Physiological response of *Hordeum brevisubulatum-Epichloë bromicola* ecotypes to mixed salt stress

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Soil salinization has become a global agricultural issue, reducing agricultural productivity, threatening livestock production and food security. Wild barley (*Hordeum brevisubulatum*) is widely distributed in lightly saline meadows in northern China and exhibits high salt tolerance. This tolerance can be further enhanced by the presence of *Epichloë bromicola*, a mutualistic fungal endophyte and this symbiotic association serves as essential germplasm for developing novel salt-tolerant grass lines. This study evaluated the salt tolerance of 16 wild barley-*E. bromicola* ecotypes under greenhouse conditions. Plants (n=15) from each ecotype were subjected to mixed salt stress (1:1 NaCl-Na_2S0_4) at different concentrations (0 mM, 400 mM, 600 mM, and 800 mM) and their growth responses assessed after 30 days. The results demonstrated that salt stress significantly increased leaf mortality, relative membrane permeability, and Na^+ content, while reducing plant height, stem diameter, tiller number, biomass, K^+ content, and the concentration of peramine, an endophyte-derived alkaloid that has insect deterrent activity. Notably, peramine content varied significantly among ecotypes, with DB, B1, B2, B3, and R showing no detectable peramine, whereas D2 exhibited the highest level (424 μg/kg). Correlation analysis revealed that peramine content was positively correlated with plant chlorophyll content, stem diameter, green leaf rate, and K^+ content, but negatively correlated with leaf mortality, withering rate, and relative membrane permeability. After 30 days of exposure to 800 mM mixed salt, mortality was detected in a subset of ecotypes. R, DB, LZ E-, D3, and E1 showing mortality rates of 100%, 33%, 33%, 33%, and 27%, respectively. A grey relational analysis ranked the ecotypes' salt tolerance in descending order: D2 > A1 > J > H > G > D1 > K > LZ E+ > B3 > B2 > B1 > E1 > D3 > LZ E- > DB > R. Cluster analysis classified them into five groups: D2 (highest tolerance), A1 (high tolerance), J/ H/ G/ D1/ K/ LZ E+ (moderate tolerance), B3/ B2/ B1 (low tolerance), and E1/ D3/ LZ E-/ DB/ R (lowest tolerance). In conclusion, D2, the most salt-tolerant ecotype, represents a valuable genetic resource for breeding high salt-resistant wild barley-*E. bromicola* symbiont lines.

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