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# Proliferation, rooting and acclimatization of micropropagated grape cv. THOMPSON SEEDLESS

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**SUMMARY**

This study describes a protocol for rapid and large scale *in vitro* propagation of the valuable *Vitis vinifera* cv. THOMPSON SEEDLESS. Culture conditions influencing shoot proliferation, rooting and acclimatization were examined. The *in vitro* shoot proliferation was studied by 3 different medium (MS, WPM and B-5) with PGR concentration. In that Murashige and Skoog medium with 1.5 mg/l BAP and 0.005 mg/l IBA showed maximum rate of proliferation. The *in vitro* rooting was observed with different level of IBA and MS medium. Rooting of grape was improved in ½MS medium with 2 mg/l IBA with 200 mg/l activated charcoal. This treatment gave quick and maximum rooting compare to different concentration of IBA. For acclimation 5 medium were studied in that more than 98 % of the rooted plantlets were successfully acclimatized within 5 days in cocopeat medium.

**Key words :** Grape, Proliferation, Rooting and acclimatizaon

Grape *Vitis vinifera* is a temperate fruit crops grown successfully in different agro-climatic zones in the world. The World production of grape is presently 65.48 million MT out of which, India accounts for 1.2 million MT of grapes making a share of 1.83 per cent of the world production and 3 per cent of total fruit production in the country (Anonymous, 2006). The major production constraints are lack of quality planting material, incidence of disease and pest. Grape holds an enviable position as a stable fruit crop as well as cash crop. Conventional propagation of grape is through cutting and grafting, but success of grafting is low. Since bud grafting is tedious time consuming procedure and mass production of quality planting material is slow.

Rapid propagation of grapevine via *in vitro* culture techniques is utilized by the commercial nursery industry in the United States. It is used for rapid clonal multiplication of pathogen free or virus in dexed plants on a continuous year round basis. The multiplication rate of most of the fruit trees is slow and in some cases difficult to propagate vegetatively on large scale. Similarly, in spite of careful realization of treatments against pest and diseases, bacterial and virus infections can not be prevented totally. The answer to these problems is expected through tissue culture propagation (Micropropagation). Shoot tip, axillary bud, leaf segment etc. ( Novak and Juvova, 1982) has been employed as explants for micropropagation. Micropropagation will not only meet the required quantity of planting material but it will also be useful to increase identified elite single plant within a short period of time. The plants multiplied by micropropagation are generally disease free and may also be used for international distribution and exchange without the risk of spreading the disease or the lengthy procedures of quarantine.

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## MATERIALS AND METHODS

The present investigations on various aspects for standardization of Micropropagation in *grape* (*Vitis Vinifera*) var. Thompson seedless were carried out at the Department of Biotechnology, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari. Shoot proliferation, rooting and acclimatization of single nodal segments containing the axillary bud were removed from vigorously growing net house grape plant. Axillary buds were washed in running tap water. Surface sterilized in mercuric chloride with 0.1 % then rinsed three times in sterilize distilled water. Axillary bud establishes in 2 mg/l BAP and multiplication in 2 mg/l BAP + 0.005 mg/l IBA in MS medium.

For proliferation three different media *viz.*, MS, WPM, B-5 were tested with different level of PGR concentration. For rooting different concentration of MS medium (Full, half, ¼) with IBA in different concentration were tested. The best rooting size of shoot also studied with different length of shoot (less than 5, 5 to 10, and more than 10 to 20 mm) and observed the rooting frequency. *In vitro* raised plant having 3-4 roots and 6 leaves were taken out from bottles. The roots were

washed thoroughly in tap water to remove adhering agar. For acclimatization different medium likes Vermiculite, Cocopeat, FYM: Soil: Sand (1:1:1), Sand and Perlite were examined. *In vitro* raised plants of grape were transplanted in plastic cup containing combination of different potting mixture. For acclimatization of *in vitro* rooted plants were placed in the culture room at 26±2$^0$C temperature. The potting mixtures were drenched with 0.05 % bavistin. The plantlets were initially kept covered for 3 days and then the covering was gradually removed within 7 days. The plantlets completely uncovered were brought outside A/C room and kept for a week at room temperature for another 2 weeks. The plantlets were successfully transplanted to soil in the field.

## RESULTS AND DISCUSSION

In the present investigation it was observed that medium formulation displayed a strong effect on the growth of shoot like length of shoot, length of internodes. After multiplication shoots were placed for proliferation in different medium. Comparing the three formulations revealed that the proliferation rate of the MS medium was superior as compared to the WPM and B-5 medium. The induction of proliferation in regenerated shoot was achieved on MS medium containing 1.5 mg/l BAP + 0.005 mg/l IBA which was found to be the best proliferation medium (Table 1) (Fig.1). In this medium rapid increase of shoot length (3.55 cm) and maximum length of internodes (1.4 cm) were observed. In most of the studies of *in vitro* culture of grape, basal media derived from the MS ( Lee and Wetzstein, 1990; Goussard, 1981; 1982) have been successfully used.

**Table 1 : Effect of different mediam on shoot proliferation of grape cv. THOMPSON SEEDLESS after incubation of 4 weeks**

| Proliferation treatments | Proliferation (%) | Length of internodes per shoot (cm) | Length of shoot (cm) |
|---|---|---|---|
| MS medium | | | |
| 1.5 mg/l BAP+0.005 mg/l IBA | 75 | 1.4 | 3.55 |
| 2 mg/l BAP + 0.1 mg/l NAA | 66.65 | 0.95 | 2.45 |
| 2 mg/l BAP + 0.2 mg/l NAA | 45.25 | 0.6 | 2.30 |
| 4 mg/l BAP + 0.1 mg/l NAA | 46.5 | 0.4 | 1.10 |
| 4 mg/l BAP + 0.2 mg/l NAA | 32 | 0.2 | 1.00 |
| 10 mg/l BAP+0.1 mg/l NAA | 15 | 0.2 | 1.00 |
| 10 mg/l BAP+0.2 mg/l NAA | 10 | 0.1 | 0.00 |
| WPM medium | | | |
| 2 mg/l BAP + 0.1 mg/l NAA | 34 | 0.5 | 1.70 |
| 2 mg/l BAP + 0.2 mg/l NAA | 30.5 | 0.4 | 1.50 |
| 4 mg/l BAP + 0.1 mg/l NAA | 11 | 0.3 | 1.50 |
| 4 mg/l BAP + 0.2 mg/l NAA | 10 | 0.2 | 1.00 |
| 10 mg/l BAP+ 0.1 mg/l NAA | 10 | 0.1 | 0.00 |
| 10 mg/l BAP+ 0.2 mg/l NAA | 6.5 | 0.1 | 0.00 |
| B-5 medium | | | |
| 2 mg/l BAP + 0.1 mg/l NAA | 31 | 0.35 | 1.80 |
| 2 mg/l BAP + 0.2 mg/l NAA | 30 | 0.32 | 1.70 |
| 4 mg/l BAP + 0.1 mg/l NAA | 15 | 0.2 | 1.00 |
| 4 mg/l BAP + 0.2 mg/l NAA | 13 | 0.1 | 1.00 |
| 10 mg/l BAP+ 0.1 mg/l NAA | 10 | 0.1 | 1.00 |
| 10 mg/l BAP+ 0.2 mg/l NAA | 8 | 0.1 | 1.00 |
| S.E. ± | 1.1 | 0.03 | |
| C.D. (P=0.05) | 3.24 | 0.07 | |

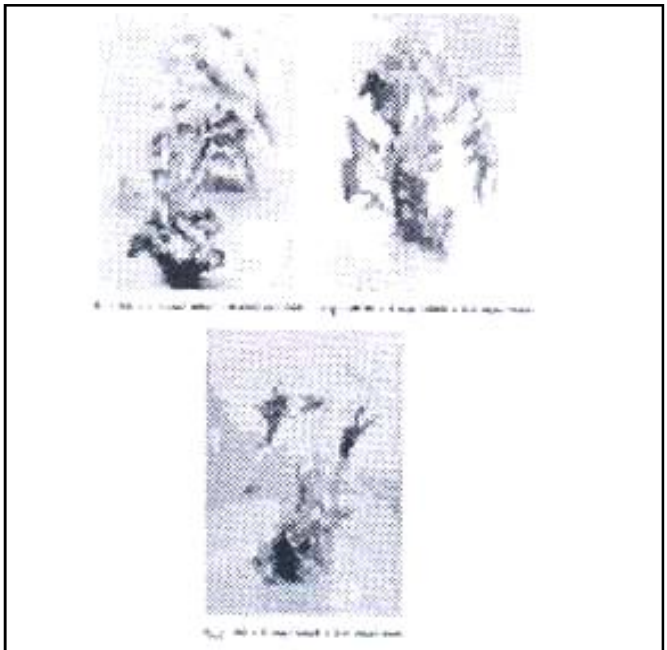

**Fig.1 : Proliferation of shoots**

Shoot obtained from culture proliferation were used for rooting studies. The individual shoots of about 2.7 cm length were transferred on rooting medium. Out of nine treatments, half strength MS medium supplemented with 2 mg/l IBA with 200 mg/l activated charcoal was found to be the most effective (Table 2) for minimum days taken for root induction (4.8), maximum length of root (7.4 cm), length of shoot (8.2 cm) and number of root/shoot (9.03) (Fig.2). Similar results were observed by Novak and Juvova (1982) and Heloir *et al*. (1997). The data on rooting response to different length of shoot are presented (Table 3). It was observed that the rooting frequency was increased with an increase in the length of shoot. Maximum rooting frequency was recorded in 10 to 20 mm length of shoot (90 per cent) (Table 3). Similar result have been reported by Lee and Wetzstein, (1990) and Singh *et al*. (2003) in grape.

*In vitro* raised plants of grape having well developed

**Table 2 : Effect of IBA and strength of medium on induction of rooting of grape cv. THOMPSON SEEDLESS after incubation of 3 weeks**

| Treatment No. | Survival of shoot (%) | Days taken for root initiation | Length of root (cm) | No. of root / shoot | Length of shoot (cm) |
|---|---|---|---|---|---|
| MS ¼ + 0.5 mg/l IBA | 70 (56.8) | 9.17 | 3.5 | 4 | 6.2 |
| MS ¼ + 1.0 mg/l IBA | 80 (63.5) | 9.03 | 4.5 | 6.13 | 7.17 |
| MS ¼ + 2.0 mg/l IBA | 75.67 (60.4) | 7.2 | 6 | 6.83 | 7.7 |
| MS ½ + 0.5 mg/l IBA | 80.33 (63.7) | 9.07 | 4.5 | 6.5 | 6 |
| MS ½ + 1.0 mg/l IBA | 74.67 (59.8) | 8 | 6.5 | 7.53 | 7.5 |
| MS ½ + 2.0 mg/l IBA | 70.33 (57.0) | 4.8 | 7.4 | 9.03 | 8.2 |
| MS + 0.5 mg/l IBA | 49.67 (44.8) | 9.5 | 4 | 1.67 | 4.7 |
| MS + 1.0 mg/l IBA | 60.33 (51.0) | 9.4 | 5.5 | 1.83 | 5.83 |
| MS + 2.0 mg/l IBA | 66.33 (54.5) | 9.3 | 6.2 | 2.6 | 5.97 |
| S.E.± | 0.89 | 0.18 | 0.12 | 0.18 | 0.12 |
| C.D. (P=0.05) | 2.64 | 0.52 | 0.35 | 0.52 | 0.36 |

Figure in paratheses are arc sine transformed value

**Fig.2 : Rooting of shoot**

**Table 3 : *In vitro* effect of the different length of shoot on rooting after incubation of 3 weeks**

| Length of shoot (mm) | Rooting frequency (%) |
|---|---|
| Less than 5 | 30 |
| 5 to 10 | 70 |
| More than 10 to 20 | 90 |

Medium - ½ MS + 2 mg/l IBA + 200 mg/l AC.

3-4 roots and 6 leaves were taken out from the culture bottles. The roots were washed thoroughly in tap water to remove the adhering agar. The rooted plantlets were then dipped in 0.05 per cent bavistin, (carbendazim 50 per cent WP) and planted in plastic pots containing different potting mixture. Maximum survival (98 %) was observed in cocopeat as compare to other potting mixture (Table 4) (Fig.3). These observations were supported by various earlier workers (Singh *et al.,* 2004; Krishna *et al.*, 2006 and Lewandowski 1991).

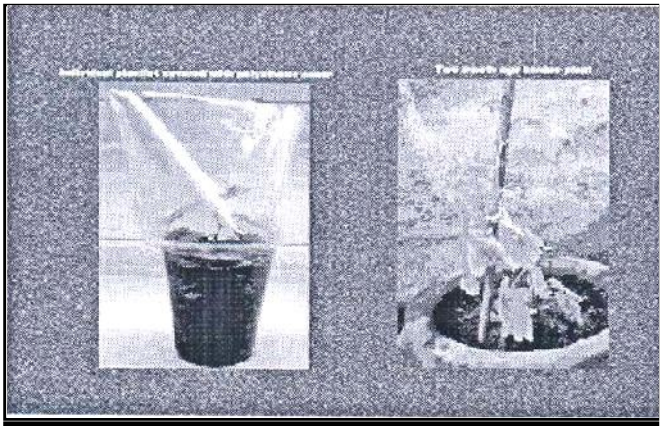

**Fig. 3 : Acclimatization of plantles**

**Table 4 : Effect of different potting mixtures on hardening of grape cv. THOMPSON SEEDLESS**

| Potting mixture | Survival of plantlets (%) | Days taken new sprouting | Length of shoot (cm) |
|---|---|---|---|
| Vermiculite | 60 (50.79) | 7 | 9 |
| Cocopeat | 98 (82.21) | 4 | 10 |
| FYM: Soil: Sand (1:1:1v/v) | 90 (71.64) | 5.5 | 9.6 |
| Sand | 30 (33.18) | 10 | 8.9 |
| Perlite | 65 (53.74) | 7 | 9.3 |
| S.E. ± | 1.66 | 0.43 | 0.16 |
| C.D. (P=0.05) | 5.01 | 1.29 | 0.50 |

Figure in paratheses are arc sine transformed value.

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