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Effect of different culture medium on subculture and differentiation of alfalfa anther callus

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Key words : alfalfa, anther callus, tissue culture, hormone, macroelement

Introduction Cultivated alfalfa (*Medicago sativa* L.) is one of the high quality legume crop, which is widely planted in the world. Alfalfa is cross-pollination plant, genotype heterozygote and genetics diversity. Method of conventional breeding would not easy to obtain pure line. But pure line can be acquired by the use of anther culture to create double haploid (DH) lines in short time. Combined anther culture with cross-breeding, heterosis utilization, transgenic technology have formed an effective breeding technology and become an important part of breeding. The effects of different medium components, hormone categories and concentrations was investigated in the experiment, to establish foundation for further optimize culture conditions and constructing doubled haploid population.

Materials and Methods The callus were induced from the alfalfa anther cultured on a double-layers medium which contains N6 macroelement, the MS microelement, MS microelement, B5 organic compound, 2,4-D 2mg/l + 6-BA 0.5mg/l. The callus was transferred to the different mediums (Table 1), and growth of the callus was observed. Culture conditions: pH of medium was 5.8, the light intensity was 2000 lx, time of illumination was 12-14 h/d, the temperature was $25 \pm 2^\circ\text{C}$.

Table 1 Medium components.

Medium	Macroelement	Auxin(mg/l)	Cytokinin(mg/l)
S2	MS	0	0
D3	SH	NAA0.05	BA0.5
D4	SH	2,4-D0.05	BA0.5
D5	SH	NAA0.05	KT1
D6	SH	NAA0.05	KT0.5
D7	SH	0	BA0.5
D8	SH	0	0
D18	N6	0	0

Note: other components of medium in the table are MS micronutrients MS microelement, MS organic compound, lactalbumin hydrolysate(LH) 1g/L, activated carbon (Ae) 1g/L.

Results and discussion

The effect of different macroelement of medium on Alfalfa anther callus Fett-Neto etc. think that the growth of the cells depend on NO_3^- as the nitrogen source and inhibit on high concentration NH_4^+ . The experiment showed: the effect of callus culture in the medium containing MS macroelement or SH macroelement was better than in the medium containing N6 macroelement in primary culture, but the medium containing N6 macroelement was the best medium to callus subculture and embryogenic callus induction from the 5th to the 9th subculture.

The Effect of hormone on growing of callus Daipeng Li etc. think that exogenous hormones could transfer development information of dedifferentiate and redifferentiation in plant callus culture, effect of exogenous hormones related with explant, the kinds and the levels of endogenous, hormones of callus, function of exogenous hormone must combined with endogenous hormones. Therefore, it should consider hormone concentration of explant and callus to adding plant exogenous hormone. The experiment showed: callus growth was similar in the D4 (0.05 mg/l 2,4-D + 0.5 mg/l BA) and D6 (0.05 mg/l NAA + 0.5mg/l KT) medium, D4 and D6 were better than D3(NAA 0.05 mg/l + BA0.5 mg/l).

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