論 文 の 内 容 の 要 旨

生産・環境生物学　専　攻
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論文題目　　Studies on the Variation of Salinity Responses and Salt-tolerant Mechanisms of *Miscanthus sinensis* Andersson
（ススキの塩ストレスに対する応答および耐塩性機構に関する研究）

The lignocellulosic crop, *Miscanthus* spp. has been identified as a good candidate for biomass production. To satisfy the need for high yield and to avoid competition with food production by growing the crop in marginal areas, we studied the responses of *M. sinensis* to salinity.

Effects of salt concentrations (0─360 mM NaCl) on seed germination, growth, photosynthesis, and shoot ion accumulation were examined. In the germination test of 17 accessions, high salt concentration inhibited seed germination in all accessions, while germination percentage varied significantly among the accessions. Two accessions showing variable salt tolerance, JM0119 (salt-tolerant) and JM0099 (salt-sensitive),

were selected for a greenhouse study on plant dry weight, leaf chlorophyll content, total leaf area, tiller number, photosynthetic rate (A), stomatal conductance (g_s), PSII operating efficiency ($\Delta F/F'_m$), and shoot ion concentration during various lengths of time. The experiment was performed twice, but for most parameters, no significant differences between runs were observed. Salinity resulted in a reduction of plant growth. A and g_s were adversely affected by all salt treatment levels, while $\Delta F/F'_m$ was not reduced until salt concentrations reached 120 mM NaCl. In contrast to the increases of shoot Na^+ and Cl^- concentrations, shoot K^+ concentration declined in the presence of salt. Overall, this study revealed a great variability for salt tolerance in *M. sinensis* germplasm. The relative advantage of JM0119 over JM0099 under saline conditions was primarily associated with larger leaf area, a greater number of tillers, greater photosynthetic capacity, and restricted Na^+ accumulation in shoots.

In order to better understand the physiological and biochemical responses of C_4 photosynthesis to salinity in *M. sinensis*, seedlings of two accessions (salt-tolerant ‘JM0119’ and salt-sensitive ‘JM0099’) were subjected to 0 (control) or 250 mM NaCl stress for two weeks. The shoot dry weight, leaf chlorophyll content, gas-exchange, chlorophyll *a* fluorescence, activities of phospho*enol*pyruvate carboxylase (PEPC), pyruvate, orthophosphate dikinase (PPDK), NADP-malic enzyme (NADP-ME), NADP-malate dehydrogenase (NADP-MDH), and ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), and contents of carbohydrates, protein, and foliar total free-amino acids were investigated on five harvest dates. Salt-induced reduction of the relative growth rate related to the inhibited photosynthetic rate. Higher photosynthetic rate, transpiration rate, chlorophyll content, the PSII operating efficiency, coefficient of photochemical quenching, and contents of soluble sugars, protein, and total free-amino acids under salinity were observed in ‘JM0119’ relative to those in ‘JM0099’. Activities of PEPC and PPDK were gradually increased under salt duration in ‘JM0119’, while they displayed single peak curves in ‘JM0099’. NADP-MDH activity enhanced gradually in both accessions under salt duration, with greater increases observed in ‘JM0099’. Salt-enhanced activity of NADP-ME was only observed in ‘JM0099’. The maximum efficiency of PSII photochemistry, activity of RuBisCO, and starch content were slightly affected by salt stress. Greater photosynthetic capacity under salt stress was mainly associated with non-stomatal factors including less chlorophyll loss, higher PSII operating efficiency, and enhanced activities of PEPC, PPDK, and NADP-MDH. Despite the repressive effect on plant photosynthesis due to the imbalanced source-sink relationship, accumulated soluble sugars provided osmotic adjustment and osmprotection in leaves. More efficient nitrate assimilation was also related to salt-tolerance owing to the greater contents of protein and total leaf free-amino acid.

The adaptation responses of genes involved in C_4 path way (*pepc*, *ppdk*, *nadp-mdh*, *nadp-me*, and *RbcS*) and genes encoding Na^+/H^+ antiporters, NHX1 and SOS1, to NaCl stress were also examined in JM0119 and JM0099. Based on the sequencing on approximate 600 bp-long cDNA fragments obtained from degenerated PCR, the cDNA sequences of genes examined were highly conserved among the relatives of *M. sinensis*. These salt-induced variation of gene expression investigated by quantitative real-time

PCR provided evidences for insights of the molecular mechanisms of salt tolerance in *M. sinensis*. Up-regulation of *pepc*, *ppdk*, *nadp-mdh*, and *nadp-me* were observed in both accessions with different patterns, while *RbcS* expression was suppressed by salt stress. Between the accessions, higher *pepc*, *ppdk*, and *RbcS* expression were related to greater photosynthetic capacity under salt regime. Expression of *nadp-mdh* and *nadp-me* may be closely related to each other and partially responsible for the changes in the activity of NADP-MDH and NADP-ME. The expression of *nhx1* was up-regulated by salt stress in JM0119 shoot and root tissues. However, it was hardly affected in JM0099 shoot tissue except for a significant increase at 100 mM salt treatment, and it was salt-suppressed in JM0099 root tissue. Thus, the remarkably higher expression of *nhx1* and *sos1* were associated with the resistance of Na^+ toxicity by regulation of Na^+ influx, efflux, and sequestration under different salt conditions.

Results in this thesis are expected to provide useful information in screening for salt-tolerant germplasm in *M. sinensis* and they may shed new light on elucidating the physiological and biochemical mechanisms associated with salt stress in this species.