Comparative *in vitro* multiplication of some grape (*Vitis vinifera*) genotypes

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ABSTRACT

The objective of the study was to standardize the protocol for effective and mass multiplication of four wine and juice making grape *(Vitis vinifera* L.*)* genotypes, viz. Pusa Navrang, Hybrid 76-1 (Hur × Cardinal), Pearl of Csaba and Julesky Muscat. Culture initiation was undertaken on Murashige and Skoog (MS) medium supplemented with 2.0, 4.0 mg/l benzyl amino purine *(*BAP) and Kinetin individually and in combination with 0.2 mg/l naphthyl acetic acid (NAA). Explant subcultured on MS medium supplemented with 2.0, 4.0 mg/l indole-3-butyric acid (IBA) singly and in combination with 200 mg/l activated charcoal (AC). The rooted plants (30-day-old) were hardened using rooting medium consisting of coco peat + vermiculite + perlite (2:1:1). Among the different growth regulators tried for highest culture establishment (73.85%), minimum day to axillary bud sprout (9.31) and rooting (16.46%), maximum root length (6.28) with 2.0 mg/l BAP + 0.2 mg/l NAA treatment. Highest culture establishment recorded in the genotype Pusa Navrang (45.34%) followed by in Julesky Muscat (44%). Multiplication rate per 5 subcultures was recorded the maximum in Pusa Navrang (5.6) followed by Pearl of Csaba (5.1), and Hybrid 76-1 (4.9). In the present investigation, the application of MS medium containing growth regulators BAP (2 mg/l) + NAA (0.2 mg/l) and IBA (2 mg/l) + AC (200 mg/l) found best for culture initiation and rapid multiplication, respectively. For hardening using glass jar with polypropylene (PP) cap was a better strategy.

Key words: Micropropagation, Protocol, Repetitive micro-cutting, *Vitis vinifera*

Grapevines (*Vitis vinifera* L.) are traditionally propagated from hardwood stem cuttings of dormant one-year-old canes (Hartmann *et al.* 2002). However, this method is rendered slow and ineffective for rapid multiplication of newly released varieties, especially when there is enormous demand by the growers. To hasten rapid multiplication of any particular variety, plant tissue culture can be employed as an alternative tool. *In vitro* propagation is an alternative method to propagate grapevines. Numerous methods for grapevine *in vitro* propagation have been described (Chee *et al.* 1984, Gray and Fisher 1985, Harris and Stevenson 1982). *In vitro* propagation (micropropagation or tissue culture) requires specialized facilities, equipment and expertise, but can potentially yield exponentially more plants, nodal tissue culture of grapevines can propagate elite or scarce varieties much quicker than traditional methods (Torregrosa *et al.* 2001). Grapevines established from micropropagated plants may display signs of plant juvenility or excessive vigour for a few years. These characteristics

diminish until the plants are indistinguishable from traditionally propagated plants after several years (Deloire *et al.* 1995 and Mullins *et al.* 1979). Hardening or Stage-IV of micropropagation is considered most critical stage of micropropagation as it is the beginning of the biochemical and physiological processes in plants, which are necessary for survival. During hardening, the *in vitro* raised plantlets are subjected to *ex vitro* conditions and resort to develop functional root system, increased absorption of water and mineral nutrients, increased photosynthesis etc. During successful acclimatization, relative humidity levels gradually decreases from the high humidly of *in vitro* culture vessels to the lower humidity of greenhouse or field conditions (Laslo *et al.* 2010).

The goal of this work was to develop a system for improved *in vitro* plantlet quality and acclimatize plantlets. The potential benefits of this research include increased plant material available for growers at a lower cost and an increase in availability of this grape for wineries.

MATERIALS AND METHODS

The experiments were conducted at the Central Tissue Culture Laboratory, LBS Building, Indian Agricultural Research Institute (IARI), New Delhi. Newly emerged vine segments from field-grown 20-year-old mother plants of the 4 grapes genotypes, viz. Pusa Navrang, Hybrid 76-1 (Hur × Cardinal), Pearl of Csaba and Julesky Muscat were

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procured from the Main Orchard, Division of Fruits and Horticultural Technology, IARI, New Delhi. The newly sprouted, just mature vegetative shoots were collected from the field-grown healthy grape vines. Nodal segments, i.e. 4 to 5 node position from the tip and downwards were excised and the leaf primordia removed and used for the culture establishment. Murashige and Skoog (1962) MS medium was used as basal medium. The collected vine segments were washed with a solution containing 3-4 drops of liquid detergent (Teepol®). The single nodal segments were excised as explant. The explants were surface sterilized under the laminar air-hood with the 0.1 % $HgCl_2$ solution having one drop of Teepol for 7 min. Thereafter, the explants were rinsed with sterilized double-distilled water thrice, air-dried and inoculated each in a single test tube containing the sterilized culture establishment nutrient medium. Then cultures were incubated in culture room and provided with a photoperiod of 16/8 h light/dark cycle at $25 \pm 2°C$.

For culture initiation MS medium supplemented with different concentrations of growth regulators was used. Growth regulator combinations tried were 2.0, 4.0 mg/l BAP and Kinetin individually and in combination with 0.2 mg/l NAA. Thereafter, the proliferated cultures in different

genotypes were subcultured using repetitive two node microcutting techniques (Singh *et al.* 2004). Murashige and Skoog medium supplemented with different concentrations of growth regulators was employed to standardize medium for *in vitro* multiplication, shoot elongation and rooting. Auxin as 2.0 and 4.0 mg/l IBA singly and in combination with 200 mg/l activated charcoal (AC) supplemented with 30 g/l sucrose and 8 g/l agar-agar was also used.

The observation on explant survival was taken at 15, 30 and 45 days after inoculation, while data on bud sprouting was observed daily post inoculation. The rooted plants (30-day-old) were hardened using potting medium consisting of coco peat + vermiculite + perlite (2:1:1) moistened with the ¼th MS medium salt solution devoid of calcium and organics in either plastic pot covered with polythene bag or glass jar caped with polypropylene cap. Then after the 30-day-old, the rooted plantlets were shifted to glasshouse conditions and different parameters were recorded at 30 and 45 days of transfer. The experiment was laid out in completely randomized design (CRD). In general, about 25 cultures were taken for each treatment. The percentage data were subjected to angular transformation before analysis. ANOVA was calculated to partition the variance as reported by Gomez and Gomez (1984).

Table 1   Effect of different plant growth regulators on culture establishment (%)

Treatment (mg/l)	15 DAI					30 DAI					45 DAI				
	PN	H 76-1	POC	JM	Mean	PN	H 76-1	POC	JM	Mean	PN	H 76-1	POC	JM	Mean
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2.0 BAP	83.23	84.50	82.50	78.32	82.14	61.1	62.6	58.77	56.97	59.86	48.20	48.47	47.20	35.23	44.78
(T$_1$)	(65.81)	(66.80)	(65.25)	(62.23)	(65.02)	(51.39)	(52.28)	(50.03)	(48.99)	(50.67)	(43.95)	(44.1)	(43.38)	(36.40)	(41.96)
4.0 BAP	85.5	85.5	86.23	79.50	84.18	67.20	65.50	62.73	58.70	63.53	49.20	49.60	40.20	38.37	44.34
(T$_2$)	(67.60)	(67.60)	(68.20)	(63.06)	(66.61)	(55.04)	(54.01)	(52.36)	(49.99)	(52.85)	(44.52)	(44.75)	(39.33)	(38.26)	(41.72)
2.0 BAP + 0.2 NAA	96.6	84.93	92.53	91.60	91.42	83.03	84.20	83.77	82.37	83.34	78.77	76.80	74.37	75.43	76.34
(T$_3$)	(79.44)	(67.16)	(74.13)	(73.14)	(73.47)	(65.66)	(66.56)	(66.21)	(65.15)	(65.89)	(62.54)	(61.19)	(59.56)	(60.27)	(60.89)
4.0 BAP + 0.2 NAA	90.50)	82.5	88.50	84.83	86.58	75.10	73.43	72.93	70.57	73.01	61.50	62.30	65.27	62.33	62.85
(T$_4$)	(72.04)	(65.25)	(70.16)	(67.07)	(68.63)	(60.04)	(58.95)	(58.63)	(57.12)	(58.69)	(51.63)	(61.19)	(53.87)	(52.12)	(52.43)
2.0 Kinetin	80.31	72.5	68.5	56.50	69.45	55.50	55.67	56.20	40.23	51.90	44.67	37.53	38.30	39.23	39.93
(T$_5$)	(63.64)	(58.35)	(55.84)	(48.72)	(56.64)	(48.14)	(48.23)	(48.54)	(39.35)	(46.07)	(41.92)	(61.19)	(38.22)	(38.77)	(39.17)
4.0 Kinetin	85.50	79.5	85.17	68.87	79.76	60.50	58.30	50.50	45.43	53.68	37.70	31.40	33.30	45.37	36.94
(T$_6$)	(67.60)	(63.06)	(67.33)	(56.07)	(63.51)	(51.04)	(49.76)	(45.27)	(42.36)	(47.11)	(37.86)	(37.76)	(35.23)	(42.32)	(37.37)
2.0 Kinetin + 0.2 NAA	76.98	73.5	80.37	82.50	78.34	59.03	59.6	63.40	61.37	60.85	47.20	40.43	41.30	48.7	44.41
(T$_7$)	(61.45)	(59.00)	(63.68)	(65.25)	(62.34)	(50.18)	(50.52)	(52.75)	(51.55)	(51.25)	(43.38)	(34.07)	(39.97)	(44.24)	(41.76)
4.0 Kinetin + 0.2 NAA	75.33	74.60	79.50	80.31	77.44	51.20	53.87	52.30	55.40	53.10	43.27	39.37	35.30	48.30	41.5
(T$_8$)	(60.20)	(59.72)	(63.06)	(63.64)	(61.65)	(45.67)	(47.20)	(46.30)	(48.08)	(46.81)	(41.11	(39.47	(36.44	(44.01	6(40.10)
Mean	74.89	70.84	73.70	69.16		56.96	57.02	55.62	52.34		45.61	42.88	41.69	43.66	
	(59.75)	(56.32)	(58.63)	(55.46)		(47.46)	(47.50)	(46.68)	(44.73)		(40.77)	(39.14)	(38.44)	(39.6)	
CD (P=0.05)															
T					0.91					0.41					0.51
G					0.60					0.27					0.34
T × G					1.81					0.82					1.01

*Transformed data: ArcSin √%\

RESULTS AND DISCUSSION

Culture initiation

For enhancing explant survival, two cytokinins (BAP and Kinetin) and auxin (NAA) were tried in different combinations in grape genotypes. Under our experimental conditions, culture establishment was significantly higher in the genotype Pusa Navrang (45.34%) followed by in Julesky Muscat (44%) (Table 1) at 45 days of inoculation. Significantly, lower culture establishment was recorded in the Pearl of Csaba (39.96%). Among the different growth regulators tried for culture establishment, the highest (73.85%) culture establishment was noted with 2.0 mg/l BAP + 0.2 mg/l NAA treatment, followed by 4.0 mg/l BAP + 0.2 mg/l NAA (72.13%), which were significantly different. The two way interaction between the genotype and growth regulator treatments showed that Pusa Navrang had significantly higher (78.77%) culture establishment with the addition of 2.0 mg/l BAP + 0.2 mg/l NAA followed by H 76-1 (76.8%). Whereas, the lowest culture establishment was noted in Pearl of Csaba (23.30%) with 4.0 mg/l kinetin. Addition of low level of auxin (0.2 mg/l NAA) further improved (max. 76.34%) the culture establishment. With the lower concentration of kinetin (2.0 mg/l Kinetin) comparatively less percentage (min. 39.93%) of explant survival was observed (Table 1). However, cytokinin supplementation was found essential for culture initiation in these grape genotypes. The addition of lowest level concentration of NAA used affects well the initiation of grapevine *in vitro* (Vasil 1990, Tapia and Read 1998, Al-Otaibi 2007). This finding may be taken place due to balance between exogenous and endogenous plant growth regulators and role of adverse effect of cytokinins on cell elongation and the positive mode of action of NAA in this context (George *et al.* 2008, Abido *et al.* 2013). Whereas, Torrey and Reinert (1961) found that NAA is working to increase the activating enzymes that break down starch and has ability to move the active site leading to increased proliferation of organogenesis. These findings confirmed the results reported by Singh *et al.* (2004) and Khawale *et al.* (2006).

Minimum 9.31 days were taken for the axillary bud sprouting with 2.0 mg/l BAP + 0.2 mg/l NAA, followed by with 4.0 mg/l BAP + 0.2 mg/l NAA (11.17 days), which were significant with each other. Whereas, the maximum days to bud sprout (19.20) were taken in 4.0 mg/l kinetin. Days taken for axillary bud sprouting was significantly lower in Pusa Navrang (11.70) followed by H 76-1 (12.58). Pearl of Csaba took the maximum 13.84 days for axillary bud sprouting among the genotypes. The interaction between genotype and growth regulator was also significant. Duration for bud sprouting was the minimum in genotype Pusa Navrang (6.50 days) followed by Pearl of Csaba (8.40 days) with 2.0 mg/l BAP + 0.2 mg/l NAA treatment (Fig 1).

The beneficial role of BAP in culture initiation was more noticeable when it was used along with low level of NAA. Days to shoot bud sprouting and root induction were
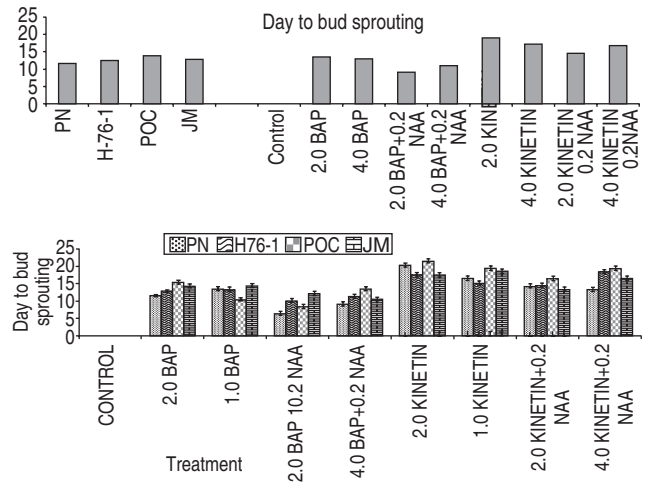

Fig 1 Effect of different plant growth regulators on day to bud sprouting

found to vary with genotype. Among the growth regulator treatments, 2.0 mg/l BAP + 0.2 mg/l NAA gave the earliest shoot sprouting (11.6 days). Earlier Mhatre *et al.* (2000) reported that NAA @ 0.09 mg/l was essential in culture initiation medium for three *V. vinifera* cultivars. Ibanez *et al.* (2005) found that the presence of BA in the culture medium was a decisive factor in axillary bud growth and elongation. In media containing 1 or 2 mg/l BA, the mean number of axillary buds and shoots developed per explants were significantly greater than in its absence. Benzyladenine (BA) has been reported to be effective in enhancing axillary bud proliferation in several *Euvitis* species (Harris and Stevenson 1982, Gray and Fisher 1985, Heloir *et al.* 1997, Dzazio *et al.* 2002, Ayman *et al.* 2011). Heloir *et al.* (1997) and Mhatre *et al.* (2000) found higher concentrations of BA growth of axillary buds were stimulated without much mortality.

Day to shoot proliferation and rooting

The genotype Pearl of Csaba taken the maximum days (23.71) for axillary shoot induction, whereas Pusa Navrang registered the minimum days (22.13) to axillary shoot induction followed by H 76-1 (22.47), which were non-significant amongst themselves (Table 2). It was clear that control registered significantly the maximum days (30.27) to shoot induction which is generally not desired in the micropropagation. The two way interaction between the treatment and genotype clearly indicated that the days required for the shoot induction were the minimum in Pusa Navrang (14.30) days, followed by H 76-1 (18) days in the treatment 2.0 mg/l IBA + 200 mg/l AC. The maximum number of days required for the shoot induction was noted in Pearl of Csaba (32.23) days with the control, which was significantly different to other genotypes. Among the growth regulator treatments, the days required for rooting was significantly minimum (16.46) in the treatment 2.0 mg/l IBA + 200 mg/l AC, followed by 4.0 mg/l IBA + 200 mg/l AC (19.06), which were significant with each other. Among

Table 2   Effect of IBA and AC on day to shoot and root sprouting

Treatment(mg/l)	Day to shoot sprouting					Day to root initiation				
	PN	H 76-1	POC	JM	Mean	PN	H 76-1	POC	JM	Mean
Control (T_0)	31.00	29.37	32.23	28.47	30.27	24.33	21.50	25.47	25.37	24.17
2.0 IBA (T_1)	24.27	22.20	22.37	23.30	23.03	20.37	20.80	21.13	21.17	20.87
4.0 IBA (T_2)	20.40	21.40	24.37	23.07	22.31	19.40	19.17	20.03	22.23	20.21
2.0 IBA + 200 AC (T_3)	14.30	18.00	18.30	21.03	17.91	13.40	14.77	17.20	20.47	16.46
4.0 IBA + 200 AC (T_4)	20.67	21.37	21.30	22.43	21.44	18.52	17.83	20.47	19.40	19.06
Mean	22.13	22.47	23.71	23.66		19.20	18.81	20.86	21.73	
CD (P=0.05)										
GR (A)					0.79					0.72
Genotype (G)					0.71					0.64
A × G					1.58					1.44

the interaction between the treatment and genotype, the root induction was earliest (13.40 days) in Pusa Navrang in the treatment 2.0 mg/l IBA + 200 mg/l AC, followed by in H 76-1 (14.77 days). The maximum number of days was required for root induction in Pearl of Csaba (25.47) in control. It was evident from the two way interaction analysis between treatment and genotype that the days taken for the root induction were minimum in Pusa Navrang, which required the minimum (13.40 days) for root initiation in the treatment 2.0 mg/l IBA + 200 mg/l AC.

The MS medium supplemented with IBA (2 mg/l) and AC (200 mg/l) gave the best shoot and root proliferation results. The addition of IBA has been found essential for *in vitro* rooting since the absence of auxin delayed rooting also it play several different steps on the proposed sequences reactions. Auxins concentration (IBA) affected the roots initiated at given sucrose concentration. In grape, the addition of low-level of auxin has been proposed earlier for *in vitro* rooting (Torregrosa and Bouquet 1995, Mhatre *et al.* 2000). Furthermore, addition of activated charcoal was not only essential for enhancing the rooting frequency, but also for improving overall root quality, important for *ex vitro* survival

of tissue culture raised plantlets. Whereas sucrose is needed for root elongation (Chee *et al.* 1984).

Effect of different plant growth regulators on in vitro shoot proliferation (%) and rooting (%)

Table 3 and Fig 3 indicate that the shoot induction (66.49%) and rooting (65.73%) was minimum in Pusa Navrang. The significantly highest shoot induction was noted in the Julesky Muscat (72.53%), whereas, the maximum rooting was noted in the Pearl of Csaba (71.01%). The growth regulators supplemented to the MS medium significantly influenced the response. The highest shoot induction (94.38%) and rooting (95.73%) were noted in 2.0 mg/l IBA + 200 mg/l AC. In control, the lowest shoot induction (25.34%) and rooting were (19.64%) noted.

Among the interaction between the growth regulator and genotypes, the highest shoot induction was in the H 76-1 (97.97%) followed by Julesky Muscat (95.20%), which were significant among themselves in the treatment 2.0 mg/l IBA + 200 mg/l AC. Significantly, the highest rooting was noted in the Pearl of Csaba (98.67%) followed by Julesky Muscat (97.47%). In the present investigation,
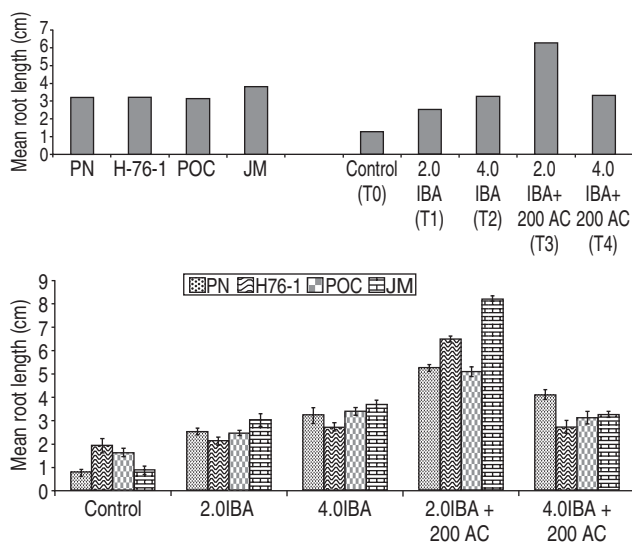

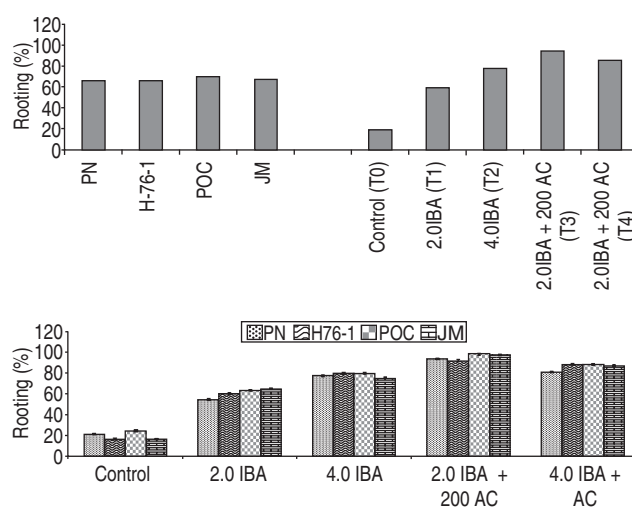Fig 2 Effect of IBA and AC on mean root length (cm)

Fig 3 Effect of IBA and AC on rooting (%)

Table 3 Effect of IBA and AC on *in vitro* shoot sprouting (%) and mean shoot length (cm) in grape genotypes

Treatment(mg/l)	Shoot sprouting (%)					Mean shoot length (cm)				
	PN	H 76-1	POC	JM	Mean	PN	H 76-1	POC	JM	Mean
Control (T$_0$)	20.43 (26.86)	25.03 (30.01)	25.43 (30.27)	30.47 (33.49)	25.34 (30.16)	2.43±0.6	3.37±0.32	3.07±0.58	4.10±0.58	3.24
2.0 IBA (T$_1$)	62.00 (51.92)	70.07 (56.81)	69.37 (56.37)	76.10 (60.72)	69.38 (56.46)	6.37±0.83	7.03±0.94	5.80±0.58	6.07±1.22	6.17
4.0 IBA (T$_2$)	80.27 (76.54)	81.47 (81.79)	74.13 (71.29)	82.43 (77.32)	79.58 (76.73)	6.67 ±0.25	5.80±0.19	6.73±0.30	7.57±0.25	6.69
2.0 IBA + 200 AC (T$_3$)	94.60 (63.61)	97.97 (64.48)	89.73 (59.41)	95.20 (65.20)	94.38 (63.18)	11.13±0.79	7.17±0.55	12.15±0.88	14.43±0.52	12
4.0 IBA + 200 AC (T$_4$)	75.15 (60.08	79.53 (63.08)	88.5 (70.16)	78.43 (62.31)	80.40 (63.91)	5.77±0.88	6.73±0.61	8.03±0.88	7.23±0.64	7.2
Mean	66.49 (55.80	70.81 (59.23)	69.43 (57.50	72.53 (59.81)		6.47	6.73	7.16	7.88	
CD (P=0.05)										
Treatment (T)					0.67					0.33
Genotype (G)					0.6					0.29
T × G					1.34					0.66

*Transformed data: ArcSin √%. PN- Pusa Navrang, H-76-1- Hybrid 76-1, POC- Pearl of Csaba and JM- Julesky Muscat.

MS medium supplemented with a lower concentration of IBA with activated charcoal was found to give the shoot sprouting as well as root sprouting percentage. With the lower concentration of IBA, i.e. 2.0 mg/l without AC comparatively less percentage of shoot and root sprouting were observed.

Helior *et al.* (1997) have reported that IBA serves as a suitable auxin for *in vitro* rooting of *V. vinifera* cv. Pinot noir and the addition of NAA does not give rise to more roots, but leads to callus formation. With NAA, the number of roots was higher and the roots were shorter compared to media containing IBA at the same concentration. Jaskani *et al.* (2008) also found higher concentration of IBA best for root formation in micro shoots MS medium without IBA showed complete failure in root formation. In *V. vinifera* cv. Perlette up to 95% rooting of micro-cuttings were obtained on MS medium supplemented with IBA and NAA (Butiuc-keul *et al.* 2009).

Effect of different plant growth regulators on mean shoot length and root length (cm)

In the interaction between the growth regulator treatment and genotype for the mean shoot length was recorded the maximum in Julesky Muscat (14.43 cm) followed by Pearl of Csaba (12.15 cm), which were significant with each other (Table 3 and Fig 2). Among the treatments, the maximum shoot lengthS (12.0 cm) were reported in the treatment 2.0 mg/l IBA + 200 mg/l AC followed by in 4.0 mg/l IBA + 200 mg/l AC (1.8). Whereas, in the control (no growth regulators), the minimum (3.24) of shoot length was recorded. Mean shoot lengths were significantly higher in Julesky Muscat (7.88) which was significantly different with each other. However, the genotypes, H 76-1 (6.73) and Pusa Navrang

(6.47) were at par with each other. Among the different treatments, the maximum root length was registered in the treatment 2.0 mg/l IBA + 200 mg/l AC (6.28) followed by 4.0 mg/l IBA + 200 mg/l AC (3.32), which were significant with each other. The control recorded significantly the lowest root length (1.30 cm). In the interaction between the growth regulator treatment and genotype for the root length was recorded the significantly maximum in Julesky Muscat (8.23 cm) with the treatment 2.0 mg/l IBA + 200 mg/l AC. The control recorded significantly the lowest root length in all the genotypes.

Among the genotype the mean root length significantly highest in Julesky Muscat (3.82 cm), followed by H 76-1 (3.21 cm) which were non-significantly different with each other. While minimum root length was reported in Pearl of Csaba (3.15 cm).

The addition of IBA has been found essential for *in vitro* rooting since the absence of IBA gives late rooting which was also poorly developed. Auxins concentration (NAA) affected the numbers of roots initiated at given sucrose concentrations as well helps to increase in shoot length. While IBA increases the length of roots (Chee *et al.* 1984, Singh *et al.* 2004, Khawale *et al.* 2006 and Jaskani *et al.* 2008).

Effect of different plant growth regulators on multiplication rate per 5 subcultures

The multiplication rate was recorded the maximum in Pusa Navrang (5.6) followed by Pearl of Csaba (5.1), and H 76-1 (4.9) of which Pearl of Csaba and H 76-1 were non-significant with each other but significantly differ to Pusa Navrang (Fig 4). Among the treatments, the maximum multiplication rate (8.5) was reported in the treatment 2.0

Table 4   Effect of different hardening strategies on plantlet survival and time taken to glasshouse transfer

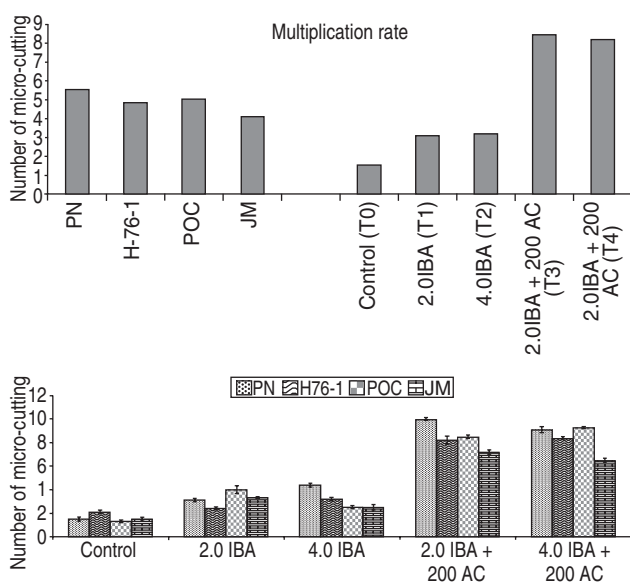| Treatment	Plantlet survival (%)					Days taken to *ex vitro* transfer				
	PN	H 76-1	POC	JM	Mean	PN	H 76-1	POC	JM	Mean
Glass jar with PP Cap (T$_1$)	82.03±0.32 (64.90)	83.60±0.25 (66.09)	89.33±0.22 (70.91)	88.90±0.12 (70.51)	85.97 (68.10)	22.87± 0.12	24.10± 0.35	22.67± 0.30	24.60± 0.29	23.56
Plastic pots with polythene cover (T$_2$)	69.40±0.31 (56.39)	62.93±0.43 (52.48)	61.37±0.55 (51.55)	60.13±0.17 (50.83)	63.46 (52.81)	28.07± 0.22	28.87± 0.12	27.77± 0.20	27.00± 0.25	27.93
Mean	75.72 (60.65)	73.27 (59.28)	75.35 (61.23)	74.52 (60.67)		25.47	26.48	25.22	25.80	
CD (P=0.05)										
Treatment (T)					0.32					0.37
Genotype (G)					0.45					0.52
T×G					0.64					0.73

*Transformed data: ArcSin √%

Fig 4   Effect of IBA and AC on multiplication rate in grape genotypes

mg/l IBA + 200 mg/l AC followed by in 4.0 mg/l IBA + 200 mg/l AC (8.3), which were both non significantly differ with each other. However, in the control (no growth regulators), the minimum (1.6) cultures after 5 subcultures proliferated. In the interaction between the growth regulator treatment and genotype for the multiplication rate per 5 subcultures showed that the maximum values in Pusa Navrang (10.0) with the treatment 2.0 mg/l IBA + 200 mg/l AC. The control recorded significantly the least multiplication rate in all the genotypes. Earlier Chee *et al.* (1984) reported inclusion of salts in medium increases the multiplication rate. Unlike the results of Mhatre *et al.* (2000) who reported *in vitro* multiplication protocol for three different grape genotypes but the utility of the protocol was not proven when tested. Singh *et al.* (2004) reported the multiplication potential and estimated in the two newly released genotypes using two-node repetitive micro-cutting technique, which ranged from 85000 to 119000 plantlets per annum.

In vitro hardening

Effect of different hardening strategies plantlet survival (%)

The plantlets in the sterilized coco peat + vermiculite + perlite (2:1:1) (T$_1$) in the glass jar were found to be the effective means of *in vitro* plantlets hardening which gave the highest survival (85.97%) (Table 4). Plastic pot with the coco peat + vermiculite + perlite (2:1:1) (T$_2$) were found non-significant since they required longer duration for acclimatization (27.93) and give minimum (63.46%) survival rate. The transparent polypropylene cap was found most effective as light reached the plantlets under the jar. Furthermore, the plantlets under the jar had higher CO_2 concentration along with higher humidity, improving the growth and recovery of the plantlet. These results are in conformity with the earlier results reported by Chee and Pool (1982) and Alizadeh *et al.* (2010).

In the present investigation, the application of MS medium containing growth regulators BAP (2 mg/l) + NAA (0.2 mg/l) and IBA (2 mg/l) + AC (200 mg/l) found best for culture initiation and rapid multiplication, respectively. For hareding using glass jar with polypropylene (PP) cap was a better strategy. Tissue culture prooved an important and advantageous technique with potential application for the production of homogeneous, true-to-the-type, virus-free planting material. Also the variability observed in *in vitro* performance of the four grape cultivars indicates that the efficiency of *in vitro* multiplication technique is much genotype dependent, allowing the selection of genotypes with high performance.

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