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Comparative *in vitro* propagation of stress tolerant grape (*Vitis* spp.) rootstocks and assessment of clonal fidelity of plantlets

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ABSTACT

Micropropagation in nine *Vitis* rootstocks using nodal segments was studied. Optimum *in vitro* culture establishment was highest on Murashige and Skoog (1962) medium (MS) with 3.0 mgl^{-1} BAP + 0.25 mgl^{-1} NAA. Rootstock 110 R gave the earliest bud sprouting (4.03 days), whereas, 1616 C and 110 R gave the highest culture establishment (71.11 & 69.67%). The shoot proliferation was most efficient on MS medium + 4.0 mgl^{-1} IBA + 0.5 mgl^{-1} BAP. Dogridge showed the maximum multiplication rate/ sub-culture (10.07), while 1613 had the minimum (5.07). Half-strength MS medium supplemented with 4.0 mgl^{-1} IBA gave good rooting parameters, while half-strength MS medium with 1.5 mgl^{-1} IBA + 1.5 mgl^{-1} NAA induced more number of roots. Dogridge and Salt Creek had the higher rooting (77.58 & 74.24%) compared to other genotypes. High *ex vitro* plantlet survival (82.75%) was noted in 1103 P in glass jars, while 1616 C plantlets took the shortest time (44.40 days) for transfer to glasshouse. Application of two marker (RAPD & ISSR) systems further confirmed the genetic stability of micropropagated plantlets. Based on the overall performance of rootstocks for *in vitro* multiplication they could be ranged as Dogridge > Salt Creek > *V. parviflora* > St. George >1616C > 1103P > 140Ru > 110R > 1613C.

Key words: Clonal fidelity, comparative multiplication, grape rootstocks, *in vitro* propagation.

INTRODUCTION

Grapevine is one of the most important fruit crops grown in India occupying an area of 1,10,000 ha with production of 1.7 MT (NHB, 11). With the erratic weather patterns and extreme abiotic stress conditions have led to reduction in productivity. The use of biotic and abiotic stress tolerant rootstocks offers a feasible option for sustainable grape production. Dogridge is considered as one of the most important rootstock adopted commercially for establishing vineyards in western India. Over dependence on this rootstock necessitated the growers to adopt other rootstocks to combat multiple edaphic problems.

Grape rootstocks are propagated by cuttings, which are slow, labour intensive and largely influenced by weather and edaphic factors. Micropropagation is an alternative method that produces genetically identical, physiologically uniform and pathogen-free planting material. Successful *in vitro* clonal propagation methods have been reported in various *Vitis* sp. and genotypes (Zhang *et al.*, 15; Alizadeh *et al.*, 2). Though success in *in vitro* propagation has been reported earlier, however, it largely dependent upon the interaction between genotype, explant source and culture medium that necessitate developing specific regeneration protocols for individual genotype (Kurmi *et al.*, 8). Further, it is important to check the genetic stability of *in vitro* regenerated plantlets. Several molecular markers were used to check the clonal fidelity in many perennial crops such as grape rootstocks (Alizadeh and Singh, 2), apple rootstocks (Harshita and Vibha, 5). Hence, in the present study, we examined the *in vitro* multiplication behaviour of nine *Vitis* rootstocks and also checked the clonal fidelity of hardened plantlets.

MATERIALS AND METHODS

Experiment was conducted at the Central Tissue Culture Laboratory, Division of Fruits and Horticultural Technology, LBS Centre, ICAR-IARI, New Delhi during 2013-2015. Nine grape rootstocks, *viz.*, Dogridge, Salt Creek, 110 Ritcher, 1103 Paulsen, 1616 Couderc, 1613 Couderc, 140 Ruggeri, St. George and *Vitis parviflora* were selected for the present study. The protocol for initiating aseptic cultures developed by Alizadeh *et al.* (3) was followed. The nodal segments were inoculated individually in test tubes on solid Murashige and Skoog (10) medium supplemented with benzyl-aminopurine (BAP) either singly or in combination with low concentration of NAA (α-naphthalene acetic acid) and then incubated at 25 ± 1°C with 16/8 h light and dark photoperiod.

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The data on growth parameters, *viz.*, days taken for bud sprouting and culture establishment were recorded. After four weeks of culture establishment, two-node micro-shoot cuttings were excised and cultured on shoot proliferation-cum rooting medium comprising full-strength MS medium supplemented with IBA (indole-3-butyric acid) or NAA alone or combinations. Multiplication parameters, *i.e.*, shoot length (cm), number of shoots explant^{-1}, number of micro-cuttings explant^{-1}, multiplication rate, multiplication cycle, number of roots explant^{-1} and root length (cm), were determined upon each sub-culture. The rooted plantlets were hardened in the glass jars with polypropylene (PP) caps or plastic pots with polythene cover containing sterilized hardening medium (peat: vermiculite: perlite; 2:1:1) moistened with half-strength MS inorganic salts. Hardened plantlets were transferred to plastic pots filled with sterilized sand, soil and FYM (farm yard manure) (2:1:1) in a glasshouse during 6-8th week of hardening depending up on the genotype. The observations on plantlet survival and days taken to *ex vitro* transfer were recorded.

For clonal fidelity analysis, total DNA was extracted from young leaves of plantlets of mother vines and randomly selected tissue cultured plantlets using acetyl trimethyl ammonium bromide (CTAB) method (Simon *et al.*, 14). The samples were diluted to a concentration of 50 ng l$^{-1.}$ A total of 20 primers each (Macrogen®, USA) were used for RAPD and ISSR analyses out of which 10 were selected based on reproducibility of the bands. The PCR reactions were carried out with 20 µl reaction mix. The PCR amplifications were performed by using following thermal profile for each marker. Amplification was confirmed and alleles were separated by running on 1.5% agrarose gel and electrophoresed in 1.0X TAE at 120 volts for 2 h for both RAPD and ISSR analyses. In the present study, 45 samples were analyzed using RAPD and ISSR primers each set including one mother plant (raised in germplasm block) of individual along with four randomly selected in *vitro* raised plantlets. The data was analysed using SAS Ver 9.3 and the mean differences were separated using Duncan's Multiple Range Test (DMRT). Cluster analysis was carried out using the SHAN module (NTSYS pc 2.02) software package (Rohlf, 12). An unweighted pair group method of arithmetic mean (UPGMA) dendrogram was generated from Jaccard's similarity values individually for RAPD and ISSR markers.

RESULTS AND DISCUSSION

The comparative *in vitro* multiplication of nine grape rootstock genotypes was found to be strongly influenced by genotype and concentration of the growth regulators used (Table 1). Significant differences were observed in the treatment combinations of BAP and NAA (3.0 mgl^{-1} BAP + 0.25 mgl^{-1} NAA and 4.0 mgl^{-1} BAP + 0.25 mgl^{-1} NAA), which showed better response than BAP alone for time taken to initial bud sprout (5.78 and 5.77 days) and culture establishment (71.50 and 67.70%). The interaction effect of growth regulators enhanced culture establishment and minimized the time to bud sprouting in grapevines (Alizadeh *et al.*, 3; Abido *et al.*, 1; Itoo *et al.*, 6), which corroborated our findings. This difference might be due to the varying balance between endogenous and exogenous plant growth regulators. All the rootstock genotypes exhibited statistically significant variation for time taken for initial bud sprout and culture establishment. Rootstock 110 R took the minimum time (4.94 days), while it was more delayed in Dogridge (8.58 days) and *V. parviflora* (8.34 days). The rootstock 1616 C showed the highest culture establishment (77.11%), while it was lowest (54.44%) in Dogridge. These results are in tune with the findings of Alizadeh *et al.* (3) and Kurmi *et al.* (8), who also suggested that the level and combination of plant growth regulator(s) effective for a particular genotype may not be effective for another genotype or species.

It was clearly noticed from Table 2 that the longest shoots (10.61 cm) were recorded in MS medium supplemented with 4.0 mgl^{-1} IBA + 0.5 mgl^{-1} BAP, which also resulted in production of significantly higher shoots (1.62) compared to PGR-free control (0.04) (Fig. 2B & 2C. 1). Though the application of IBA alone induced shoot proliferation in grape rootstocks, the addition of BAP in the culture media enhanced the shoot growth and also increased the number of shoots explant^{-1}. The highest shoot length was recorded in *Vitis parviflora* (8.92 cm) and Dogridge (8.52 cm), while it was lowest in 1613C (5.61 cm). These findings were confirmed with the earlier results of Mukherjee *et al*. (9), who also found that addition of BAP in MS medium gave better shoot proliferation in rootstock DeGrassette and higher average number of proliferated shoots explant^{-1} in grape rootstocks (El-Agamy *et al.*, 4). The addition of auxin increased the enzyme activity that could breakdown starch and thus increased shoot proliferation of organogenesis.

The micro-cutting multiplication rate and multiplication cycle were monitored upto eight successive sub-cultures in the established cultures. Irrespective of the genotype, lower multiplication rate was observed till 3rd sub-culture, which thereafter gradually increased (Fig. 1A). In general, the multiplication rate in term of number of micro-cuttings explant^{-1} increased with the increase in number

Table 1. Effect of different plant growth regulators on time to shoot bud sprout and culture establishment in grape rootstock genotypes.

Treatment (mgl^{-1})	Days to bud sprouting						Mean	Culture establishment (%)						Mean
Genotype	2.0 BAP	3.0 BAP	4.0 BAP	2.0 BAP + 0.25 NAA	3.0 BAP+ 0.25 NAA	4.0 BAP+ 0.25 NAA		2.0 BAP	3.0 BAP	4.0 BAP	2.0 BAP + 0.25 NAA	3.0 BAP + 0.25 NAA	4.0 BAP + 0.25 NAA	
Dogridge	9.76	8.80	8.83	8.37	7.73	7.97	8.58[a]	46.67 (43.04)*	48.90 (44.37)	53.33 (46.89)	62.22 (52.14)	62.22 (52.14)	53.34 (46.92)	54.44[e] (47.58)
Salt Creek	8.23	7.10	6.67	6.40	5.90	5.53	6.64[c]	55.56 (48.23)	56.90 (48.97)	57.78 (49.55)	61.34 (51.60)	68.90 (56.16)	66.67 (54.83)	61.34[cd] (51.56)
110 Richter	5.90	4.87	5.23	5.20	4.43	4.03	4.94[f]	53.34 (47.75)	66.67 (54.83)	73.34 (59.15)	66.67 (55.37)	75.57 (60.45)	77.78 (61.94)	69.67[ab] (56.56)
1103 Paulsen	5.63	5.83	5.53	4.93	4.70	4.93	5.26[ef]	55.56 (48.18)	62.23 (52.20)	63.34 (52.86)	64.45 (53.70)	66.67 (55.17)	73.34 (59.05)	64.77[bc] (53.55)
1616 Couderc	6.20	5.53	5.00	4.97	4.60	4.53	5.14[ef]	57.78 (49.51)	66.67 (54.83)	75.56 (60.45)	71.11 (57.55)	80.00 (63.44)	73.33 (59.05)	71.11[a] (57.48)
1613 Couderc	8.63	8.03	7.30	7.10	6.80	6.90	7.46[b]	55.55 (48.22)	64.44 (53.44)	71.11 (57.55)	68.90 (56.16)	75.56 (60.45)	67.78 (55.49)	62.50[abc] (55.24)
140 Ruggeri	5.93	6.17	6.00	5.33	5.00	5.07	5.58[e]	56.89 (48.97)	65.56 (54.35)	67.00 (55.03)	71.34 (57.69)	73.55 (59.19)	72.22 (58.39)	68.10[ab] (55.61)
St. George	7.43	6.33	6.17	5.87	5.53	5.03	6.06[d]	61.12 (51.51)	58.34 (50.35)	66.67 (54.85)	61.11 (51.94)	75.00 (60.21)	63.88 (53.19)	64.35[bc] (53.67)
V. parviflora	9.76	8.90	8.00	8.13	7.33	7.90	8.34[a]	50.00 (45.00)	50.00 (45.08)	55.67 (48.26)	60.00 (50.81)	62.22 (52.14)	57.89 (49.62)	56.70[de] (48.45)
Mean	7.50[a]	6.84[b]	6.53[cb]	6.26[c]	5.78[d]	5.77[d]		54.90[d] (47.81)	60.33[c] (50.94)	65.20[b] (53.85)	66.20[b] (54.45)	71.50[a] (57.73)	67.70[ab] (55.37)	
LSD ($p \leq 0.05$)														
Treatment (T)							0.35							2.77
Genotype (G)							0.42							3.40
T × G							0.82							5.10

*Mean values of multiplication parameters within each column followed by the same letter(s) are not significantly different according to the Duncan's multiple range test ($p \leq 0.05$); Data in parentheses are transformed values

of sub-cultures, which was completely genotype dependent. The mean number of two-node micro-cuttings sub-culture^{-1} ranged from minimum of 5.07 to maximum of 10.7 in the selected genotypes. Irrespective of the PGR treatments, Dogridge recorded the maximum mean number of micro-cuttings explant^{-1} (10.07) followed by Salt Creek (8.67) and *V. parviflora* (8.16), while 1613C recorded the minimum (5.07). The multiplication cycle ranged from earliest (45 days) to most delayed (57 days) for different rootstocks. The rootstocks 1103 P and 110 R had shorter multiplication cycle (45.86 and 46.20 days), whereas it was most delayed in *V. parviflora* (57.02 days) followed by 1613 C (56.70 days).

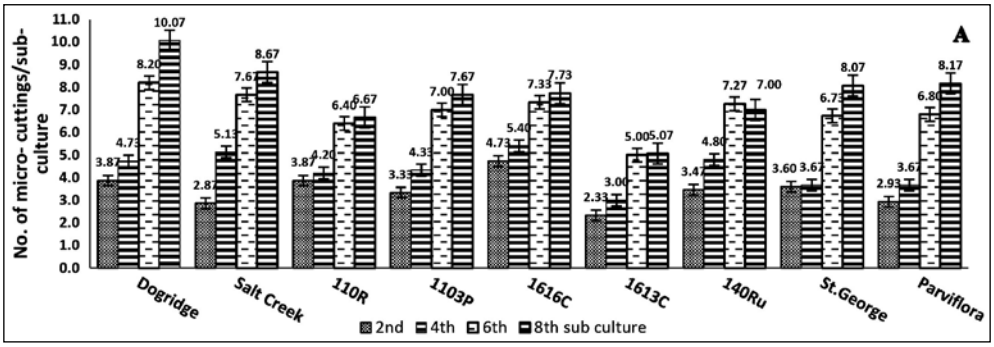
Rootstocks Dogridge, Salt Creek and *V. parviflora* were more responsive with regard to shoot length, number of shoots explant^{-1} and multiplication rate under *in vitro* conditions. Earlier also it was proposed that *in vitro* shoot proliferation of grape rootstock genotypes is largely due to the interaction of cytokinin and genotype (Alizadeh *et al.*, 3; El-Agamy *et al.*, 4).

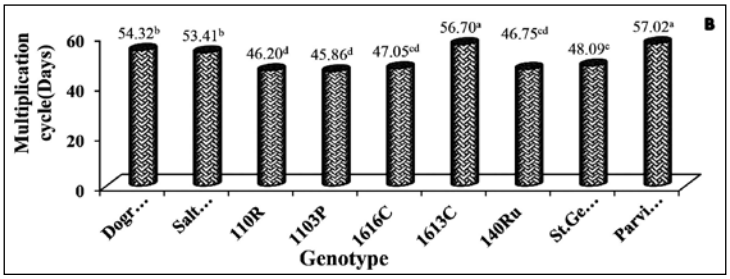
Rooting of micro-shoots in grape rootstocks was comparatively better in the medium supplemented with IBA alone (2.0 or 4.0 mgl^{-1}) or in combination with BAP (0.5 mgl^{-1}), while low success and delayed rooting was observed in control (MS without PGRs) (Table 2). The media supplemented with higher concentrations of IBA and NAA recorded more number of roots but it also led to callus formation (Fig. 2C.2). It was clearly observed from the data and Fig. 2B that maximum root length (9.57 cm) and rooting (84.10%) were recorded in MS medium + 4.0 mgl^{-1} IBA but maximum number of roots per shootlet (10.6) was noticed on MS medium + 1.5 mgl^{-1} IBA + NAA (1.5 mgl^{-1}) + BAP (1.0

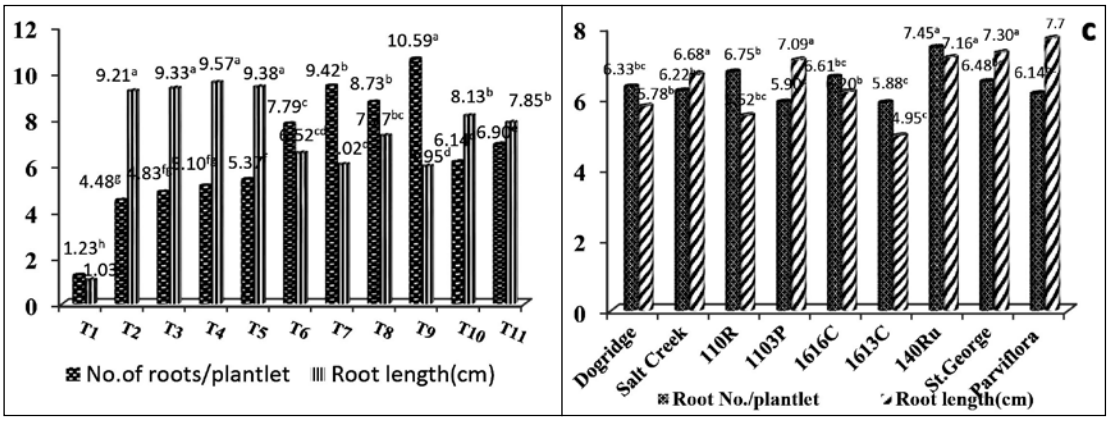
Table 2. Effect of different plant growth regulators on shoot length (cm) and rooting (%) in grape rootstock genotypes.

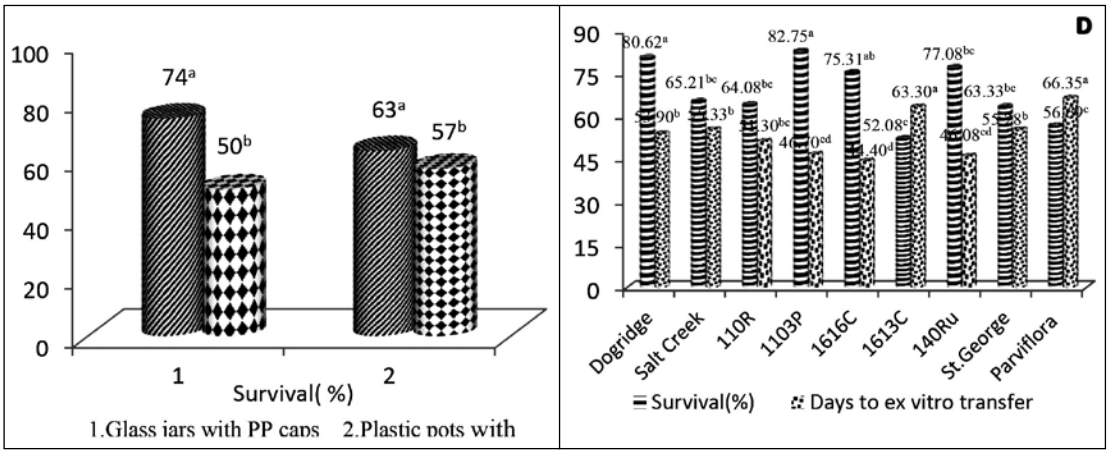
Treatment (mgl^{-1})	Shoot length (cm)									Mean	Rooting* (%)									Mean
Genotype	Dogridge	Salt Creek	110 R	1103 P	1616 C	1613 C	140 Ru	St. George	*V. parviflora*		Dogridge	Salt Creek	110 R	1103 P	1616 C	1613 C	140 Ru	St . George	*V. parviflora*	
0.0 (control)	3.77	3.84	3.03	2.28	1.80	2.13	2.43	3.07	2.37	2.74^{d}	23.34	26.67	20.00	20.00	30.00	20.00	20.00	23.34	23.34	22.97f
2.0 IBA	9.65	8.56	6.82	13.47	8.16	6.95	12.01	11.16	12.68	9.94^{a}	93.34	93.34	80.00	90.00	90.00	73.34	83.34	83.34	70.00	84.07^{a}
4.0 IBA	9.89	9.54	7.61	11.63	9.52	7.15	11.07	11.39	12.81	10.07^{a}	86.67	86.67	76.67	86.67	86.67	76.67	83.34	83.34	73.34	82.22^{a}
2.0 IBA + 0.5 BAP	10.81	9.69	9.52	11.12	11.13	7.71	10.94	12.93	10.45	10.47^{a}	96.67	90.00	76.67	80.00	86.67	80.00	80.00	83.34	83.34	84.10^{a}
4.0 IBA + 0.5 BAP	11.76	9.92	10.03	11.47	11.18	8.44	10.91	10.07	11.66	10.61^{a}	93.34	83.34	80.00	80.00	83.34	66.67	70.00	76.67	80.00	79.25^{a}
1.0 IBA + 1.0 NAA	8.66	9.19	7.73	4.20	4.94	5.01	4.51	4.95	7.95	6.35^{c}	80.00	70.00	60.00	66.67	73.34	56.67	63.34	60.00	60.00	65.56^{cd}
1.5 IBA + 1.5 NAA	6.97	4.55	5.06	6.02	5.83	4.05	5.91	6.12	7.65	5.79^{c}	83.34	73.34	66.67	63.34	63.34	46.67	53.34	56.67	56.67	62.60^{de}
1.0 IBA + 1.0 NAA + 1.0 BAP	7.57	6.13	5.56	4.77	8.66	4.63	4.74	4.52	8.00	6.06^{c}	73.34	70.00	76.67	73.34	73.34	63.33	70.00	70.00	70.00	71.11^{bc}
1.5 IBA + 1.5 NAA + 1.0 BAP	7.12	6.82	4.64	4.09	7.58	4.30	4.71	6.09	6.94	5.81^{c}	63.34	63.34	63.34	60.00	60.00	50.00	50.00	56.67	53.34	57.78^{e}
2.0 IBA + 0.25 NAA + 0.5 BAP	8.73	8.85	7.07	8.47	6.73	6.03	9.51	8.90	9.53	8.20^{b}	83.34	80.00	73.34	73.34	80.00	70.00	76.67	80.00	73.34	76.67^{b}
4.0 IBA + 0.5 NAA + 0.5 BAP	8.81	7.14	6.20	7.73	7.21	5.35	8.86	8.30	8.10	7.49^{b}	76.67	80.00	76.67	73.34	76.67	63.34	70.00	73.34	56.67	71.85^{b}
Mean	8.52^{ab}	7.67^{c}	6.67^{d}	7.75^{bc}	7.52^{c}	5.61^{e}	7.78^{bc}	8.00^{bc}	8.92^{a}		77.58^{a}	74.24^{ab}	68.18^{d}	69.70^{cd}	73.03^{bc}	60.61^{e}	65.45^{de}	67.88^{d}	63.64^{de}	
LSD ($p \leq 0.05$)																				
Treatment (T)										1.02										3.70
Genotype (G)										1.42										3.35
T × G										2.08										7.15

Mean values of parameters within each column and row followed by the same letter(s) are not significantly different according to the DMRT ($P \leq 0.05$); *transformed data









*Mean values of parameters followed by the same letter(s) are not significantly different according to the DMRT ($p \leq 0.05$)

Fig. 1. A. Comparison of rate of multiplication rate; B. multiplication cycle of grape rootstocks; C. Effect of plant growth regulators on *in vitro* rooting; D. Effect of hardening strategies on plantlet survival and days taken for *ex vitro* transfer in grape rootstocks.

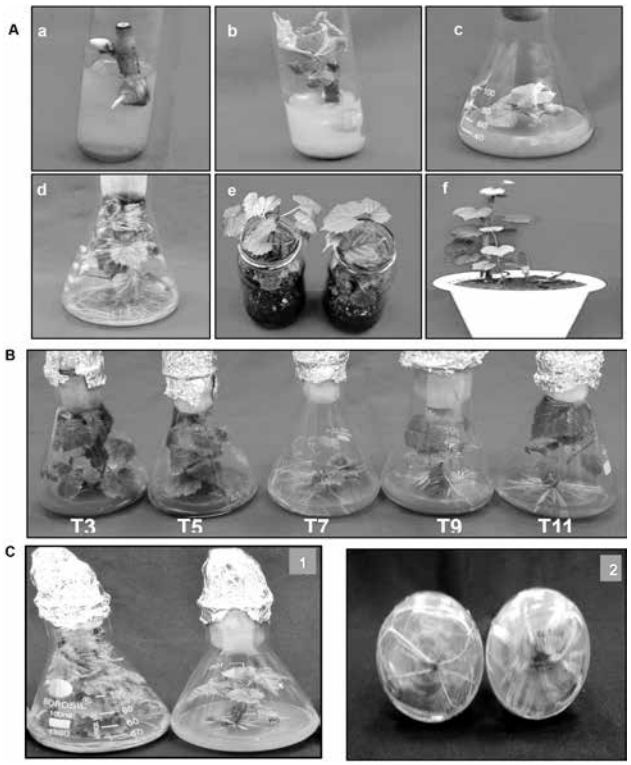


Fig. 2. A. Protocol for *in vitro* propagation of grape rootstocks. (a, b, c). Culture initiation stage, (d) Shoot-cum-root multiplication, (e). Hardening stage, and (f) Hardened plantlet; B. Performance of grape rootstock Dogridge on various treatment combinations. [T_3 = IBA (4.0 mgl^{-1}), T_5 = IBA (4.0 mgl^{-1}) + BAP (0.5 mgl^{-1}), T_7 = IBA (1.5 mgl^{-1}) + NAA (1.5 mgl^{-1}), T_9 = IBA (1.5 mgl^{-1}) + NAA (1.5 mgl^{-1}) + BAP (1.0 mgl^{-1}), T_{11} = IBA (4.0 mgl^{-1}) + NAA (0.5 mgl^{-1}) + BAP (0.5 mgl^{-1}); C. 1. Shoot growth on best treatment with lowest; 2. Root No. on medium supplemented with IBA alone and in combination with NAA.

mgl^{-1}). These results were in conformity with those of Abido *et al.* (1). The duration for root initiation (Table 3, Fig. 2) revealed that though Dogridge (16.28 days) and Salt Creek (15.50 days) responded late but had higher rooting success (77.58 and 74.24%). The highest number of roots micro-shoot^{-1} was recorded in 140 Ru (7.45), while it was least in 1613 C (5.88). Maximum root length was observed in the species *V. parviflora* (7.71 cm) and St. George (7.29 cm), while it was minimum in 1613 C (4.95 cm). These difference are expected as the result of genotypes and to some extent influence of PGR for *in vitro* rhizogenesis.

There was significant difference among the plantlet hardening strategies. The earliest time taken for *ex vitro* transfer and maximum plantlet survival was noted in glass jars with PP caps (Fig. 1D) as there was low desiccation and minimal microbial infection. Highest hardening success was achieved with the rootstock 1103 P (82.75%) cultured in glass jars. Rootstock 1616C plantlets were earliest to harden (44.40 days). Similar strategy has earlier been reported by Singh *et al.* (13) for *V. vinifera* cultivars and Alizadeh *et al.* (3) in some grape rootstocks. It was observed that in glass jars, the elongation of plantlet was better, which had positive influence on acclimatization.

Clonal fidelity or genetic uniformity of micropropagated plantlets, showed 1,655 bands in RAPD analysis out of which 1,341 bands showed sufficient polymorphism (Table 3). The scorable bands for each primer ranged from 4 (U13, U20) to 12 (J07) with band size ranging from 200 to 1500 bp. Maximum number of 310 bands were amplified with primer OPA15 with band size of 200 to 1500 bp, while minimum number (110 bands) were obtained with primers OPG 14 & OPU 20 within the size range of 300 to 550 bp and 210 to 550 bp, respectively. Monomorphic pattern of the bands indicated that there was no genetic variation in the *in vitro* regenerated plantlets compared to mother plants. In ISSR analysis, a total of 1600 amplified bands were produced with 10 ISSR primers with a band size ranging from 200 to 1500 bp. About 1209 bands showed sufficient polymorphism (Table 4). Primer UBC 824 amplified the maximum number of 290 bands with band size 300-2000 bp, while primer UBC 873 amplified the lowest number of 69 bands (300-1100 bp). According to pooled data analysis of two marker systems, 138 distinct and scorable bands were generated ranging from 200 to 2000 bp (Table 5). A total of 3,255 bands were generated with the both the markers and all were found to be monomorphic, corroborating high degree of clonal fidelity of micropropagated

Table 3. Details of oligo-nucleotide decamer primers used for assessing clonal fidelity of grape rootstock plantlets.

Primer No.	Sequence (5′-3′)	Total amplified bands	Polymorphic bands	Band size (bp)
OPA15	TTCCGAACCC	310	130	200-1500
OPJ01	CCCGGCATAA	250	240	200-1100
OPJ07	CCTCTCGACA	140	130	300-1500
OPG14	GGATGAGACC	110	110	300-550
OPH19	CTGACCAGCC	148	120	300-1000
OPP02	TCGGCACGCA	121	121	300-1500
OPP09	AGGTGACCGT	186	150	250-1200
OPU13	GGCTGGTTCC	160	110	400-1000
OPU16	CTGCGCTGGA	120	120	200-500
OPU20	ACAGCCCCCA	110	110	210-550

Table 4. List of different ISSR primers used for detecting clonal stability in tissue cultured grape rootstock plantlets.

UBC Primer No.	Primer sequence (5′-3′)	Total amplified bands	Polymorphic bands	Band size (bp)
UBC 807	AGA GAG AGA GAG AGA GT	71	25	300-850
UBC 809	AGA GAG AGA GAG AGA GG	190	100	300-1500
UBC 824	TCT CTC TCT CTC TCT CG	290	200	300-2000
UBC 858	TGT GTG TGT GTG TGT GRT	125	125	300-1000
UBC 859	TGT GTG TGT GTG TGT GRC	225	135	200-850
UBC 860	TGT GTG TGT GTG TGT GRA	130	130	380-1000
UBC 861	ACC ACC ACC ACC ACC ACC	145	145	300-1500
UBC 862	AGC AGC AGC AGC AGC AGC	100	100	300-1150
UBC 868	GAA GAA GAA GAA GAA GAA	255	180	300-1000
UBC 873	GAC AGA CAG ACA GAC A	69	69	300-1100

Table 5. Comparative data obtained by RAPD, ISSR and pooled analyses of *in vitro* grape plantlets for evaluation of clonal fidelity.

Particulars	RAPD	ISSR	Pooled analysis
No. of primers used	10	10	20
Scorable band classes per prime	4-12	3-11	3-11
Total No. of bands obtained	1655	1600	3255
Av. No. of bands per primer	7.0	6.7	6.9
Band size (bp)	200-1500	200-1500	200-1500
In vitro induced variation	Nil	Nil	Nil

grape rootstocks in the present study. The UPGMA dendrogram generated for ISSR (Fig. 3A) and RAPD (Fig. 3B) further confirmed the true-to-type nature of *in vitro* derived plantlets with their respective mother plants. Similarity matrix based on Jaccard's coefficient revealed that the pairwise value between the mother plant and the plantlets was 1, indicating 100 per cent similarity. This finding was in conformity with those reported earlier (Khawale *et al.*, 7; Alizadeh and Singh, 2; Zhang *et al*., 15) in grape rootstocks, and Harshita and Dhawan (5) in apple rootstock.

The protocols standardized for *in vitro* multiplication of the above grape rootstock genotypes (Fig. 2A) can be used commercially with minimum possibility of any *in vitro* induced variability.

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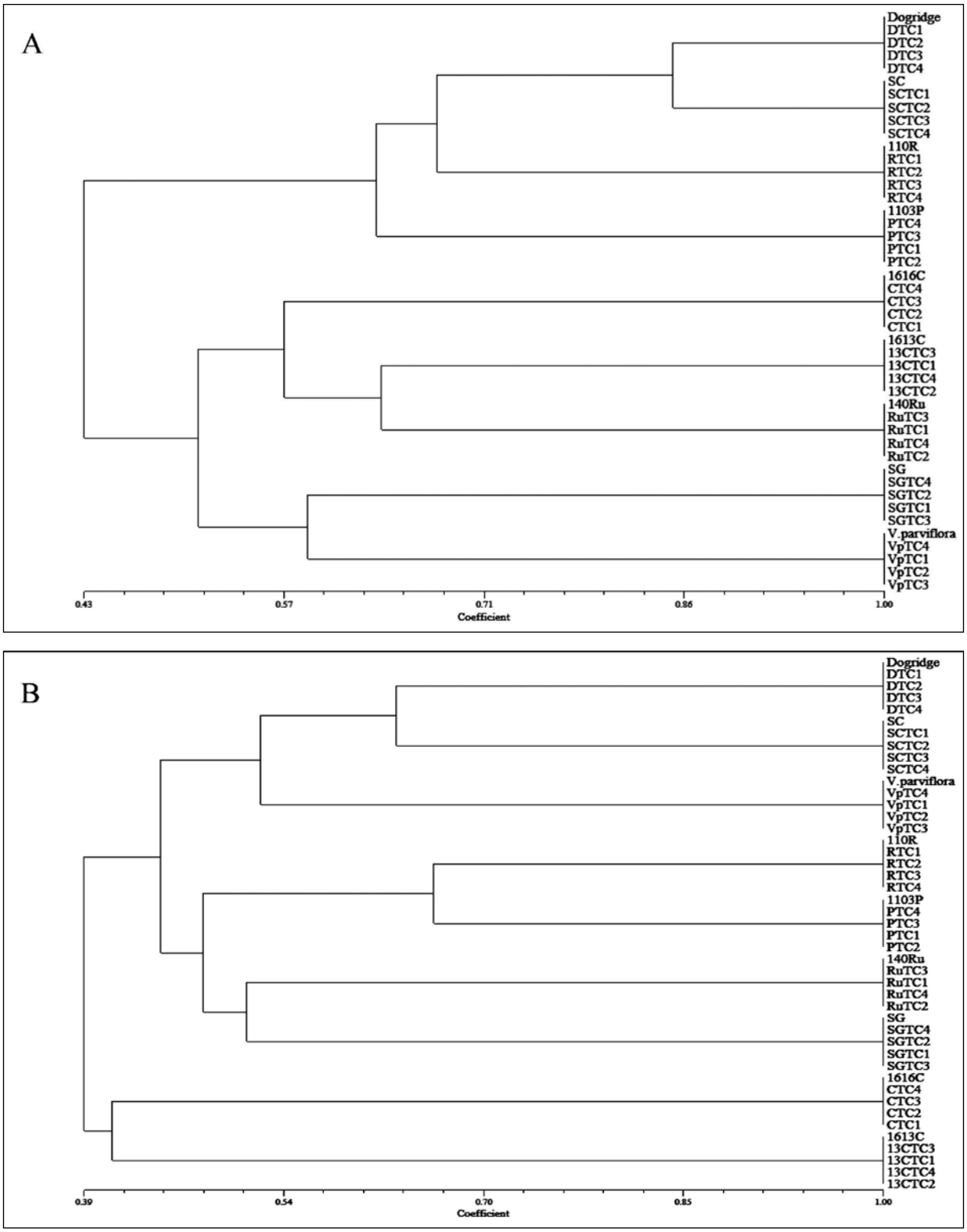

Fig. 3. Dendrogram obtained based on data A. ISSR and B. RAPD corroborating high levels of similarity among in mother plants and their respective *in vitro* raised plantlets (Dogridge (DTC1 to DTC4), Salt Creek (SCTC- SCTC4), 110 Richter (RTC1-RTC4), 1103 Paulsen (PCT1 –PCT4), 1616 Couderc (CTC1-CTC4), 1613 Couderc (13CTC1-13CTC4), 140 Ruggeri (RuTC1-RuTC4), St. George (SGTC1-SGTC4), and *V. parviflora* (PvTC1- PvTC4).

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