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Enhanced tolerance to heat stress under water deficit in transgenic *Agrostis mongolica* Roshev. overexpressing *ABF3* gene

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Objectives

Here we report an efficient *Agrobacterium*-mediated transformation procedure for *Agrostis mongolica* Roshev. and a development the transgenic plants tolerant to heat stress under water deficit condition.

Materials and Methods

1. Material

Plant - *Agrostis mongolica* Roshev.

Agrobacterium strain - EHA 105/pCUMB carries a target gene ABA-responsive element binding factor3 (*ABF3*), reporter genes green fluorescence protein - β-glucuronidase (*gfp-gus*), and a selectable marker gene hygromycin phosphotransferase (*hph*).

2. Methods

Type 3 callus induction, Transformation, Factors effect on transformation efficiency, Regeneration/Selection, Southern blot analysis, Northern blot analysis, Stress test

Results and Discussion

The most important factor to insure success in this study was could select regenerable and reproducible callus type of *A. mongolica*. Pre-cultivate for 3 days on $CaCl_2$ free medium and co-cultivate for 10 days on 100-150 mg/L acetosyringone, pH 5.8 medium were increased the transformation efficiency. The transformation efficiency was 49.2% and GFP was intensively expressed from the hygromycin-resistant callus and seedlings. The result of Southern blot analysis showed that the ABF3 transgene was stably integrated into the genome of transgenic plants. Of the tested five transgenic lines two gave one copy number, suggesting single transgene integrationand three gave two copy numbers, indicating two transgene integrations. Northern blot analysis confirmed that ABF3 was highly expressed in transgenic plants, gene silence was not observed. Transgenic plants showed normal growth in terms of morphologies ofwhole plant. Interestingly, both of the transgenic and wild-type plants were not flowered for more than 3 years growing in the open field. The stomata of the transgenic plants had smaller openings than did the wild-type plant and water content of leaves of transgenic plants remained about 3-4 fold higher than wild-type plants after water deficit treatment. The transgenic plants showed about 2 fold higher tolerant to heat stress under water deficit condition than those of wild-type plants.

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