Research on the Sterilization Method for Endophytic Fungi of Tall Fescue Seeds

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Introduction Seed explant is usually used in tissue culture of tall fescue. However, the fungal endophyte *Neotyphodium coenophialum* that symbioses with tall fescue may cause high contamination rates in the course of callus culture (Hinton & Bacon, 1985; Wang & Ge, 2005). So, an effective sterilization method of seeds is necessary for establishment of regeneration systems of tall fescue using tissue culture.

Materials and methods The seed germination abilities of *Festuca arundinacea* Schreb. cv. Arid III and Crossfire II were evaluated, and repeated three times. The presence of endophytic fungi was detected by the aniline blue staining method in 100 seeds. The seeds of tall fescue were sterilized by marinating in different water temperatures (40℃, 50℃, 60℃) for different times (10 min, 20 min, 30 min), then the seeds were soaked in 75% ethanol for 5 min, and 5.25% (w/v) sodium hypochlorite for 15 min, then washed five-times in sterile water. The sterilized seeds were inoculated in MS medium (pH 5.8), at 25℃ in dark. Germination rate, contamination rate and callus induction rate of seeds were counted 30 days later. The data were analyzed by t-test, the least significant difference (LSD) test, or two-factor variance analysis. The Rank-Sum Ratio (RSR) was used to determine statistically significant differences in sterilization effect in the 10 controls (SAS Software, Cary, NC, USA).

Results and discussion The positive rate of endophytic fungi of Arid III or Crossfire II was 67% or 56%, respectively. Germination rate of Arid III was 89.0%, that of Crossfire II was 84.0%. Germination rate of Arid III seeds soaked in 75% ethanol for 5 min, and 5.25% (w/v) sodium hypochlorite for 15 min was 82.9%, that of Crossfire II was 81.8%. The result of t test showed that there was no significant difference in seeds germination rates of Arid III and Crossfire II before and after sterile treatment. After eliminating the effect of ethanol and sodium hypochlorite on the vigour seeds, the results reflect the actual effect of the different water temperature and soaked time on the growth of callus or endophytic fungi. The results of the least significant difference showed that the germination rate and callus induction rate of Arid III and Crossfire II seeds were distinct difference among various controls ($P<0.01$). Two-factor variance analysis showed that the water temperature and soaked time had the most significant influence on the germination rate, contamination rate and callus induction rate of Arid III and Crossfire II seeds ($P<0.01$). RSR results ranged between 0.3-0.7. Based on these results, the most optimal method of sterilization was marinating in 50℃ water for 10 min, and dipping in 70% alcohol about 5 min, then in sodium hypochlorite solution about 15 min.

Conclusions The sterilization method can decrease the contamination rate of seeds, and increase the callus induction rate under the high germination rate of seeds, which may play an important role in solving problem of high contamination rate and low callus induction rate in tissue culture. Research about sterilization methods has benefits for the establishment of regeneration systems of tall fescue.

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