therefore, all plants studied here are noted as *Setaria viridis* due to the taxonomical difficulty.

Occurrence of *S. viridis* in coastal strand communities in the close proximity to the high tide line suggests that the plants are definitely inundated with splash of seawater. In particular, tropical cyclonic storm systems, such as typhoon and hurricane, cause profound impacts on coastal plants as the result of strong wind, which defoliates and breaks branches (Griffiths et al., 2003 and Griffiths, 2006). Salt spray associated with windstorm causes extensive necrotic damages to leaves of coastal plants (Lowry et al., 2008). As the coastal plants in Japan generally suffer severe damage from several typhoons every year, they should certainly develop adaptive traits to the catastrophic damage. Salinity tolerance is one of the most important adaptive traits to seashore, and consists of two phases, salt spray tolerance and soil salinity tolerance. Itoh et al. (2014b) reported that coastal plants of *S.*

viridis exhibited the higher salinity tolerance than inland plants collected from orchards and vegetable gardens when they were sprayed or were cultured with NaCl solution. It is meaningful for elucidation of its adaptation to coast habitats to confirm the high salinity tolerance using various methods to measure the salinity tolerance.

Ahmad and Wainwright (1977) demonstrated that two methods of measurement of salinity tolerance, rooting technique and growth analysis, were reliable for evaluation soil salinity tolerance of *Agrostis stolonifera*. The rooting technique is a modified method of determining tolerance to heavy metal by rooting tillers in culture solution and is suitable for screening of populations for tolerance. However, it was pointed out that the rooting technique was not as reliable for measuring salt tolerance as growth analysis measuring plant weight in culture solution (Tiku and Snaydon, 1971). These two methods are necessarily compared for determining the soil salinity tolerance of *S. viridis*.

Salt spray is known to cause leaf necrosis (Boyce, 1954). Lowry et al. (2008) readily proved that percentage leaf damage and the date of death after sequential sprayings of NaCl solution were reliable for determining the salt spray tolerance of *Mimulus guttatus*. This method appears simple and labor-saving due to the least procedures before scoring the damage from saline solution. However, it is difficult to determine the percentage leaf necrosis precisely on narrow leaves of *S. viridis*. Growth analysis is another way for assessing the salt spray tolerance. When plants are sprayed with NaCl solution, they are damaged due to leaf necrosis, resulted in growth inhibition. Measurement of growth inhibition of the plant sprayed at various rates of NaCl is laborious and time-consuming but is suitable for determining the rates of NaCl which damage the plant. Both the sequential sprays of NaCl solution and single spray at varying rates of the NaCl are applicable for evaluating salt spray tolerance.

The present study was undertaken to elucidate differences in soil salinity tolerance and salt spray tolerance between the coastal and inland plants of *S. viridis* with four test methods. Coastal plants of this species that usually grow in the habitats above the high tide line have lower possibility to be submerged in seawater but are likely exposed to seawater splash. Therefore, it is necessary to evaluate soil salinity tolerance and salt spray tolerance separately. The soil salinity tolerance was examined with rooting technique in water culture and with growth analysis in soil culture. Two types of seedlings which were different in age and size were grown in water and soil treated with NaCl for measurement of top and root growth. Single and sequential sprayings of NaCl solution were performed on 3 to 4-leaf seedlings to quantify growth inhibition and leaf necrosis from NaCl. Based on the results of these examinations on different-aged plants, mode of adaptation of *S. viridis* to coast habitats was discussed by comparing coastal plants in salinity tolerance with inland individuals. The significance of test methods for salinity tolerance was also discussed.

Ⅱ. Materials and Methods

Plants of *Setaria viridis were* individually collected from 22 populations in 12 locations along coastlines of central Japan from 2001 to 2011 (Itoh et al. 2014a). They were grown in three coast habitats (sandy seashore, pebbly seashore and rocky cliff) and arable lands (garden and orchard). Pairs of the coastal plants and the inland ones were made in five of 12 locations. The coastal plants were collected within 50 meters of the seaside in horizontal distance while the inland plants within a kilometer of the nearest seaside. Ten juvenile plants were randomly collected from each population and were transplanted into 11 cm diam. pots at Shikoku Gakuin University to obtain their seeds. The seeds of each plant were assigned to an individual (synonymous with a line) of each population. The selfed seeds obtained in a paper bag were individually stored in a refrigerator at 5°C. As a procedure, in this study, the seeds stored in a refrigerator were buried in the soil for two winter months, January and February, to break seed dormancy and subsequently stored in the refrigerator prior to the experimental use. Seven coastal and seven inland plants were examined for salinity tolerances. Ten of the 14 plants were derived from 5 locations in which pairs of coastal and inland plants were sampled. The remained four plants were from a pebbly beach, a rocky cliff and two orchards respectively.

1. Soil salinity tolerance

1.1. Water culture

Soil salinity tolerance of seedlings was assessed with a so-called rooting technique in May, 2012. Two different-sized seedlings, namely seedlings at 1-day post germination with ca. 1mm root elongated and 5-leaf seedlings of which roots were clipped beforehand, were examined in water culture with NaCl solution at 0, 15.7, 31.3, 62.5 and 125 mM/l. After they grew in NaCl solution, the length of the longest root of each plant was measured and was calculated a ratio to that of the same line grown in control without NaCl added.

Ten seedlings at 1-day post germination of each plant from coast and inland habitats incubated at 25℃ were placed on two layers of filter papers in a 9 cm-diam. petri-dish filled with 10 ml NaCl solution. After the seedlings were allowed to grow for 10 days in the petri-dish with a lid on to avoid evaporation in an incubator at 25℃, root of each seedling was measured. Large seedlings studied here were grown in 2-fl. oz. pots up to 5-leaf stage in a greenhouse prior to the examination for salinity tolerance initiated from May 23. Four replicates of the large seedling without roots were separately placed into four 400 ml plastic vessels filled with NaCl solutions. The vessels were placed in a naturally lit greenhouse and were topped up with distilled water each day. The NaCl solutions were changed every four days for two weeks. After the plants rooted, the length of the longest root of each plant was measured.

1.2. Soil culture

Plants grown in soil culture were also examined for determining soil salinity tolerance in May, 2013. Plant materials and sizes in this experiment were the same as those in water culture. Seedlings at 1-day post germination in an incubator at 25℃ were transplanted in 1-fl. oz. pots filled with peat soil for horticultural use on

May 3 and were sub-irrigated in plastic square pans placed in naturally lit greenhouse. Liquid fertilizer was applied into the soil to set each pot to have N 80 P 80 K 80 kg/ha. Half of a total of 420 plants grown in the pots were treated with NaCl solution just after transplanting them into the potsoil while the other half was grown up to 3 to 4-leaf stage before the treatment. Three replicates of individuals from coast and inland habitats were placed in the greenhouse with randomized complete block.

As soil treatments, NaCl solutions of 5 ml at 0, 62.5, 125, 250 and 500 mM/l were individually poured into the 30-ml pot soil three times every other day to set the plants to grow in the soils containing NaCl at 0, 31.3, 62.5, 125 and 250 mM/l per pot. Two different-sized seedlings, 1-day post germination and 3 to 4-leaf seedlings, of which roots were imposed with NaCl in the soil were raised in the greenhouse for three and two weeks respectively. After top and underground growth of each plant were harvested, they were dried in an oven at 80°C for two days for weighing. Ratio of treated plant to untreated control in weight was calculated as soil salinity tolerance.

2. Salt spray tolerance

Salt spray usually damage plant growth through causing necrosis on leaves and branches of plants (Boyce 1954). While serious necrosis on leaves results in death, plants having slight necrosis often recover and grow as well as untreated plant. Thus, the damage from necrosis can necessarily be evaluated not only by necrosis percentage but also by plant growth. Pilot tests for examination of the effect of seawater on the growth of *S. viridis* in greenhouse exhibited that 7-leaf inland plants died while coastal plants did not, when they were sprayed with seawater (approximately 500 mM/l of NaCl) at the water volume of 1000 l /ha (Figure 1). Similar results were observed on seedlings at 3 to 4-leaf stage at which they were turned out to be autotrophic. The seedlings at this stage were used for the experiments of salt spray tolerance because large number of plants can be examined at once in a small greenhouse.

Figure 1. Photograph showing growth of plants from a rocky cliff (a and b) and from an orchard (c and d) when they were sprayed with water (a and c) and seawater (b and d). Plant in a circle was the same as d.

2.1. Single treatment

Seedlings at 3 to 4-leaf stage from coast and inland habitats were examined with single spraying of NaCl solution in May to determine the growth inhibition rate of NaCl for plants. Three replicates of each plant were sprayed overhead with NaCl solutions of 1000 l/ha at 0, 250, 500, 1000, 2000 and 4000 mM/l of NaCl. The NaCl of 500mM/l is nearly equivalent to seawater in net weight of NaCl, and that of 4000 mM/l is close to a saturated solution of NaCl. After top growth of each replicate was harvested two weeks after spraying, they were dried in an oven at 80°C for two days and were weighed. When the plant in 1-fl. oz. pot is sprayed overhead at the highest rate of NaCl, the concentration of NaCl of the pot soil is estimated less than 7 mM/l, assuming that NaCl solutions infiltrate into a whole soil equally. The effect of NaCl sprayed should be negligible on root growth, according to the results of soil treatments of NaCl.

2.2. Sequential treatments

Necrosis on leaves was observed with sequential sprayings of NaCl solution at every three days for 30 days. The rate of NaCl for spraying was increased with time, beginning from the 1st spray at 125 mM/l followed by the 2nd at 250, the 3rd at 500, the 4th at 1000 and the 5th and after that at 2000 mM/l. The necrosis percentage was scored by eyesight rating 10 times just before sequential spraying. The increasing rates of NaCl were adopted to cause necrosis on the aged seedlings which were usually more tolerant to salt spray than younger ones. Three replicates of each plant derived from coast and inland habitats were grown up to 3 to 4-leaf stage for this treatment as described in the experiment for single treatment. Beside necrosis percentage, the date of necrosis appeared, necrosis percentage and the date of death (complete leaf necrosis) were recorded every three days for each replicate.

3. Observation of leaf tissues

Leaf tissues of coastal and inland plants of *S. viridis* were observed by staining with safranin. Sixth leaf blade of a plant grown in pot was clipped and was sliced around the middle of the leaf blade for observation.

Ⅲ. Results and Discussion

1. Soil salinity tolerance

1.1. Water culture

Root elongation of *S. viridis* in water culture was greatly inhibited in both small and large-sized seedlings even at 31.3 mM/l of NaCl (equivalent to one eighth of seawater in net weight of NaCl) in water culture (Figure 2). Although no significant difference in rooting was observed between plants from coast and inland habitats, the coastal plants tended to have rather greater rooting at 31.3 and 62.5 mM/l

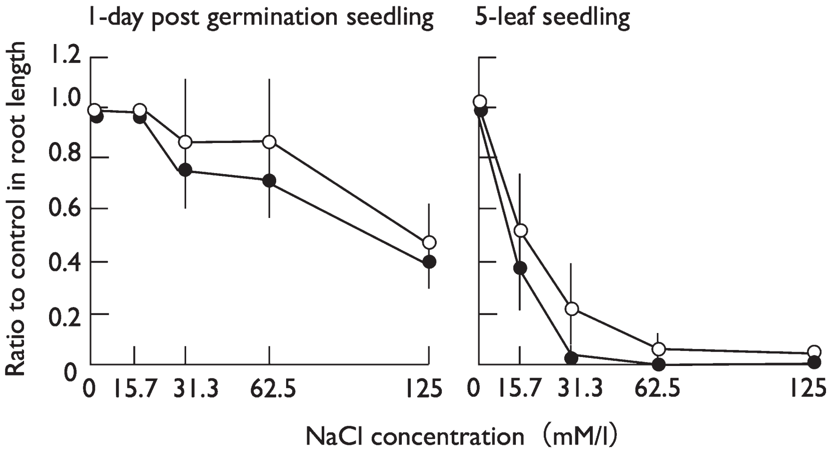

Figure 2. Soil salinity tolerances of 1-day post germination seedling and 5-leaf seedling growing in NaCl solution. Ratio to control in root length was calculated as soil salinity tolerance. Vertical bars indicate S.E. ○, coastal plant, ●, inland plant.

of NaCl than inland ones. In the experiment on larger seedlings of which roots were clipped prior to water culture, the coastal plants had 80% inhibition in root elongation even at 31.3 mM/l of NaCl while inland individuals never rooted. These results suggest very low potentiality for *S. viridis* plants to grow in saline soils.

1.2. Soil culture

Plant growth of *S. viridis* in response to varied salt concentrations was studied in soil culture this time to make it clear that this species cannot grow in saline soils. Figure 3 shows relationships between top and root growth of plants growing in saline soil and under salt spray. Significantly positive relationships between top and root growth were found in both soil salinity ($r=0.828$, $p<0.01$) and salt spray ($r=0.879$, $p<0.01$). The results suggest that the increase of top biomass of *S. viridis* synchronized with the root growth when either leaves or roots were damaged with

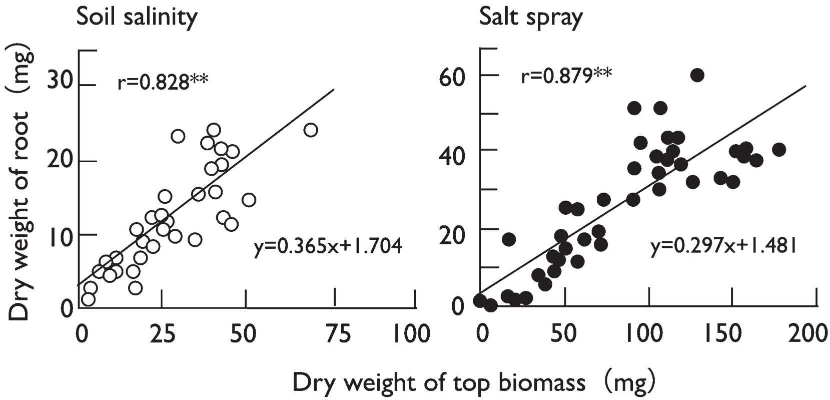

Figure 3. Relationships between top and root growth in dry weight of plant treated with NaCl solution. ○, soil salinity, ●, salt spray.

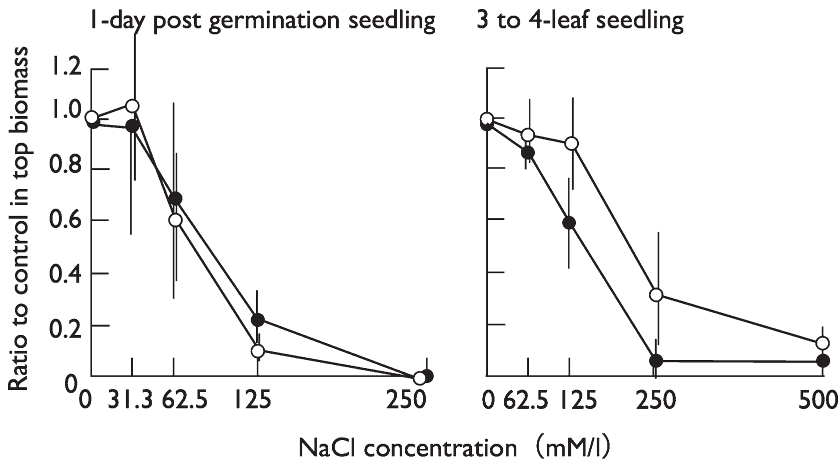

Figure 4. Soil salinity tolerances of 1-day post germination seedling and 3 to 4-leaf seedling growing in pot soil to which NaCl solution was applied. Ratio to control in top biomass was calculated as soil salinity tolerance. Vertical bars indicate S.E. ○, coastal plant, ●, inland plant.

saline water. Consequently, the measurement of top biomass inhibition appears to be suitable for evaluating the salinity tolerance.

Figure 4 shows the top biomass inhibitions by NaCl of 1-day post germination seedlings and 3 to 4-leaf seedlings of which roots in pot were treated with NaCl solution. No significant differences were observed in soil salinity tolerance between the coastal and inland plants, irrespective of seedling size. The large seedling at 3 to 4-leaf stage was twice more tolerant than the seedling just after germination. The large seedling was inhibited in growth at 125 mM/l of NaCl while the inhibition on small one was found at 62.5 mM/l. As seen in the water culture experiments, the coastal plants in soil culture tended to be more tolerant in averaged plant growth than inland individuals. This was observed on large-sized seedlings sprayed at 125 and 250 mM/l. This rate of NaCl inhibiting plant growth was much lower than the seawater salinity. These results suggest that any *S. viridis* plant may be unable to grow in saline habitats such as salt marsh land.

2. Salt spray tolerance

Salt spray tolerance was evaluated on 3 to 4-leaf seedling with two different treatments of NaCl solution, single and sequential treatments. Plant damages were observed on growth basis for single treatment and on injury basis for sequential treatments because the necrosis caused by NaCl spraying does not necessarily result in growth inhibition. Dry weight and plant length of *S. viridis* plants sprayed at various rates of NaCl were measured to determine the rate of NaCl which affects the growth. Sequential treatments were carried out to find the rates of NaCl causing necrosis on leaves and killing the plants (complete leaf necrosis).

2.1. Single treatment

Coastal plant of *S. viridis* showed significantly higher salt spray tolerance than inland individual at 250 to 2000 mM/l of NaCl (Figure 5). The result was clearly observed in both plant length and top biomass. The inland plant was damaged seriously at 125 mM/l, and was almost dead at 2000 mM/l while the coastal

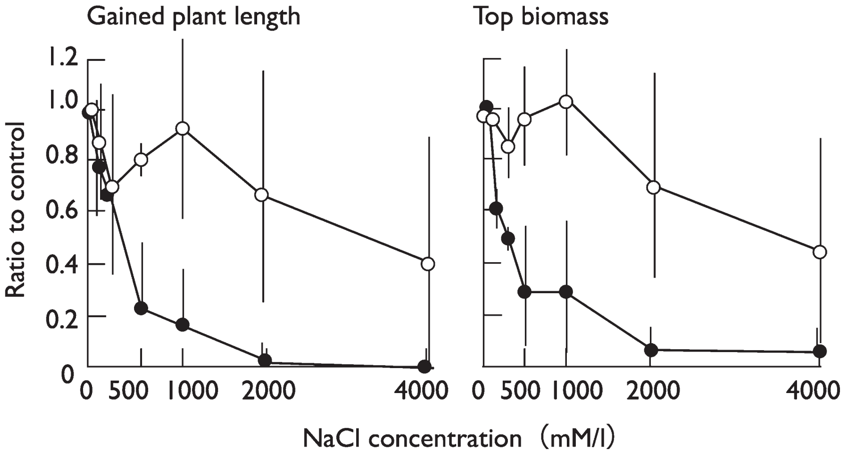

Figure 5. Salt spray tolerance of 3 to 4-leaf seedling sprayed with NaCl solution. Ratio to control in plant length and top biomass was calculated as salt spray tolerance. Vertical bars indicate S.E. ○, coastal plant, ●, inland plant.

individual had no damage in top biomass even at 1000 mM/l of NaCl and some injury at 2000 mM/l. The standard error of coastal plant increased at 2000 and 4000 mM/l of NaCl in both plant length and top biomass. This was resulted from wide variation in salt spray tolerance among coastal plants, hence the salt spray tolerances of all 14 individuals from coast and inland habitats were plotted against the rates of NaCl in Figure 6. Six of seven coastal plants exhibited higher salt spray tolerance than all seven inland lines at any rate of NaCl, and were never damaged even at 2000 mM/l which was four times higher in net weight of NaCl than seawater. Exceptionally, one plant from a sandy seashore provided higher salt spray tolerance than inland ones at 250, 500 and 1000 mM/l of NaCl, but decreased its tolerance at 2000 mM/l as low as that of the inland lines. The results obtained here indicate that salt spray tolerance varied among coastal populations.

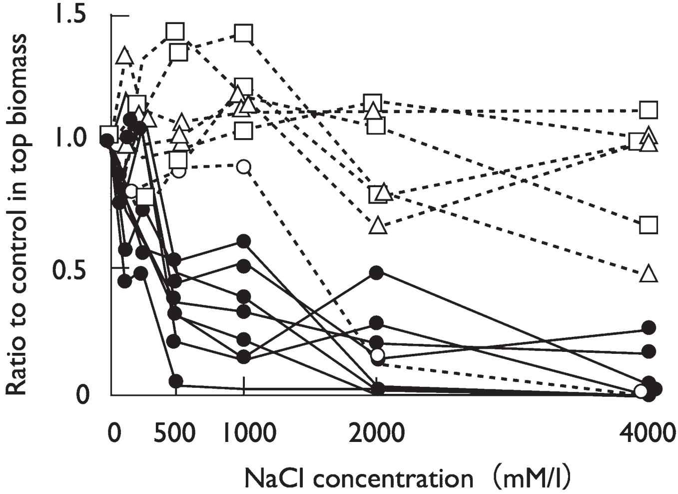

Figure 6. Salt spray tolerance of individual seedlings at 3 to 4-leaf stage sprayed with NaCl solution. Ratio to control in top biomass was calculated as salt spray tolerance. ○, sandy shore plant, □, pebbly shore plants, △, rocky cliff plants, ●, inland plants.

The coastal plants from pebbly shore and rocky cliff possess extremely higher salt spray tolerance than the individuals from gardens and orchards. The sandy shore plants appear to be intermediate in salt spray tolerance between those two groups of plants.

2.2. Sequential treatments

As the great difference in salt spray tolerance between coastal and inland plants was observed on growth basis, necrosis caused by NaCl solution was assessed with 11 sequential treatments of NaCl for 30 days (Figure 7). The amount of NaCl on stems and leaves accumulated from 0.75 g/㎡ (equivalent to 125 mM/l) on the first day to 83.5 g/㎡ on the last day of the treatments. Significant difference was found in percentage leaf necrosis between the coastal and inland plants. Six coastal plants

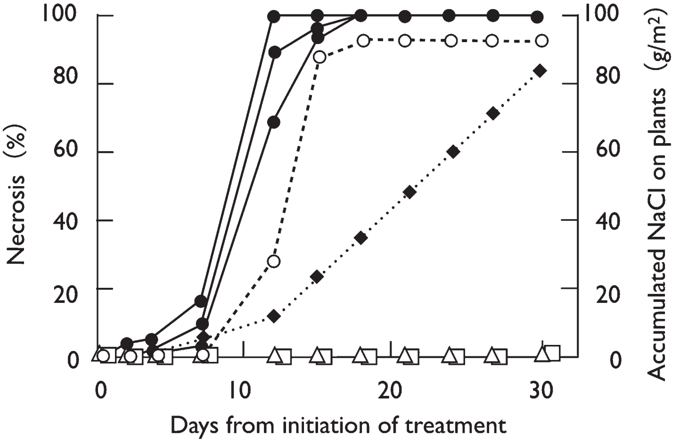

Figure 7. Salt spray tolerance of individual seedling at 3 to 4-leaf stage sequentially sprayed with NaCl solution. Percentage of leaf necrosis represented salt spray tolerance. ○, sandy shore plant, □, pebbly shore plants, △, rocky cliff plants, ●, inland plants, ◆, amount of accumulated NaCl on plant.

except for one from a sandy shore didn't show any evidence of damage from accumulated NaCl even at 83.5 g/m^2 while all seven inland individuals from gardens and orchards died at 23.5 g/m^2 on the 15th day. The inland plants provided a slight necrosis at 0.75 g/m^2 on the 4th day. A sandy shore plant was intermediate and showed higher tolerance than any inland plant at 11.25 g/m^2 on the 12th day. The order of salt spray tolerance among plants observed here was same as that from the single treatment experiment. Figure 8 shows the relationship between growth inhibition when plant was sprayed at 3 g/m^2 (=500 mM/l) of NaCl (approximately equivalent to seawater) and amount of accumulated NaCl providing plant leaves with necrosis when it was sprayed sequentially. Both the growth inhibition from NaCl and the amount of NaCl causing necrosis appeared to be reliable representatives for

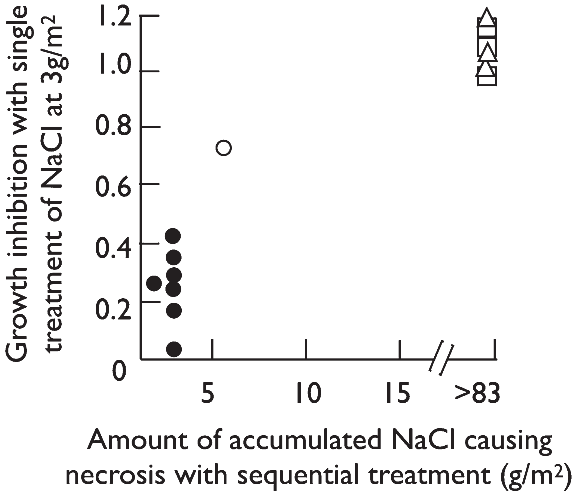

Figure 8. Relationship between amount of accumulated NaCl causing leaf necrosis with sequential treatments and growth inhibition from NaCl at 3 g/㎡ with a single treatment. The growth inhibition was on the same scale as salt spray tolerance at 500 mM/l in Figure 6.

salt spray tolerance because those two provided the similar results. The single treatment of various rates of NaCl solution is useful for determining the concentration of NaCl inhibiting plant growth while the sequential treatments of NaCl solution can be employed to find the order of salt spray tolerance among plants.

The coastal plants derived from pebbly shore and rocky cliff possess extremely high salt spray tolerance, and never showed any necrosis even when they were dressed intermittently seven times with NaCl at 12 g/㎡. This suggests that the coastal plants may survive strong selection pressure of repeated splash of seawater. Contrarily, the inland individuals from gardens and orchards were injured at 0.75 g/㎡ of NaCl causing slight necrosis on their leaves and were recovered later just like untreated control. The necrosis caused by NaCl at 1.5 g/㎡ (equivalent to half of seawater in NaCl) on these plants often resulted in serious damage in plant growth. Therefore, the inland plants may be eliminated from seashores due to its low salinity tolerance. Plants derived from sandy shore in Seto Inland Sea which are intermediate in salt spray tolerance may have an advantage to survive sea soft wind over sandy seashore.

3. Observation of leaf tissues

As shown in Figure 9, leaf tissues were clearly different in structure between the coastal and inland plants of *S. viridis*. The differences in leaf tissue are genetical because leaf tissues are sampled from the plants growing in pots under uniform conditions (Willweber-Kishimoto, 1962). Sclerenchyma developed between vascular bundle and epidermis were much larger in the coastal plant than the inland one (a and b in Figure 9). The sclerenchyma tissues were found at every two vascular bundles forming veins on both upper and lower surfaces of leaves. Many spinules lie on the vein formed with sclerenchyma on the upper surface of leaves. Although the leaf surface of the coastal plants feels waxier than the inland individuals when touching with fingers, the difference in amount of cuticle on leaf surface was not clear (c and d in Figure 9). The results obtained here suggest that leaves of the coastal plants are protected from salt spray by thicker sclerenchyma which makes plants hard and stiff to protect them. The cuticle on leaf surface might also play a role to enhance the salt spray tolerance although the difference was unclear in this observation. Further examinations will be required to conclude the role of the cuticle for salt spray tolerance.

Ahmad and Wainright (1977) demonstrate that the rooting technique which evaluates the salinity tolerance by measuring the root elongation in NaCl solution is a reliable and labor-saving method in *Agrostis stolonifera* although the best way for salinity tolerance is to measure the effect of the salt on the relative growth. This implies that non-growth analysis such as a rooting technique readily assesses salinity tolerance as well as growth analysis. This was agreed with the results in *Setaria viridis* in this study. The two methods were consistent in soil salinity tolerance and

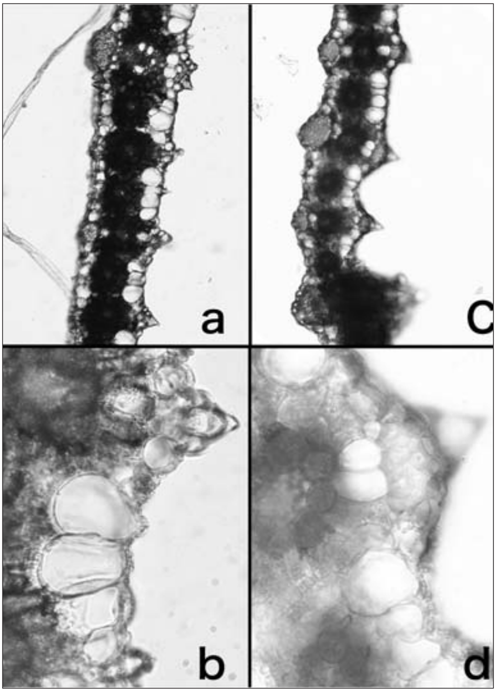

Figure 9. Photographs showing leaf blade tissues of a coastal plant from rocky cliff (c and d) and of an inland plant from orchard (a and b). Sclerenchyma was observed between vascular bundle and epidermis (a and c, magnification; x 100). Cuticles under spinules were slightly stained with safranin (b and d, magnification; x 400).

indicated that even coastal plant is not advantageous for growing in saline habitats over inland plant.

For salt spray tolerance, growth and non-growth analyses were performed by measuring growth inhibition and necrosis caused by NaCl solution. Observing the symptom of necrosis is an easy and labor-saving way, and is suitable for the screening of populations for salt spray tolerance. As in Figure 8, the date necrosis occurred on plant leaves exhibited the same order of salt spray tolerance among plants as that obtained from growth analysis, and was regarded as a reliable method for salt spray tolerance of *S. viridis*. With scoring necrosis, the salt spray tolerance of plant can be estimated from the date when necrosis appeared on plant, death date (complete necrosis) and duration from the beginning of necrosis to death. The date of necrosis occurred may be the best trait to find susceptibility to salt spray because subtle necrosis often disappears before long, and brings no damage on plant growth. The death date observed with sequential treatments of increasing rates of NaCl may be one of the most persuasive traits proving plant adaptation to salt splash. Salt splash may not be a selection pressure at all for the coastal individuals from pebbly shore and rocky cliff because they never died even when they were sequentially sprayed up to 83 g/m^2 of NaCl in total. In addition, scoring necrosis appears to be suitable for obtaining offspring of the treated plants because plants showing subtle necrosis can survive to attain maturity. After recording the date necrosis occurred, the individual can be back into pot so that they can produce seeds for further study. Thus, the observation of necrosis is suitable for breeding of the salt spray tolerant plant.

Variation in salinity tolerance within a species has been mostly reported for soil salinity tolerance to clarify intraspecific differentiation of wild plants in coastal zone (Ahmad and Wainright, 1977 and Flowers and Colmer, 2008) and to breed a new variety of agricultural plants in saline soils (Mano and Takeda, 1997). Very few report the intraspecific variation in salt spray tolerance except for *Erigeron* and *Mimulus* species (Boyce, 1954 and Lowry et al., 2008). An explicit instance of adaptation to coastal habitat is found on two plant forms, coastal perennial and inland annual form of *Mimulus guttatus* (Hall and Willis, 2006 and Lowry et al., 2008 and 2009). It is concluded that salt spray over the coast injures immigrants of the inland annual form while the coastal form survives with the high salt spray tolerance. When two forms are examined in salt spray tolerance with sequential treatments of NaCl solution, the coastal form also died eventually, following prompt death of the inland annual. However, in the present study, the coastal plants of *S. viridis* from pebbly shore and rocky cliff possess extremely high salt spray tolerance, and survive the repeated treatments almost intact at the highest rate of NaCl. This indicates that the plants of *S. viridis* can grow in the habitats in close proximity to the seaside. In fact, the coastal populations often inhabit the coastal strand just above the upper tidal zone which is subjected to not only ordinary salt spray but also repeated splash of seawater when catastrophic storm such as a typhoon hit the coast. The repeated salt splash of seawater may have played a role of strong selection to evolve the coastal plant of *S. viridis* which never die with sequential sprays of NaCl solution. The plant from a sandy shore of Seto Inland Sea is intermediate in salt spray tolerance. It may survive sea soft wind of the inland sea. The coastal plants of *S. viridis* are certainly prominent examples for proving that salt spray tolerance plays an important role for plants to adapt to coastal strand.

Ⅳ. Conclusions

Soil salinity tolerance of plant has been focused primarily due to the importance of agricultural production in saline soil and of plant conservation in salt marsh land. This paper proved the significance of salt spray tolerance of plant which grew in coastal strand just above the high tide line. The salt spray tolerance was readily evaluated by both plant growth inhibition from NaCl solution at various concentrations and necrosis percentage on plant leaves affected by NaCl spray. The measurement of growth inhibition is suitable for determining the rate of NaCl of salt spray tolerance, whereas the scoring leaf necrosis is a convenient way to find the order of salt spray tolerance among plants. Extremely high salt spray tolerance of coastal plants of *S. viridis* enable them to survive the habitats in close proximity to the high tide line of seawater.

References

- Ahmad I. and Wainwright S.J. 1977 Tolerance to salt, partial anaerobiosis, and osmotic stress in *Agrostis stolonifera*. *New Phytologist* 79, 605-612

- Boyce S. G. 1954 The salt spray community. *Ecological Monograph* 24, 29-67.
- Clausen, J. 1951 *Stages in the evolution of plant species*. Cornell Univ. Press, Ithaca, NY.
- Cordeau S, Petit S, Reboud X. and Chauvel B. 2012 Sown grass strips harbour high weed diversity but decrease weed richness in adjacent crops. *Weed Research* 52, 88-97.
- Dekker, J. 2003a The foxtail (*Setaria*) species-group. *Weed Science* 51, 641-646.
- Dekker J. 2003b The evolutionary biology of the foxtail (*Setaria*) species-group. In: Principles and Practices in Weed Management: *Weed Biology and Management*; Inderjit (Ed.). Kluwer Academic Publishers, The Netherlands.
- Flowers T. J. and Colmer T. D. 2008 Salinity tolerance in halophytes. *New Phytologist* 179,945-963
- Griffiths M.E. 2006 Salt spray and edaphic factors maintain dwarf stature and community composition in coastal sandplain heathlands. *Plant Ecology* 186, 69-86.
- Griffiths M.E, Keith R.P. and Orians C.M. 2003 Salt spray differentially affects water status, necrosis, and growth in coastal sandplain heathland species. *American Journal of Botany* 90, 1188-1196.
- Hall, M. C., and Willis J. H. 2006 Divergent selection on flowering time contributes to local adaptation in *Mimulus guttatus* populations. *Evolution* 60, 2466-2477.
- Itoh M, Nakashima Y. and Oki Y. 2014a Variation in morphology of *Setaria viridis*. Comparison between seaside populations and inland populations. *Weed Research, Japan* 59 Supplement 53. (In Japanese)
- Itoh M, Nakashima Y. and Oki Y. 2014b Variation in salt tolerance of *Setaria viridis*. Comparison between seaside populations and inland populations. *Weed Research, Japan* 59 Supplement, 54. (In Japanese)
- Lowry D. B, Hall M.C, Salt D.E. and Willis J.H. 2009 Genetic and physiological basis of adaptive salt tolerance divergence between coastal and inland *Mimulus guttatus, New Phytologist* 183,776-788.
- Lowry D. B, Rockwood R.C. and Willis J.H. 2008 Ecological reproductive isolation of coast and inland races of *Mimulus guttatus*. *Evolution* 62, 2196-2214
- Mano Y. and Takeda K. 1997 Heritability of salt tolerance at germination based on parent-offspring and selection response in barley (*Hordeum vulgare* L.). *Breeding Science* 47, 353-358.

- Marshall, E.J.P. 1989 Distribution patterns of plants associated with arable field edges. *Journal of Applied Ecology* 26, 247-257.
- Marshall E. J. P, Brown V. K, Boatman N.D, Lutman P.J.W, Square G.R. and Ward L.K. 2003 The role of weeds in supporting biological diversity within crop fields. *Weed Research* 43, 77-89.
- Osada T. 1989 *Illustrated grasses of Japan*. Heibonsya Press. Tokyo
- Petit S, Stuart R.C, Gillespie M.K. and Barr C.J. 2003 Field boundaries in Great Britain: stock and change between 1984, 1990 and 1998. *Journal of Environmental Management* 67, 229-238.
- Tiku B.L. and Snaydon R.W. 1971 Salinity tolerance within the grass species *Agrostis stolonifera* L. *Plant and* Soil 35, 421
- Wang R.L, J. Wendel and Dekker J. 1995 Weedy adaptation in Setaria spp.: I. Isozyme analysis of the genetic diversity and population genetic structure in *S. viridis. American Journal Botany* 82, 308-317.
- Willweber-Kishimoto E. 1962 Interspecific relationships in the genus *Setaria. Contributionsfrom Biological Laboratory, Kyoto University*, Pp. 1-41.