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An efficient and reliable protocol on *in vitro* propagation of 'Colt' (*Prunus avium* F 299/2 x *Prunus pseudocerasus* Lind.) rootstock of cherry

M.A. Mir[1], Ikra Manzoor[1]*, Ume Kulsum[2], Manpreet Kaur[3], Bisma Gulzar[1] and Suhail Manzoor[4]

[1]Division of Fruit Science, Faculty of Horticulture, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir, Shalimar, Srinagar-190025, India. [2]Division of Agricultural Statistics, Faculty of Horticulture, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir, Shalimar, Srinagar-190025, India. [3]Division of Botany, Punjab Agricultural University Ludhiana-141027, India. [4]Division of Plant Pathology, Faculty of Horticulture, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir, Shalimar, Srinagar-190025, India.
[*]E-mail: manzoorikra@gmail.com

Abstract

The 'Colt' is a triploid hybrid of *Prunus avium* and *P. pseudocerasus* grown as a cherry rootstock because of its resistance to cherry stem pitting (a debilitating virus disease), *Phytophthora* root rot, bacterial canker and gopher damage. The current study was carried out using two types of explants: forced (F_1) and unforced (F_2) shoots tips. Five types of sterilants were used to sterilize explants: 10% sodium hypochlorite (NaOCl) for 10 minutes (S1), 0.1% mercuric chloride ($HgCl_2$) for 5 minutes (S2), ethyl alcohol for 10 seconds (S3), (S1) + (S3) and (S2) + (S3). During culture establishment, six growth regulator regimes *viz.,* BA (benzyl adenine) (0.20), (0.40) and (0.60) and BA+IBA (Indole-3-butyric acid) (0.20+0.01), (0.40+0.01), and (0.60+0.01) mg/L, two media *i.e.*, MS (Murashige and Skoog, 1962) and WPM (Woody Plant Medium) were utilized. During shoot proliferation, MS & WPM culture medium with growth regulators *viz*., BA (0.20), (0.40) and (0.60), BA+IBA (0.20+0.01), (0.40+0.01) and (0.60+0.01) mg/L were used, respectively. During rhizogenesis in different media (MS & WPM), various levels of IBA (0.50, 1.00, 1.50 and 2.00 mg/L) were used. The superior rooted plants were hardened in sand & cocopeat, respectively. In this experiment, maximum culture asepsis (%) obtained in unforced shoot tips(F_2) with (S_5), maximum explant survival (%) in forced shoot tips (F_1) with (S_4), and highest establishment (%) with MS medium, BA (0.6) mg/L & in forced shoot tips (F_1). Significantly higher shoot proliferation is obtained with MS medium with BA (0.6) mg/L, maximum shoot number & length in WPM medium with BA (0.2) mg/L. A significantly higher rooting percentage was obtained in an MS medium with IBA (1.00) mg/L and maximum *ex vitro* survival (%) in cocopeat.

Key words: Colt, cherry, forced, unforced, cocopeat, hardening

Introduction

Cherry (*Prunus avium*) small stone fruits belonging to the family Rosaceae are high in K, Ca, Vitamins (A and C), Na, protein, carbohydrates, Mg, Fe, and fat, as well as antioxidants and anti-inflammatory compounds (Kelley *et al.*(2018). Cherries are thought to have originated In Asia Minor, between the Black Sea and the Caspian Sea (Webster, 1996). Turkey was the world's largest producer of cherries, producing approximately 874 thousand metric tons (Kassaye and Bekele, 2015). Cherry is the most abundantly grown temperate fruit in the world. Cherry trees, like the majority of fruit crops, are propagated by grafting the scion wood onto rootstock. Seedling rootstocks are not uniform, and tree vigour and bearing age vary greatly.

Furthermore, in recent years, the average land holding of orchardists has decreased, resulting in the replacement of traditional cherry orchards with standard trees with wider spacing with high-density planting systems with dwarfing clonal rootstocks (Kumar *et al.,* 2020). Nowadays, dwarf rootstocks of sweet cherry are used in the production of intensive orchards (Drkenda *et al.,* 2012). Compared to standard rootstocks, dwarf and semi-dwarf rootstocks are particularly helpful in increasing fruit quality and efficiency (Fallahpour *et al.,* 2015). In today's intensified fruit production conditions, there is a high demand for dwarf rootstocks, enabling restricted plant growth, resulting in trees with a compact crown, thus promoting easy harvesting and maintenance (Orlova *et al.,* 2019). Aside from the high productivity of grafted varieties, the advantages of modern clonal rootstocks include genetic homogeneity, compatibility with varieties, good fixation in soil and almost complete absence of root growth, resistance to changing environmental abiotic and biotic stress and ease of vegetative propagation (Tsafouros and Roussos, 2019). The rootstock is an integral part of any orchard management system. Dwarfing cherry rootstocks and newer cultivars have opened up new opportunities for developing high-density sweet cherry orchards. Colt' (*P.s avium* F 299/2 x *P. pseudocerasus* Lind) cherry rootstock belonging to the family Rosaceae originated in Kent, England, by H.M. Tyderman, late

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of East Malling, England. The East Malling Research Station in Maidstone, Kent, England, was used for pollination, evaluation, and testing (https://fps.ucdavis.edu/treedetails.cfm?v=3468). The 'Colt' cherry is a triploid hybrid of *P. avium* and *P. pseudocerasus* L. grown as a cherry rootstock (James *et al.,* 1984).

Because of its resistance to cherry stem pitting, a debilitating virus disease, 'Colt' has been widely planted. It is resistant to Phytophthora root rot, bacterial canker, and gopher damage but susceptible to crown gall. 'Colt' performs well in replant situations where cherries follow cherries on non-fumigated sites in the Pacific Northwest (PNW) (Long, 1995).

Without healthy planting material, fruit crop cultivation is currently impossible. Clonal micropropagation is a popular approach for commercially producing fruit stocks (Stanisavljevic *et al.,* 2017). Growing stone fruit crops on clonal rootstocks makes it easier to obtain sterile, virus-free planting stock (Orlova *et al.,* 2019). The advantages of clonal micropropagation of plants are relatively short reproductive phase and a higher yield of marketable produce (Hosseinpour *et al.,* 2015).

The clonal planting stock is commercially propagated using traditional methods such as mound layering. These methods face drawbacks like complete season dependence, lower multiplication ratio, the need for a lot of space and labour, and yield of unprofitable planting material. *In vitro* propagation techniques, on the other hand, provide an effective method of producing high-quality true-to-type planting material in less time and space (Lal *et al.,* 2022).

Over the last two decades, many researchers have used micro-propagation under suitable in-vitro conditions to produce healthy, true-to-type, disease-resistant plants and with desirable traits in cherry scions and rootstocks. Different genotypes require different micropropagation systems, such as explant type, culture establishment, sterilization techniques, rooting media, and hardening procedures. Furthermore, the levels and types of plant growth regulators differ and exhibit different responses with different explant types (Kumar *et al.,* 2020; Moghadam *et al.,* 2021). The present study aimed to develop a reliable and efficient protocol for fast *in vitro* propagation of cherry rootstock "Colt'' to boost/increase production.

Materials and methods

Sterilization and preparation of plant material: The current experiment was performed in the Tissue Culture Laboratory, Division of Fruit Science, SKUAST-Kashmir, Shalimar. The current study used two classes of explants: forced and unforced. The forced wood was harvested from mature standing stock plants of "colt" from the start of dormancy in November to December. To force the dormant wood 15-20 cm long shoot segments with latent buds (subterminal or apical potion) with 10-15 mm diameter were excised from "colt" cherry trees in bearing stage. This dormant wood was pre-treated with Captan at 0.2% concentration before being stored in poly-bags at 4 ± 3 ◦C until further use. Forcing was carried out following the procedure outlined by Dalal *et al.* (1992).

During the active growing season, active shoot tips (unforced) of 2-3 cm length were taken from mature cherry rootstocks of "colt" grown in the experimental block of SKUAST-K, Shalimar. Unforced explants were placed in flasks containing normal tap water and transported to the lab, where they were washed vigorously and kept under running water for at least 1 hour. In this experiment, five types of sterilants were used to sterilize explants: 10% sodium hypochlorite (NaOCl) for 10 minutes (S1), 0.1% HgCl2 for 5 min (S2), ethanol for 10 sec (S3), (S1)+ (S3) & (S2)+ (S3). Percent culture asepsis & Explant survival (%) was estimated four weeks after sterilization as follows:

Explant survival (%) = (Number of explants survived/Total number of explants sterilized) x 100

Culture asepsis (%) = (number of aseptic explants obtained /total number of explants sterilized) x 100

Culture media and culture conditions: After sterilization with $HgCl_2$/NaOCl, the explants were flushed in sterile water, patted dry with sterile tissue paper and cut with a sterile blade into 1.5-2.0 cm cuttings with a single bud. The prepared segments were then inoculated on MS medium (Murashige and Skoog, 1962) or Woody plant medium (Lloyd and McCown, 1980) containing macro and micro-salts and vitamins for establishment. Sucrose was kept at a concentration of 3%, myoinositol at 100 mg/L, and Plant Growth Regulators (PGR's) [auxins and cytokinins] at different concentrations and combinations *viz*; BA (0.20), (0.40)&(0.60) & BA+IBA concentrations of (0.20+0.01), (0.40+0.01) and (0.60+0.01) mg L^{-1}. Agar was used to solidify the media, which was kept at 5.7 pH. The culture vessels (test tubes or flasks) containing the prepared media were then sterilized in an autoclave at 121°C and 15 psi pressure for 15- 20 minutes.

Culture establishment: The cultures were incubated under controlled temperature and light conditions in a culture/growth room at 24°C & light: dark cycle of 16:8 hours. After 30 days of inoculation, the percentage of established cultures was calculated using the best explant type for future experiments.

Established cultures (%) = (Number of explants cultures established/ Total number of cultures inoculated) x 100

Proliferation: The sterile forced shoot tip cultures that established the primary culture medium were further split into nodal segments (0.5-1.0 cm long) for subculture. Such segments were carefully inserted on MS & WPM culture medium with BA (6-Benzylamino Purine) for proliferation at BA (0.20), (0.40), & (0.60); BA+IBA (0.020+0.01), (0.40+0.01), and (0.60+0.01) mg/L. The proliferation coefficients, plus the number and length of shoots (mm), were calculated after a post-4-week interval. The cultures were maintained at 25°C, an illumination level of 2000 Lx and light/dark cycles of 16/8 h.

Proliferation percentage (%) = (Number of buds regenerated /number of buds inoculated) × 100

Rooting: The sterile proliferated shoot tip cultures were rhizogenized in different media (MS & WPM) with varying levels of IBA (0.50), (1.00), (1.50), and (2.00) mg/L, respectively. The rooting coefficients and the number and length of roots (mm) were calculated post 4-week intervals during which the cultures were maintained at a temperature of 25°C, an illumination level of 2000 Lx and light/dark cycles of 16/8 h.

Rooting percentage (%) = (number of explants rooted/number of explants inoculated) × 100.

Acclimatization: The successfully developed plantlets were carefully removed from the culture flasks using long forceps to prevent plant injury. They were then washed with sterile water to remove the agar adhering to the roots. These plantlets were treated with 0.2 gl-1 Mancozeb solution (Aria, Iran) and placed in

covered jars with 100% cocopeat and sand. The pot tops were wrapped with clear plastic and grown in a shaded poly-house at 23°C, and to maintain humidity (90%), they were sprayed with water once a day. The plastic covers were removed after three weeks of plantation and the delicate plantlets were watered twice daily. After adaptation, the plants were transferred to larger pots with the same medium, which were then moved into a shade house with indirect sunlight & lower RH.

Survival percentage = (Number of the plants surviving after transplantation/number of plants transplanted) × 100.

Statistical analysis: The data recorded for different parameters during the entire course of experimentation were statistically analyzed using OPSTAT (v.6.8) under a completely randomized design (CRD) with three replications.

Results

Culture asepsis and explant establishment: For sterilization of explants, five types of sterilants have been used in this experiment *viz.,* 10% sodium hypochlorite (NaOCl) for 10 minutes (S_1), $HgCl_2$ for 5 minutes at 0.1% concentration (S_2), Ethyl alcohol for 10-second duration (S_3), 10% sodium hypochlorite (NaOCl) for 10 minutes followed by Ethyl alcohol for 10-second duration (S_4) & $HgCl_2$ (0.1%) for 5 minutes followed by Ethyl alcohol for 10 seconds (S_5) with two types of explants *viz*., forced (F_1) & unforced shoot tips(F_2). After about four weeks of sterilization, culture asepsis (%) & explant survival (%) were estimated with maximum culture asepsis (%) obtained in unforced shoot tips(F_2) with 0.1% $HgCl_2$ for 5 minutes + Ethyl alcohol for 10 seconds (S_5) (60.66 %) followed by forced shoot tips(F_1) with10% sodium hypochlorite (NaOCl) for 10 minutes + Ethyl alcohol for 10 seconds (S_4) (60.66 %)(55.80%) as shown in (Table 1). In this experiment, maximum explant survival (%) (60.33 %) was obtained in forced shoot tips (F_1) with 10% sodium hypochlorite (NaOCl) for 10 minutes, followed by Ethyl alcohol for 10 seconds (S_4) followed by unforced shoot tips (F_2) with 10% sodium hypochlorite (NaOCl) for 10 minutes (S_1) (50.66 %) as shown in (Table 1).

Table 1. Influence of sterilants and explant origin on Culture asepsis (%) & Explant survival (%) of cherry rootstock "Colt"(*P. avium* F 299/2 x *P. pseudocerasus* Lind)

Sterilants (S)	Culture asepsis (%)		Explant survival (%)	
	F_1	F_2	F_1	F_2
S_1	16.16	44.83	20.33	50.66
S_2	13.13	33.16	15.93	13.13
S_3	52.60	23.50	12.90	30.33
S_4	55.80	22.53	60.33	22.93
S_5	41.16	60.66	13.26	10.30
C.D($P \leq 0.05$)	1.08		0.98	

*The bold values respresented highest Culture asepsis (%) and Explant survival (%)

Six growth regulator regimes *viz.,* BA (benzyl adenine 0.20, 0.40 and 0.60) and BA+IBA (Indole-3-butyric acid) (0.20+0.01), (0.40+0.01), and (0.60+0.01) mg/L, two media *i.e.*, MS & WPM & two types of explants *viz*., forced (F_1) & unforced shoot tips (F_2) were utilized to standardize explant establishment in "Colt" rootstock of cherry. In this study, as shown in Table 2, highest

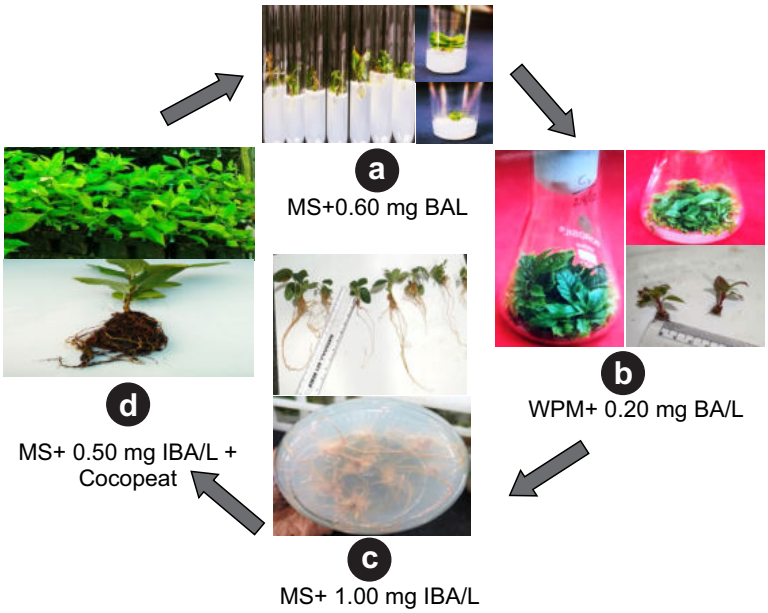

Fig.1. *In vitro* propagation of 'Colt' (*P. avium* F 299/2 x *P. pseudocerasus* Lind) rootstock of cherry. a) Culture establishment., b) Shooting ., c) Rooting., d) Hardening.

establishment percentage (83.33%) with MS medium, BA (0.6) mg/L & in forced shoot tips (F_1), followed by WPM medium, BA (0.2 and 0.4) mg/L and forced shoot tips (F_1) with (81.00%) depicted in (Fig. 1 a)

Table 2. Influence of growth regulators, media and explants source on Established cultures (%) of cherry rootstock "Colt" (*P. avium* F 299/2 x *P. pseudocerasus* Lind)

Growth regulator	Media	Explant	
		F1	F2
BA (0.20)	MS	75.00	64.33
	WPM	81.00	75.66
BA (0.40)	MS	65.00	54.66
	WPM	81.00	75.33
BA (0.60)	MS	83.33	76.66
	WPM	75.00	64.00
BA+IBA (0.20+0.01)	MS	61.00	57.66
	WPM	65.33	61.66
BA +IBA (0.40 + 0.01)	MS	57.00	46.66
	WPM	63.00	58.66
BA+IBA (0.60 + 0.01)	MS	68.33	59.66
	WPM	64.33	56.33
C.D($P \leq 0.05$)			1.00

Shoot proliferation: Since for subculture, the sterile forced shoot tip cultures that established successfully on the primary culture medium were cut into 0.5-1.0 cm long stem segments. These micro-shoots were then inoculated in MS & WPM supplemented with growth regulators *viz*., BA (0.20), (0.40) & (0.60), BA+IBA (0.20+0.01), (0.40+0.01) and (0.60+0.01) mg/L, respectively for shoot proliferation. Significantly higher shoot proliferation (92.00%) is obtained with MS medium with BA (0.6) mg/L followed by WPM medium with BA (0.2) mg/L (87.00%), (Table 3).

Maximum shoot number (5.83) in WPM medium with BA (0.2) mg/L followed by MS medium, BA (0.4) mg/L (Table 3) & maximum shoot length (16.16 mm) in WPM medium with BA (0.2) mg/L followed by WPM medium with BA+IBA (0.20+0.01) mg/L with (14.16mm) (Table 3, Fig. 1b).

Rhizogenesis and Hardening: The rooting characteristics of cherry rootstock "Colt" (rooting %, root number and root length (mm)) were observed after the sterile proliferated shoot tip cultures were subjected to

Table 3. Influence of sterilants and explant origin on proliferation (%), Number of shoots & Shoot length (mm) in forced explants of cherry rootstock "Colt"(*P. avium* F 299/2 x *P. pseudocerasus* Lind)

Plant Growth Regulators (P) (mg/L)	Proliferation (%)		Number of shoots		Shoot length (mm)	
	MS	WPM	MS	WPM	MS	WPM
BA (0.20)	82.00	87.00	3.83	5.83	13.16	16.16
BA (0.40)	72.33	85.00	4.33	3.30	10.26	9.06
BA (0.60)	92.00	76.16	4.16	3.63	10.16	7.70
BA+IBA (0.20+0.01)	79.00	81.33	3.16	5.06	12.33	14.16
BA+IBA (0.40+0.01)	68.66	80.66	3.88	2.85	9.16	8.43
BA+IBA (0.60+0.01)	86.33	71.66	3.06	2.91	8.26	6.76
C.D($P \leq 0.05$)	1.10		0.35		0.40	

*The bold values represented highest Proliferation (%), Number of shoots & Shoot length (mm)

Table 4. Influence of growth regulators and media on Rooting(%), Number of roots, Root length (mm) in forced explants of cherry rootstock "Colt" (*P. avium* F 299/2 x *P. pseudocerasus*

Plant growth regulators (P) (mg/L)	Rooting (%)		Number of roots		Root length (mm)	
	MS	WPM	MS	WPM	MS	WPM
IBA (0.50)	32.66	55.66	1.87	3.81	27.66	42.63
IBA (1.00)	90.66	23.66	5.92	0.99	70.16	21.00
IBA (1.50)	24.33	45.66	1.71	2.73	21.46	40.10
IBA (2.00)	85.66	20.00	4.75	0.91	61.63	18.66
C.D.($P \leq 0.05$)	2.71		0.04		1.22	

*The bold values represented highest rooting(%), number of roots, Root length (mm)

Table 5. Effect of growth regulators and media on *ex vitro* survival (%) in forced explants of cherry rootstock "Colt" (*P. avium* F 299/2 x *P. pseudocerasus* Lind)

Desired combination	H_1 (Cocopeat)	H_2 (Sand)
MS+ IBA (0.50mg/L)	74.97	58.33
MS+ IBA (1.00mg/L)	41.66	25.00
MS+IBA (1.50mg/L)	50.00	33.33
MS+ IBA (2.00mg/L)	25.02	16.69
C.D($P \leq 0.05$)	N/A	

*The bold values represented highest *ex vitro* survival (%)

rhizogenesis using different media (MS & WPM) with various levels of IBA (0.50), (1.00) (1.50), and (2.00) mg/L, respectively. A significantly higher rooting percentage (90.66 %) was attained in MS medium with IBA (1.00) mg L^{-1} followed by 2.00 mg/L IBA (85.66 %) (Table 4). The highest root number (5.92) was achieved with 1.00 mg/L IBA followed by 2.00 mg/L IBA (4.75) (Table 4). Also, maximum root length (70.16 mm) was achieved with 1.00 mg/L IBA followed by 2.00 mg/L IBA (61.63) (Table 4, Fig. 1c).

The plantlets with maximum rooting rate (%) were carefully removed from the culture vessels and cleaned thoroughly under running tap water to remove any residual agar adhering to roots. Those plantlets were then placed in covered jars containing cocopeat & sand (100%), respectively. Observations on the survival percentage of rooted plantlets were recorded after 4 weeks. In this experiment, it was found that maximum *ex vitro* survival (%) was observed in plantlets hardened with (74.97 %) success in cocopeat followed by sand (58.33 %) (Table 5, Fig. 1d).

Discussion

Culture asepsis (%), Explant survival (%) and Culture establishment (%): The present research has been done to standardize a protocol on *the in vitro* propagation of cherry rootstock 'Colt' (*P. avium* F 299/2 x *P. pseudocerasus* Lind). During sterilization regime maximum culture asepsis (%) was obtained in unforced shoot tips (F_2) with 0.1% $HgCl_2$ for 5 minutes followed by ethanol for 10 seconds (S_5) (60.66 %) which is supported by various researchers (Table 1), In the 'Oblacinska' rootstock of sour cherry, a minimum rate of contamination (up to 27%) was noticed in unforced (dormant buds) collected during November and December(Doric *et al.,* 2015).In peach explants, maximum aseptic cultures 63.34% were obtained with 70 % ethyl alcohol for 30 seconds, along with 0.1 % $HgCl_2$ 3 minutes and 1% NaOCl for 3 minutes (Bisht *et al.,* 2016).In quince (*Cydonia oblonga*) cv. SKAU-016optimum culture asepsis (55.99%) has been noticed with 0.1 % $HgCl_2$ for 10 minutes and 70% ethanol for 10 seconds (Basu *et al.,* 2017). In sweet cherry (*P. avium* L.) rootstocks Gisela 12 and Maxma 14, more culture asepsis (%), was obtained in commercial bleach (25%) and 0.01% and 0.03% of HgCl2 (*w/v*) (Jafarlou *et al.,* 2021). Low rates of contamination (26.58%) have been found in 0.1% HgCl2 and 70% Ethyl alcohol (70%) for 4 minutes in (*Musa paradisiaca* L.) var "Udhayam"(Yadav *et al.,* 2021). The mixture of 0.1% HgCl2 for 10 min and 70 % ethyl alcohol showed maximum success during sterilization in all three explants *viz.,* shoot tip (89.58%), nodal segment (89.57%) and leaf explants (83.33%) (Farooq *et al.,* 2021). A study was conducted by Sawant *et al.* (2021), where a protocol on surface sterilization was developed for 22 varieties of rice (*Oryza sativa* L.) using 1.0% $HgCl_2$ for 10 min followed by 70% ethyl alcohol for 1 minute with 86.67% aseptic cultures. As in this experiment Table 1, maximum explant survival (%) (60.33 %) was obtained in forced shoot tips (F_1) with 10% sodium hypochlorite (NaOCl) for 10 minutes, followed by ethanol for 10 seconds (S_4), which is supported by Dar *et al.* (2010) as in sour cherry (*Prunus cerasus* L.) rootstock, forced dormant cuttings showed higher rates of survival. In the forced explants in sweet cherry (*P. avium* L.), rootstock "Mazzard" showed a maximum survival rate (Peer *et al.,* 2011). The survival (90%) in apricot (*Prunus armeniaca* L.) is affected via 1.5 % NaOCl for 10 minutes (Faraq *et al.,* 2012). The forced explants in sweet cherry (*P. avium* L.) cv. "Bigarreau Noir Grossa" showed a maximum survival rate (Peer *et al.,* 2013). Also, Mihaljevic *et al.* (2013) showed that NaOCl gave explants survival (80%) in "Oblacinska" sour cherry.

Wolella (2017) showed a survival rate (97%) with 2% NaOCl for 15 minutes during *in vitro* studies on plum (*Prunus domestica* L.) *cv. Stanley.* In cherry rootstock CAB-6P, maximum explants survival (97 %) was noticed with NaOCl treatment (Stanisavljevic *et al.,* 2017). A protocol was developed to sterilize nodal explants in thirteen peach accessions *viz*., Flame crust, Early green, Florida king, Spring crust, 669 No.2., No.2, Early grand, A7, Clark king, 4.5, Chaki pakora, A4 and A2. During this study, it was found that NaOCl 28% for 15 minutes and 70% Ethyl alcohol for 1 minute gave complete survival (100%) in four accessions *viz*., 669 No.2, Early green, 4.5, A7, and Clark king as reported by Irum *et al.* (2020). In *Juglans regia* L. cv. Sulaiman (Persian walnut) forced shoot tips depicted the highest rates of survival (74.5 ±2.4%) (Bhat *et al.,* 2022). In this experiment (Table 2), the highest establishment percentage (83.33%) was achieved using MS medium supplemented with 0.6 mg/L BA (6-benzyl amino purine) in forced shoot tips (F1), as shown in Figure 1a. This result aligns with the findings of Snir (1982), who reported maximum bud establishment in *in vitro* cultures of four sweet cherry (*P. avium L.*) cultivars using MS medium with 1 mg/L BAP. Similarly, Pruski (2007) demonstrated that the *in vitro* establishment of cultures

in Mongolian cherry (*Prunus fruticosa L.*) and Nanking cherry (*Prunus tomentosa* L.) was enhanced when MSMO (Murashige and Skoog Minimal Organic) solid medium was fortified with either 4.44 µM or 8.88 µM BA. In MS medium supplemented with 1.5 mg/L BAP (6-Benzylaminopurine), the highest culture establishment has been found on in rough lemon (*Citrus jambhiri* Lush.)(Kour and Singh, 2012). Farag *et al.*(2012) reported that MS medium was best for culture establishment in apricot (*Prunus armeniaca* L.). In pomegranate, the highest culture establishment (68.5 %) has been found in MS medium treated with 1.0 mg/L BAP (Singh and Patel, 2016). Thakur *et al.* (2016) reported that during *in vitro* propagation in Gisela-5 rootstock (*Prunus cerasus* x *Prunus canescens*) 0.1 % HgCl2 for 5 minutes has been found best for establishment of buds July (70%) which is followed by February (51.50%), compared to 50(%) in May on MS medium fortified with 0.5 mg/L BAP. Lizzarraga *et al.* (2017) found that in 8 cultivars of apple culture establishment (%) protocol was developed using MS medium fortified in BA (1.0 mg/L). Highest establishment of cultures was obtained with MS medium augmented with 1.5 mg/L of BAP using tips of shoots (97.90%) and segments of nodes (95.74%), respectively (Farooq *et al.,* 2021).

Significantly higher shoot proliferation (92.00%) is obtained with MS medium with 0.6 mg/L BAP Table 3, supported by Larraburu *et al.* (2007), who used BA with MS medium for *in vitro* studies of Photinia maximum proliferation success was found for 24 months. In jojoba, maximum internodal and apical cuttings proliferate on MS medium (Lambardi *et al.,* 2013). In chokeberry during *in vitro* culture, MS medium has been used for initiation and proliferation, respectively (Litwińczuk, 2013). In *in vitro* studies of paradise tree (*Melia azedarach*), the maximum multiplication rate (of micro-shoots) was observed on MS medium amended with BAP (0.5 mg/L) (Mroginski and Rey, 2013). Ray *et al.*(2013) reported that MS basal medium fortified with different plant growth regulator combinations upon various stages of development in *Cordyline terminalis* gave maximum regeneration (95 ± 2.8). Renu and Singh (2018) noticed the highest proliferation of shoots using MS basal medium in Red Fuji apple cultivar (*Malus* × *domestica* Borkh.). In MS medium, the highest multiplication of shoots was found in Gisela 12 followed by Maxma 14 (6.2 shoots/explant & 4.4 shoots/explant, respectively) (Jafarlou *et al.,* 2021).

Hosseinpour *et al.* (2015) reported that in M × M60 cherry rootstock BAP is important for on proliferation and elongation in shoots. During tissue culture of 'Gisela 5' cherry rootstock, BA(0.5mg/L) had the highest proliferation rates (Borsoi *et al.,* 2020) since N6-benzyladenine (BA) is a cytokinin used mostly during initiation and proliferation stages of 'Gisela 5' rootstock of cherry at varying concentrations (Thakur *et al.,* 2012).

Maximum shoot number (5.83) in WPM medium with BA (0.2) mg/L and maximum shoot length (16.16 mm) in WPM medium with BA (0.2) mg/L Table 3 Figure 1b as supported by Sisko (2011) that reported WPM resulted in the maximum rate of shoot multiplication in 'Gisela 5' cherry rootstock (4.2 shoots/ explant). Agahye *et al.* (2013) found that in Gisela 6, the semi-dwarf rootstock of cherry WPM media showed the maximum height of shoots. Fallapour *et al.* (2015) reported similar results while micropropagation of 'Gisela 5' (rootstock of cherry); WPM showed the maximum number of shoots. N6-benzyl adenine (BA) is one of the effective cytokinins for elongation of shoots in various woody plants *viz.,* Saponari *et al.* (1999)in *Prunus cerasus*, Mansseri-Lamrioui *et al.*(2009) in *Prunus insigna,* and Sharma *et al.*(2017) in *Prunus avium*.

Rhizogenesis and hardening: A significantly higher rooting percentage (90.66 %) was recorded using MS basal medium with 1.00 mg/L of IBA. The highest root number (5.92) is obtained in MS medium supplemented with 1.00 mg/L of IBA . Also, the maximum root length (70.16 mm) was reached in MS medium augmented with 1.00 mg/L of IBA (Table 4, Figure 1c). This conforms with the study of various workers for *in vitro* propagation of different rootstocks of cherry (Sisko, 2011; Canli and Demir, 2014; Sarropoulou *et al.,* 2014; Xu *et al.,* 2015; Zamanipour *et al.,* 2015). Muna *et al.* (2000) found the highest rate of rooting (%) Maxama-14 rootstock of cherry at minimal IBA levels MS medium.In *Diospyros kaki* L. *cv*. "Kaki tipo" is culture of shoots in a growth medium containing IBA (1 mg/L) (Scaramucci, 2007). Similarly, Mir *et al* .(2010) and Aghaye *et al.*(2013) depicted that during *in vitro* rhizogenesis in ½ MS medium fortified in IBA in Mazzard, Mahaleb and Gisela 6 cherry rootstocks. In jojoba, maximum rooting is found in ½ MS medium with 14.7 µ M IBA (Lambardi *et al.,* 2013). In paradise tree (*Melia azedarach*) maximum rooting was found in MS medium with IBA (0.1 mg/L) during *in vitro* studies (Mroginski and Rey, 2013). Also, in Tetra (*Prunus empyrean*), the rootstock of cherry maximum rooting was found in medium with IBA than IAA (Sadeghi *et al.,* 2015). In ½ MS medium with IBA (1 mg/L), sour cherry (*Prunus cerasus* L.) 'Oblacinska' rooting percentages of 71.3% and 81.3% was obtained in 'OV 17' and 'OV 32'accessions, respectively, (Doric *et al.,* 2015). During tissue culture in cherry birch (*Betulalenta* L.), it was found that 80% of shoots formed roots in ½ MS medium fortified with 10 µM of IBA (Rathwell *et al.,* 2016) . *In vitro* rhizogenesis in clonal rootstock of cherry Gisela 5 (*Prunus cerasus* × *Prunus canescens*), showed maximum rooting (100%) on IBA (0.5 mg/L) with MS medium(Kumar *et al.,* 2020). During *in vitro* rooting of MA×MA60 semi-dwarf sweet cherry rootstock, the maximum rooting percentage (75%) and Number of roots (5.33) was found in MS medium (Moghadam *et al.,* 2021). Under *in vitro* studies in Gisela 6 semi-dwarf cherry rootstock**,** maximum rooting (100%) was in Gisela 12 and (88.88%) Maxma 14 in half MS +1.00 mg/L of IBA (Jafarlou *et al.,* 2021). During this experiment, it was found that maximum *ex vitro* survival (%) was observed in plantlets hardened with (74.97 %) success in cocopeat (Table 5, Figure 1d) . Various authors favour our research, *viz*., Kumar *et al.*(2016), who have published their research in Apple CV. Golden Delicious, Cuko *et al.* (2017) in two cvs. of apple *viz.*, Golden Delicious and Starking Delicious, Batool *et al.*(2020) in banana, Kumar *et al.*(2020) in Gisela 5 (cherry rootstock) & Mon *et al.*(2021) in banana with superior survival (%). Coco peat refers to the fiber of coir with pith of husk of coconut at the base, giving ventilation to roots for proper growth (Van Son, 2007). Cocopeat has pores that aid in giving channels for aeration and drainage Well-rooted plantlets were hardened with cocopeat in 'Bhagwa' variety of pomegranate with excellent results(Desai *et al.,* 2018).

This study establishes an optimized three-month protocol for *in vitro* propagation of the 'Colt' cherry rootstock (*P. avium* F 299/2 x *P. pseudocerasus* Lind). Maximum culture asepsis (60.66%) was achieved using unforced shoot tips treated with 0.1% $HgCl_2$ and ethyl alcohol, while forced shoot tips treated with NaOCl and ethyl alcohol showed the highest explant survival (60.33%). The

best establishment rate (83.33%) occurred on MS medium with 0.6 mg/L BA using forced shoot tips.Shoot proliferation (92%), maximum shoot number (5.83), and length (16.16 mm) were achieved on MS and WPM media with BA. Rooting was most efficient (90.66%) on MS medium with 1.0 mg/L IBA, yielding the longest roots (70.16 mm). Hardened plantlets had a 74.97% ex vitro survival rate. This protocol offers a rapid and effective propagation method for 'Colt' rootstock.

The study revealed that different sterilization methods influenced the sterilization of explants, growth regulators enhanced the average number of shoots per explant, and various media improved rooting of local 'Colt' cherry rootstock. In general terms, the MS medium with BA (0.6 mg/L) was the most appropriate for plant development in the case of forced shoot tips whereas in forced shoot tips had the highest *ex vitro* efficiency cocopeat.

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References

Aghaye, R. N.M., A. Yadollahi, A. Moeini and S. Sepahvand, 2013. *In vitro* culture of Gisela 6 semi-dwarf rootstock. *J. Biodivers. Environ. Sci*., 7: 57-64.

Basu, Y.A., M.A. Mir, K.M. Bhatt and B.A. Mir, 2017. *In vitro* propagation of Cydonia oblonga cv. SKAU-016. *Int J CurrMicrobiol App Sci*., 6(9): 1865-1873.

Batool, T.S., M.S. Shoaib, P.A. Baloch, J. Akhtar and Q.M. Ali 2020 . Effects of different potting media during hardening of tissue-cultured raised banana plants. *Int J BiolBiotechnol*., 17: 83-86.

Bhat, S.N., A. Khalil, N. Nazir, M.A. Mir, I. Khan, S.S. Mubashir, M.S. Dar, S.H. Wani and M.A. Hossain, 2022 . *In vitro* Prevention of Browning in Persian Walnut (*Juglans regia* L.) cv. Sulaiman. *Int. J. Plant Biol.,* 13(3): 330-342.

Bisht, T.S., P.N. Singh and L. Rawat, 2016. Effect of explant type and surface sterilants on asepsis and survival culture for callus induction in peach. *J. hill agric*., 7(1): 36-40.

Borsai, O., D. Clapa, A. Magdea, M. Harta, A. Andrecan and V. Mitre, 2020. Effects of different culture media and plant growth regulators on micropropagation of **'**Gisela 5' cherry **r**ootstock. *Scientific Papers*., 64(1), 33-40.

Bosnjak, A.M., S. Keresa, I.H. Jercic and M. Baric, 2012. The effect of cytokinin type and explant orientation on axillary shoot proliferation and *in vitro* rooting of 'Gisela 5'cherry rootstock. *J Food Agric Environ*., *10*(3&4): 616-620.

Canli, F.A. and F. Demir, 2014. *In vitro* multiplication and rooting of 'F12-1'(*Prunus avium* L.) and 'Maxma 14'(*Prunus mahaleb* L.× *P. avium* L.) rootstocks. *Indian J Hortic*., 71(2): 145-150.

Çuko, B., V. Sota and E. Kongjika, 2017. Stabilisation of *in vitro* rhizogenesis of two apple (*Malus domestica* L.) cultivars during micropropagation. *Albanian J. Agric. Sci*., 257-262.

Dalal, M.A., B.B. Sharma and M.S. Rao, 1992. Studies on stock plant treatment and initiation culture mode in control of oxidative browning in *in vitro* cultures of grapevine. *Sci. Hortic*., 51(1-2): 35-41.

Dar, H. A., S.R. Singh, A.S. Sundouri, M.K. Sharma and K.K Srivastava, 2010. Micropropagation of sour cherry rootstocks by employing forced and unforced explants. *Indian J Hortic*., 67(4): 9-14.

Desai, P., G. Patil, B. Dholiya, S. Desai, F. Patel and S. Narayanan, 2018. Development of an efficient micropropagation protocol through axillary shoot proliferation for pomegranate variety 'Bhagwa'. *Ann. Agrar. Sci*., 16(4): 444-450.

Doric, D., V. Ognjanov, G. Barac, M. Ljubojevic, A. Pranjic, K . Dugalic and S. Ercisli, 2015. Use of *in vitro* propagation of Obla? inska? sour cherry in rootstock breeding. *Turk. J. Biol*., 39(4): 575-581.

Drkenda, P., A. Spahic, and A. Begic-Akagic, 2012 . Testing of'GiSela 5'and'Santa Lucia 64'cherry rootstocks in Bosnia and Herzegovina. *Acta Agric. Slov*., 99(2): 129-136

Fallahpour, M., S.M. Miri and N. Bouzari, 2015. Propagation Of 'Gisela 5'rootstock as affected by mineral composition of media and plant growth regulators. *J. Hortic. Res*., 23(1): 57-64.

Farag, K.M., N.M. Nagy, H.I. Soliman and M.E. Ahmed, 2012. *In vitro* propagation of apricot (*Prunus armeniaca* L.) growing at Saint Catherine valleys in Sinaipeninsula, Egypt. *J Agric. Env Sci DamUniv Egypt*., 11: 1-35.

Farooq, I., Z.A.Qadri, Z.A. Rather, I.T. Nazki, N. Banday, S. Rafiq, K.Z. Masoodi, A. Noureldeen and S. Mansoor, 2021. Optimization of an improved, efficient and rapid *in vitro* micropropagation protocol for *Petunia hybrida*Vilm. Cv."Bravo". *Saudi J. Biol. Sci*., 28(7): 3701-3709.

Foundation Plant Services *Prunus* Encyclopedia, https://fps.ucdavis.edu/treedetails.cfm?v=3468

Hosseinpour, B., N. Bouzari, Z. Didar, M. Masoumian, S.A. Ghaemmaghami, A. Ebrahimi, S.M. Mirabbasi S.M and A. Farvardin, 2015. High frequency *in vitro* propagation of M× M60, a cherry rootstock: the effects of culture media and growth regulators. *Iran. J. Genet. Plant. Breed*., 4(2): 28-36.

Irum, S., A.H. Shah, S. Erum, F.A. Khan, N. Asim, A. Khan, H. Qamar, W. Khan, U. Khan and S.A. Jan, 2020. Optimization studies for *in-vitro* culture establishment of selected peach (*Prunus persica* L.) germplasm. *Fresenius Environ. Bull*., 29(12 A): 11573-11580.

Jafarlou, A. M., S.P. Pirivatlo, B. Salehi and A.H.H. Mogbli, 2021. Establishing an efficient *in vitro* propagation system for sweet cherry rootstocks Gisela 12 and Maxma14 and assessment of genetic homogeneity by ISSR markers. *J Crop Sci Biotechnol*., 24(4): 449-460.

James, D. J., J.A. Passey, and S.B. Malhotra, 1984. Organogenesis in callus derived from stem and leaf tissues of apple and cherry rootstocks. *Plant Cell Tissue Organ Cult*., 3: 333-341.

Kassaye, E. and B.D. Bekele, B. D. 2015. *In vitro* optimization of the protocol for micropropagation of plum (*Prunus salicina* l. Var. Methley) from nodal explants. *Biotechnol Int.*, 8(4): 137-148.

Kelley, D. S., Y. Adkins and K.D. Laugero, K. D. 2018. A review of the health benefits of cherries. *Nutrients*, 10(3): 368.

Kour, K. and B. Singh, 2012. *In vitro* multiplication of rough lemon (*Citrus jambhiri* Lush.). *IOSR J. Agric. Vet. Sci*., 1(4): 5-9.

Kumar, R. S., C. Joshi and T.K. Nailwal, 2016. Callus induction and plant regeneration from leaf explants of apple (*Pyrus malus* L.) cv. golden delicious. *Int. J. Curr. Microbial. Appl. Sci*., 5(2): 502-510.

Kumar, A., V. Sharma and M. Thakur, 2020. *In vitro* rooting and hardening of clonal cherry rootstock Gisela 5 (*Prunus cerasus* × *Prunus canescens*). *Int. J. Agric. Sci*., 90(5): 1032-1035.

Lal, M., M. Jamwal, H. Singh, P. Bakshi, M. Sharma, B. Kumar and S.K. Sinha, 2022. Comparitive economics of *in vitro* grown plantlets of clonal apple MM 106 and MM 111 rootstocks. *AMA- AGR MECH ASIA AF*., 53: 6161-6171.

Lambardi, M., E.A. Ozudogru and S.M. Jain, 2013. *Protocols for micropropagation of selected economically-important horticultural plants* (Vol. 994). Hertfordshire, Humana Press.

Larraburu, E.E., S.M. Carletti, E.A. Rodríguez Cáceres and B.E. Llorente, 2007. Micropropagation of photinia employing rhizobacteria to promote root development. *Plant Cell Rep*., 26: 711-717.

Litwińczuk, W. 2013. Micropropagation of chokeberry by *in vitro* axillary shoot proliferation. *Methods Mol Biol*., 11013: 179-186.

Lizarraga, A., M. Fraga, J. Ascasibar and M.L. Gonzalez, 2017. *In vitro* propagation and recovery of eight apple and two pear cultivars held in a germplasm bank. *Am. J. Plant Sci*., 8(9): 2238-2254.

Lloyd, G. and B. McCown, 1980. Commercially-feasible micropropagation of mountain laurel, Kalmia latifolia, by use of shoot-tip culture. *Comb. Proc. Intl. Plant Prop. Soc.,* 30: 421-427.

Long, L. 1995. Colt rootstock may be answer for cherry replant problems. *Good Fruit Grower*, 46(4): 43-44.

Mansseri-Lamrioui, A., A. Louerguioui and A. Abousalim, 2009. Effect of the medium culture on the micro cutting of material resulting from adult cuttings of wild cherry trees (*Prunus avium* L.) and of *in vitro* germination. *Eur. Res. J*., 25:345-352.

Mihaljevic, I., K. Dugalic, V. Tomas, M. Viljevac, A. Pranjic, Z. Cmelik, B. Puskar and Z. Jurkovic, 2013. *In vitro* sterilization procedures for micropropagation of 'Oblacinska'sour cherry. *Indian J. Agric. Sci*., 58(2): 117-126.

Mir, J. I., N. Ahmed, M.K. Verma, M. Ahmad and S. Lal, 2010. *In-vitro* multiplication of cherry rootstocks. *Indian J. Hortic*., 67(4): 29-33.

Moghadam, E. G., T. Amuzegar, A. Asgharzadeh and M. Zamanipour, 2021. Study of factors affecting on the micropropagation of the maxma60 sweet cherry semi-dwarf rootstock. *Iranian J. Hortic. Sci*.., 52(3): 767-777.

Mon, Y. Y., K. T. Oo, K.K. Linn, Z.M. Lynn, Z. Hlaing and N.C.K. Ko, 2021. Evaluation of plant growth parameters by *in vitro* and *ex vitro* inoculation of micropropagated banana plantlets with rhizospheric and endophytic bacterial inoculum. *J. Sci. Innov. Res*., 10(1): 16-22.

Mroginski, L. A. and H.Y. Rey, 2013. Micropropagation of paradise tree (*Melia azedarach*) by *in vitro* culture of axillary buds. *Methods Mol Biol*., 11013: 223-230.

Muna, A. S., A.K. Ahmad, K. Mahmoud, and K.A. Rahman, 2000. *In vitro* propagation of a semi-dwarfing cherry rootstock. *Plant Cell Tissue Organ Cult.,* 59(3): 203-208.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Plant Physiol*., 15(3): 473-497.

Orlova, L. G., S.N. Shcheglov, and A.P. Kuznetsova, 2019. Study of new rootstocks for cherries in the nursery. *Fruit Growing Viticulture South Russia*, 58(4): 46-57.

Peer, F. A., K.D. Farooqui, K.R. Dar, M.Y. Bhat, G. Hussain and Z.A. Rather, 2011. *In vitro* microropogation of sweet cherry (*Prunus avium* L.) rootstock cv.'Mazzard'. *Appl. Biol. Res*., 13(1): 10-16.

Peer, F. A., Z.A. Rather, K.R. Dar, M.A. Mir and G. Hussain, 2013. Studies on *in vitro* propagation of sweet cherry cv. Bigarreau Noir Grossa. *Indian J Hortic, .* 70(3): 317-322.

Pruski, K. 2007. Tissue culture propagation of Mongolian cherry (*Prunus fruticosa* L.) and Nanking cherry (*Prunus tomentosa* L.). In *Protocols for micropropagation of woody trees and fruits*. pp.391-407.

Rathwell, R., M.R. Shukla, A.M.P. Jones and and P.K. Saxena, 2016. *In vitro* propagation of cherry birch (*Betula lenta* L.). *Can. J. Plant Sci*., 96(4): 571-578.

Ray, T., P. Saha and S.C. Roy, 2013. Micropropagation of Cordyline terminalis. *Methods Mol. Biol*., 11013:269-277.

Renu, B. A. and R. Singh, 2018. Optimization of micropropagation from nodal segments of apple (*Malus* × *domestica*. Borkh) cultivars Golden Delicious and Red Fuji. *Curr. Appl. Sci. Technol*., 31(3): 1-9.

Sadeghi, F., A. Yadollahi, M.J. Kermani and M. Eftekhari, 2015. Optimizing culture media for *in vitro* proliferation and rooting of Tetra (*Prunus empyrean* 3) rootstock. *J. Genet. Eng. Biotechnol*., 13(1): 19-23.

Saponari, M., G. Bottalico and V. Savino, 1999. *In vitro* propagation of *Prunus mahaleb* and its sanitation from Prune dwarf virus. *Adv. Hortic. Sci*., 13: 56-60.

Sarropoulou, V., K. Dimassi-Theriou and I. Therios, 2014) L-arginine impact on cherry rootstock rooting and biochemical characteristics in tissue culture. *Turk J. Agric. For*., 38(6): 887-897.

Sawant, G. B., S.V. Sawardekar, M.Y. Patil and S.M. Jadhav, 2021. Surface sterilization and callus induction potential of various indica rice (*Oryza sativa* L.) varieties. *Appl. Biol. Res*., 23(2): 147-156

Scaramucci, S. 2007. Study on *in vitro* rooting of *Diospyros kaki* L. *J. Fruit Vegetable Growing*, 69(12): 52-59.

Sharma, V., M. Thakur and A. Kumar, 2017. An efficient method for *in vitro* propagation of Gisela 5 (*Prunus cerasus* x *Prunus canescens*)-clonal cherry rootstock. *Int. J. Curr. Microbiol. Appl. Sci*., 6(8): 2617-24.

Sisko, M. 2011. *In vitro* propagation of Gisela 5 (*Prunus cerasus*× *P.canescens*). *Agricultura* (*Slovenia*), 8(1): 31-34.

Snir, I. 1982. *In vitro* Propagation of Sweet Cherry Cultivars . *HortScience*., 17(2): 192-193.

Stanisavljevic, A., D.Bosnjak, I. Stolfa, R. Vukovic, T. Kujundzic and M. Drenjancevic, 2017. Sterilization of different explant types in micropropagation of CAB-6P and Gisela 6 cherry rootstock. *Poljoprivreda*, 23(2): 31-37.

Thakur, M., V. Sharma, D.P. Sharma, G. Kumari and M. Vivek, 2016. *In vitro* propagation of virus indexed Gisela-5 (*Prunus cerasus* x *Prunus canescens*)-clonal cherry rootstock. *J. Crop Sci*., 2(2): 87-99.

Tsafouros, A. and P.A. Roussos 2019. First report of Krymsk® 5 (cv. VSL 2) cherry rootstock *in vitro* propagation: Studying the effect of cytokinins, auxins and endogenous sugars. *Not Bot Horti Agrobot ClujNapoca*, 47(1): 152-161.

Van Son, N. 2007. Response of gerbera (*Gerbera jamesonii* Bolus) varieties to micro-propagation. *Master of Science Thesis in Horticulture (Agriculture). University of Agricultural Sciences, Dharwad, India*.

Xu, J., I.K. Kang, C.K. Kim, J.S. Han, and C. Choi, 2015. Optimization of apical tip culture condition for *in vitro* propagation of'GiSela 5'dwarf cherry rootstock. *J. Plant Biotechnol*., 42*(*1): 49-54.

Yadav, A., Y. Prasad, M. Kumar, S. Pandey, R. Maurya and P. Pandey, 2021. Effects of Mercuric chloride and Ethanol for surface sterilization under *in vitro* plant growth in banana (*Musa paradisiaca* L.) variety "Udhayam". *J Appl. Pharmcog. Phytochem*., 10(1): 2281-2283.

Webster, A. D. 1996. The taxonomic classification of sweet and sour cherries and a brief history of their cultivation.pp.3-24 In: *Cherries: Crop Physiology, Production and Uses*.

Wolella, E. K. 2017. Surface sterilization and *in vitro* propagation of *Prunus domestica* L. cv. Stanley using axillary buds as explants. *J. Biotech. Res*., 8: 18.

Zamanipour, M., E.G. Moghadam, A. Tehranifar and B. Abedi, 2015. The effects of media, plant growth regulators and apex size on the success of meristem culture in *Prunus avium* cv pishras-e-mashhad. *Indian J. Fundam. Appl. Life Sci.*, 5: 924-929.

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