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IN VITRO SHOOT INDUCTION OF THREE GRAPE (*VITISVINIFERA* L.) VARIETIES USING NODAL AND AXILLARY EXPLANTS

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ABSTRACT
Nodal and axillary explants bearing two buds each from three varieties of grape (*Vitisvinifera* L.), viz., Crimson seedless, Bangalore Blue and Red Globe were micro propagated for induction of shoots on basal MS (Murashige and Skoog, 1962) medium. Nodal-bud cuttings performed well in all the three varieties: Crimson Seedless T_2V_1 (71.43), followed by Bangalore Blue T_2V_2 (27.27) and Red Globe T_2V_3 (20.00), whereas, axillary-bud cuttings of just Crimson Seedless T_1V_1 (15.29) survived, and, the other two varieties, Bangalore Blue T_1V_2 (0.00) and Red Globe T_1V_3 (0.00), failed to survive. Hence, from the present findings, it is concluded that nodal-bud cuttings from 'Crimson Seedless' grape variety are suitable for *in vitro* shoot induction on MS medium.

INTRODUCTION

Grape *(Vitisvinifera* L.) is a perennial, deciduous, woody vine cultivated in the tropical, sub-tropical and temperate regions of the world.Propagation by conventional means is restricted to a particular season and is dependent on availability of planting material.Conventional asexual propagation methods are difficult due to the long juvenility, greater time taken and plant material requirement for propagation and season-dependency (Jaiswal and Amin, 1992). Grapevine is conventionally propagated using dormant hardwood cuttings (36 to 46cm long), collected from well-developed current season's canes during the winter (Thomas, 2001). These are planted in the nursery during spring, and one season's growth gives plants for transplanting to the vineyard (Hartmann *et al*., 1997). This is a season-bound and slow process. Non-conventional propagation methods,*viz.,* plant tissue culture, has emerged as a powerful tool for propagation and improvement which is adopted as an established method for commercial propagation under limited space, time and controlled conditions throughout the year, An added advantage is the ability to produce disease-free planting material under aseptic conditions. In micro propagation of the grape,the degree of response is highly dependent on genotype, as, various *Vitis* species/ cultivars/ hybrids respond differently to specific culture conditions (Qiu *et al*., 2004). Hence, there is an acute need to develop a protocol for shoot induction in different varieties and for studying the response of different explants for micro propagation for commercial propagation,entailing limited time and space. Mhatre *et al*. (2000)used nodal-bud cuttings bearing a single axillary bud, from three cultivars *viz*., Thompson Seedless, Sonaka and Tas-e-Ganesh, to initiate shoot cultures on G16 medium containing adenine sulphate, monobasic sodium phosphate, BAP and NAA, and developed a turf of multiple shoots.*In vitro* propagation of *Vitis* offers opportunities for increasing plant material available for cultivation. Cultures were established and maintained *in vitro* on MS medium, better shooting and shoot proliferation (80%) was obtained by sub-culture of micro cuttings on MS medium supplemented with 5μM BA (Jaskani *et al*., 2008) in'Muscat of Alexandria' cv. from shoot tips and internode segments. Maximum rate of shoot proliferation was obtained on MS medium containing 3.0mg/l BAP + 0.2mg/l NAA (Abido *et al*., 2013). Therefore, the present investigation was undertaken on micro propagation of three important cultivars of *Vitisvinifera* L., viz., Crimson Seedless, Bangalore Blue and Red Globe, using axillary-bud and nodal-bud cuttings as explants, with an objective of identifying a suitable explant type and variety for *in vitro* shoot induction.

MATERIALS AND METHODS

Source of explant and types of explant

The study was carried out at ICAR-IIHR, Bangalore, during 2014 in the Division of Biotechnology. Axillary and nodal-bud cuttings bearing two buds each were collected during the rainy season (July - August) from nine- to ten-year old vines of different varieties, *viz*., Crimson Seedless, Bangalore Blue and Red Globe.

Explant preparation

Explants placed in moist paper towels in the laboratory to avoid desiccation of explants. The explants (1.0cm to 1.5cm), axillary-bud cuttings are prepared by removing all the extraneous tendrils and leaves and the nodal cuttings with two buds are prepared.

Media sterilization

Murashige and Skoog (1962) (MS) medium supplemented with 2 mg/l biotin, 1 mg/l thiamine-hydrochloride and 2 mg/ lbenzyladenine, and, added Sucrose 30gl^{-1} as carbon source, medium pH was adjusted to 5.8 and 2.5 gl^{-1}Gelrite was added as a solidifying agent in the medium; 17.5 ml of medium was poured into each test-tube (15cm length, 2.5cm dia), and the medium was sterilized in an autoclave at 121°C, 15 psi pressure for 20 min.

Surface-sterilization of the explant

As a preliminary treatment, the explants were thoroughly washed under running tap water three times to remove all dust and debris adhered to its surface and washed with detergent.

Inoculation procedures

Sterilization steps were carried out aseptically under a laminar air-flow cabinet, which was sterilized by turning on its UV light for 45 minutes and wiped down with 95% alcohol for 15 minutes. *Inoculation of explants*: Explants were treated with 'Tween-20' surfactant solution (1 to 2 drops in 100ml distilled water), followed by pre-soaking in a broad-spectrum systemic fungicide, Bavistin,@ 250 mg^{-1}for 15 min. and surface-sterilized with 0.1% $HgCl_2$for 10 min., followed by three rinses in sterile distilled-water. Explants were then inoculated onto the semisolid medium under a laminar air-flow work station, and placed for incubation in a growth room facilitated with 3000Lux light intensity under a photoperiod of 16h light/ 8h dark, and maintained at a temperature of 25 ± 2ºC.

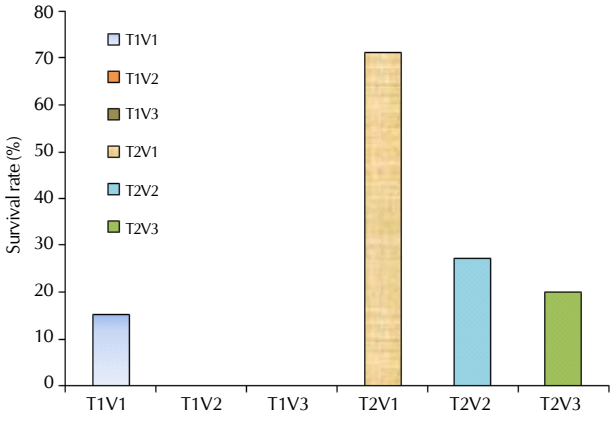

Figure 1: Percentage survival rate of three different grape varieties generated from Nodal and Axillary bud cuttings as explants

RESULTS AND DISCUSSION

Effect of variety onnodal-bud and axillary-bud cuttings cultured *in vitro*

Selection of a suitable explant is a crucial step for efficient initiation of micro propagationin grapevine and other woody species (Grenan, 1992). In the present investigation, among the explants used, nodal-bud cuttings regenerated well and showed good shoot-induction capacity (Fig 2) with appreciable results (Table 1 & Fig. 1) in all the three varieties,*viz*., Crimson Seedless T_2V_1 (71.43), Bangalore Blue T_2V_2 (27.27), and Red Globe T_2V_3 (20.00), compared to the axillary-bud cuttings (Fig 3);whereas, axillary-bud cuttings regenerated only in Crimson Seedless (Table 1 & Fig. 1): T_1V_1 (15.29) responded well, and the other two varieties [Bangalore Blue T_1V_2 (0.00) and Red Globe T_1V_3 (0.00)] failed to survive. ANOVA generated in this study showed a highly significant difference between varieties and explants with F-value of 67.12, along with co-efficient of variation 2.69 and R-square value of 0.99 (Table 2). *In vitro* regeneration of the important commercial varieties is very important for rapid multiplication of planting material round-the-year in a smaller space and shorter time, besides the benefit of aseptic, disease-free planting material production. In present study, different varieties were micro propagated using nodal and axillary-bud cuttings as explants. Here, nodal explants regenerated very well, along with good shoot-induction capacity compared to the axillary-bud cuttings,in all the three varieties studied,*viz*., Red Globe, Crimson Seedless and Bangalore Blue. This could be due to the hardy nature of the nodal explants, as, these are considered the best propagating material for *in vitro* shoot and root proliferation. This is in line with Terregrosa *et al.* (2000) where nodal-tissue culture of grapevines was used for propagating elite or scarce varieties much quicker than traditional methods. Our findings are similar to those of Banilas and Korkas (2010) who found that nodal-culture provided

Table 1: Mean survival rate of three different grape varieties from two types of explants

Sr. no.	Treatment	Treatment detail	Mean survival rate ± Std. Dev.
1	T1V1	Crimson Seedless from apical shoot	15.29(23.11 ± 0.18d)
2	T1V2	Bangalore Blue from apical shoot	0.00(0.00 ± 0.00e)
3	T1V3	Red Globe from apical shoot	0.00(0.00 ± 0.00e)
4	T2V1	Crimson Seedless from nodal shoot	71.43(57.47 ± 1.39a)
5	T2V2	Bangalore Blue from nodal shoot	27.27(31.31 ± 0.27b)
6	T2V3	Red Globe from nodal shoot	20.00(26.27 ± 0.45c)

Factor 1: Explants; T1 = Apical shoot, T2 = Nodal shoot; Factor 2: Varieties; V1 = Crimson Seedless, V2 = Bangalore Blue, V3 = Red Globe; Values in parenthesis are arc sine transformed values.

Figure 2: Nodal bud cuttings used as explants.

Figure 3: Axillary bud cuttings used as explants

Table 2:ANOVA for different varieties and nodal, axillary bud cuttings as explants.

Sr. no.	Source	F value	Pr > F
1	Explants	11333.5	< .0001
2	Varieties	3620.59	< .0001
3	Explants x Varieties	67.12	< .0001
Co eff. Var.	2.65		
R-Square	0.999		

certain advantages for rapid micro propagation of grape cv. Agiorgitiko and, potentially, can be used for other grapevine cultivars. Also, Mhatre *et al*. (2000) used nodal segments ofcvs. Thompson Seedless, Sonaka and Tas-e-Ganesh as initial explants for micro propagation, and produced three shoots each in a span of three to four weeks.

In the present investigation, among the three varieties studied,*viz*., Red Globe, Crimson Seedless and Bangalore Blue, 'Crimson Seedless' proliferated well, with good survival percentage in both nodal-bud and axillary-bud cuttings. Similar results were obtained in*in vitro* propagation of 'Crimson seedless' via axillary-bud proliferation from single-node segments; nodal segments cultured on MS basal medium showed maximum bud-break and shoot initiation in 90% and 85.7% of the explants, respectively (Nookaraju *et al*., 2010).

In the present experiment, along with the explante type and variety, 6-BAP played its own role in induction of shoots in the grape cvs. Red Globe, Crimson Seedless and Bangalore Blue, similar to the findings of Kinfe (2001).*In vitro* shoot regeneration from nodal segments cultured on semisolid medium supplemented with 5μM BAP, 15 g/l sucrose for 'Bordo' and 45 g/l sucrose for 'Chardonnay' showed better results, according to Cristina *et al* (2013). Lee and Wetzstein (1990)also reported work on nodal segments cultured on Murashige and Skoog (MS) (1962) Basal medium supplemented with BAP at different concentrations. Among these, the highest percentage of survival (rate) of cultured nodal explants was obtained on 0.5 mg/l BAP in all the three varieties of grapes studied, and maximum percentage success (96%) was obtained in'Chenin Blanc'. Cultivars UngniBlanc and Canonann on scored the same percentage of survival rate (88%), less by 8% seen in 'Chenin Blanc'. Usman *et al* (2005) found maximum number of shoots to be induced on 1.5 to 2.0mg/l of BAP, yielding 90% shoot induction in single-node-cutting explants in guava. Similarly, mulberry nodal-explants inoculated on MS medium fortified with BAP (1.0 mg/l), TDZ (0.1 mg/l) and NAA (0.25 mg/l) showed maximum regeneration of 85.67% (Sanjeevan *et al*., 2011).

Analysis of Variance (ANOVA) for different varieties in nodal and axillary-bud cuttings

ANOVA generated in this study showed a highly significant difference between varieties and the explants type, with F-value of 67.12 along with co-efficient of variation 2.69, and, with R-square value of 0.99 (Table 2).

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