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Short Communications

In vitro rooting and hardening of clonal cherry rootstock Gisela 5 (*Prunus cerasus* × *Prunus canescens*)

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Cherry occupies an important position among temperate fruits worldwide. Cherry fruits have been a favourite human food for centuries. The fruits are undoubtedly attractive in appearance, on account of their bright, shiny skin colour, subtle flavour and sweetness which is of great appeal to the consumers. Cherries are usually grown in the cold climates at an altitude of about 1600 to 2700 m above the mean sea level requiring 1000-1500 hours of chilling period during winters.

Like majority of fruit crops cherry trees are also propagated by grafting the scion wood on rootstock. Seedling rootstocks are not uniform and show great variability in tree vigour and bearing age. These difficulties can be overcome by the use of dwarfing clonal rootstocks. A new series of dwarfing rootstocks for cherry, known as 'Gisela' or the Geissen series were developed in Germany. Gisela 5 is considered as very useful and economically important dwarfing rootstocks for intensive sweet cherry growing in temperate conditions. It was developed from the cross between *Prunus cerasus* and *Prunus canescens*. Propagation of Gisela 5 is not efficient through conventional methods. In these circumstances, *in vitro* propagation of rootstocks can be a viable alternative to conventional propagation. Therefore, the present study was conducted to develop a protocol for high efficiency rooting and hardening of Gisela 5 cherry rootstock for its commercial exploitation.

The *in vitro* multiplied shoot cultures maintained in the Department of Biotechnology, YSP UHF, Nauni, Solan, were used as source of micro shoots during the year 2017-18 (Sharma *et al*. 2017). Micro shoots from 4 weeks old cultures were used to make cuttings of approximately 2-3 cm long for root induction. The lower leaves were removed from micro shoots and cultured for rooting.

In single step procedure of rooting, shoots were cultured on full and half strength MS medium supplemented with different concentrations of auxins, viz. IBA, IAA and NAA. The cultures were incubated in dark for 24 hours and then transferred under fluorescent light. In two step procedure, shoots were kept in different concentrations of auxins in half strength MS broth for 24 hr under dark conditions and then transferred to auxin free half strength MS medium and kept under light for root induction and elongation. Additives like activated charcoal (0.1%) and phloroglucinol (0.2%) were also added to the rooting medium. In all rooting experiments 4 g/l agar was used for solidification of medium which allows easy removal of plantlets for hardening. After rooting, the rooted shoots were washed gently under running tap water for 1-2 hr to remove agar adhering to it. Plantlets were treated with fungicide solution (0.5% Carbendazime) for 30 minutes. Small pots were filled with different potting mixture, i.e. Soil: Sand: FYM (1:1:1); Cocopeat; Sand; Sand: FYM (1:1); Cocopeat: Sand (1:1) and treated plantlets were transplanted in it. Plantlets were watered and covered with glass jars in order to maintain high relative humidity (80%) around the plantlets. After 15 days when the plants showed initial signs of establishment, humidity was decreased by removing the glass jars for few minutes. Thereafter, the plants were hardened by removing the glass jars for increased time intervals, hence, reducing the relative humidity gradually. They were irrigated whenever required and sprayed with fungicide solution (0.1% carbendazime) to check the fungal attack. After 4-5 weeks, glass jars were removed completely, depending upon the growth of plantlets. Eight weeks old hardened plantlets were transferred to big earthen pots containing Soil: Sand: FYM (1:1:1). Survival and growth of plants were observed after 2-3 weeks of transfer.

The induction of roots *in vitro* is an important step in micropropagation of plants. In our studies, effect of different auxins, i.e. IBA, IAA, NAA and antioxidants, i.e. activated charcoal and phloroglucinol on *in vitro* rooting was evaluated.

Rooting was induced on entire range of IBA concentrations tested however, it was observed that 0.5 mg/l IBA in full strength MS medium proved to be the best with early root initiation after 13 days and 100% rooting of shoots within one month (Table 1 & Fig 1). Our observation is

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Table 1 Effect of different concentrations of IBA, NAA and IAA on *in vitro* rooting of Gisela 5 on full strength and half strength MS medium

Growth regulator	MS medium	Concentration (mg/l)	Days taken for root initiation	Per cent rooting	Average root length (cm)	Average no. of roots per shoot
IBA	Full strength	0.5	13	100.00 (90.00)	6.2	10
		1.0	20	50.00 (44.98)	4.5	7
		1.5	20	40.11 (39.28)	5.2	8
		2.0	14	10.00 (18.42)	4.2	6
	Half strength	0.5	2.0	30.00 (33.19)	5.7	6
		1.0	22	10.08 (18.50)	4.3	6
		1.5	22	50.01 (44.99)	5.5	5
		2.0	21	20.00 (26.55)	5.8	4
NAA	Full strength	0.5	24	20.01 (26.56)	5.6	3
		1.0	26	30.01 (33.20)	4.4	2
		1.5	24	10.01 (18.44)	4.6	3
		2.0	22	20.05 (26.58)	4.3	1
	Half strength	0.5	23	10.01 (18.43)	5.6	3
		1.0	21	20.03 (26.57)	4.3	4
		1.5	24	20.00 (26.60)	4.5	3
		2.0	21	40.03 (39.23)	5.7	3
IAA	Full strength	0.5	23	30.00 (33.19)	5.4	2
		1.0	22	10.00 (18.43)	4.3	3
		1.5	26	30.04 (33.22)	5.5	3
		2.0	26	20.07 (26.60)	5.2	2
	Half strength	0.5	22	20.08 (26.61)	4.7	5
		1.0	21	20.06 (26.60)	5.3	4
		1.5	25	30.04 (33.22)	6.5	3
		2.0	26	40.09 (39.26)	4.2	6
$CD_{0.05}$				0.740 (0.544)		

Fig 1 *In vitro* rooting of Gisela 5 in different medium

supported by findings of many workers who used different concentrations of IBA for rooting of *in vitro* micro shoots during micropropagation of various cherry rootstocks (Buyukdemirci, 2008; Sisko, 2011; Canli and Demir, 2014; Sarropoulou *et al.*, 2014; Xu *et al.,* 2015; Zamanipour *et al.*, 2015). Similarly, Mir *et al*. (2010) and Aghaye *et al*. (2013) achieved *in vitro* rooting in half strength MS medium supplemented with IBA in Mazzard, Mahaleb and Gisela 6 rootstocks. Very less rooting was observed when different concentrations of NAA (40% rooting) and IAA (30% rooting) were used. Sisko (2011) reported low

Table 2 Effect of different concentrations of IBA on *in vitro* rooting of Gisela 5 in two step procedure

Growth regulators	Concentration of IBA (mg/l)	Days taken for root initiation	Per cent rooting	Average root length (cm)	Average no. of roots per shoot
IBA	0.1	21	60.00(50.74)	4.5	3
	0.2	23	10.00(18.43)	5.2	2
	0.3	22	10.00(18.42)	5.4	3
	0.4	21	60.03(50.76)	6.6	3
	0.5	22	20.00(26.55)	4.5	2
NAA	0.1	30	30.00(33.20)	4.5	2
	0.2	28	10.11(18.52)	4.7	2
	0.3	29	20.07(26.60)	3.5	1
	0.4	28	20.03(26.57)	5.6	1
	0.5	27	10.00(18.42)	4.4	2
IAA	0.1	25	30.03(33.21)	3.3	2
	0.2	26	20.03(26.57)	4.5	1
	0.3	23	10.07(18.49)	5.2	2
	0.4	24	10.08(18.50)	4.5	1
	0.5	23	10.00(18.42)	3.6	2
$CD_{0.05}$			0.912(0.721)		

percentage rooting with 1mg/l NAA as compared to IBA in Gisela 5. Similar to our studies rooting efficiency in Tetra (*Prunus empyrean*) rootstock was highest on medium containing IBA as compared to IAA (Sedeghi *et al*., 2015). The lower efficiency of IAA may be because it is metabolised by peroxidise enzyme rapidly as compared to other auxins.

When the micro shoots of Gisela 5 were precultured on MS broth fortified with IBA at concentrations of 0.1 mg/l and 0.4 mg/l for 24 hours before transferring to solid MS medium resulted in highest root induction (Table 2). Our results are in analogy with Thakur *et al*. (2016) who demonstrated efficient rooting in Gisela 5 using IBA in two step rooting procedure. Similarly, Muna *et al*. (2000) reported maximum rooting efficiency in cherry rootstock Maxama-14 at low concentration of IBA in liquid MS medium.

When activated charcoal (0.4%) and pholoroglucinol (0.1%) were added to half strength MS medium along with different IBA concentrations, did not show any increase in rooting percentage. Maximum rooting of 40.06% was observed in MS medium fortified with 0.5 mg/l IBA and activated charcoal (Fig 1). Similarly, no rooting was observed when phloroglucinol was added to the MS medium along with IBA. Our results are similar to Soni *et al*. (2011) who reported inhibition of callus formation in apple rootstock Merton 793 when activated charcoal was added to the rooting medium. Addition of phloroglucinol was non beneficial in rooting of apple rootstock MM 106 (Sharma *et al*. 2000) which is similar to our results.

100% survival was observed on potting mixture comprising of soil: sand: FYM (1:1:1). In cocopeat, 40% plantlets survived. After 4 weeks of hardening, these plants were transferred to bigger earthern pots filled with soil and FYM for further growth (Fig 2). It was observed that better

Fig 2 Hardened plantlets of Gisela 5 after 4, 6 and 12 weeks of transfer.

root and shoot development prior to hardening determined the survival of *in vitro* raised plants which is supported by Minaev *et al.* (2003)**.**

So, we developed a technique for *in vitro* rooting and hardening of Gisela 5 (*Prunus cerasus* × *Prunus canescens*)- cherry rootstock, so as to produce the planting material in large number for commercial exploitation of this rootstock.

SUMMARY

A technique for *in vitro* rooting and hardening of Gisela 5 (*Prunus cerasus* x *Prunus canescens*) cherry rootstock has been developed. *In vitro* shoot cultures maintained by routine subculturing were used as source of microshoots for rooting experiments. In single step rooting procedure, maximum rooting (100%) was achieved on full strength MS medium fortified with 0.5 mg/l IBA with thin, long roots devoid of callus. In two step procedure of rooting, maximum rooting (60.00%) was observed after 24 hours dark incubation in half strength MS broth fortified with 0.1 and 0.4 mg/l IBA, followed by transfer to semisolid half strength MS basal medium. Addition of activated charcoal (0.4%) in half strength MS medium fortified with 0.5 mg/l IBA showed 40.06% rooting without callus, whereas in phloroglucinol (0.1%) supplemented medium only callus was observed at the bases of the shoots which did not lead to root formation. The *in vitro* rooted plantlets were successfully hardened in sand: soil: FYM (1:1:1) with 100 per cent survival in the month of August. After two months these plants were transferred to bigger pots containing sand: soil: FYM (1:1:1) and are showing normal growth without any morphological variations.

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