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Effect of culturing time, position of node on explant establishment and Surface sterilization procedure for *in vitro* micropropagation of grape rootstocks

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ABSTRACT

The current study, titled "Studies on *in vitro* Surface sterilization and disinfection procedure for *in vitro* propagation of grape rootstocks was conducted at the Tissue Culture Laboratory, ICAR-National Research Centre for Grapes Pune, from 2018-19 and 2019-2020. maximum explant establishment (82.3 %) and (80.3 %) were recorded in Dogridge and 110 R, respectively. When compared to May, June and July, the month of 'April' is ideal for culturing nodal cuttings. Both rootstocks had a similar pattern in terms of the month-to-month establishment. Carbendazim @ 1 g/l for 2 hours was shown to be the most effective in reducing contamination and promoting plant establishment in Dogridge (11.6 %) and 110R (13.3) rootstocks respectively. In Dogridge and 110 R rootstocks, charcoal @ 1g/l was found to be effective in reducing browning (12.3 and 12.6 %) and contamination rate (11.3 and 11.0 %). In Dogridge and 110 R. Surface sterilization with mercuric chloride for 7-10 minutes was shown to be effective in reducing contamination rate for nodal explants in Dogridge (19.6 %) and 110 R (22.6 %).

Key words : *Micropropagation, Grape rootstocks, Surface sterilization*

Introduction

Grapevine (*Vitis* spp.) is one of the most widely grown fruit crops in the world, (Reynolds, 2017). Due to its widespread use in the production of wine and table grapes, as well as the nutritional benefits of grape metabolites. It is a very profitable crop, leads to an increase in demand from both the national and international market. As a result, it is necessary to expand the grape growing area. Hence, to fulfil these gaps there is an intense need for the production of huge planting material. In order to obtain high number of plants it is necessary to establish proper conditions of in vitro culture (Anupa *et al*., 2021). *In vitro* regeneration depends upon various factors, but the most significant is the season in which the tissue was collected for implantation (Murashige, 1974). Plant parts are a repository for a wide range of contaminants, and therefore obtaining plant material that is free of contaminants is very difficult. Woody plants are grown in soil for many years in ambient conditions and are routinely infected both internally and externally with microorganisms that are frequently difficult to control *in vitro* (Skirvin, 1983). As a result, explants must be surface sterilized before culturing. This is accomplished through the use of a variety of surface sterilizing agents. The type, concentration and duration of disinfection treatment are determined by the contamination level and the hardiness of the explant.

(Scientist - Fruit Science)

Numerous sterilizing agents have been used, including calcium hypochlorite, chlorine water, bleaching water, mercuric chloride and hydrogen peroxide. Keeping the shoot tips and nodal segments submerged in running water for an hour before sterilization with a single surface was effective. Additionally, this procedure resulted in the leaching of water-soluble phenols and other growth inhibitors. Selecting an appropriate explant and standardizing an effective disinfection protocol are critical components of a successful micropropagation technique. Explants and methods of culturing influence micropropagation success. Shoot tip and axillary bud explants are critical in grape and are frequently used for direct organogenesis due to their operability and genotype stability (Torregrosa *et al*., 2001; Mhatre *et al*., 2000; Ikten and Read, 2010; Krizan *et al*. 2012; Ruma, 2014; Wafa, 2015 and Theivanai *et al.* (2020).

Materials and Methods

Nodal cuttings bearing single buds of Dogridge and 110 R rootstocks were collected from nursery mother block, at ICAR-NRC for Grapes, Pune. Explants were placed in wet paper towels in the lab to prevent desiccation. Nodal explants were cultured throughout the year and mean monthly (January to December) plant establishment was recorded. Nodal cuttings bearing single buds of Dogridge and 110 R rootstocks were collected from the nursery of ICAR-NRC Grapes, Pune, Maharashtra, the nodal cuttings (2-3 cm) with single buds are made and treated with a 5 per cent (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 completely washed with running tap water for 30 minutes. Nodal cuttings were given 1 mg/l of Bavistin/ carbendazim pretreatment for different time interval. nodal cuttings are treated with fungicide Bavistin/carbendazim for different time interval from 1 to 4 hours, surface sterilization for nodal explants under laminar by disinfectants sodium hypochlorite and Mercuric chloride was done on rootstocks.

From the investigations percentage of explants that survived on grape rootstocks was significantly impacted by culturing time (Table 1) shows the data collected on explant establishment in April it was found the highest explant establishment in Dogridge and 110 R respectively, (82.3 %) and (80.3 %) which was followed by May in Dogridge and 110 R (79.0 %) and (73.0 %), respectively. For the month-by-month establishment, both rootstocks showed a similar pattern and significantly different. The month of January had the lowest explant establishment of all the months studied. These results indicate that there is influence of external environment on explant collected and percent contamination of explant survival by using nodal explants on rootstocks Degrossette, 110 R and 1613 C rootstocks. (Singh, 2014; Singh *et al*., 2002). The highest percentage of culture establishment in April may be attributable to reduced contamination compared to June and July because the spread of bacterial and fungal infection is normally quite high during the wet season. It is possible that polyphenol content is related to explant survival in grape genotypes. Because of the lower polyphenol content in vines during this time of year, explant survival was better when explants were cultivated in mid-April. These findings are consistent with those obtained for the cultivars Pusa Urvashi, Perlette, Centennial Seedless and Pusa Seedless.

Table 1. Effect of culturing time on percent explants survival on grape rootstocks

Inoculation (month) year (2019)	Explant survival/Explant establishment (%)	
	Dogridge	110 R
January	55.6 [f]	54.3 [gf]
February	62.6 [d]	61.6 [d]
March	72.6 [c]	70.3 [c]
April	82.3 [a]	80.3 [a]
May	79.0 [b]	73.0 [b]
June	58.3 [e]	56.3 [ef]
July	61.6 [d]	57.3[e]
August	58.3 [g]	50.3 [h]
September	61.3 [d]	55.3[ef]
October	62.0 [d]	57.6 [e]
November	61.3 [d]	56.3 [f]
December	55.6 [f]	56.3[f]
S.Em±	1.9	2.0
CD @ 5 %	2.2	2.4
CV (%)	2.1	2.1

From the findings, Table 2 shows the effect of pretreatment with carbendazim fungicide on surface sterilization of nodal explants at various time intervals in rootstocks Dogridge and 110R. treatment shows significant differences in reducing contamination per cent with bavistin @ 1g/l for 2 hours had the lowest contamination rate in rootstocks

Dogridge (11.6 %) and 110R (13.3 %), respectively. These findings show that surface sterilization for 2 hours reduced contamination, while treatment for more than 3 hours causes desiccation of plant tissues and failure in plant establishment. These findings show that surface sterilization for 2 hours reduces contamination, while treatment for more than 3 hours causes desiccation of plant tissues and failure in plant establishment.

Table 2. Effect of pre-treatment (carbendazim) and time interval on explant establishment in rootstocks

Treatment (carbendazim g/l)	Per cent contamination (%)	
	Dogridge	110 R
1 hour 30 min	52.0 [a]	51.6[a]
1 hour 45min	24.3 [b]	26.0 [b]
2 hour	11.6 [f]	13.3 [e]
2 hour 30 min	20.3 [dc]	22.6 [c]
2 hour 45min	21.3 [c]	22.0 [c]
3 hour	20.0 [cde]	22.0 [c]
3 hour 30 min	18.0 [de]	17.6 [d]
3 hour 45min	19.0 [de]	14.6 [e]
4 hour	19.6 [cde]	15.6 [de]
S.Em±	1.7	1.6
CD @ 5 %	2.2	2.9
CV (%)	2.1	2.1

Theivanai *et al.* (2020) conducted similar research on grape rootstock, Dogridge and two types of explants *viz*., axillary buds and shoot tips. The explants were pre - treated for 30 minutes with Carbendazim (0.3 and 0.5 %) prior to surface sterilization for 30 minutes, the axillary buds were pretreated with 0.5 per cent carbendazim resulted in the highest survival rate (68.33 %), the least pollution (15 %), and the lowest mortality (16.67 %).

Surface sterilization of explants is required to eliminate contamination while causing the least amount of damage to plant cells. The chemicals used for sterilization are chosen based on the type of explant to be utilized for micropropagation. The time gap between carbendazim pre - treatments in the current investigation was increased since the type of explant used in the investigation was nodal cuttings.

Fungal contamination of grapevine explants is a severe issue, and field explants are frequently infected with bacteria and fungi. As a result, surface disinfection of the explants is required before sterile culture establishment.

The present results indicated that minimum contamination was observed when explants treated with mercuric chloride Table 3 shows that surface sterilization of nodal cuttings under laminar airflow is done for 3 to 10 minutes with sodium hypochlorite and mercuric chloride. Mercuric chloride for 7-10 minutes has been proven to be effective in decreasing contamination. Mercuric chloride for 7-10 minutes was found to be significantly different from other treatments in both Dogridge (19.6 %), 110 R (22.6 %) and (22.6), (23.0 %) for 10 and 7 min respectively. Mercuric chloride for 7-10 min was found to be significantly different from other treatments in both Dogridge (19.6 %) and 110 R (22.6 %) and (23.0 %) for 7 and 10 min, respectively, and the highest contamination per cent was seen in sodium hypochlorite @0.05 for 3 min in both Dogridge (70. 6 %) and 110 R (70.3 6 %). Similar results were by several workers (Theivanai *et al*., 2020; Ruma, 2014 and Wafa, 2015; Zhang *et al*., 2010; Krizan *et al*., 2012 and Jamwal *et al*., 2013; Mhatre *et al*., 2000; Singh *et al*., 2004; Gray and Benton, 1991). In grape, a combination of 0.5 percent carbendazim and 70% ethanol, followed by 0.1 percent mercuric chloride, was used for sterilizing the explant for 7-10 minutes. Although mercuric chloride is exceedingly effective, it is highly poisonous to both plant and animal tissues and must be handled with caution. Because of the element's phytotoxicity, multiple rinses are required before inoculation to eliminate all traces of the element from the explants. As a result, sodium hypochlorite, which has a similar effect but is less hazardous, was attempted for surface sterilization.

Table 3. Standardization of time interval for surface sterilization for nodal explants under laminar by disinfectants on rootstocks

Treatments	Dogridge		110 R	
	Sodium hypochlorite @ 0.05%	Mercuric chloride @0.01 %	Sodium hypochlorite @ 0.05%	Mercuric chloride @0.01 %
3 min	70.6[f]	63.3[f]	70.3[f]	61.0[f]
4 min	55.3[c]	51.3[e]	56.0[e]	53.6[e]
5 min	51.0[b]	51.3[e]	50.3[a]	49.0[e]
7 min	63.3[d]	23.3[b]	62.0[d]	23.0[b]
10 min	67.0[e]	19.6[a]	63.6[e]	22.6[a]
12 min	55.3[c]	36.0[c]	55.3[c]	36.0[c]
14 min	51.0[b]	45.3[d]	51.0[b]	45.3[d]
18 min	50.3[a]	30.0[d]	50.3[a]	43.0[d]
S.Em±	1.15	1.5	0.9	1.5
CD @5%	3.6	4.1	2.8	5.8
CV (%)	3.25	3.7	2.9	3.6

The use of charcoal on media effectively reduced browning and it was significantly influenced in reducing contamination (Table 4). The lowest browning (11.5 and 11.7 %) was detected with charcoal 1g/l in Dogridge and 110 R rootstocks. Without charcoal, browning percentage was recorded the highest in Dogridge and 110 R respectively (35.0 and 35.1 %).Charcoal on media significantly reduced contamination and it was effective in reducing contamination (Table 4). The lowest contamination (11.3 and 11.0 %) was recorded with charcoal @ 1g/lit in Dogridge and 110 R rootstocks. Without charcoal, browning percentage (37.0 and 38.0 %) contamination (39.3 and 36.6 %) was recorded the highest in Dogridge and 110 R, respectively. similar studies found by When activated charcoal was utilized @ 2.0-2.5 g/l, eleven virus and viroid free cultivars were grown on activated charcoal (AC) containing media in the case of five viruses and two viroids (Olah, 2017) decreases or eliminates the browning process (Reustle and Natter, 1994).

Activated charcoal has unique physical and chemical properties since due to its porous structure AC has an extremely large inner surface with high adsorbing capacity. This adsorbing property makes AC beneficial in several fields of plant tissue culture as well (Thomas and Schiefelbein, 2008). AC is widely used not only in plant tissue culture (Pan and Vanstaden, 1998; Thomas and Schiefelbein, 2008) but also in media for the isolation and maintenance of various microorganisms, as well as for the isolation of DNA from various soils. The selectivity of adsorbing harmful chemicals and secondary metabolites accumulated in the media is linked to its beneficial effects. AC has the ability to change the pH of a medium and create a darker environment akin to soil conditions. Adsorption of AC may also lower inhibitory chemicals such as ethylene and phenolics, hence boosting plant development. Its radical impact is a reduction in the concentration of plant growth regulators (PGRs), vitamins, and metal ions, as well as a reduction or elimination of the browning process (Olah, 2017; Dev *et al*., 2019; Ahmadian *et al*., 2013; Atlas, 2010; Dussert *et al*., 1992; Pan and Vanstaden, 1998; Reustle and Natter 1994).

Table 4. Effect of with and without charcoal on browning on Dogridge and 110 R rootstocks

Treatments	Browning (%)		Contamination (%)	
Rootstocks	Dogridge	110 R	Dogridge	110 R
With charcoal	11.5	11.7	11.71	12.0
Withoutcharcoal	35.0	35.1	36.71	36.7
S.Em±	0.9	0.9	1.10	1.0
CD @ 5 %	2.6	3.08	3.4	3.3
CV (%)	9.7	11.2	12.1	11.6

Table 5. Standardization of position of node on success of nodal cuttings on rootstocks

Nodal position	Explant establishment (%)	
	Dogridge	110 R
1st node	31.0 [f]	31.3 [e]
2nd node	41.0 [e]	43.3[d]
3rd node	60.3[c]	54.0 [c]
4th node	89.6 [a]	84.0 [a]
5th node	66.0 [b]	71.0 [b]
6th node	54.0 [d]	54.0 [c]
S.Em±	4.7	5.2
CD @ 5 %	7.27	8.7
CV (%)	2.2	2.1

The position of explant was significantly influenced the per cent establishment on rootstocks. Table 5 revealed that maximum explant establishment was recorded on 4th node (89. 10 %) and (80.3 %) in Dogridge and 110 R respectively, it was followed by 5th node (66.0 %) and (71 %) in Dogridge and 110 R respectively while the lowest was recorded in the 1st node (31.0 %) in both Dogridge and 110 R.

Anupa *et al*. (2016) found that establishment of nodal cuttings was significant andshown high capacity for shoot induction in variety Crimson Seedless (71.43 %) when compared to axillary bud (15.29 %)

Conclusion

The percentage of explant establishment of grape is highlydepending upon time, season and surface sterilization of explant for *invitro* micro propagation. The present findings will help in micro propagation of grape.

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