

An efficient system for protoplast culture from alfalfa (*Medicago sativa*) suitable for plant transformation and regeneration.

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Medicago inc is committed to the development of cost-efficient methods for the use of alfalfa as a cellular factory for pharmaceuticals. The species has been chosen for its high protein content, high yield, numerous environmental benefits and perennial characteristics. Although Agrobacterium-mediated transformation has proven efficient on regenerative genotypes, the method has limitations in the context of recombinant product development. Although use of more direct DNA transfer methods is appealing, it is limited by the inefficiency of plant regeneration from isolated cells or protoplasts. We have developed an improved method to purify and regenerate protoplasts from leaf tissue of alfalfa. With this improved system which comprises new medium composition for digestion, purification and initial development, first cell division occurs at day 7 and ten-cell microcolonies have formed within 2 weeks of protoplast isolation. Within 3 to 4 weeks, 20 % of protoplasts have developed colonies of 50-100 cells. These colonies are transferred to regeneration conditions in which 5% of protoplasts develop into microcalli within 6 weeks. Most microcalli then form somatic embryos from which plantlets are obtained 15 weeks after protoplast isolation. Performance of this method was established on protoplast which were isolated at the earliest stage in jellifying polysaccharide films. Results on colony and microcallus development from protoplasts will be discussed as well as applications of this method for high throughput genetic transformation.