USE OF ANTIOXIDANTS FOR CONTROL OF PHENOLIC EXUDATION IN SHOOT TIP CULTURE OF HILL BANANA (*MUSA* SPP.) CV. VIRUPAKSHI.

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Abstract

Hill Banana (Cultivar Virupakshi) is one of the highly priced Banana variety of Tamil Nadu grown in the Pulney hills of Dindigul region. Fusarium wilt disease has resulted in reduction in yield and also in the cultivable area. *In-vitro* propagation of disease free plant is a method to overcome this problem, but interrupted by the excess exudation of phenols.Phenols will darken the tissue culture medium, finally leading to death if not controlled. In the present study, Citric acid and Ascorbic acid at two different concentrations viz., 50 and 100 ppm were used.Explants were either soaked in the antioxidants or they were incorporated into the medium. Soaking of explants for 30 minutes in an anti-oxidant solution prior to their culture caused the maximum reduction in phenol content on 24th DAI from 0.112 per cent and 0.110 to 0.075 per cent and 0.044 per cent at 50 mg and 100 mg concentrations respectively.

Key words: Antioxidant,Citric acid, Phenols,Tissue culture, Virupakshi

I. INTRODUCTION

Bananas and Plantains (*Musa* spp.) forms the subsistence crop of the farmers as it provides security for food and income. Production and productivity of Indian bananas have increased over years owing to the adoption of improved production technologies like quality planting materials, high density planting system, fertigation, integrated nutrient, pest and disease management etc. But still some of the problems like Fusarium wilt, bunchy top virus, Sigatoka leaf spot and weevils are a cause of concern. Some of the elite varieties like Hill Banana, Nanjanagud Rasthali are slowly getting extinct because of Fusarium wilt. Perennial system of cultivation of these varieties has resulted in disease build up, which is responsible for the slow decrease in the yield and finally the death of the plants. Therefore it is of prime importance to rejuvenate these varieties and reintroduce them in their natural habitats and there by prevent the varieties getting extinct.

Hill Banana (Cultivar Virupakshi) is one of the highly priced Banana variety of Tamil Nadu grown in the Pulney hills of Dindigul region. It is locally called as “Malaivazhai” and is known for its fragrance, taste and medicinal values. Perennial system of cultivation of this cultivar in the hilly regions has resulted in build up of Fusarium wilt disease. This has resulted in reduction in yield and also in the cultivable area from 18000 hectares in 1980 to just 2000 hectares in 2008. Some efforts have been taken to rejuvenate this variety, but with little success. Production of quality planting material through tissue culture has gained momentum in the recent past as a strategy to conserve this cultivar. However, *in-vitro* propagation of banana is quite often interrupted by the excess exudation of phenols as reported in banana cv. Williams[1]. Even in Hill banana the major problem of micro propagation is high mortality of cultured explants due to blackening of the medium by the phenolic exudates.

Phenols are chemical compounds that include a wide range of plant compounds which posses in common, an aromatic ring bearing one or more hydroxyl constituents. Phenolic substances tend to be water soluble since they most frequently occur combined with sugar as glycosides and are usually located in the cell vacuoles. Phenols are collectively called polyphenols. These form a problem in in-vitro culture of banana explants, accompanied by the darkening of medium, an attribute of the phenolic compounds exuded from the plant tissue and accumulating in the culture medium resulting in darkening of the medium. The browning of the surface of the explants is due to the oxidation of phenolic compounds resulting in the formation of quinines which is highly reactive to the plant tissue. However, these

phenolic compounds are actively responsible for certain browning reactions and astringency of the fruit and its responsible for high mortality rate (lethal browning) in third generation of tissue culture [2].

The most reactive phenolases and hydrolases in living tissues act upon phenols resulting in the formation of hydroquinones. These hydroquinones then become cyclic/polymerized and/or oxidize proteins to form increasingly melanic compounds [3] responsible for the browning of tissues. Quinones may also be produced from phenols through the action of peroxidase enzymes, which could catalyse their oxidation in the presence of peroxide. The resultant product of the reaction between some amines and quinines causes deamination of glycine, which also results in browning [4]. Phenols and other free radicals are mainly released during the wounding process and they inhibit the activity of growth promoting enzymes. Substantially, these compounds arrest the growth of shoot tips in culture [5]. The influence of phenolic compounds on growth and development has also been reviewed by [6].

Banana Explants are susceptible to tissue browning and elimination or minimization of this process is an essential prerequisite to successful culture establishment. Therefore identification of a suitable treatment to minimize tissue browning in the explants with particular emphasis on the use of antioxidants is the main objective of this study. Hence, the present study was undertaken in a commercially important cv. Virupakshi(AAB). Sudarsano and Goldy [7]reported that frequent subcultures prevented tissue browning caused by the action of phenols. Similarly use of antioxidants along with aminoacids also prevented browning of tissues in *Musa* spp. as reported by Jarret *et al*. [8] and Mante and Tepper [9].But such frequent transfers and addition of aminoacids along with antioxidants is both time consuming and expensive procedure, which cannot be undertaken in a commercial situation. Therefore, in the present study, low cost antioxidants like ascorbic acid and citric acids were evaluated for their effect in controlling the phenolic exudation. The current study reveals the dynamics of phenolic exudates in shoot tip culture of cv.Virupakshi as influenced by the exogenous antioxidants.

II. MATERIALS AND METHODS

The present study on the effect of antioxidants in curtailing the phenolic exudation in banana was conducted in Hill banana cv.Virupakshi. Citric acid (CA) and ascorbic acid (AA) were used as antioxidants. Shoot tips (5 cm^3) of this cultivar were extracted from 2-3 months old suckers and brought to the lab in 0.1 per cent cetrimide solution. Equal proportion of Citric acid and Ascorbic acid at two different concentrations viz., 50 and 100 ppm were used. One set of explants were soaked for 30 minutes in an anti-oxidant solution prior to its culture (T_2 and T_4) while in the other set, the anti-oxidants were incorporated into the culture medium (T_1 and T_3). The shoot tips were then trimmed to a size of 2 cm^3 and washed with 0.5% detergent solution of Tween-20 and then washed well with distilled water so as to remove the detergent. Later the explants were taken to the laminar air flow chamber where they were surface sterilized for 10 min in 5 per cent sodium hypochlorite and for 5 minutes in 0.1 per cent mercuric chloride with sterile water rinsing in between. After the final rinse with the sterile water, outer layers were removed to get a cube of 1.5 cm.

The axenic explants were then initiated on MS [10] medium containing 3.0 mg BAP, 30g sucrose and 2g phytagel per liter. The cultures were incubated at $25 + 2°C$ with 70 per cent relative humidity. Artificial illumination was provided through cool white fluorescent lamps and the light intensity was maintained at 1600 lux with a photoperiod of 14/10 hr. light/dark cycle. Care was taken so that explants were trimmed approximately to a constant size of 2.0g before initiation and similarly the medium was poured at a constant volume of 15ml per culture tube. Phenol content of the explants along with the medium was estimated at regular intervals of six days i.e., 0^{th}, 6^{th}, 12^{th}, 18^{th}, 24^{th} and 30^{th} day after inoculation (DAI). The standard colorimetric method of Malik and Singh [11] was adopted for phenol estimation. The experiment was laid out in a Completely Randomized Design (CRD) with five treatments and four replications. The studies were concentrated on the initial period of one month, which is very crucial for the cessation of apical dominance leading to the production of adventitious buds.

III. RESULTS AND DISCUSSION

The interference of the phenols with morphogenesis is a well established fact and they develop browning around the explants due to their

accumulation. Phenol induced suppression of tissue growth has been well documented in Tobacco[6], arrest of vegetative growth in cashew[5] and toxic effects on cashew explants[12]. The effect of exogenous antioxidants in controlling the levels of phenolic exudates on *in-vitro* shoot tip culture of banana (*Musa* spp.) cv. Virupakshi is presented in Fig. 1.

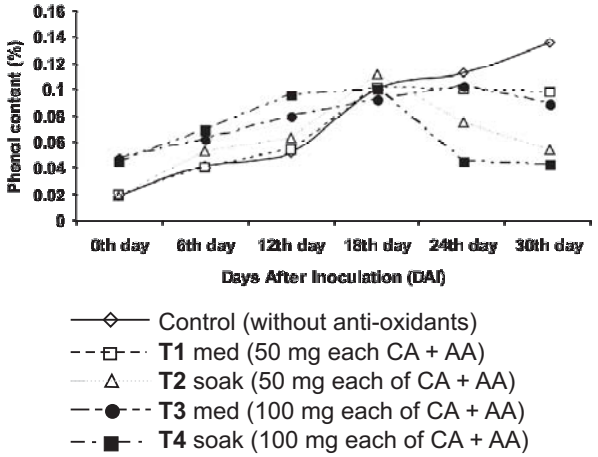

Fig. 1: Effect of exogenous antioxidants in controlling the levels of phenolic exudates during the *in-vitro* shoot tip culture of banana (*Musa* spp.) cv. Virupakshi.

Although the initial levels of phenolics were high, it declined over time. The phenol content on various Days After Inoculation (DAI) was statistically significant among the treatments except for 18 DAI. The mean values of the phenolic contents are shown in Table. 1.

In control, the phenol content increased gradually and reached the maximum of 0.136% on 30th DAI, which could be attributed to the absence of antioxidant treatments. In T_1 and T_3 where the antioxidants were incorporated into the culture medium, the phenol content increased gradually and later decreased indicating that inclusion of antioxidants in the culture medium is quite effective in controlling the phenolic exudation as reported earlier in banana [9],[13]. However, the reduction in phenol content was more pronounced (i.e., from 0.102 to 0.089%) at higher concentrations (100 mg l^{-1}) as against the lower concentrations of antioxidants (50 mg l^{-1}). This gives an indication that the effect could further be improved by increasing the concentration of antioxidants to an optimum level.

While in T_2 and T_4 where the explants were soaked for 30 minutes in an anti-oxidant solution prior to its culture, the phenol content increased gradually up to 18th DAI and then declined drastically on 24th DAI (Day After Inoculation) i.e., from 0.112 to 0.075% in T_2 and from 0.100 to 0.044% in T_4. The present results are in agreement with the findings of Amin and Jaiswal [14] in Guava. This drastic reduction in phenol content beyond 18 DAI could be attributed to the following reasons.

1. Exudation of phenols generally ceases once after shoot multiplication begins.

2. Rapid oxidation of ascorbic acid in the medium to dehydro-ascorbic acid, which would have been

Table 1: Effect of exogenous antioxidants in controlling the phenolic exudates(per cent) during *in-vitro* shoot tip culture of banana (*Musa* spp.) cv. Virupakshi

Treatments	0th day	6th day	12th day	18th day	24th day	30th day
Control (without antioxidants)	0.019	0.041	0.052	0.100	0.113	0.136
T1 - medium (50 mg each of CA + AA)	0.020	0.040	0.054	0.101	0.100	0.098
T2 - soaking (50 mg each of CA + AA)	0.018	0.053	0.063	0.112	0.075	0.055
T3 - medium (100 mg each of CA + AA)	0.048	0.062	0.079	0.093	0.102	0.089
T4 - soaking (100 mg each of CA + AA)	0.045	0.070	0.095	0.100	0.044	0.042
CD (p=0.05)	0.004	0.010	0.009	NS	0.012	0.016
CV%	9.180	12.276	8.516	10.141	8.946	12.001

absorbed by the tissues leading to the control of phenolic exudation in the later days of culture.

3. Soaking of explants in a mixture of ascorbic acid and citric acid exposes them to reducing agents that prevent blackening through the oxidation of phenols and also lowers the pH as the PPO activity is greatest only at a pH of 6.5 and above [15].

4. The conjugational properties of the phenolic compounds with sugars, amino acids and proteins.

From the above results, it is concluded that (i) the activity of ascorbic acid and citric acid in preventing the phenolic exudation was reduced when they were incorporated in the culture medium. This might be attributed to the thermo-labile nature of ascorbic acid, which would have lost its effect on autoclaving of the medium and (ii) accumulation of phenolics during *in-vitro* culture may not only depend on the strength of the medium employed, but also on the key enzymes involved in the biosynthesis of phenolics, substrate availability, exposure to oxygen and other physiological conditions [6].

IV. CONCLUSION

The effect of antioxidants in controlling the phenolic exudation was studied in the *in-vitro* shoot tip culture of banana cv. Virupakshi . Equal proportion of citric and ascorbic acids were tried at two different concentrations viz., 50 and 100 mg l^{-1} and the explants were treated with antioxidants either prior to inoculation or incorporated into the culture medium. Observations on the phenolic content were recorded at regular intervals of six days up to a period of one month after inoculation. The phenol content on various DAI was statistically significant among the treatments except for 18 DAI. Soaking of explants for 30 minutes in an anti-oxidant solution prior to their culture caused the maximum reduction in phenol content on 24^{th} DAI from 0.112 per cent and 0.110 to 0.075 per cent and 0.044 per cent at 50 mg and 100 mg concentrations respectively. In cases, where the antioxidants were incorporated into the culture medium, the reduction on 24^{th} DAI was too low. Results showed that the soaking of explants in an antioxidant mixture prior to their culture was effective at both the concentrations in controlling the phenolic exudation.

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