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Development of Anther Culture Protocol for Selected Brinjal Germplasm (*Solanum melongena* L.)

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Key words: Anther Culture, Brinjal (*Solanum melongena* L.), Callus induction, Double haploid, Plant growth regulators

Abstract

Selected anthers from seven brinjal germplasm namely; Anjalee, Padagoda, EGH-5, EGH-13, EGH-16, V-5, and 8104 were cultured on MS medium containing different hormonal supplements for callus induction and *in vitro* regeneration. Various hormonal treatments (T1 to T4) for callus induction consisted 1.0 mg/l Kn and 2.0 mg/l BAP with different concentrations (1, 3, 5 and 0 mg/l) of NAA. The highest percentage of callus induction (83.33 ± 16.67, $p < 0.05$) was obtained in T2 from the hybrid germplasm EGH-13. Significantly the highest percentage of root formation (83.33 ± 16.67, $p < 0.05$) was obtained on 1 mg/l BAP from EGH-13. MS supplemented with 1 mg/l BAP was found to produce shoot (6.67 ± 6.67, ($p < 0.05$) only for germplasm Padagoda. All other germplasm failed to produced shoots on any medium having different combination of hormonal supplements.

Introduction

Brinjal (*Solanum melongena* L.), is an important agricultural crop belonging to the family Solanaceae which is valued for its nutritional and economic benefits. It is the fifth most economically important solanaceous plant after potatoes, tomatoes, pepper, and tobacco (Taher et al. 2017). Due to the uses and the demand for the brinjal crop as a vegetable, it is required a method for production development. Production of brinjal can further be increased if improved cultural practices are combined with good quality seeds. The quality seed production techniques of brinjal with the conventional breeding techniques required a large time period to complete the breeding cycle and its processes. Plant tissue culture techniques have emerged as a pivotal strategy to improve plant breeding in recent years (Hussain et al. 2012). Among these techniques, anther culture stands out as a technique for producing haploid plants, facilitating the development of pure lines in

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breeding programmes. Anther culture involves the *in vitro* culture of anthers to induce microspore development into haploid plants. This technique allows for the production of double haploids, which are homozygous and can significantly reduce the time required for traditional breeding methods (Hussain et al. 2012). A study has demonstrated that the anther culture can lead to a higher frequency of haploid plant regeneration in brinjal compared to conventional breeding strategies (Pagalla et al. 2020). Anther culture offers the advantage of minimizing the impact of environmental factors on plant growth and development, providing a controlled environment for microspore development. This leads to increased efficiency in breeding programmes, allowing for the rapid evaluation of genetic traits and quicker generation of new germplasm (Germana 2011). Genotype, donor plant culture conditions, stage of development of microspore, pre-treatment of flower buds or anthers, culture medium composition and culture conditions affect the culture response (Kumar et al. 2003). Salas et al. (2011) conducted anther culture study in Spain on 11 different brinjal varieties. However, for the locally available brinjal germplasm of Sri Lanka, there are no reported anther culture studies. Thus, the study was focused on developing a suitable anther culture medium for selected brinjal germplasm.

Materials and Methods

In this study, seven different brinjal germplasm named, Anjalee, Padagoda, EGH-5, EGH-13, EGH-16, V-5, and 8104 were used to test anther culture ability and test better technology with media combinations. Anjalee, EGH - 5, EGH -13, and EGH - 16 were the F_1 hybrid varieties; V-5 and 8104 were parental lines and Padagoda was an open-pollinated variety. The anthers from all germplasm were obtained from the unopened flower buds which were nearly 10 to 15 mm long, in morning hours around 7 from the Horticultural Crop Research and Development Institute (HORDI), Gannoruwa, Peradeniya, Sri Lanka. MS basal media with different concentrations of plant growth hormones NAA and 2 mg/l BAP, as well as 1 mg/l Kn, were used (Table 1). The pH of the medium was maintained between 5.7 and 5.8 with 1N NaOH /HCl. Media-containing jars were sterilised using an autoclave at 121°C under 1.06 kg/cm^3 steam pressure for 20 min.

Table 1. Callus induction growth hormone compositions.

	T1	T2	T3	T4 (Control)
BAP	2 mg/l	2 mg/l	2 mg/l	2 mg/l
NAA	1 mg/l	3 mg/l	5 mg/l	0 mg/l
Kn	1 mg/l	1 mg/l	1 mg/l	1 mg/l

The flower buds were subjected to pre-surface sterilization with 2-3 drops of liquid detergent (Teepol) under running water for 30 minutes. Pre-sterilized flower buds were wrapped using aluminium foil and kept in the refrigerator for 24 hrs at 4°C temperature

for cold treatment. Pre-surface sterilised flower buds were taken out from the refrigerator 30 min before culturing and kept until the flower buds came to room temperature. Then the flower buds were immersed in 70% ethyl alcohol for 5 min. Next, they were transferred into distilled water, and they were immersed for 3 min. Then sterilised flower buds were transferred into a new, pre- sterilised Petri dish.

The sterilised flower buds were transferred to the laminar flow. The flower buds and anthers were sterilised using different surface sterilisation methods as mentioned below. The flower buds were immersed in 50% Clorox for 15 min and they were transferred into distilled water and kept for 3 min. Then the flower buds were placed over a sterile Petri dish to excise the anthers by using a scalpel and forceps. Anthers were immersed in a fungicide called "Daconil" for 5 min and then they were washed with distilled water. Next, fungicide treated anthers were immersed in 40% Clorox for 3 min and then they were transferred into distilled water and kept for 3 min. The *in vitro* culturing process was carried out under aseptic conditions. Cultures were incubated at 23 ± 1°C under 16 hrs photoperiod using white fluorescent lights (1000 Lux) in a culture room. The experiment was done as all treatments from each germplasm were replicated 3 times with 10 anthers in each replicate. After the formation of the callus, they were subcultured on MS medium with different concentrations of BAP such as 0.5, 1.0 and 2 mg/l and responses to regeneration media were observed. The average number of shoots or changes in different calli were observed after four weeks of subculturing. Then the emerging shoots were transferred to MS medium supplemented with 1 mg/l of BAP for root formation.

The experiment was arranged as a two-factor Completely Randomized Design (CRD) with three treatments and a control. The data was statistically analysed using nonparametric statistics in ANOVA using R studio with a 95% confidence interval. Mean separation was done by LSD test at 0.05 significant level.

Results and Discussion

The formation of callus was observed within 14 days after culturing the anthers of seven different brinjal germplasm (Fig. 1). However, Khatun et al. (2006) reported that approximately 28 days were taken to initiate callus from the anthers of six Bangladeshi eggplant varieties. In this study, the local brinjal germplasm were found to produce callus approximately within 14 days. Factors such as the physiology of the mother plant (Germana 2011) and the correct stage of microspore development (Pagalla 2023) may be the reason for these particular results, as Sri Lanka has a climate that suits brinjal.

According to Fig. 2, the investigation revealed that the percentage of callus formation exhibits a significant effect ($p < 0.05$). The maximum callus formation percentage (83.33 ± 16.67) was observed in the treatment where 3 mg/l NAA (T2) was applied with 2 mg/l BAP and 1 mg/l Kn supplemented MS medium from F_1 hybrid variety EGH- 13. While the minimum percentage of callus formation was observed and recorded in hormonal composition T4 where no NAA was applied for all the selected brinjal germplasm. Only

germplasm V-5 showed callus initiation in this treatment. According to Kumar et al. (2003), 11.2-20% of callus initiation was performed on 0.5 mg/l of 2,4-D, 1 mg/l of BA, and 2 mg/l of NAA in brinjal.

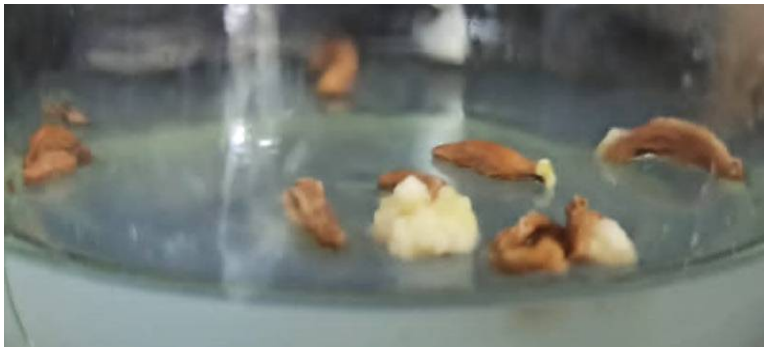

Fig. 1. Callus formed on anthers after 14 days of culturing.

Bhattacharya et al. (2019) reported that MS salts + Vitamins + NAA-5 mg/l + BAP-2 mg/l + 3% Sucrose was recommended as the best medium for brinjal anther culture. In contrast according to the results of this study MS salts + Vitamins + NAA-5 mg/l + BAP-2 mg/l + Kn-1 mg/l + 3% Sucrose comparatively showed lower results in all selected brinjal germplasm. The investigation revealed that a high concentration of 5 mg/l of NAA reduces the callus formation percentage in selected brinjal germplasm.

Although the anther culture has not been applied for locally available selected brinjal germplasm, but some studies reported that brinjal hybrids gave better results in anther culture (Rotino 2016). Accordingly, this study also proved that hybrids have a higher potential to form calli in anther culture.

Though we transferred the callus to various concentrations of BAP for the induction of shoots, spontaneous roots were produced from the callus. However, the investigation revealed (Fig. 3) that the percentage of root formation from callus exhibits a significant effect ($p < 0.05$). This may be due to the residuals of NAA remaining in callus tissues. A similar incident was recorded in the study conducted by Khatun et al (2006) with brinjal. This study recorded maximum rooting for MS medium 2.5 mg/l NAA + 2 mg/l BAP. In this experiment, the maximum root formation percentage (83.33 ± 16.67) was observed in the treatment with MS + 1 mg/l BAP (RT2) from hybrid variety EGH-13.

When considering the responses of selected germplasm to anther culture, the investigation revealed that from all germplasm the maximum root formation (32.36 ± 11.17) was observed from hybrid variety EGH-13 (Fig. 4), while the minimum root formation (3.52 ± 2.38) was observed from germplasm 8104. The hybrid varieties, EGH-5, EGH-13, EGH-16, and Anjalee gave comparatively better results in root formation on calli than the other germplasm.

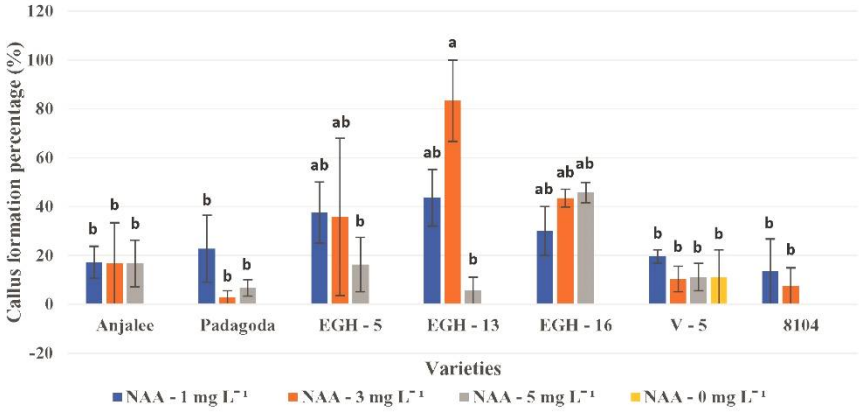

Fig. 2. Effects of different treatments on callus induction in different brinjal germplasm on MS with 2 mg/l BAP, 1 mg/l Kn and NAA (0-5 mg/l). Bars denoted by the same letters are not significantly different from each other based on the least significant difference at 0.05 level of probability.

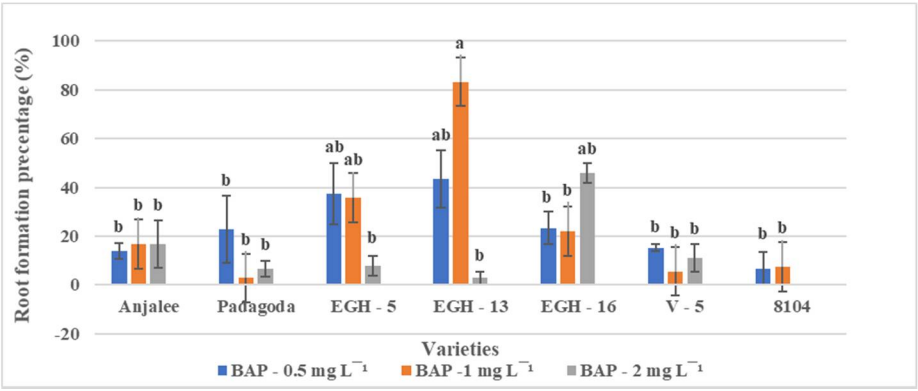

Fig. 3. Effects of different treatments on root formation in different brinjal germplasm. Bars denoted by the same letters are not significantly different from each other based on the least significant difference at 0.05 level of probability.

Only germplasm 'Padagoda' formed shoots on MS medium with 1 mg/l BAP after 4 weeks of subculturing (Fig. 5). Padagoda has not formed shoots on medium with 0.5 mg/l BAP and 2 mg/l BAP. Other germplasm did not form shoots on any medium mentioned above. They formed roots on each medium throughout the observation period of 4 weeks after the subculture. Similarly, a study conducted by Khatun et al. (2006) recorded no shoots on the MS medium with 2.5 mg/l NAA and 2 mg/l BAP for 35 days of period for Dohazari, ISD-006, Laffa S Ishurdi L, IPM L and Jessore L brinjal varieties, only root formation was recorded. In this experiment shoot formation was observed with 6.67% for padagoda, but root formation was observed in every germplasm.

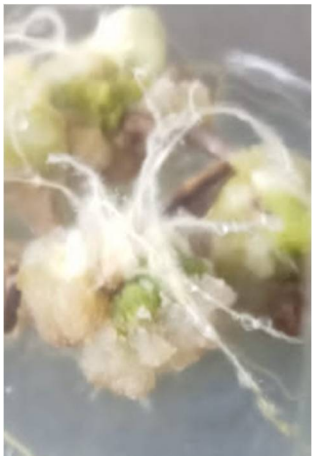

Fig. 4. Root formation on calli after 1-2 weeks of subculturing in EGH-13.

Fig. 5. Sprouted shoots after 4 weeks of subculture in germplasm Padagoda.

According to Foo et al. (2018), the shoot formation with 2 mg/l Kn produced 65% of shoot formation which was higher results than with BAP in tested brinjal seeds. Their investigation shows that only some germplasm are responsible for the shoot formation under the same condition in brinjal anther culture.

It can be concluded that the anthers with the correct physiological stage and favourable environmental conditions have the potential to succeed in anther culture in brinjal. Among selected germplasm, hybrid germplasm; EGH-13, EGH-16, EGH-5, and Anjalee had high potential towards callus induction and root formation. The MS medium with 1 mg/l Kn, 2 mg/l BAP, and 3 mg/l NAA has been given the best result in callus induction from Hybrid germplasm EGH-13. The MS medium with 1 mg/l BAP has been given the highest root formation from the same variety only the variety Padagoda responded to shoot formation on MS medium with 1 mg/l BAP.

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