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Effects of plant growth regulators on plant regeneration through somatic embryogenesis of *Medicago sativa* L

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Objectives

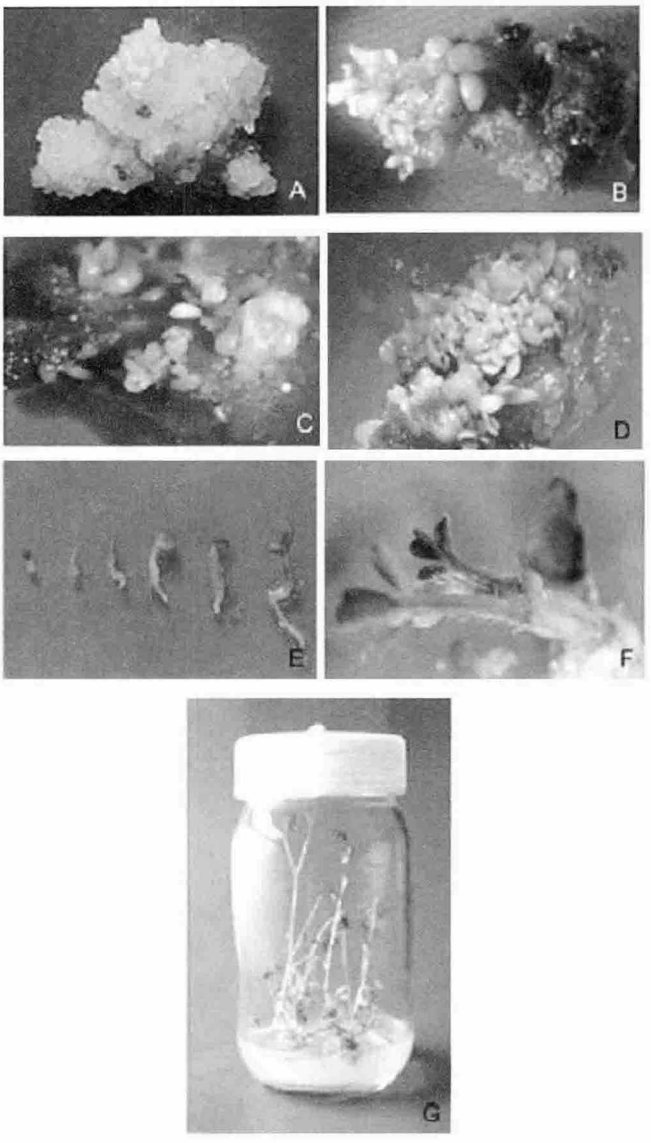
To confirm a successful transformation system of the alfalfa, we investigated the effects of plant growth regulators and kanamycin concentration on plant regeneration through somatic embryogenesis of *Medicago sativa* L.

Materials and Methods

1. The effect of plant growth regulators (PGRs) on embryogenic callus induction was tested using MS medium containing various PGRs (NAA, 2, 4-D, IAA, BA, kinetin and zeatin).
2. The response of explant on embryogenic callus induction was examined using hypocotyl and cotyledon segments of alfalfa seedlings. Hypocotyl and cotyledon segments from 7-day-old seedlings were placed on the surface of solid MS medium supplemented with 2,4-D and kinetin, NAA and BA, IAA and zeatin combination, respectively.
3. The effect of cytokinin on plant regeneration from embryogenic callus was examined. The cytokinins included BA, kinetin and zeatin. The embryogenic calli derived from explants were transferred to MS medium supplemented with BA, kinetin and zeatin using concentration of 0.01, 0.1 or 0.5 mg1-1, respectively.
4. In order to examine effective kanamycin concentration for transformation, hypocotyl segments were cultured on MS medium supplemented with 0.01 mg1-1 IAA, 1.0 mg1-1 zeatin and kanamycin (0, 25, 50, 75 or 100 mg1-1).

Results and Discussion

An efficient plant regeneration system of alfalfa (*Medicago sativa* L.) through somatic embryogenesis was established. Embryogenic callus was obtained by culture of hypocotyl segments on MS medium with 0.01 mg1-1 IAA and 1.0 mg1-1 zeatin after 45 days of culture. They were converted to the somatic embryo when embryogenic calli were transferred to MS medium without hormone and MS medium containing various cytokinin (BA, kinetin and zeatin). Most of somatic embryos developed into plantlet, especially, normal plantlets were developed on MS medium supplemented with 0.1 mg1-1 kinetin. Also secondary embryos were appeared on surface of primary embryo but they were showed abnormal growth. Adequate concentration of kanamycin for selection was 50 mg1-1. Regenerated plantlets were transplanted to pot containing vermiculite and perlite for the further anaylsis.



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