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SUMMARY OF DOCTORAL THESIS

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Title: Studies on the Salt Tolerance Mechanism of Halophytes Growing in Xinjiang, China

（中国新疆ウイグル自治区における塩生植物の耐塩メカニズムに関する研究　）

Progressive soil salinization is a serious issue in worldwide and is a major cause of desertification in arid regions. Extensive degradation of irrigated lands due to salinization poses both environmental and socio-economic problems in Central Asia and northwestern China. To combat soil salinization, various methods have been used. A biological method, phytoremediation, comes into employ to decrease the trend of soil salinization and ameliorate degraded land effectively. A method using native halophytes has drawn attention and been given high priority in several countries. The Xinjiang region of China is very rich in halophyte species. These halophytes might allow the utilization of salt-affected soils. For appropriate and the successful use of halophytes to ameliorate salt-affected soil, it is necessary to determine the mechanism(s) by which halophytes tolerate saline conditions. Therefore, the objective of this study is to gain a precise understanding of the salt-tolerance mechanism of halophytes growing in the Xinjiang region. To achieve this objective, this study conducted field investigations and greenhouse experiments. On the field investigations, the cation concentrations and distributions among plant parts and osmolyte accumulation in leaves were determined in five halophytes (*Tamarix hispida*, *Halocnemum strobilaceum*, *Kalidium foliatum*, *Karelinia caspica*, and *Phragmites australis*) growing around Aiding Lake, Xinjiang, China. The responses of *Elaeagnus* species, a pioneer, multipurpose tree species, to various salinities were examined in greenhouse experiments.

The construction of study was therefore arranged as follows：The first chapter introduces salinization problem in worldwide, rehabilitation methods for salt affected areas, importance of halophytes use as phytoremediation in saline regions and objectives of this study. Chapter II presents the results of a study of the cation content of five halophytes growing under saline conditions, thus providing basic information on the differences in salt-regulation mechanisms among species. Chapter III presents the results of a study of the osmolyte contents of the five halophytes, providing a better understanding of the physiological strategies of halophytes to cope with saline environments. In Chapter IV, the photosynthetic performance of *Elaeagnus angustifolia* L saplings are discussed to gain a better understanding of the physiological behavior of this species under saline conditions. In chapter V, the salt tolerance and response of different osmolytes to salt stress in *Elaeagnus oxycarpa* seedlings are discussed to elucidate the salt-tolerance mechanism of this tree species. And finally, main achievements in this study are summarized and ecological applications are offered in the last chapter.

In *Tamarix hispida*, the Na^+ concentrations were highest in leaves, exceeding levels in stems and roots (Chapter II). Moreover, *Tamarix* species transport absorbs ions from the roots to the leaves and can utilize saline groundwater by excreting excess salt through salt glands on the leaves. *T. hispida* accumulated several osmolytes in the leaves, particularly including pinitol, γ-butyro betaine, and amino acids (Chapter III). Combined with the ability to excrete salt and osmoregulation, these are advantages for survival during droughts and in saline environments.

Leaf or stem succulence is an adaptive feature that contributes to the regulation of internal ion concentrations in many halophytes. In such plants, the salt taken into the cytoplasm is retained in vacuoles of the leaf mesophyll. The succulent semi-shrub species *H. strobilaceum* and *K. foliatum* had steep gradients in Na^+ concentration from the leaves to the roots and stems, with Na^+ concentrations more than ten times those in leaves than in stems (Chapter II). The high water content in leaves of *H. strobilaceum* and *K. foliatum* allows them to adapt to high-salt conditions by diluting the high Na^+ levels via water retention in vacuoles. Therefore, our results indicate that the large quantities of Na^+ in the leaves of succulent euhalophytes are likely to be an essential part of the osmoregulatory mechanism, rather than nutritional. Moreover, to accommodate the ionic balance in the vacuoles, and to protect metabolic processes in the cytoplasm, osmotic adjustment between the vacuole and cytoplasm is achieved via the accumulation of organic solutes in the cytoplasm. In *H. strobilaceum* and *K. foliatum*, this protective role is fulfilled by the accumulation of high concentrations of GB in leaves (Chapter III).

Unlike the other three halophytes, the pseudo-halophytes *Karelinia caspica* and *P. australis* were able to regulate individual cation concentrations. *Karelinia caspica* accumulated more Na^+ in leaves than in roots (Chapter II); however, it has neither succulent leaves nor salt glands. Surprisingly, *K. caspica* accumulated large amounts of mannitol in leaves, implying that the accumulation of mannitol is critical for *K. caspica* to maintain favorable water status at the same level found in succulent euhalophytes, which may allow *K. caspica* to survive in high saline conditions. By contrast, *Phragmites australis* effectively excluded Na^+ from the root zone, but accumulated large quantities of K^+ in all tissues. Regarding organic osmolytes, *P. australis* accumulated high concentrations of soluble carbohydrates, mainly sucrose, and amino acids such as proline and alanine. This suggests that the high tissue ion selectivity, with high concentrations of K^+ in all tissues, and high concentrations of osmolytes, plays an important role in survival under saline conditions.

To examine *Elaeagnus* species' physiological behavior, the photosynthetic performance of 1-year-old *Elaeagnus angustifolia* L. saplings exposed to salt stress for 1 week (NaCl concentrations: 0, 200, 400, and 600 mM) was investigated (Chapter IV). In addition, the growth, photosynthesis, and osmolyte accumulation in *Elaeagnus oxycarpa* saplings were affected by salt treatments (0, 50, 100, 200, or 300 mM) for 30 days (Chapters V). The two *Elaeagnus* species were highly tolerant of salinity. The ability of *Elaeagnus* species to cope with severe salt stress primarily involved the control of gas exchange. The maintenance of PSII functions at 200 mM salinity is important for the growth of *Elaeagnus* species in saline soil. In particular, the salt tolerance of *E. oxycarpa* appears to be achieved via an array of mechanisms, including the exclusion or compartmentalization of most Na^+ in the root system, and the maintenance of osmotic balance by retaining nutrients (*e.g.*, K^+ and Ca^{2+}) at low and moderate salinity (50 and 100 mM NaCl). *E. oxycarpa* can synthesize and accumulate a set of osmolytes in leaves; sucrose, β-alanine betaine, proline, and glycine increased markedly at 200 and 300 mM NaCl, and contributed significantly to osmotic adjustment under severe stress.

From those findings, I conclude that (1)the five halophytic species growing in Xinjiang have different mechanisms for regulating salt in salt tolerance and achieved good osmoregulation by accumulating various osmolytes in their leaves. Therefore, if the total concentration of organic osmolytes in each halophyte is considered, it is theoretically sufficient for osmotic adjustment under high-saline conditions. (2)*Elaeagnus* species, which are distributed widely in arid saline areas in central Asia and northwestern China, posses various features essential for salt-tolerance to maintain growth under high salinity. These species accumulate Na^+ in shoots and leaves, which are shed at the end of the growing season, eliminating the absorbed salt from the plant body. This strategy makes this species a potential candidate for plantations for the phytoremediation of saline areas.