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## The effect factors on regeneration system of tissue culture using mature embryo of wheatgrass

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**Key words :** wheatgrass , genotype , tissue culture , mature embryo , ABA

**Introduction** It is the development trend that breeds new variety with good quality , high yield and multiresistance by transgenic technology in modern wheatgrass breeding . It is precondition of transgene that found a highly-effect and stabilized regeneration system for tissue culture . Through the experiment on tissue culture using mature embryo of differentials genotype wheatgrass to select excellent genotype of wheatgrass that was suitable for transform exogenous gene . The effect factors of callus induction and regenerated frequency were studied in the regeneration system .

**Materials and methods** The seeds of *A . cristatum* cv . Fairway (Fairway) , *A . desertorum* cv . Nordan (Nordan) , *A . mongolicum* Keng and *A . cristatum* × *A . desertorum* cv . Mengnong (Mengnong) were chosen to sterilize and embryos of them were embedded culture medium to induced the callus . Cultures were incubated in the dark at 26°C for 14 days followed by a secondary culture for 20 days that repeated twice or thrice . Then the good callus was picked out and cultivate in differential medium in the light for 16 hours . The cultures were switched to enrooting medium until the seedlings could survive perfectly .

**Results** Effect of different genotype wheatgrass on inducing callus from mature embryo : All materials for experiment could be induced to form callus in same medium . The time on appearing and forming of callus were little different . But there were much different in inducing rate and morphology of callus among 4 materials . The percentage of callus induction for *A . mongolicum* Keng was higher 20% than others . The percentage of callus induction for Nordan was 67 . 5% , lowest among 4 materials . In addition , the morphology of callus of *A . mongolicum* Keng . possessed compact and dense structure were best than others possessed soft and clearing structure (Table 1) .

**Table 1** Effect of different genotype wheatgrass on inducing callus from mature embryo .

Varieties	Inoculate No .	Time of appearing callus(d)	Time of forming callus(d)	Callus No . (piece)	Percentage of callus induction (%)	morphology of callus
Fairway	400	4	10	307	76 . 8	white ,soft ,water
Nordan	400	4	10	270	67 . 5	white ,soft ,water
<i>A . mongolicum</i> Keng	400	3	8	380	95	White ,compact
Mengnong	400	3	9	296	74	white ,soft ,water

Differentiation and enrooting ability of callus from mature embryo for different genotype wheatgrass : There are remarkably different in percentage of differentiation callus from mature embryo among 4 materials . The seedlings were appeared after the inducted callus of *A . mongolicum* Keng switched to differentiation for 15 days , but to Mengnong and Fairway , its need 20 days . The seedlings were appeared after the inducted callus of Nordan switched to differentiation for 30 days . And the Differentiation percentage of callus of *A . mongolicum* Keng , Mengnong , Fairway and Nordan were 72% , 58% , 52% and 41% respectively . When the seedlings is 2-3cm , its were inoculated in enrooting medium . The roots of all materials could be growed and they were robust .

Effect of ABA on morphology and differentiation ability of callus from mature embryo of wheatgrass : The secondary culture was done in medium appended ABA . The result showed that ABA can improve the morphology of callus and enhance the differentiation ability on 4 materials . There were different effects to morphology of different callus by different ABA concentration . When ABA concentration was 0 . 1mg/L , it was little effect to callus . But when the ABA concentration was 0 . 5mg/L , the quality of all callus was decline . The optimal ABA concentration was 0 . 3 mg/L , it could improve the quality of callus evidently .

**Conclusions** The callus from mature embryo of *A . mongolicum* Keng was higher than others in the percentage of callus induction and differentiation callus . *A . mongolicum* Keng was a perfect host to transgene . The ABA (0 . 3 mg/L) can remarkably improve the quality of callus .

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