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In vitro studies on sterilization of clonal cherry rootstock "GiSelA 5" (*Prunus avium* x *Prunus cerasus* L.)

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Abstract
'GiSelA 5' rootstock of cherry is an outcome of (*Prunus cerasus* x *Prunus canescens* L.). It bears prominent features like great cold resistance, tolerance to a modest quantity of virus, and resistance to bacterial canker. This study optimizes a reliable protocol for *in vitro* sterilization for propagation of 'GiSelA 5' clonal rootstock of cherry.In this experiment, five separate sterilisation procedures *viz*., 1% sodium hypochlorite (NaClO) for five minutes (S1), 10% mercuric chloride (HgCl2) for ten minutes (S2), 70% ethyl alcohol for ten seconds (S3), (S4) : (S1) + (S3) and S5: (S2) + (S3) were followed. The results depicted that maximum culture asepsis (%) was found in shoot tips (E1) sterilized with (S4) regime while explant survival (%) was obtained with (S1) regime in shoot tips (E1). This study yields valuable insights into a dependable and effective method for sterilization for producing high-quality plant material for commercial plantations of cherry.

Keywords: Cherry, sterilization, canker, virus, shoot tips

Introduction
Cherries are a delightful variety of stone fruit found in the Rosaceae family's genus Prunus. Prunus avium, commonly known as the sweet cherry (or sometimes termed the wild cherry), and *Prunus cerasus*, also known as the sour cherry, provide the majority of edible cherries. Cherry culture is claimed to have originated in Asia Minor, which is the area between the Black and Caspian seas (Webster, 1996) [1].
Commercial pruning rootstocks are made from stem cuttings or seeds. Segregation takes place during the creation of rootstocks from seeds. Plants are unable to develop regularly and the traits of the mother plant are not preserved. Cuttings can also be employed in the common clonal propagation method of creating rootstocks, which produces homogenous propagules. However, cutting-based multiplication may be difficult for some *Prunus genotypes* because to their weak rooting ability (Fachinello, 2000) [2], and it does not guarantee healthy, disease-free plants (Holtz *et al*. 1995) [3]. The 'GiSelA 5' sweet cherry rootstock is grown from greenwood, soft, or hardwood cuttings (Exadaktylou *et al*. 2009) [4]. Conventional propagation methods yield lower efficiency for these clonal rootstocks (Bosnjak *et al*. 2012) [5]. However, because micropropagation is not season-dependent and yields clean, disease- and virus-free planting material, it has also been shown to be an effective alternative propagation technique in the development of cherry rootstock. (Thakur *et al*. (2016) [6]; Sharma *et al*. (2017) [7]; Borsoi *et al*. (2020) [8]; Tsafouros and Roussos (2024) [9]. Developing an effective *in vitro* sterilization regime for 'GiSelA 5' rootstock was the aim of the current investigation.

Materials and Methods
Sterilization and Preparation of planting material
The current study was conducted at the Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir, Shalimar, at the Tissue Culture Laboratory, Division of Fruit Science. Two types of explants, namely shoot tips (E1) and nodal segments (E2), were used in this investigation. From April 2022 to September 2023, shoot cuttings from the appropriate mother plants of the 'GiSelA 5' rootstock were obtained. These cuttings were then kept in glass jars with regular tap water to prevent wilting until they were processed further in the lab. Then, without harming the buds in the axil and terminal positions, shoot cuttings were defoliated.

Five sterilant regimes—0.1% HgCl2 for five minutes (S1), 5% NaOCl for ten minutes (S2), 70% ethanol for ten seconds (S3), (S4): (S1)+ (S3), and (S5): (S2)+ (S3)—have been chosen for the investigation procured from HiMedia company in order to sterilise the explant material. The following parameters were noted following four weeks of sterilisation: culture asepsis (%) and explant survival (%):

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

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

Culture media and conditions

After being sterilized with HgCl2/NaOCl, the explant material was cleaned with distilled water, dried with sterile tissue paper, and cut into 1.5–2.0 cm shoots with a single bud. These shoots were then placed for inoculation using MS medium (Murashige and Skoog, 1962) [10] that was enriched with macro and micro-salts, vitamins, and other nutrients to establish the cultures. The culture vessels that were filled with media were placed in an autoclave for 15 to 20 minutes at 121 °C and 15 psi of pressure to sterilise them. The cultures are being incubated in a growth room with a controlled environment (temperature and light) at a temperature of 24 °C and a photoperiod of 16:8 hours.

Data Analysis

The data calculated had been analyzed during this research following OPSTAT (v.6.8) software with CD(p<0.05) under a completely randomized design (CRD) with three number of replications.

Results and Discussions

Culture asepsis (%)

Five sterilant regimes were used in the current investigation during the sterilization step in explants: HgCl2 (0.1%) for five minutes (S1), NaOCl (5%) for ten minutes (S2), ethanol for ten seconds (S3), (S4): (S1) + (S3), and (S5): (S2) + (S3). Shoot tip (E1) and nodal segments (E2) were the explants. The parameters of culture asepsis (%) and explant survival (%) were measured thirty days after sterilisation. The highest percentage of culture asepsis (96.66%) was observed in shoot tips (E1) inoculated on MS medium containing HgCl2 (0.1%) for five minutes plus 70% ethanol (10 seconds) (S4), followed by nodal segments (E2) using MS medium containing HgCl2 (0.1%). 5 minutes plus 70% ethanol for 10 seconds (S4) combined with 93.33% culture asepsis (Table 1), (“Figure 1A, 2A”). Our study is confirmed by (Bisht *et al*. 2016) [11] who reported that peach explants exhibited the greatest aseptic cultures of 63.34% after 30 seconds of 70% ethyl alcohol addition and three minutes of 0.1% HgCl2 addition. The application of 0.1% HgCl2 for five minutes produced the greatest proportion of aseptic cultures in ‘GiSelA 5’ rootstock (Sharma *et al*. 2017) [7].As per (Doric *et al*. 2015) [12], *in vitro* experiments carried out on Oblačinskam sour cherries demonstrated that the maximum proportion of asepsis was noted when dormant twigs were exposed to the fungicide Previcur (5%) for 30 minutes, followed by a 5-minute immersion in 0.1% mercuric chloride, and a 1-minute immersion in 70% ethanol with 0.1% Tween. According to (Yadav *et al*. 2021) [13], after 4 minutes at 70% ethanol and 0.1% HgCl2, *Musa paradisiaca* L. var. "Udhayam" displayed modest rates of contamination (26.58%). According to (Antony *et al*., 2015) [14], the highest rate of asepsis and bud rupture was observed while sterilising explants with 0.1% HgCl2 for five minutes when working with teak (*Tectona grandis*). Applying 0.05 percent HgCl2 for five minutes was shown to be the most effective method for maximally sterilising the surface of walnut embryos when fruit crops were micro propagated (Lal *et al*. 2022) [15]. (Hossini *et al*. 2010) [16] and (Muna *et al*. 1999) [17] showed a successful reduction of contamination during the *in vitro* establishment of cherry rootstocks after using HgCl2 treatment.

Explant Survival (%)

The current investigation indicated that the highest explant survival (93.33%) was achieved with shoot tips (E1) and HgCl2 (0.1%) for five minutes (S1). This was followed by nodal segments (E2) and WPM medium (90%) with 70% ethanol and 10 seconds (S3) (Table 1), (“Figure 1B,2B”).Our study is supported by many workers, Kumar *et al*. (2019) [18] who demonstrated a significant improvement in culture survival percentage (73.2%) and uniform and strong shoot growth in sugarcane variety Co. 0118 explants treated with 0.1% HgCl2s for 5 minutes as compared to other treatments. Similar findings were found by Sawant and Tawar (2011) [19] in their investigation on commercial sugarcane micropropagation. Additionally, *in vitro* cultures of Curculigo latifolia and sugarcane, respectively, Babaei *et al*. (2013) [20] and Tiwari *et al*. (2012) [21] found that 0.1% HgCl2 for 5 minutes was an effective therapy for explant surface sterilisation with negligible tissue necrosis. According to Hashim *et al*. (2021) [22], surface sterilising explants with 0.2% HgCl2 produced the highest percentage of explant viability in the instance of *Clinacanthus nutans* grown *in vitro*.

Table 1. Effect of sterilants and explant type on Culture asepsis (%) & Explant survival (%) of cherry rootstock ‘ GiSelA 5’ (*Prunus cerasus* x *Prunus canescens* L.)

Sterilant (S)	Culture asepsis (%)				Explant survival (%)			
	M_1 (MS)		M_2 (WPM)		M_1M_2 (MS) (WPM)		M_1M_1 (MS) (WPM)	
	E1	**E2**	**E1**	**E2**	**E1**	**E2**	**E1**	**E2**
S1	83.33	80.00	80.00	76.66	93.33	90.00	86.66	83.33
S2	50.00	46.66	43.33	36.66	53.33	50.00	46.66ss	46.66
S3	66.66	63.33	60.00	56.66	83.33	80.00	80.00	76.66
S4	96.66	93.33	90.00	86.66	63.00	60.00	60.00	56.66
S5	80.00	76.66	73.33	70.00	76.66	73.33	70.00	66.66
CD (p<0.05) 0.64					CD (p<0.05) 0.75			

The bold values represented highest Culture asepsis (%) &Explant survival (%)

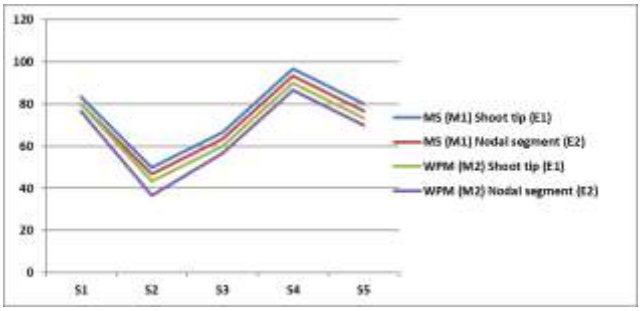

Fig 1A: Effect of sterilants and explant type on Culture asepsis (%)

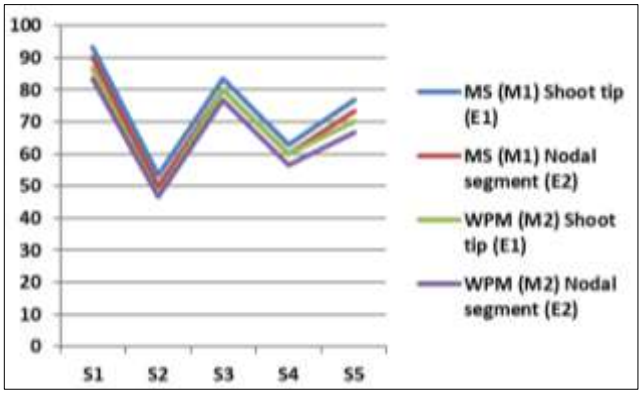

Fig 1B: Effect of sterilants and explant type on Explant survival (%)

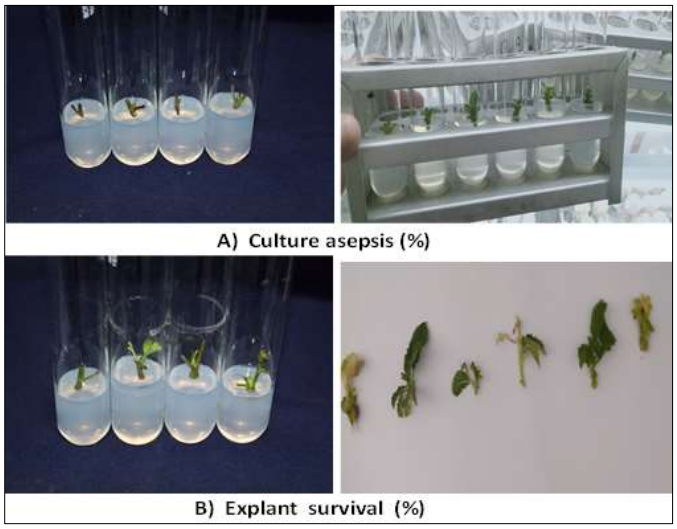

Fig 2: *In vitro* sterilization of clonal rootstock of cherry 'GiSelA 5' (*Prunus cerasus* x *Prunus canescens* L.)

Conclusion

This work demonstrates the most effective method for the *in vitro* sterilization of the 'GiSelA-5' (*Prunus cerasus* x *Prunus canescens*) cherry rootstock. The shortest amount of time—six months—was spent developing this strategy. The maximum culture asepsis (%) was achieved in shoot tips (E1) using 0.1% HgCl2 for 5 minutes plus ethyl alcohol (70%) for 10 seconds (S4) (94.99%), whereas shoot tips (E1) had the most percentage of explant survival (%) after using 0.1% HgCl2 as the only sterilising treatment for five minutes (S1). This inclusive sterilization protocol provides a reliable and efficient way to advance the propagation of the 'GiSelA 5' cherry clonal rootstock under *in vitro* conditions.

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