

In-Vitro Propagation Of Grapes Cv. Flame Seedless

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Abstract:

The present investigation entitled "In-Vitro Propagation of Grapes cv. Flame seedless" were carried out in the biotechnology laboratory and experimental field of Horticulture, faculty of agriculture, Guru Kashi University, Bathinda. Out of different sterilizing treatments the highest survival rate (60.00%) and lowest contamination percentage (40.00%) were observed in treatment containing Bavistin (0.3%) + Ethanol (70%) + HgCl₂ (0.1% for 3 min). For shoot proliferation the maximum shooting percentage (94.00%), highest number of shoots/explants (4.75) and maximum length of shoot/explant (3.75cm) were observed in MS media fortified with BA 2.0mg/l + KIN 0.1mg/l. The Maximum rooting percentage (80.00%), highest number of roots (4.25%) and maximum length of root (6.75 cm) were significantly obtained from treatment fortified with MS Media+ IBA 2.0 mg/l.

Keywords: Grapes, In-Vitro, MS Media, Plant growth regulators, Shoots

INTRODUCTION:

Grapes (*Vitis vinifera* L.) belongs to family Vitaceae is perennial woody fruit crop growing in the tropical, subtropical and temperate regions (Anupa et al., 2016). It is a globally cultivated commercial fruit crop, is native to warm and temperate zones. The *Vitis* genus is widely distributed between 25° E and 50° N latitude in Eastern Asia, Europe, Middle East and North America (Sajid et al., 2006). It is most refreshing and nourishing fruit of the world. Grapes are used for wine production, fresh fruit and juice production. From the total grape production about 70% is used for wine production, 27% as fresh fruit and the remaining 2% as dried fruit (Munir et al., 2015). Grape is native to Armenia and Russia. Grape was introduced in India during 1300 AD by invaders from Iran and Afghanistan. The major producers of grapes are Italy, France, Spain, USA, Turkey, China and Argentina (Anonymous 2010). India has occupied ninth rank with 3.31 percent share in world grape production (Anonymous 2011). India has several diverse grape growing regions. Maharashtra is India's top producer accounting for nearly 67 percent of the country's grapes and Karnataka is the distant second one with 28 percent grape production (NHB 2023-24). Tamil Nadu, Andhra Pradesh, Mizoram, Punjab and Jammu & Kashmir account for the rest of India's grape production. In past, Punjab occupies 441 ha area under grapes with an annual production of 12.5 thousand MT (Anonymous 2012). Punjab has highest per hectare productivity which is around three times than productivity of other states. The grapes can be successfully grown in arid-irrigated zone of Punjab. Micropropagation is the art and science of in vitro plant multiplication. Micropropagation in grapevine was first performed by in vitro culture of micro-cuttings (Jean et. al., 1998). However, more recently introduction of bud proliferation has been shown to provide an alternative pathway to grapevine micropropagation (Aazami et. al., 2010). This developed technique should result in clonal multiplication and uniform plants, normal yield and healthy plants. The propagation of grapes via micropropagation or tissue culture approach has been commercialized around the world. Micropropagation of selected *Vitis* genotype using the culture of intact or fragmented shoot apical meristems, axillary bud micro cuttings or through adventitious bud formation (Kurmi et. al., 2015). All types of grapevines do not give similar types of response for specific medium composition. The degree of response is highly dependent on the particular genotype, culture environment, culture medium and hormonal treatment. Hence, it is vital to develop new protocol for rapid multiplication of the available grape's varieties found in Ethiopia. Thus, this study aims to develop a protocol for multiple shoot regeneration from node through tissue culture.

MATERIAL AND METHODS:

The experiment was conducted in the biotechnology laboratory and fruit research farm of Department of Horticulture, Faculty of Agriculture, Guru Kashi University, Talwandi Sabo.

Plant Material: Grape rootstock "Flame Seedless" was selected for these studies. The explants were collected from vines grown on Y-trellis system in the orchard. The explants were prepared into appropriate size. After preparation the explants were washed with 2-3 drops of labolene with distilled water 15-20 minutes under tap water. **Surface Sterilization for Explants:** Surface sterilization is the most important step in preparation of explants for micropropagation, because controlling fungal and bacterial contamination of plant from field sources is very difficult. Three sterilizing agents: Bavistin (0.3% for 30 min), Ethanol (70% for 2 min), Mercuric Chloride (HgCl_2) 0.1% for (1,2,3,4 min) were used. The contamination usually decreases the propagation rate in in vitro cultures, causing reduced chlorophyll formation or rooting is seriously diminished so the plant material used for the culture was treated with an appropriate sterilizing agent to inactivate the microbes present on the surface. **Sterilization of media:** The media was sterilized by autoclaving at 121°C temperature, 15 psi pressure for 30-35 minutes. All the components of medium were added before autoclaving, except antibiotics. Then the media was poured in the pre-autoclaved cultured tubes and jars. **Inoculation and Incubation:** The surface sterilized explants were inoculated on MS media supplemented with various concentrations of BA, KIN and IBA under the hood of laminar air flow cabinet. The brown cut ends of explants were removed before inoculation to avoid toxic effects of sterilant and browning. After inoculation, the culture jars and test tubes were incubated in incubation room at $25\pm 2^\circ\text{C}$ temperature in dark (24 hours) for 30-40 days and were subsequently exposed to 16 hours photoperiod for regeneration. About 3-4 weeks period was required for shoot proliferation. Subculture again for further multiple shoots induction. Regenerated multiple shoots were cut and individual shoots were placed in MS medium containing different concentrations of IBA and IAA for root induction. **Data analysis:** The collected raw data during experiment trial was transferred on excel sheet in Microsoft Excel. The experimental design used was CRD (completely randomized design) with four replications of each treatment. The data was analysed using the software OPSTAT developed by CCSHAU, Hisar.

Treatment details:

Table 1: Different treatment used for surface sterilization of explants cv. Flame Seedless

Treatment no.	Concentration of treatment (%)
S ₁	control
S ₂	Bavistin (0.3%) + Ethanol (70%) + HgCl_2 (0.1% for 1 min)
S ₃	Bavistin (0.3%) + Ethanol (70%) + HgCl_2 (0.1% for 2 min)
S ₄	Bavistin (0.3%) + Ethanol (70%) + HgCl_2 (0.1% for 3 min)
S ₅	Bavistin (0.3%) + Ethanol (70%) + HgCl_2 (0.1% for 4 min)

Table 2: Standardization of shoot proliferation

Treatment no.	Treatment (mg/l)
T ₁	MS Media (no growth regulator)
T ₂	MS Media + BA 1.0 + KIN 0.5
T ₃	MS Media + BA 1.5 + KIN 0.5
T ₄	MS Media + BA 2.0 + KIN 0.1
T ₅	MS Media + BA 2.5 + KIN 0.1
T ₆	MS Media + BA 3.0 + KIN 0.1

Table 3: Standardization of Rooting

Treatment no.	Treatment (mg/l)
G ₁	MS Media (no growth regulator)
G ₂	MS Media + IBA 1.0
G ₃	MS Media + IBA 1.5
G ₄	MS Media + IBA 2.0
G ₅	MS Media + IBA 2.5
G ₆	MS Media + IBA 3.0

RESULTS:

Different treatment used for surface sterilization of explants cv. Flame Seedless

From the above surface sterilizing treatments, the highest survival rate (60.00%) and lowest contamination percentage (40.00%) were observed in treatment containing Bavistin (0.3%) + Ethanol (70%) + HgCl₂ (0.1% for 3 min). The lowest survival rate (00.00%) and highest contamination percentage (100%) were observed in control.

Standardization of shoot proliferation

Out of six treatments the maximum shooting percentage (94.00%), highest number of shoots/explants (4.75) and maximum length of shoot/explant (3.75cm) were observed in MS media fortified with BA 2.0mg/l + KN 0.1mg/l. Lowest shooting percentage (10.50%), minimum number of shoots/explants (0.25) and minimum length of shoot (0.25 cm) were obtained in treatment that contained MS basal media.

Standardization of rooting

Maximum rooting percentage (80.00%), highest number of roots (4.25%) and maximum length of root (6.75 cm) were significantly obtained from treatment fortified with MS Media+ IBA 2.0 mg/l. Minimum rooting percentage (39.25%), lowest number of roots per plantlet (1.00) and minimum length of root (1.25cm) by MS basal media.

DISCUSSIONS:

Surface sterilization

Contamination percentage

Rout et al. (2000), surface sterilisation has a crucial role in lowering the microbial load, as demonstrated by the control treatment (S₁), which had the greatest contamination (100%). Depending on exposure duration, the combination of Bavistin, ethanol, and HgCl₂ demonstrated differing degrees of contamination reduction. Treatment S₄ (HgCl₂ for 3 minutes) had the lowest contamination (40%), suggesting that this treatment was the most successful in lowering contamination without damaging the explant tissue. This supports the results of Yadav et al. (2018), who highlighted the efficacy of short-duration mercuric chloride treatments for surface sterilization. However, prolonged exposure (S₅, 4 minutes) resulted in increased contamination (65.25%), possibly due to tissue damage that made the explants more susceptible to latent infections, similar to findings by Thomas and Ravindra (2000).

Survival percentage

The highest survival rate (60%) was likewise seen in S₄, indicating that the 3-minute HgCl₂ treatment achieves the best possible balance between tissue viability and contamination reduction. Even though there was less contamination, survival was lower (33.25%) after a shorter treatment (S₂, 1 minute) than the control, indicating insufficient sterilisation. Longer exposure (S₅, 4 minutes) lowered survival to 34.75%, suggesting that over-sterilization can be phytotoxic and supporting Anbazhagan et al. (2010)'s findings that over-sterilant exposure reduced explant viability. These results highlight how crucial it is to balance tissue survival and microbial control by maximising sterilisation time. Both too much and too little sterilisation have a detrimental effect on results.

Shoot proliferation

The impact of varying KN (Kinetin) and BA (Benzyladenine) concentrations on shoot growth in MS medium. The lowest shooting percentage (10.50%), number of shoots (0.25), and shoot length (0.25 cm) were observed in the growth regulator-free treatment (T₁). Treatment T₄ (MS + BA 2.0 + KN 0.1 mg/l) had the highest shooting percentage (94.00%), number of shoots (4.75), and shoot length (3.75 cm), indicating the best shoot proliferation. According to Ahmad et al. (2020) and Nasiruddin et al. (2021), where appropriate cytokinin levels promoted shoot proliferation in diverse plant species, this suggests the synergistic action of BA and KN in boosting shoot multiplication. Reduced proliferation was the outcome of either lower or greater BA concentrations, demonstrating the significance of hormone balance (Rout et al., 2006).

In vitro rooting

The data presented in Table 3 shows the effect of Indole-3-butyric acid (IBA) on in vitro rooting. The control treatment (G₁), which lacked growth regulators, showed the lowest rooting percentage (39.25%),

number of roots (1.00), and root length (1.25 cm). Supplementation with IBA significantly improved these parameters. The highest rooting percentage (80.00%), root number (4.25), and root length (6.75 cm) were recorded at 2.0 mg/L IBA (G₄). Similar findings were reported by Hossain et al. (2013) and Rout (2006), who also observed enhanced rooting with optimal concentrations of IBA in tissue culture. However, increasing the IBA concentration beyond 2.0 mg/L (as in G₅ and G₆) resulted in a decline in rooting efficiency, indicating that excessively high auxin levels may inhibit root induction (Ali et al., 2020).

Table 4: Effect of surface sterilization agents on survival and contamination of Grapes Var. Flame Seedless

Treatment no.	Concentration of treatment (%)	Contamination (%)	Survival (%)
S ₁	control	100	0.0
S ₂	Bavistin (0.3%) + Ethanol (70%) +HgCl ₂ (0.1% for 1 min)	66.75	33.25
S ₃	Bavistin (0.3%) + Ethanol (70%) +HgCl ₂ (0.1% for 2 min)	54.75	45.25
S ₄	Bavistin (0.3%) + Ethanol (70%) +HgCl ₂ (0.1% for 3 min)	40.00	60.00
S ₅	Bavistin (0.3%) + Ethanol (70%) +HgCl ₂ (0.1% for 4 min)	65.25	34.75
	C.D. at 5%	0.59	0.59
	SE(m)±	0.19	0.19
	C.V.	0.59	1.18

Table 5: Effect of growth hormones on shoot proliferation

Treatment no.	Treatment (mg/l)	Shooting (%)	No. of shoots	Length of shoots (cm)
T ₁	MS Media (no growth regulator)	10.50	0.25	0.25
T ₂	MS Media + BA 1.0 + KN 0.5	68.75	1.00	1.75
T ₃	MS Media + BA 1.5 + KN 0.5	75.25	1.75	1.75
T ₄	MS Media + BA 2.0 + KN 0.1	94.00	4.75	3.75
T ₅	MS Media + BA 2.5 + KN 0.1	80.25	2.25	2.00
T ₆	MS Media + BA 3.0 + KN 0.1	72.00	1.25	1.00
	C.D. at 5%	0.63	0.68	0.61
	SE(m)	0.21	0.23	0.20
	C.V.	0.63	24.34	23.32

Table 6: Effect of growth hormones on Rooting

Treatment no.	Treatment (mg/l)	Rooting (%)	No. of Roots	Length of Roots (cm)
G ₁	MS Media (no growth regulator)	39.25	1.00	1.25
G ₂	MS Media + IBA 1.0	46.75	2.00	3.00
G ₃	MS Media + IBA 1.5	58.25	3.25	4.75
G ₄	MS Media + IBA 2.0	80.00	4.25	6.75
G ₅	MS Media + IBA 2.5	62.00	3.75	5.00
G ₆	MS Media + IBA 3.0	52.75	2.50	4.25
	C.D. at 5%	0.61	0.63	0.61
	SE(m)	0.20	0.21	0.20
	C.V.	0.72	15.22	9.80

CONCLUSION

The highest survival rate and lowest contamination percentage were observed in treatment containing Bavistin (0.3%) + Ethanol (70%) + HgCl₂ (0.1% for 3 min). The maximum shooting percentage, highest number of shoots/explants and maximum length of shoot/explant were observed in MS media fortified with BA 2.0mg/l + KN 0.1mg/l. Maximum rooting percentage, highest number of roots and maximum length of root were significantly obtained from treatment fortified with MS Media+ IBA 2.0mg/l.

Scope For The Future

To increase the rate of multiplication for upcoming breeding initiatives, more research utilising various explants is required.

Author Declaration

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

Conflict Of Interests

The authors have not declared any conflict of interests.

Author Disclaimer

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during writing or editing of this manuscript.

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Data Availability Statement

No data was used for the research described in the article.

Competing Interests

Authors have declared that no competing interests exist.



PLATE-1: Explants on treatment S₁ (control) shown fungal contamination of explants



PLATE-2: In vitro shoot regeneration

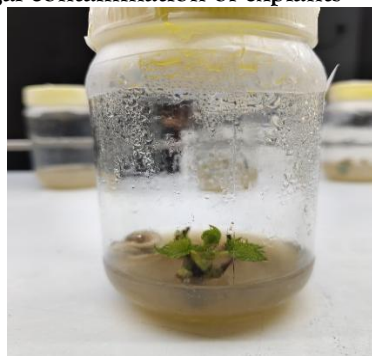


PLATE-3: In vitro rooting

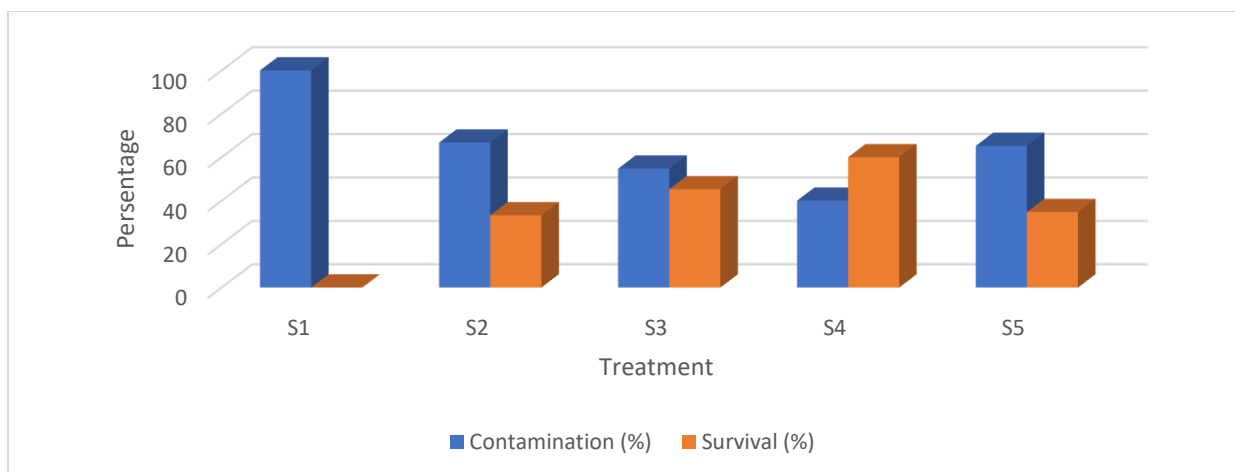


Figure-1: Influence of surface sterilization agents on survival and contamination of explant

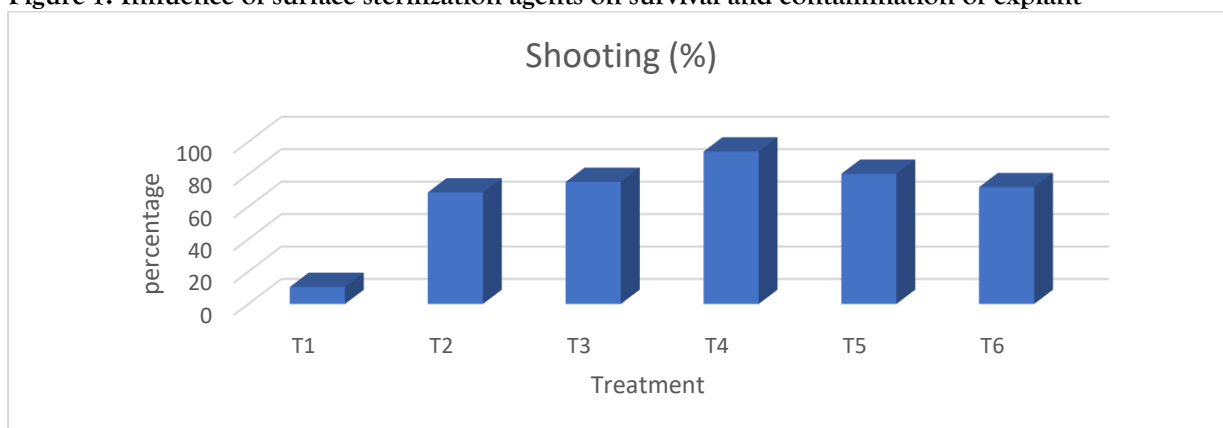


Figure-2: Influence of growth hormones on shooting (%)

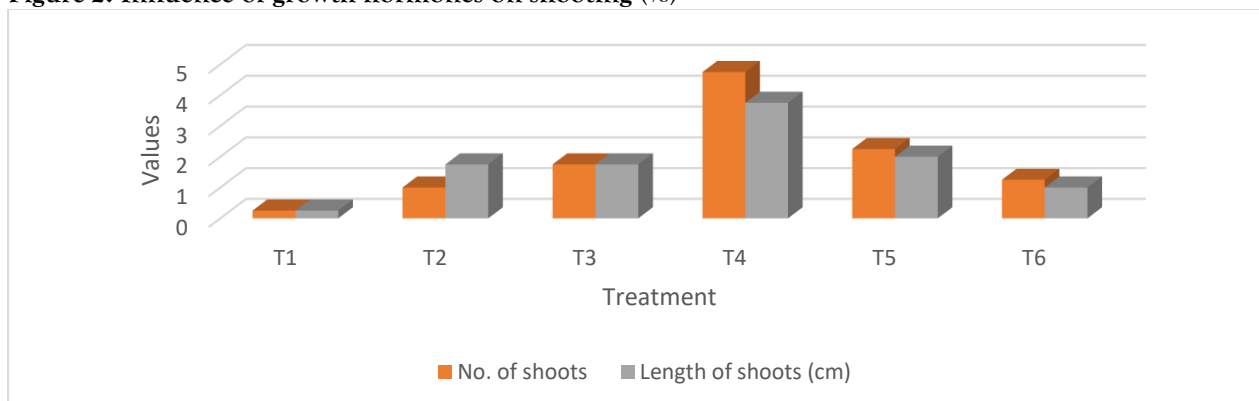


Figure-3: Influence of growth hormones on number of shoots and length of shoots (cm)

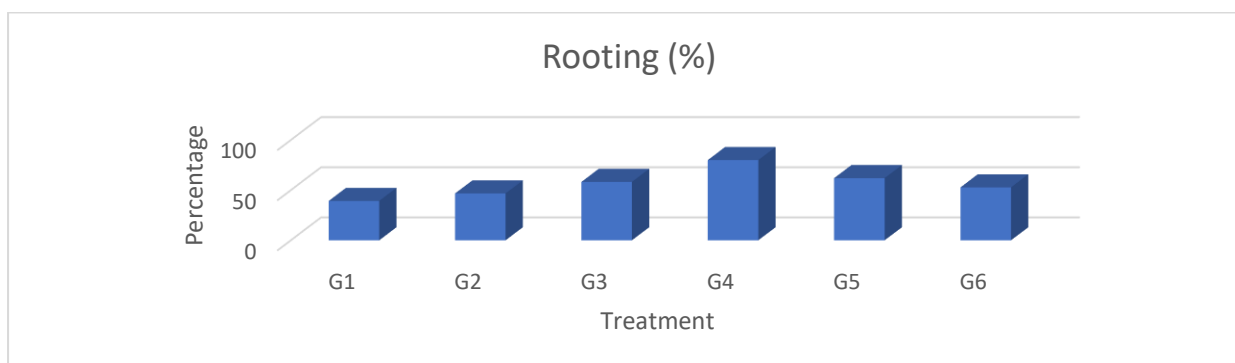


Figure-4: Influence of growth hormones on rooting (%)

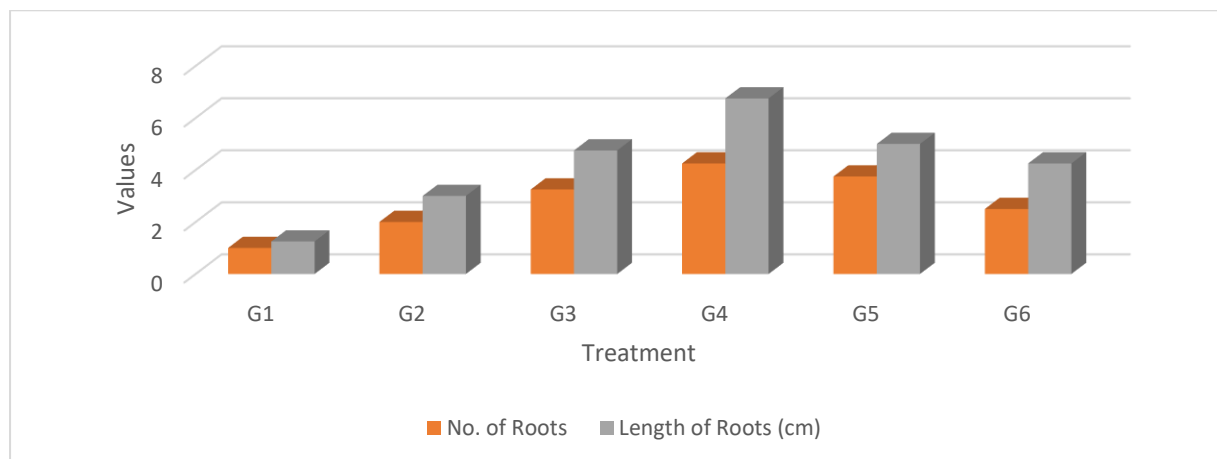


Figure-5: Influence of growth hormones on number of roots and length of roots (cm)

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