Effect of Explants and Hormone Levels on Callus Induction in Tomato (*Lycopersicon esculentum* mill.): Variety Thilina

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Abstract

Tomato (*Lycopersicon esculentum* Mill) is a major vegetable crop that has achieved tremendous popularity over the last century and it is grown in almost every country of the world, including Sri Lanka. An efficient *in vitro* propagation technique is required for the genetic improvement of this crop via gene transfer technology. The initiation of callus cultures is the first step for an efficient *in vitro* propagation technique of this crop as well as other crops. To improve Sri Lankan tomato varieties genetically, this study was conducted to investigate the influence of explants and hormone levels on callus induction of tomato (*Lycopersicon esculentum* Mill): Variety Thilina as the pioneer step. Five different hormone combinations on Murashige and Skoog's (MS) basal medium with 0.1 mg/l NAA (1-Naphthalene acetic Acid) + 1.0, 1.5, 2.0, 2.5, 3.0 mg/l BAP (Benzyl Adenine) as well as three explant types: hypocotyl, leaves and root were employed. Completely Randomized Design (CRD) was used as experimental design with 5 replicates. Statistical analysis was carried out using the Student Newman-Kuells Means Separation Test. Fresh weights of callus were evaluated after one month of culture establishment. Maximum callus production from hypocotyls and leaves were obtained on MS medium supplemented with NAA 0.1 mg/l and BAP 2.0 mg/l while root explants did not produce calli. Hypocotyls recorded to be better explants for the callus production (0.33 g/ explants).

Key words: *Lycopersicon esculentum*, Callus, Explants, MS basal medium, BAP

Introduction

Tomato (*Lycopersicon esculentum* Mill) is an important Solanaceous vegetable growing throughout the world for its versatile uses. It is one of the protective foods as it possesses appreciable quantities of vitamins, minerals and sometimes rightly referred to as 'poor man's orange' (Devi *et al.*, 2008). For geneticists, it has became a model plant due to the well investigated and relatively small genome (0.7–1.0 pg). On the other hand, breeders due to its considerable economic value, continuously search for new genomes determining a higher quality as well as resistance to diseases and unfavorable environmental conditions. With this aim, callus cultures are often used, as they are commonly considered an important source of genetic variability. The initiation of callus cultures and their use in future organogenesis or embryogenesis depends on numerous factors. Thus each time they need to be determined experimentally (Rzepka-Plevneš *et al.*, 2006). Furthermore, Sri Lankan tomato varieties less subjected to *in vitro* tissue culture techniques. To attain sustainable tomato productions, constraints such as labor cost, time consuming, have been addressed by conventional breeding and enhanced management but it has resulted in limited commercial success. The integration of modern biotechnology like tissue culture into breeding programs may provide powerful tools to overcome these limitations (Osman *et al.*, 2010). Therefore, *in vitro* tissue culture techniques are required to improve Sri Lankan crop varieties as well as worldwide crop varieties. Investigation of callus induction ability is an opening pathway to establish tissue culture technique for tomato variety Thilina as well as other crops. Therefore, the present study aimed to determine the interactive effect of the type of explant and hormone composition of the MS basal medium on the induction and growth of the callus.

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Materials and Methods

Plant source

Seeds of Tomato were purchased from the Seed and planting material Division, Department of Agriculture, Sri Lanka.

Establishment of aseptic cultures

Seeds were surface-sterilized by washing under running tap water, again washed in soapy water. Seeds were immersed in 70% ethanol for 3 minutes and rinsed three times with distilled water. Then seeds were disinfected with 20% Clorox (5.25 a.i. Sodium hypochloride) for 20 minutes. Sterilized seeds were then rinsed three times with sterilized distilled water and inoculated onto solid nutrient medium containing MS (Murashige and Skoog's, 1962) salts (half-normal concentration of macro- and micronutrients) with 3% sucrose. The pH of the medium was adjusted to 5.86 with 1N NaOH or 1N HCl solution. The medium was solidified with 0.5 % Agar prior to autoclaving at 1.4 $kgcm^{-2}$ for 20 minutes. The seeds were cultured under light in an air-conditioned room for 10 days (Dahanayake *et al.*, 2010).

Callus production ability of different type of explants in different media

Leaf, hypocotyl and root explants of aseptic plantlets were cultured on MS half strength basal medium. Five different media were used supplemented with 0.1 mg/l NAA and 1.0, 1.5, 2.0, 2.5, 3.0 mg/l 2,4-D to investigate the callus production with the 15 days old seedlings. In cultures, leaves were cut into sections (0.5 cm^2) and placed on media with the adaxial surface toward the media, while hypocotyl and roots were cut into about 5 mm and cultured by laying randomly on the media. Five replicates were used from each explant and cultures were kept under continuous light.

Data collection and analysis

Experiment was arranged according to the Completely Randomized Design (CRD). Callus induction and regeneration were evaluated 30 days after initiation. Number of explants with callus in different treatment and diameters of callus were recorded. All experiments had five replicates, each with five explants per bottle. Statistical analysis was carried out using the Student Newman-Kuels Means Separation Test of SAS software package (version 9.1.3).

Table 1. Effect of α-naphthalene acetic acid (0.1 mg/l NAA) and different combinations of Benzyladenine (BA) on the callus induction of tomato (Lycopersicon esculentum Mill; Variety Thilina).

Treatments	BAP mg/l	Fresh weight of callus per explant (g)		
		Hypocotyl	Leaves	Root
1	1.0	0.13880^{c}	0.01966^{c}	0.000
2	1.5	0.28628^{ab}	0.03590^{c}	0.000
3	2.0	0.33156^{a}	0.09178^{a}	0.000
4	2.5	0.20256^{abc}	0.06940^{b}	0.000
5	3.0	0.18320^{bc}	0.07456^{ab}	0.000

Means fallowed by the same lower case letters in each column are not significantly different at 5% level in Duncan's Multiple Range Test

Results and Discussion

Calli were initiated within 7 – 14 days directly on the cut surfaces of hypocotyls, leaves and root explants cultured on MS basal medium supplemented with auxins (NAA) and cytokinins (BAP) in different combinations (Table 1).

Callus response was distinctly affected by the types of explants (Leaves, Hypocotyls, and Roots) and growth regulators used. The highest mean weight (0.33 g/explant) of callus per explant was obtained on hypocotyl explant cultured on MS medium supplemented with 2.0 mg/l BAP and 0.1 mg/l NAA. Next highest mean weight (0.29 g/explant) was obtained on hypocotyl explant cultured on MS medium supplemented with 1.5 mg/l BAP and 0.1 mg/l NAA. The lowest mean weight (0.14 g/explant) of callus revealed by MS medium supplemented with BAP and 0.1 mg/l NAA. Mean weights were raised up to 2.0 mg/l BAP concentration and then it was decreased in both hypocotyl and leaves explants. Root explant was not produced callus in each and every BAP concentration and leaves explants were displayed callus in each and every concentration but mean weight were less than the mean weight of hypocotyls. Therefore, hypocotyl explant can be considered the best explant for all BAP concentrations. Nevertheless, 2.0 mg/l BAP with 0.1 mg/l NAA was the best hormonal concentration for the hypocotyl explant to obtain maximum weight of callus. Highest mean weight of callus (0.09 g/explant) from leaves explant was obtained from medium supplemented with 2.0 mg/l BAP as well as hypocotyl explants. Although sufficient callus was induced on hypocotyl and leave explants by all growth regulators used, but no callus production observed from root explants.

Callus cultures constitute currently an important source of genetic variability, which is of considerable importance in the breeding of many species of cultivated plants. Callus induction depends on numerous factors - different for different cultivars and species (Rzepka-Plevneš *et al.*, 2006). The *in vitro* morphogenetic responses of cultured plants are affected by different components of the culture media and explants. Therefore, it is important to evaluate their effects on plant callus induction (Osman *et al.*, 2010).

Osman *et al.* (2010) reported that callus response was markedly affected by the types (Hypocotyls and leaves) of explant and growth regulators used. Moreover, Nikam and Shitole (1998) reported that the growth regulator requirements for callus induction vary depending on the source of explant. According to Chaudhry *et al.* (2010), callus induction was observed from both leaf disc segments and hypocotyls. Similarly hypocotyl and leaves explants were obtained callus in the present study. There were no any reports on callus from root explants until now as well as in the present study. Hypocotyl explant was the best explant to obtain maximum fresh weight of callus (Osman *et al.*, 2010) and same results were observed in the present study.

In vitro callus induction depends on the endogenous concentration of plant growth regulator as well as exogenously supplied growth regulator (Osman *et al.*, 2010). The highest callus was obtained on hypocotyls explants cultured on MS medium supplemented with 0.5mg/l NAA and the same explant by using 0.1 mg/l NAA with 0.5 mg/l BAP. Furthermore, for the cotyledon explants, the highest callus was obtained on MS medium supplemented with 2.0 or 3.0 mg/l NAA. However, MS basal medium supplemented with 2.0mg/l BAP and 0.1mg/l NAA was the best hormonal concentration for both hypocotyl and leaves explant to obtain highest callus fresh weight.

As conveyed Rzepka-Plevneš *et* al., (2006) best medium for the culture of tomato cultivar 'Maskotka' on MS basal medium supplemented with 2.0 $mg.dm^{-3}$ of IAA and 1.0 $mg.dm^{-3}$ of BAP in leaves explant. However NAA (0.1 mg/l) and BAP (2.0 mg/l) were used in the present study to obtain callus from leaves explant in Thilina, a Sri Lankan variety. According to them higher IAA and lower BAP concentration were induced the maximum callus from leave explant. However, lower NAA and higher BAP were induced maximum callus from leaves explants in the present study.As reported by Devi *et al.,* (2008), the MS medium supplemented with 3.0mg/l BAP and 2.5 mg/l IAA optimum for callus induction in Tomato. However BAP (2.0 mg/l) and NAA (0.1 mg/l) were obtained optimum fresh weight of callus from our study.

Maximum callus production was noted on MS basal medium supplemented with 0.1 mg/l NAA and 2.0 mg/l BAP from both explants hypocotyl and leaf of Tomato Variety Thilina. Hypocotyl explants were better for callus production comparing to leaf explants.

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