Induction of Microspore Embryogenesis for Producing Homozygous Lines in Cassava (*Manihot esculenta* Crantz.) Through Anther Culture

Dissanayake D.M.L.S.[a], Attanayaka D.P.S.T.G.[b], Perera P.I.P.[a*]

[a]Department of Horticulture and Landscape Gardening,
[b]Department of Biotechnology, Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Makandura, Gonawila, 60170, Sri Lanka
*Corresponding author (email: panawalageindra@gmail.com)

Abstract

Cassava (*Manihot esculenta* Crantz.) is the third carbohydrate source in the tropics which is an important raw material for food, feed and starch based industries. Cassava breeding program is mainly targeted to improve the tuber yield, starch and tolerant to biotic and abiotic stresses. Cassava is an out breeding, highly heterozygous crop in which the improvement is limited through conventional breeding programs. Haploid culture is one of the breeding techniques that the homozygous lines are produced by the induction of embryogenesis in gametophyte cells. Microspore embryogenesis through anther and microspore culture are two of the haploid culture techniques where the anther culture is the most commonly used techniques for the production of homozygous lines from the heterozygous parental crops. Several factors affect for the induction of microspore embryogenesis such as pollen development stage, anther wall factors, pretreatment condition, culture medium, culture density and genotype. The present study was undertaken to optimize the above conditions affecting for microspore embryogenesis in a local genotype of cassava "Kirikawadi". Murashige and Skoog liquid medium supplemented with 0.005 mg/L additional $CuSO_4.5H_2O$ was used as the basal medium. Two different incubation temperatures, 37 and 40 °C for four different incubation time periods (6, 14, 24 and 48 hrs) were tested against the none pre-treated anthers. Numbers of anthers were cultured with different densities (20, 40, 60, 80 and 100) in the petri plates (90 x 100 mm) to test the optimum density. In order to optimize the sucrose level four concentrations (2, 9, 12 and 15 %) were tested. Type of plant growth regulator and its concentration in the culture medium plays an important role, thus, different concentrations of auxins and cytokinins such as 2, 4-Dichlorophenoxyacetic acid (2,4-D; 0, 5, 12, 24 and 48 mg/l) Naphthaleneacetic acid (NAA; 0, 1, 5, 10 mg/L) and cytokinins (6- Benzylaminopurine (BAP), 6-purine (2iP), Kinetin; 0, 5, 10 15 mg/L) were tested for efficient induction of microspore embryogenesis. Each treatment consisted of three petri plates and all the experiments were repeated

three times. Cultures were observed under the stereo microscope at two weeks interval. Responsive anthers were observed under the Scanning Electron Microscope (SEM) to identify the origin of the pro-embryo. Significance of the treatments in different experiments was determined by analyzing the collected data using SAS statistical package.

Anther enlargement was observed as an initial response after two weeks of culture initiation; however, there was no any significant difference among the treatments. Globular proembryo formation through the anther wall was observed after four weeks of culture initiation. Anthers containing those pro-embryos were counted as the responsive anthers. Origin of pro-embryos was found to be from the interior part of the anther through the SEM observation whereas the absence of any physical attachment to the anther wall further indicated the interior origin. Significant treatment effect on pro-embryo development was identified in all the experiments. Cultures incubated at 37 °C for 14 hrs gave the better performance for pro-embryo formation. Sucrose at 2% concentration gave the higher response for pro-embryo formation. Cultures with 20 anthers per petri plate were identified as the best anther density for efficient induction. Two different 2,4-D concentration (5 and 12 mg/l) gave the best pro-embryo formation. Among auxins 2,4-D with 5 mg/l and among cytokinins, BAP with 5 mg/l showed the greatest anther response. The optimized conditions will be used for further experimentation to produce a precise doubled haploid plant production system in cultured cassava anthers.

Keywords: Microspore embryogenesis; Pro-embryos; Auxin; Cytokinin