学位論文要旨

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題目: Physiological and Ecological Studies of Polymorphic Seed of Two Halophytes: Atriplex centralasiatica and Suaeda salsa.

(塩生植物Atriplex centralasiaticaおよびSuaeda salsaの多型種子の生理生態学的特性)

About 0.95 billion hectares of the world's soils are saline. Salinity is one of a major environmental factor limiting plant growth and productivity in saline soils of arid and semi-arid regions. There have been some attempts to increase crop yield by enhancing salinity tolerance. However, breeding and selection for higher salinity tolerance has brought only limited successes so far. Recently, some attempts have been made to select potentially from the numerous halophytic species some economic halophytes that can be used for on a large scale production in an economically feasible manner. In the process, the saline soils used would also get amended through halophyte planting.

Suaeda salsa and *Atriplex centralasiatica* are two important annual herbaceous halophytes belonging to the family Chenopodiaceae and widely distributed in China. These two species have economic potential as a source of oil, food, vegetables and fodder and are useful for vegetation rehabilitation in saline soils. In order to use them as a crop, the first essential step is to know the germination characteristics of the seeds of these species in saline environment, as the seed germination is a critical stage for establishment of halophytic vegetation in saline areas.

Previous studies have shown that S. salsa and Α. *centralasiatica* produce dimorphic/polymorphic seeds (brown seeds and black seeds) on the same plant. However, the physiology and ecology of germination of dimorphic/polymorphic seeds under salinity is not well understand. The objectives of this study are to investigate 1) the ecological aspect namely, how environmental factors (salinity, light, storage, temperature ect.) affect germination of the two halophytes, and 2) the physiology aspect namely, how plant hormones affect seed germination and how endogenous hormones were affected by environmental factors of the dimorphic seeds. Findings from the study are given in the following sections:

1. Germination behavior of dimorphic seeds of Suaeda salsa under saline conditions

It is very important to examine the effect of storage, environmental factors and hormonal treatment on germination of *S. salsa* in salinity. Therefore, the effect of storage, cold-wet stratification (4 °C for two days after water imbibition), application of gibberellins (GA₁ and GA₄) and temperature regimes (5/15, 10/20, 15/25, 20/30, 25/35 °C) on germination of dimorphic seeds of *Suaeda salsa* was investigated under NaCl stress conditions. The results showed that after one year of storage under room temperature, germination percentage of brown seeds decreased while that of black ones increased both in distilled water and in saline solution (300mM NaCl). Stratification and GA₄ treatments improved black seeds' germination, but showed little effect on the germination of brown seeds. However, treatment with GA₁ failed to promote seed germination of both kinds of seeds. Brown seeds under all the conditions showed higher germination percentage than black seeds. Maximum germination percentage was noted in distilled water at 15/25°C and 20/30°C temperature regime for both kinds of seeds. The highest rate of germination was obtained in distilled water at 20/30°C for brown seeds, and at 15/25°C for black seeds.

2. Germination characters of dimorphic seeds of A. centralasiatica under saline conditions

The effect of light, temperature, fruit type (presence of bracteoles), seed color, storage, cold-wet stratification, seed coat scarification and plant hormones on germination of polymorphic seeds of *A*. *centralasiatica* was investigated under saline conditions. Results showed that the effect of fruit types

was not significant on seed germination. Multi-factorial analysis of variance revealed significant differences in the effects of seed color, temperature, salinity, light and their interactions on seed germination. Maximum germination occurred in distilled water, and an increase in NaCl concentration progressively inhibited germination in all the four types of seeds. A temperature regime of 15/25°C resulted in the maximum germination. Brown seeds had a higher germination percentage than black seeds in mild temperature regime and high salinity; while black seeds had a higher germination percentage than brown seeds in higher temperature and lower salinity. Germination of black seeds was sensitive to light in NaCl solution but not of brown seeds. Attached bracteoles inhibited the germination of both black and brown seeds. The black seeds were more sensitive to presence of bracteoles than brown ones. After one-year storage, germination percentage of brown seeds decreased significantly; while that of black seeds increased in salinity stress. Cold-wet stratification promoted seed germination rate, however, it did not affect final germination percentage of both seeds. Seed coat scarification and fluridone (Abscisic acid, ABA biosynthesis inhibitor) treatments improved germination of both black and brown seeds under salinity stress. Gibberellins, 1-aminocy-cloproane-1-carboxylate (ACC, the immediate precursor of ethylene), 6-benzyladenine (BA) showed little effect on the germination of both kinds of seeds under salinity. ABA and paclobutrazol (inhibitor of gibberellin biosynthesis) did not affect seed germination in distilled water, however, ABA inhibited germination in the presence of salinity. Inclusion, by differential germination of the polymorphic sees, A. centralasiatica showed a bet-hedging ecological strategy, that ensure the establishment of plant stands under highly variable environmental conditions. ABA was the main plant hormone that affected seed germination under salinity stress.

3. Effect of salinity on levels of endogenous gibberellins and abscisic acid during germination of dimorphic seeds of *S. salsa*

In order to clarify the role of hormones in regulating germination of S. salsa dimorphic seeds under salinity, we investigated the effect of salinity on gibberellins (GAs) and abscisic acid (ABA) profiles during germination. Content of endogenous ABA in dry brown seeds was about 2.7 times that of black seeds, and after imbibition it decreased faster in the brown seeds than the black seeds. Salt (NaCl) stress slightly prevented decrease of ABA content in germinating seeds and fluridone (ABA biosynthesis inhibitor) alleviated seed germination in the presence of salinity stress. Bioactive GAs (GA₄/GA₁) and their biosynthetic precursors (GA₁₂, GA₁₅, GA₂₄, GA₉, GA₅₃, GA₄₄, GA₁₉, GA_{20}) were higher in brown seeds than black seeds both under dry condition as well as when germinating in water and salt solution. However, deactivated GA forms (GA_{51} and GA_{34}) were higher in black seeds than brown seeds when dry and also when germinating in water and salt solution. The dimorphic seeds showed significant difference in biosynthesis and inactivation of bioactive GA₄, which might result in difference in germination in water and salinity. In early stage of germination, GA₄ declined in response to salinity in both types of seeds. In the presence of GA biosynthesis inhibitor (paclobutrazol), GA4 was more active than GA1 in promoting seed germination. Salt also decreased sensitivity of both seeds to GA4, brown seeds were more sensitive to GA_4 than black seeds. Salinity might affect on one hand the content of GA_4 by inhibiting its biosynthesis and promoting its deactivation and by affecting sensitivity to GA₄ and on the other hand biosynthesis of ABA, which affected seed germination.

4. Conclusion

Based on the above results, it can be concluded that the dimorphic/polymorphic seeds of *S. salsa* and *A. centralasiatica* exhibit distinct differences in dormancy, germination, dispersal strategies and storage characteristics. Brown seeds are more tolerant than black seeds during germination under salinity. Brown seeds showed higher dispersal performance than black seeds, and would germination in spring; black seeds showed seed bank features and would germinate in summer, next year or later. This is a kind of bet-hedging ecological strategy and permits the two species to successfully inhabit the harsh saline habitats. In practice, it is suitable to sow brown seeds in spring and black ones in summer, as well as to use black seeds for storage. Plant hormones (ABA and GAs) are involved in regulation of seed germination and dormancy of dimorphic/polymorphic seeds of the two halophytes. ABA inhibited seed germination of both kinds of seeds under salinity, while GAs (especially GA₄) could alleviate salinity stress in some cases. The results also indicated that salinity stress inhibited seed germination mainly through affecting GA biosynthesis and ABA catabolism. The reason for difference in germination behaviors of the black and brown seeds of *S. salsa* under salinity could in part to be the difference in biosynthesis and deactivation of active GAs (GA₁ or GA₄) and catabolism of ABA in the two kinds of seeds.