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IN VITRO PROPAGATION OF GRAPE ROOTSTOCK, DOGRIDGE AND 110 R: EFFECTS OF PGRS AND MEDIUM COMPOSITIONS

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Abstract – The objective of this study's is to develop a protocol for in vitro propagation of a grape rootstock. Dogridge and 110-R was chosen for their drought and salinity tolerance. In vitro culture can yield a large number of identical plantlets that can be preserved and used for a variety of purposes. In order to obtain high number of plants it is necessary to establish proper conditions of in vitro culture. Considering all the variables, the culture medium MS medium supplemented with 6 BAP @ 3.5 mg/l showed the best growth rates and development for shoots through nodal cutting and in vitro multiplication rate for dogridge and 110 R grape rootstock. Maximum rooting percentage and root growth was recorded with use of IBA+NAA (0.5+0.1 mg/l) in dogridge (68.3) and 110R (66.3).

INTRODUCTION

The *Vitis* spp. is one of the world's most cultivated fruits, reflecting its enormous economic importance (Reynolds, 2017). Because of its widespread application in the production of wine and table grapes, as well as the nutritional benefits of grape metabolites. The grapevine has gained scientific attention and has become a model woody species in plant biotechnology. Grape is one of the world's largest fruit crops, and according to the International Organization of Vine and Wine (OIV), global grape production in 2019 was 77.8 million tonnes, with wine grapes accounting for 57%, table grapes accounting for 36%, and dried grapes accounting for 7% (OIV, 2019).

Rootstocks are the major planting material required for vineyard establishment, Rootstocks a play a major role in improving scion performance, Fight against the stress-related problems. In India, grape growers have been introduced and are used from several countries (Upadhyay*et al.,* 2007). Its increased demand for propagation by the grape growers, leads to shortage of planting material and it is highly impossible to meet the required demand by conventional propagation methods. Hence, to fulfill these gaps there is intense need for production of huge planting material. Asexual propagation methods are difficult due to its season dependency and time consuming. Grapevine is traditionally propagated by utilising dormant hardwood (35-45 cm long) sequences collected in the winter in well advanced current season canes. They are planted in the nursery in the springtime and plants are transplanted into the viticulture in one season (Hartmann *et al*., 1997). This is a slow seasonal multiplication process. Rootstocks play an major role in grape propagation in order to overcome problems related to the soil such as salinity, drought and they are also tolerant to major pest and diseases *viz*., downy mildew, powdery mildew and anthracnose. However, rootstocks are in larger demand by the growers, and they are very difficult to multiply in larger scale rapidly because of problem of dormancy in winter season.

Hence, to overcome these problems this study was taken to tackle the major issues related to propagation under large scale, culture of plant tissue emerged as a strong propagation and crop improvement tools.Adopted and set up method for commercial propagation under controlled conditions in limited time and space across the year that also enables the production in aseptic conditions of disease-free planting materials (Hammschlag *et al*., 1955) along with rapid multiplication.In previous *Vitis* spp in vitro propagation protocols, requirements for medium-sized compositions varied widely. For example, MS

(Murashige and Skoog, 1962) and 1/2 MS were equivalent *in vitro* to *V. rotundifolia*, while WPM (Llyod and McCown, 1980) were the producer of stunted soots (Gray and Benton, 1991). Lu (2005) found however that the WPM media was higher than MS or NN. In addition, Mhatre *et al.* (2000) reported that BAP was found to be optimal for *V. vinifera* shoot multiplication. However, Protocols for tissue culture are available depending on how much the particular genotype reacts, since various Vitis species, cultivars or hybrids react differently to certain conditions of culture (Qiu *et al*., 2004).

Taking the above reports into consideration, we examined the impacts on in-vitro shooting and rooting in dogridge and 110R grape rootstocks by using different medium composition and plants growth regulators in search of an efficient procedure for micro propagation.

MATERIALS AND METHODS

Actively growing, young Axillary apical buds and nodal bud cuttings bearing single buds of dogridge and 110 R rootstocks were collected from vineyard of ICAR- NRC on Grape Pune, Maharashtra. Explants placed in moist paper towels in the laboratory to avoid desiccation of explants. The explants (1.0 cm to 1.5 cm), axillary-bud cuttings are prepared by removing all the extraneous tendrils and leaves and the nodal cuttings with single buds are prepared and treated with 5% (v/v) aqueous solution of a liquid detergent 'Tween-20' surfactant solution (1 to 2 drops in 100 ml distilled water) for 5 minutes, then thoroughly wash with running tap water for 30 minutes. The shoots were sterilised under aseptic conditions, immersing them for 20 seconds in a water solution of 75 per cent ethanol, then for 10 minutes in 0,1 per cent $HgCl_2$, with occasional swirling. Axillary apical buds and single nodal buds of dogridge and 110-R rootstocks were inoculated on Murashige and Skoog (1962) (MS) medium, which was supplemented with Sucrose 30 gl/1 as a carbon source, activated charcoal (1 g/l), and various growth regulator combinations. MS was used as a basal medium for culture initiation as well as a shoot proliferation/rooting medium. The following concentration of BAP (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, and 4.5 mg/l), IBA (0.5,1.0, 1.5, 2.0, 2.5, 3.0) for rooting and IBA + NAA (0.5,1.0, 1.5, 2.0, 2.5, 3.0 with 0.1 and 0.2) for culture initiation medium were selected for study. The medium's pH was adjusted to 5.8 and 8 mg/l agar was added as a solidifying agent; 30 ml of medium was poured into each glass bottle and sterilized in an autoclave (121°C, 15 psi pressure for 20 minutes). Sterilization was done aseptically in a laminar air-flow cabinet, which was sterilized by turning on its UV light for 45 minutes, wiping it down with 95 percent alcohol for 15 minutes, and surface-sterilizing it with 0.1 percent HgCl2 for 7 to 10 minutes, followed by three rinses in sterile distilled water.

Explants were then inoculated onto the solid medium using a laminar air-flow work station and incubated in a growth room with 3000 Lux light intensity, a photoperiod of 16 hours light/ 8 hours dark, and a temperature of 25±2 °C. The in vitro shoot proliferated 30-day-old plantlets were transferred for further shooting media, and after 30 days, plantlets were transferred to rooting media. In vitro rooted plants were pre-hardened for 48 hours in half Murasigue and skoog (MS) liquid media before being transferred for primary hardening in perlite and coco peat (1:1) ratio for 15 -25 days at room temperature before being shifted to secondary hardening media containing soil, sand, and compost (2:1:1) ratio. Observations were recorded in 15-day intervals. The experiment was laid out in completely randomized design (CRD) with 5 replicates. Data were analyzed using one-way ANOVA with the help of a statistical software package, SAS/STAT, India

RESULTS

Effect of medium composition on shoot growth and proliferation

The effect of different media and growth regulators combinations by axillary and nodal bud cuttings of rootstocks dogridge and 110 R, for shoot induction, is studied in the current findings. Because the most effective MS medium among the four media compositions tested was found, this medium was used in all following subcultures or tests. The results show that among different concentrations of 6 BAP cultured on different medium, MS medium supplemented with 6 BAP @ 3.5 mg/l on nodal cuttings of dogridge (84.6 percent) and 110 R (81.6 percent) showed significant results, followed by treatment 6 BAP @ 3.0 mg/l on nodal cuttings of dogridge (76.6 percent) and 110 R (71.3 percent) respectively, and the lowest was observed in treatment 6 BAP @ 3.0 mg/l on no (Table 1). Whereas axillary cuttings failed to establish as growth regulator concentrations were increased.

Table 1. Effect of different media and PGRs on shoot induction by nodal and axillary cuttings

Treatments	Nodal bud cuttings								Axillary bud cuttings							
	MS Media		½ MS Media		NN Media		WPM media		MS Media		½ MS Media		NN Media		WPM media	
MS+6 BAP (mg/l)	DR	110 R	DR	110 R	DR	110 R	DR	110 R	DR	110 R	DR	110 R	DR	110 R	DR	110 R
0.5	22.0	20.6	20.7	15.2	10.8	10.6	12.9	12.2	20.6	19.6	15.3	13.4	12.5	12.2	14.5	14.2
1.0	35.3	32.3	20.5	10.5	12.4	11.3	11.2	11.3	27.3	25.3	19.8	18.7	18.3	18.2	20.2	20.1
1.5	54.6	49.0	25.2	15.6	15.7	13.8	12.9	12.8	20.6	30.6	20.1	20	20	19.7	22	21.8
2.0	60.3	61.7	30.0	20	25.6	24.2	30.8	30.4	38.3	34.3	24.6	23.7	22.3	20.2	25.4	25.3
2.5	73.0	62.6	32.3	28.3	27.6	26.5	35.6	34.2	32.6	30.6	30.1	29.9	27.2	28.5	29.2	28.8
3.0	76.6	71.3	35.2	30.1	31.3	30.3	39.5	37	20.6	21.6	22.1	21.5	21.2	20.4	20.2	20.1
3.5	84.6	81.6	36.0	35.8	32.5	30.7	40.2	39	18.6	18.6	15.5	15.1	14.8	14.1	18	18
4.0	55.0	53.0	33.3	32.7	32.2	31.9	36.4	35.3	10.0	12.3	10.7	10.2	10	10	12.5	12.1
4.5	50.6	50.5	32.0	33.0	31.0	29.0	33.0	33.1	5.6	2.3	0.0	0.0	0.0	0.0	5.0	5.0
LSD @ 0.05%	2.2	2	1.2	1.2	1.1	1.3	1.2	1.1	1.8	1.2	1.4	1.3	1.3	1.3	1.2	1.2

Standardization of selection of explants on per cent success of nodal cuttings

According to the data presented in Fig. 1, the maximum explants establishment was observed (89.6 percent) and (80.3 percent) in dogridge and 110 R, respectively, while the lowest was observed in the first node (31.0 percent) in dogridge and 110 R. When compared to tender green and slightly matured green nodes, medium matured green nodes are better for plant establishment. The fourth and fifth nodes are ideal for the establishment of explants via nodal cuttings. These findings could be attributed to the presence of a good food reserve for plant establishment in the 4th and 5th node of nodal cuttings for micro propagation. *In vitro* cultures of two grape rootstocks, Dogridge (*Vitis champini*) and H-144 (*Vitis vinifera* ×*V. labrusca*), were initiated using single node segments.

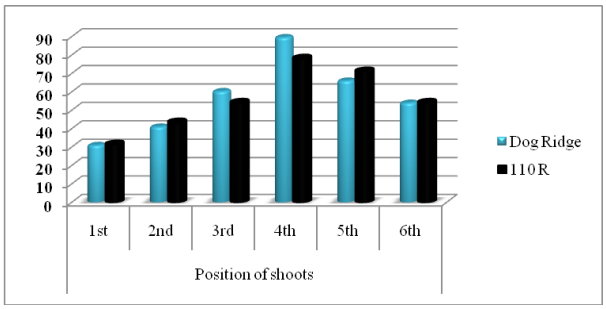

Fig. 1. Standardization of position of explant on per cent success of nodal cuttings

The results show that among different concentrations of 6 BAP, significant results were obtained from treatment MS medium supplemented with 6 BAP @ 3.5 mg/l nodal cuttings of Dogridge (4.3) and 110 R (3.4) multiple shoots, sprouting in 8 days in both dogridge (8 days) and 110 R (3.4) multiple shoots (8 days). The lowest values were found in dogridge 6 BAP at 4.5 mg/l (0.3) and 110 R (0.3), respectively. Shoot length and number of leaves were highest in 6 BAP @ 3.5 mg/l (3.4) and (3.3) cm and (7.0) and (6.0) no. of leaves in dogridge and 110 R, respectively, and lowest in 6 BAP @ 0.5 mg/l and 4.5 mg/l, the multiple shoots that regenerated from nodal cuttings were sub cultured through separating individual shoots. In the subsequent sub-culturing and later on when the shoots reached around 4.5 to 5.0 cm in 6-8 weeks individual shootings were excised into 1-1.5 cm single nodal segments with axillary bud. MS medium enriched with 0.5 mg/l. The maximum number of shoots per explant (3.63), which was

significantly higher than 3.49 at BAP (0.5 mg/l).

Effect of medium composition on Rooting

To induce rooting in rootstocks, the effect of growth regulators on percent rooting on average 3 to 4 cm shoots was studied. The highest percent of rooting was observed with IBA+NAA (0.5+0.1 mg/l) in dogridge (68.3) and 110R (66.3), followed by IBA+NAA (1.0+0.1 mg/l) in Dogridge (56.0) and 110R (54.0). The lowest was found in growth regulator combination IBA +NAA(2.0+0.2 mg/l) in Dogridge (1.6) and 110 R (4.6) in 1.5+0.2 mg/l whereas no rooting was found in IBA +NAA(3+0.2 mg/l) in Dogridge (0.0) and 110 R (0.0). Less no. of days to root was took in same growth regulator combinations in both Dogridge (27.6) and 110 R (30.0). The root length, number of primary roots, and number of secondary roots are significantly higher in the Growth Regulator treatments. The highest value was observed for root length and secondary roots in MS medium supplemented with IBA+NAA (0.5+0.1 mg/l) in Dogridge (4.0) and 110R (3.7), primary roots in dogridge (2.6) and 110R (2.6), and secondary roots in Dogridge (12.6) and 110R (11.6), respectively, while the lowest was observed in 1.5+0.2 mg/l in Dogridge (1.1) and 110 R (0.8) (Table 3). *In vitro* rooted plants were subjected for pre hardening for 48 hours in half Murasigue and Skoog

Table 2. Effect of different concentration of 6 BAP on shoot growth and proliferation of nodal cutting

Treatments	No. of days to shoot (Days)		No. of shoots/ explant		Shoots length (cm)		No. of leaves	
6 BAP (mg/l)	DR	110 R	DR	110 R	DR	110 R	DR	110 R
0.5	13.3a	14.3a	1.3 d	1.0 de	1.0 de	1.0 e	2.6 b	2.6 b
1.0	11.6 b	12.3b	1.6cd	0.6 ef	0.6f	0.4 f	2.6 b	2.6b
1.5	11.0b c	11.3 c	1.6cd	1.1 d	1.1d	1.0 e	2.3bc	2.3bc
2.0	10.3 c	10.0 d	1.0 de	1.4 cd	1.4 cd	1.2 de	2.0cb	2.0 bc
2.5	9.0 d	9.0 e	3.0 b	1.9 bc	1.9 cb	1.6 cb	2.6b	2.6 b
3.0	8.3d	8.3 e f	2.0 c	2.3 b	2.3b	2.03cb	3.3b	3.3 b
3.5	8.0 d	8.0 f	4.3 a	3.4 a	3.4 a	3.3a	7.0 a	6.0 a
4.0	8.0 d	8.0 f	1.3 cd	2.4 b	2.4b	2.2 b	3.3b	3.3b
4.5	8.6 d	8.6 e	0.3 e	0.3 f	0.3	0.3f	1.0	1.0c
LSD @ 0.05%	**1.0**	**0.9**	**0.6**	**0.5**	**0.5**	**0.4**	**1.4**	**1.4**

Table 3. Effect of different concentrations of PGR on rooting parameters

Treatments	Rooting percentage		No. of days to root		Root length (cm)		No. of primary roots		No. of secondary roots	
MS+IBA+NAA	DR	110R	DR	110R	DR	110R	DR	110R	DR	110R
0.5 IBA	53.3 b	49.6 c	31.0 c	33.3 d	0.7 ef	0.7 ef	1.1 bcd	2.0 ab	9.0 bc	7.6 bcd
1.0 IBA	43.3 c	41.3 d	31.0 c	32.6 de	1.0 de	0.8 de	1.40 bc	2.3 a	10.0 b	8.6 b
1.5 IBA	31.6 de	28.3 ef	34.0 b	34.3 cd	1.2 d	1.1 d	1.3 bc	1.3 bc	8.3 cd	8.3 cb
2.0 IBA	21.0 f	19.0 h	34.6 ab	35.6 bc	0.5 gf	0.3 fgh	1.0 bcd	1.3 bc	7.3 de	6.6 ed
2.5 IBA	6.6 g	6.6 I	36.0 ab	37.0 ab	0.3 fgh	0.2 gh	0.3 de	0.3 de	1.6 f	1.3 f
3.0 IBA	0.0 h	0.0 j	0.0 e	0.0 g	0.0 h	0.0 h	0.0 de	0.0 e	0.0 g	0.0 f
0.5 IBA + 0.1NAA	68.3 a	66.3 a	27.6 d	30.0 f	4.0 a	3.7 a	2.6 a	2.6 a	12.6 a	11.6 a
1.0 IBA + 0.1 NAA	56.0 b	54.0 b	31.3 c	31.0 ef	3.3 b	3.0 b	2.6 b	1.3 bc	12.0 a	11.3 a
1.5 IBA + 0.1 NAA	31.3 de	29.0 ef	31.3 c	33.6 cd	0.7 ef	0.7 ef	0.6 cde	1.0 cd	8.0 cde	7.3 bcd
2.0 IBA + 0.1 NAA	29.3 e	27.6 f	34.0 ab	35.6 bc	0.3 fgh	0.2 gh	1.6 b	1.3 bc	6.6 e	5.6 e
2.5 IBA + 0.1 NAA	20.6 f	18.0 h	36.6 a	38.0 a	0.2	0.3 fgh	0.0 e	0.0 e	0.0 g	0.0 f
3.0 IBA + 0.1 NAA	0.0 h	0.0 j	0.0 e	0.0	0.0 h	0.0 h	1.6 b	1.3 bc	8.3 cd	7.3 bcd
0.5 IBA + 0.2 NAA	32.6 d	31.6 e	29.0 cd	0.0 g	2.1c	1.9 c	0.6 cde	1.0 cd	7.3 de	6.3 de
1.0 IBA + 0.2 NAA	20.6 f	23.0 g	31.3 c	29.6 f	0.5 gf	0.5 efg	1.6 b	1.3bc	8.0 cde	7.0 cde
1.5 IBA + 0.2 NAA	6.6 g	4.6 I	34.0 b	32.3 de	1.1gf	0.8 de	0.0 e	0.0 e	0.0 g	0.0 f
2.0 IBA + 0.2 NAA	1.6 h	0.0 j	0.0 e	34.0 cd	0.0 h	0.0 h	0.0 e	0.0 e	0.0 g	0.0 f
2.5 IBA + 0.2 NAA	0.0 h	0.0 j	0.0 e	0.0 g	0.0 h	0.0 h	0.0 e	0.0 e	0.0 g	0.0 f
3.0 IBA + 0.2 NAA	0.0 h	0.0 j	0.0 e	0.0 g	0.0 h	0.0 h	0.0 e	0.0 e	0.0 g	0.0 f
LSD @0.05 %	2.8	3.7	2.6	2.2	0.4	0.3	0.9	0.9	1.47	1.6

(MS) liquid media then they are transferred for primary hardening in perlite and coco peat (1:1) ratio for 15 -25 days at room temperature and shifted for secondary hardening media containing soil, sand and compost (2:1:1) ratio.

DISCUSSION

In this study the MS medium was higher for in vitro growth and shoot proliferation than several other media. WPM was previously found to be more effective in shooting than either the medium MS or NN on *V. thunbergii*, as the reduced nitrogen content of WPM is one possible explanation (Lu, 2005). Furthermore, the use of MS medium with 1/2 nitrate in grapevine rootstock shoots proliferation has been reported to be effective (Matsumoto and Sakai, 2003).

MS Medium with of 3.0 mg/l BAP + 0.2 mg/l NAA Muscats Alexandria have been found best to be the most proliferated shootings were obtained from shoot tips and internode segments Abido *et al*. (2013). Direct, *in vitro* regeneration with nodal segments can effectively multiply two genotypes of grapes (Alizadeh *et al.*, 2018) by the end of March, the second and third bottom node sections of new development shootings were also taken by AI-Aizari *et al.* (2020). The media composition necessary for the micro propagation of different Vitis species varied by genotype, all this studies showed clearly.

BAP was more effective in the current study for the shoot growth and production of new explants per explant inoculated. Previous research showed clearly that BAP is the most effective cytokine to cause *Vitis vinifera* shooting (Heloir *et al*., 1997). More TDZ-containing medium shootings, but the shooting did not lengthen in the current study. The ability of TDZ to inhibit shooting length can be linked to its high cytokine activity (Huettman and Preece, 1993).

However, the highest rooting percentage and root growth were observed when IBA+NAA (0.5+0.1 mg/l) was used in dogridge (68.3) and 110R. (66.3) Abido *et al*. (2013) Found that rooting has been tested for different IBA and NAA levels. The rooting percentage, number of roots/shot and root length of shoots in Muscat of Alexandria were higher in the MS Medium, supplemented by 1.0 mg/l IBA+0.5mg/l NAA (87 percent, 3.4, and 4.5 cm, respectively). Mhatre *et al*. (2000) reported that using 0.1 mg/l IAA was ideal to induce roots, as well as to remove shooting and calli sponges in grapevines. Shoots which were *in vitro* rooted in IBA were most likely not restored by ex vitrum because of the large frequency of formation of massive callus. Similar findings were made in Primelea spp, where it could be attributed to a massive increase in callus and therefore a poor vascular relationship between roots and roots that could result in the failure of in-vitro IBAs to reestablish ex vitrium (Offord and Tyler, 2009).

CONCLUSION

In this study, among the growth regulator combinations used, 6 BAP @ 3. 5 mg/ lit found significant for shoot induction where as for root induction combination of IBA and NAA (0.5+0.1 mg/l) was found significant. Hence, this protocol can be used for in vitro micro propagation of grape rootstock undrer asceptic condition.

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