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Optimization of somatic embryogenesis in suspension cultures of horsegram [*Macrotyloma uniflorum* (Lam.) Verdc.]—A hardy grain legume

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Abstract

Cell suspension cultures were established from immature cotyledon derived calli from drought tolerant legume horsegram [*Macrotyloma uniflorum* (Lam.) Verdc.]. Embryogenic callus could be originated from cut slices of the immature cotyledons on MS solid medium [Murashige, T. Skoog, K., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15, 473–497] augmented with 1.0 μM zeatin and 4.5 μM NAA. Numerous somatic embryoids (26.4%) appeared on MS liquid basal nutrient medium with 5.6 μM NAA and with absence of zeatin during 3 weeks culture. Sustained cell division resulted in the formation of cell aggregates, and then progressed to globular, heart and further if they differentiate properly to torpedo and cotyledonary stages within 5 weeks. Transfer of individual embryos on to a fresh MS

Abbreviations: MS, Murashige and Skoog medium; NAA, α-naphthaleneacetic acid; 2,4-D, 2,4-dichlorophenoxyacetic acid; KIN, kinetin; GA_3, gibberellic acid; PCV, packed cell volume; FAA, formalin acetic acid alcohol

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basal medium with no plant growth regulators was able to achieve complete maturation. Only a relatively few number of embryos developed into root/shoot when transferred to 0.9 μM GA_3, 15 g/l^{-1} sucrose and 2.4 g/l^{-1} gelrite containing medium. Substitution of sucrose associated with the use of L-glutamine gave, in the range of concentrations tested, the strongest enhancement of the embryo growth and development. About 5% of somatic embryos were converted into true-to-type fertile plants.

Keywords: Embryogenic callus; Cell suspension culture; Somatic embryos; Immature cotyledon; *Macrotyloma uniflorum*

1. Introduction

Pulses can furnish an eminent source of dietary protein constituents for human consumption as a big benefit in a balanced energy and protein diet for those who live in developing countries, especially when intake from animal or fish sources is limited or insufficient. Horsegram [*Macrotyloma uniflorum* (Lam.) Verdc.] is one of the important early domesticated and enriched nutritious crops cultivated mainly in hot dry and tropical countries in a post-rainy season, where the seeds are parched and then eaten after boiling or frying, either whole or as a meal (Purseglove, 1974). Moreover, the dry seeds are boiled, pounded with salt and fermented to produce a sauce similar to soy sauce from *Glycine max*. Unfortunately, the uncertainty in horsegram is its yield stability and vulnerability to yellow mosaic disease causing about (30–50%) yield loss (Muniyappa et al., 1975; Smartt, 1990). Legume improvement by conventional breeding methods has limitations mainly due to the insects-pests interference, for which no source of resistance is available. Genetic engineering could alleviate these problems by incorporating useful genes from unrelated sources into elite genetic backgrounds. An efficient regeneration system for legume is a prerequisite for the genetic transformation to achieve desirable traits with improved productivity. An efficient regeneration system through somatic embryos has provided a foundation for crop improvement that includes somatic hybridization, in vitro selection and genetic transformation, which allows us to modify one single property of the plant. Only a relatively small number of reports are available, i.e. callus induction (Varisai Mohamed and Jayabalan, 1996) and, direct shoot regeneration from shoot tip and cotyledonary node explants (Sounder Raj et al., 1989; Varisai Mohamed et al., 1998, 1999). Although, the formation of somatic embryos in horsegram has been reported recently by the current authors (Varisai Mohamed et al., 2004). However, to our knowledge, no report on regeneration of plants via somatic embryogenesis from immature cotyledon has been yet described. The present investigation reports a detailed study of embryo development and plant formation through somatic embryogenesis using cell suspension cultures from immature cotyledon explants as well as some data on their embryogenic growth characteristics are presented. This established system might also be used to select useful traits at the cellular level and to obtain improved plants or genetically stable cells.