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MICROPROPAGATION OF GRAPE VARIETY VITIS VINIFERA L-BANGALORE BLUE

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ABSTRACT:

Micropropagation has been a well-established methodology for in vitro regeneration of plants. *In-vitro* micropropagation of V*itis vinifera* provides opportunities for increasing plant material for cultivation. Cultures were established and maintained *in vitro* on MS medium (Murashige and Skoog) supplemented with BAP for shoot induction. Plant regeneration of V*itis vinifera* L. was achieved using axillary buds as explants. The MS medium was supplemented with 6.0 - 8.0 µM/L of BAP growth regulators showed variable responses. Formation of roots directly from internodes was seen in 1.5 µM/L of NAA with 0.1µM/L of BAP. Further it was found that the strength of half MS was a better basal medium for induction of roots from nodal segment with auxiliary.

KEYWORDS:

***VITIS VINIFERA,* MS MEDIUM, SHOOT INDUCTION, BAP: 6-BENZYLAMINOPURINE, NAA: 1-NAPHTHALENE ACETIC ACID**

INTRODUCTION

Vitis vinifera L. (Vitaceae) is a perennial, woody vine, usually climbing by tendrils. Since ancient times, the different parts of this plant have been used because of many biological activities in folk medicine. The seeds and the leaves of the grapevine are used in herbal medicine, and its fruits are put to use as a dietary additive. The leaves of plant are wealthy in tannins, flavonoids and procyanidins. Additionally, the leaves also contain organic acids, lipids, enzymes and vitamins. Its leaves are consumed in some traditional foods and used for diarrhoea, emesis and varicose treatment. Grape leaves have been used to stop bleeding, inflammation, and pain, such as the kind brought on by haemorrhoids in the conventional medicine. Grape seed and skin contain several active components, embracing flavonoids, polyphenol, anthocyanins, proanthocyanidins, procyanidines, and the stilbene derivative resveratrol. Grape seed extracts, in particular, has been reported to possess a broad span of pharmacological and therapeutic effects such as antioxidative, anti-inflammatory, and antimicrobial activities, as well as having cardioprotective, hepatoprotective, and neuroprotective effects. A traditional Chinese medicine, this plant is extensively used to relieve arthritis, eye irritation and hepatitis.

A low survival rate by stem cuttings in *Vitis vinifera* restricts its mass propagation via conventional methods. Therefore, an efficient in vitro propagation system for producing this plant is required to further clarify its potential medicinal values and germplasm conservation.

In vitro, propagation has received considerable attention in the last few years. The most important technique of micropropagation, reported by various researchers, is the meristem proliferation in which apical buds or nodal segments having an axillary bud are cultured to regenerate multiple shoots without any intervening callus phase. Thus, the present investigation was undertaken to develop an efficient protocol for *in vitro* micropropagation of *Vitis vinifera* through shoot tip culture and to introduce this species in natural habitat.

MATERIALS AND METHODS

EXPLANTS PREPARATION

This programme was carried out from date November 2006 to February 2007. Explant was collected from in Division of Hoticulture, G.K.V.K.U.A.S., Bangalore and washed thoroughly under tap water and washed with Active 80 soap water to remove the adhering particles. Then the explant was treated with fungicide (1000 ppm Bavistin) and Bactericide (100 ppm citrimide) for 25-30 minutes. It followed by successive three washing with distilled water to make the material free from disinfectant chemicals followed by treatment. Surface sterilization was carried out with 0.1% $HgCl_2$ for 10 min followed by gentle shaking. After surface sterilization, the segmented parts were thoroughly washed for several times with sterilized distilled water. Now the explant is ready for inoculation on required medium.

INOCULATION IN CULTURE MEDIUM AND CONDITION

It then explants were transferred in 100 ml glass bottle with 15 ml, basal media (MS) supplemented with different concentration of BAP(6-benzylaminopurine) 1.0, 2.0, 4.0, 6.0, and 8.0 µM/L for Micropropagation of *Vitis vinifera*. 100 ml glass bottles were incubated at 25±2 ºC under the warm fluorescent light with intensity varied from 2000-3000 lux. pH was adjusted to 5.8 prior to autoclaving. 100 ml glass bottles were incubated at

25+-with 16th photoperiod. The similar sterilization techniques were repotted by Evans et al. (1983), Pierik (1987).

ROOTING OF SHOOTS AND TRANSFER OF PLANTLETS TO SOIL

Shoots of 2-3 cm in height with two or three leaflets derived from cultures were transferred to MS medium containing different concentration of NAA + 20g sucrose or IAA or IBA or 200mg activated charcoal separately for rooting [NAA 0.0, 1.0, 2.0, 3.0 µM/L and BAP 0.1 +NAA 1.5 µM/L]. The rooted shoots were transferred to MS medium for further elongation of the roots. The rooted plants were washed with water to remove media then transferred to pots containing autoclaved vermiculite soil and sand (1:2:1), and covered with polyethylene bags for one week to maintain high humidity and subsequently exposed to low air humidity for increasing period and finally polyethylene bags were removed. These hardened plants after that transferred to the greenhouse.

RESULTS AND DISCUSSION:

The selection of suitable explants is a crucial step for efficient initiation of micropropagation of grapevine as well as other woody species (Grenan 1992).Use of larger explants is desirable as they are easier to dissect and have much higher survival and growth rate than smaller explants. In grapevine,Yu and Meredith (1986) reported that survival rate and shoot production was greater with axillary buds than shoot tips and better result found on those explants, which are grown in shade as compare to under the full sun.

Plant regeneration of *Vitis vinifera* was achieved using auxiliary buds as explants. Nodal segments from dormant shoots were cultured on MS media having BA (0, 5 or 10 µM). None of the explants showed shoot formation (Muhammad J. Jaskani, 2008). The MS (Murashige and Skoog 1962) Mediums was supplemented with BAP (1.0 to 8.0 µM/L) growth regulators showed variable responses. The percentage of initial explants responding to culture varied according to the concentration of cytokinin (Table 1). A sprouting rate of 100% was obtained for axillary-buds cultured in presence of 6.0 and 8.0 µM/L BAP. In the culture media containing BAP the number of shoots per explant increased with increasing concentrations up to 6.0 µM/L and then fell. The maximum number of axillary-buds developed per explant was about nine when 6.0-8.0 µM/L BAP.

TABLE 1: EFFECT OF DIFFERENT CONCENTRATION OF CYTOKININ ON MULTIPLE SHOOT FORMATION

MS MEDIUM + GROWTH HORMONE	SPROUTING (%)	NO OF SHOOTS/ CULTURE
MS+BAP (1.0 µM/L)	88	1.2 ± 0.4
MS+BAP (2.0 µM/L)	85	1.8 ± 0.7
MS+BAP (4.0 µM/L)	95	1.9 ± 1.1
MS+BAP (6.0 µM/L)	100	2.5 ± 1.0
MS+BAP (8.0 µM/L)	100	2.0 ± 0.4

Micro shoots were transferred on half strength MS medium supplemented with IAA, NAA or IBA for root induction. Formation of roots directly from internodes was seen in 1.5 µM/L of NAA with 0.1µM/L of BAP (table 2). Further it was found that the strength of half MS was a better basal medium for induction of roots from nodal segment with auxiliary. Root quality decreased at the highest level of NAA tested. NAA had a significant effect on root formation on half-strength MS salts. Further roots formed at the highest level of NAA tested. We also observed greater amounts of callus on explants rooted on the highest level of NAA, and we noted that the roots were brittle and broke easily when the in vitro plantlets were transferred to soil. At 0 and 1 µM/L NAA, explants formed similar numbers of roots, and these roots did not differ significantly in length between the two treatments.

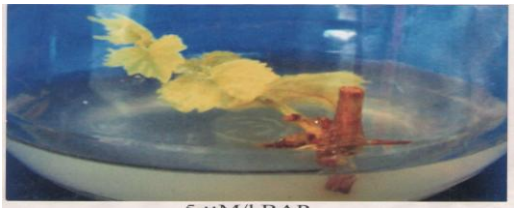
Helior *et al.* (1997) showed that IBA is a suitable auxin, while other types of auxins(e.g., NAA) may lead to callus formation. Muhammad Jaskani *et al,* (2008) reported that media having 10 µM IBA proved the best for root formation in micro shoots while in the absent of IBA shoots showed complete failure in root formation. Hicks & Dorey (1998) also reported roots at high frequency on MS plus IBA but level of IBA were different.

TABLE 2: EFFECT OF DIFFERENT CONCENTRATION OF AUXIN ((NAA)) ON MULTIPLE ROOT FORMATION

MS MEDIUM HALF STRENGTH+ GROWTH HORMONE	EXPLANT ROOTED (%)	NO OF ROOTS/ CULTURE
NAA(0.0 µM/L)	80	2.2 ± 0.5
NAA (1.0 µM/L)	85	2.3 ± 0.8
BAP(0.1) +NAA(1.5 µM/L)	100	2.9 ± 1.1
NAA(2.0µM/L)	100	2.8 ± 1.0
NAA(3.0µM/L)	100	2.5 ± 1.0

The present study describes a method for table grape (*Vitis vinifera*) propagation. Both shoot proliferation, and root induction can be accomplished using BAP and NAA respectivily. This method of clonal propagation is characterized by higher regeneration efficiency, achieved through simple in vitro manipulations.

ROOTING IN VITIS VINIFERAL



MULTIPLICATION IN V*ITIS VINIFERA*L

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