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PROTOCOL OF OPTIMIZATION TOMATO TISSUE CULTURE FOR THE MICROPROPAGATION AND RECOMBINANT PROTEIN PRODUCTION (FERON GENOTYPE)

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ABSTRACT

For years, plants used for the production of recombinant proteins and as molecular farming is known. However, the transformation of tomatoes is still not A common method and repeatable for all digits. In this study, we've optimized the tissue culture of tomato genotype (Feron genotype) with two cotyledon and hypocotyl explants in 6 different culture medium whit different hormones. In the meantime, culture medium supplemented whit benzyl amino purine (BAP) mg/L3 and Zeatin mg/L1 in addition whit 0.5 mg/L indole acetic acid (IAA) showed the highest regeneration rate. Interaction among culture medium and explants on callus induction and regeneration were observed. Among the explants cotyledon was the best regeneration.

Keywords: Tomatoes, optimize tissue culture, the role of hormones

INTRODUCTION

Molecular farming, it involves the use of plants, and potentially also animals, as the means to produce valuable compounds such as recombinant proteins. Farming is a new innovative and revolutionary in the field of genes cloning. In the meantime plants are considered tools cheap and simple technology to produce recombinant proteins.

Plants and animals have similar processing so that many animal proteins produced in plants,the Post-translational modificationare correctly and completely active [1]. The list of plant species that by DNA vector (*Agrobacterium* methods) and other techniques are transgenic, continuously developing and currently ability to transformation to more than 120 plant species in at least 35 families has been expanded [2]. Plant cell culture technology for the commercial production of natural products known but as an alternative to the culture of the whole plant can be used in the field. Pharmaceutical proteins such as interleukin-value, the human t-PA antibodies as a pilot project in plants via tissue culture and cell culture technologies have been produced. Currently, transgenic plants are considered an important field of research so the first step in the production of recombinant proteins in plant and molecular farming is optimization of plant tissue culture [3]. Tomato is an important vegetable in the world that is available to all people of the world especially in tropical and subtropical areas that can be harvested all year round and due to a variety of vitamins, carotene, amino acids useful, sugar and minerals plays an important role in human health however, tomato plants are an important model for improving other dicotyledonous plants [4] that this issue related to small genome of this plant [5]. However, tomato transformation method is not a common and repeatable for all its digits [6]. So far to optimization of tissue culture and transformation of tomato a different explants such as leaf discs, cotyledon and protoplast are used also for direct regeneration of transgenic plants various concentrations of hormones is used according to the tomatoes must be optimized [7, 8]. In this study, tissue culture and tissue culture optimization tomato genotypes Feronusing two tiny samples cotyledon and hypocotyl were tested in six different hormonal environments[9].

MATERIALS AND METHODS

Seed culture, Tomato seeds by putting in 70% ethanol for 3 min and active chlorine for 7 minutes were sterilized and were washed three times with sterile water. For germination sterilized seeds were placed on germination medium (GM) Table 1. After 8 days at 25 ° C whit humidity 70% and 8/16 h photo period ($90\mu molm^{-2}seg^{-1}$)seedlings were growth. Cotyledon and hypocotyl sterile isolated and were placed on pre-culture medium in 1, 2, 3 and 4 days treatments in the dark at 25^{oC} temperature [10]. Optimization of Culture medium, to select the Culture medium appropriate for callus

induction and shoot direct regeneration different levels of three hormones BAP, IAA and Zeatin were used. Select the type of hormone was performed using the results from other researchers [11].

Table 1: Composition of Culture medium

	GM	PM	CM	RM	RTM
MS	+	+	+	+	+
Sucrose	30g/lit	30g/lit	30g/lit	30g/lit	30g/lit
Agar	8g/lit	8g/lit	8g/lit	8g/lit	8g/lit
Thiamine –HCL				0.4mg/lit	
NAA		1mg/lit	1mg/lit		
BAP		1mg/lit	1mg/lit		
IAA				0.5mg/li	
Zeatin ribozaid				0.5mg/li	

RESULTS AND DISCUSSION

After preliminary experiments were used for callusing and regeneration culture medium suitable for direct regeneration from cotyledon and hypocotyl explants were optimized.

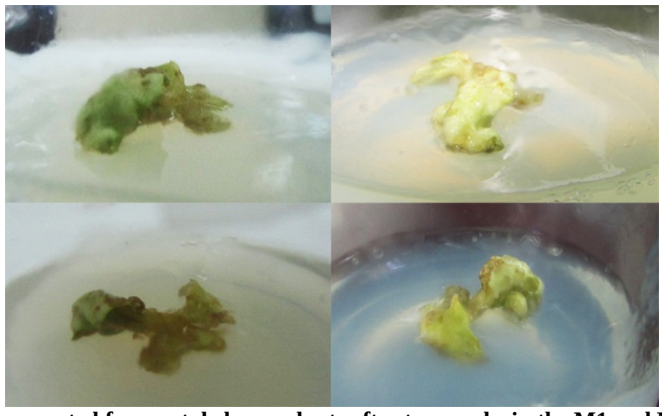

Figure 1: Callus generated from cotyledon explants after two weeks in the M1 and M2 after 2 weeks

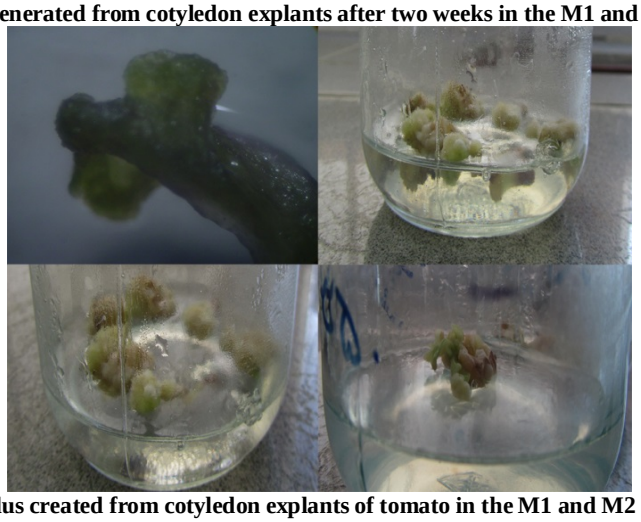

Figure 2: Callus created from cotyledon explants of tomato in the M1 and M2 after 4 weeks

The results of placement test hormone levels showed that different treatments were statistically significant differences. Regarding the effect culture medium on callusing, the results showed a significant difference between the different culture media. The best results in response to callusing were obtained in the 1M and 2M while the minimum response was obtained at 3M medium.

Table 2 combines the best medium for induction callusing

	Culture environment					
M6	**M5**	**M4**	**M3**	**M2**	**M1**	
---	**---**	**2**	**2**	**1**	**1**	**BAP**
0.5	**---**	**---**	**0.5**	**---**	**1**	**NAA**
---	**0.5**	**0.5**	**---**	**1**	**---**	**IAA**
1	**1**	**---**	**---**	**---**	**---**	**Zeatin**

The effect of different genotypes in the culture medium for the callus number was significant. Feron genotype in the M1 and M2 culture medium had 100% callusing but in the M5 and M6 culture medium had the lowest percentage of callusing. Seedlings Regeneration, use of hormone Z1I0.5 and 4.0 mg/L of thiamine was the best medium for shoot induction or direct regeneration in cherry genotype. Most of the seedlings were in the cotyledon explants. Some researchers for shoot induction used from a complex combination in this way that they used other compounds, such as Polyvinyl pyrrolidone (Pvp), Dithiothreitol (DDT) and glucose [12]. Sherry et al. used from of different concentrations of hormones Zeatin whit IAAand also Palysty et al. were used 2.5 mg levels of BAP hormone for the shoot induction [13, 14]. Some of researchers were used of Zeatin of 1 mg/l for 4 weeks to shoot elongation [15]. For Rooting, Some researchers Believes use of IAA is necessary and some have deemed it necessary hormone-free environment. In this study, we saw appropriate the use of 2 mg per liter of IBA. Tomato plant is one of the main products in high yield per hectare, especially in the greenhouse considering the short period of its growth as the bio-reactors for the production of recombinant proteins is appropriate. In this study, from Zeatin was used to improve regeneration of tomato plant that regeneration rate can be greatly increased and used from IAA as a natural auxin that had an important role in direct Regeneration of plant [16]. Thiamine (in 0.4 mg/L) was one of the main issues in increasing rate of regeneration.

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