盐胁迫下野菊离子的吸收转运情况及相关基因的表达分析

罗玠晔

（东北林业大学园林学院 哈尔滨 150040）

摘 要：【目的】明确抗盐能力不同的野菊（Chrysanthemum indicum）株系在盐胁迫情况下的离子分布运输情况及相关基因的变化规律，从更深层次上认识野菊的抗盐机理，为后续研发和培育新的抗盐品种提供参考。【方法】以前期筛选的 2 个高抗株系（H1、H2）、2 个中抗株系（M1、M2）和 2 个低抗株系（L1、L2）的扦插苗为实验材料，置于 NaCl 浓度为 150mmol/L 的霍格兰营养液中进行盐胁迫处理。测定 6 个离子指标：根、茎、叶的 Na+、K+ 离子的含量；并计算各株系中根、茎、叶 K+/Na+ 的值和不同部位离子选择性运输能力（SK+，Na+）。分析盐胁迫对抗盐能力不同的野菊株系各部位离子含量、比例及运输情况的影响。采用生物信息学方法对野菊进行 NHX 家族成员、 HKT 基因鉴定。并利用 qRT-PCR 技术，测定盐胁迫下抗盐能力不同的野菊株系在根、茎、叶中 NHX、HKT 基因不同时间点的表达水平。【结果】与对照组相比，野菊各株系根、茎、叶的 Na+ 含量显著增加， K+ 含量及 K+/Na+ 值均显著降低；多数情况下，野菊高、中抗株系各部位间的 SK+，Na+ 高于对照组。随着株系抗盐能力的降低，根、茎和叶中的 Na+ 含量呈持续上升趋势，而 K+ 含量及 K+/Na+ 值则呈持续下降趋势，各部位间的 SK+，Na+ 值均呈现先升高后下降的趋势。共鉴定出 6 个野菊 NHX 家族成员（CiNHX1-6）， 1 个野菊 HKT 基因（CiHKT1）。在盐胁迫后，野菊 CiNHX2 基因在高抗株系根部的表达上调幅度明显高于低抗株系，而该基因在 H1、H2 株系根、茎部中有着相同的表达模式。同样， CiNHX4 基因在高抗株系根、叶部的相对表达量也大幅上调，该基因在 H1、H2 株系根、叶部中有着相同的表达模式。 CiNHX1、CiNHX3、CiNHX5、CiNHX6 在各株系的根、茎、叶中的表达水平并无显著变化，且总体上表达水平较低，其中 CiNHX3 在根部的表达量略高， CiNHX1、CiNHX6 在叶部的表达量略高。野菊 CiHKT1 基因在根、叶部的表达受盐胁迫的影响较为明显，高抗株系的相对表达量上调幅度明显。【结论】野菊抗盐能力越强，其离子吸收、转运、分布情况受盐胁迫影响越小；盐胁迫下高抗株系能有效地外排 Na+ 并选择性地吸收 K+ ，稳定体内的离子含量与分布；中抗株系受胁迫影响相对较大，但可以通过选择性运输 K+ 来防止 Na+ 的过量对地上部分造成毒害。 CiNHX2、CiNHX4、CiHKT1 与野菊株系抗盐能力强弱密切相关，是野菊抵御盐胁迫的关键基因。

关键词：野菊；盐胁迫；离子平衡；NHX 基因；HKT 基因

Ion Uptake, Transportation and the Expression of Related Genes in Chrysanthemum indicum Under Salt Stress

Abstract:【Objective】The aim of this study was to elucidate the ion distribution and transport characteristics of various Chrysanthemum indicum strains with differing salt tolerate abilities under salt stress. Additionally, the study also aimed to investigate the changes in related genes. In order to gain a deeper understanding of the salt tolerance mechanism of Dendranthema indicum and provide a reference for the subsequent development and cultivation of new salt-tolerant varieties. 【Method】The previously selected two high resistant strains (H1, H2), two middle resistant strains (M1, M2), and two low resistant strains (L1, L2) were used as experimental materials,

and were subjected to salt stress treatment in Hoagland nutrient solution with 150mmol/L NaCl. The content of Na+ and K+ ions in the roots, stems, and leaves were measured, the K+/Na+ ratio in the roots, stems, and leaves, and the K+/Na+ selectivity ratio (SK+，Na+) in different parts of the strains were calculated. The effects of salt stress on the ion content, ratio, and transport in different parts of C. indicum strains with different salt resistance abilities were analyzed. The NHX family members and HKT genes of C. indicum were identified by bioinformatics method. The expression levels of NHX and HKT genes at different time points in the roots, stems, and leaves of C. indicum strains with different salt resistance abilities under salt stress were determined by qRT-PCR. 【Result】Compared with the control group, the Na+ content in the roots, stems, and leaves of various strains varied significantly increased, and the K+ content and K+/Na+ ratio were significantly reduced. In most cases, SK+，Na+ in various parts of high and medium resistant strains were higher than that in the control group. With the decrease of salt resistance, the Na+ content in roots, stems and leaves of various strains was increasing, while the K+ content and K+/Na+ ratio were decreasing. SK+，Na+ in each part were increasing first and then decreasing. A total of 6 members of the C. indicum NHX family (CiNHX1-6) and 1 C. indicum HKT gene (CiHKT1) were identified. After salt stress,the expression of the CiNHX2 gene in the roots of high-resistant strains is significantly higher than in low-resistant strains, while the expression pattern of this gene is the same in the roots and stems of H1 and H2 strains. Similarly, the expression of the CiNHX4 gene in the roots and leaves of high-resistant strains is significantly upregulated, while the expression pattern of this gene is the same in the roots and leaves of H1 and H2 strains. The expression levels of CiNHX1, CiNHX3, CiNHX5, and CiNHX6 in the roots, stems, and leaves of each strain did not show significant changes, and the overall expression level was low. The expression level of CiNHX3 in the roots was slightly higher, and the expression levels of CiNHX1 and CiNHX6 in the leaves were slightly higher. The expression of the CiHKT1 gene in the roots and leaves of wild C. indicum was significantly affected by salt stress, and the relative expression level in high-resistant strains was significantly upregulated. 【Conclusion】The stronger the salt resistance of C. indicum is, the less its ion absorption, transport and distribution are affected by salt stress. Under salt stress,the high resistant strains can effectively excrete Na+ and selectively absorb K+ thereby stabilizing the ion content and distribution in body. The medium resistant strains are relatively affected by stress, but excessive Na+ toxicity to the aboveground part can be prevented by selectively transporting K+. CiNHX2, CiNHX4, and CiHKT1 genes are closely related to the salt tolerance of wild C. indicum strains, and are the key genes for wild C.indicum to resist salt stress.

Keyword: Chrysanthemum indicum ; Salt Stress ; ion homeostasis ; NHX gene ; HKT gene