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An efficient *in vitro* propagation of clonal cherry rootstock Gisela-6 and validation of genetic stability through SCoT markers

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ABSTRACT

Gisela 6 or G6 (*Prunus cerasus* × *P. canescens*), a dwarfing rootstock, is vital in cherry cultivation due to its disease resistance and wider adaptability. However, traditional propagation methods face challenges in maintaining a consistent supply of quality G6 rootstocks due to variability in plant characteristics and disease susceptibility. To work over this, several combinations and concentrations of growth regulators were used to optimize the *in vitro* propagation of G6 rootstock, which was successfully multiplied following a 12-day period on MS medium, enriched with 0.5 mg/L BA and 0.3 mg/L GA_3. The highest successful shoot multiplication (1:6) on MS medium supplemented with 0.7 mg/L BA, 0.7 mg/L GA_3, and 0.1 mg/L IBA was observed after the fourth sub-culture. The two-step method that included growing on half-strength basal MS medium, following 48 h of culture on half-strength liquid MS medium supplemented with 0.8 mg/L IBA produced the maximum rooting (90.45%). Of the total plantlets, 60% got rooted, and survived after putting in pots, containing soil, sand, and FYM (1:1:1). The regenerated G6 plantlets were validated through 36 Start Codon Targeted Polymorphism (SCoT) markers, which revealed a high level of similarity (94%) between *in vitro* propagated and mother plants. The optimized *in vitro* propagation protocol, along with genetic stability assessment using SCoT markers, ensured consistent production of the true-to-the type G6 planting material, addressing challenges in commercial cultivation, and advancing rootstock propagation for sustainable cherry production.

Key words: *Prunus cerasus* × *Prunus canescens,* PCR, rootstocks, SCoT.

INTRODUCTION

Cherry is a popular temperate stone fruit, prized for its high nutritional content and excellent flavour is being grown all over the world. With a yearly global production of 2,765.83 thousand tonnes from 454.66 thousand ha, Turkey, Chile, United States, Uzbekistan, and Spain are the top producers. Cherry was introduced in India by British settlers in Shimla as well as Kashmir hills, and is now cultivated commercially with total production of 10,934.45 t from an area of 3,557 ha (FAOSTAT, 9).

The major aspect that restrains cherry cultivation in India is lack of suitable rootstock adaptable to diverse agro-climatic conditions. Seedling rootstocks, being non-identical and prolonged juvenility, lead to late bearing. To maneuver with this, dwarf clonal rootstocks work better than strong seedling rootstocks, offering good yield, less labour intensive, low production costs and simple orchard maintenance. The “Gisela” or “Geissen series” of dwarfing rootstocks for cherries has been developed from Germany. Of these, Gisela 6 (G6) has become a key rootstock due to its disease resistance, wide soil adaptability and compatibility with multiple cherry cultivars. Cherry trees are commercially propagated through grafting of scion wood on clonal or seedling rootstocks. However, these methods have drawbacks such as low rooting potential, non-uniformity and do not guarantee healthy and disease-free plants (Janick and Moore, 12), making it difficult to maintain a consistent supply of quality G6 rootstocks.

A significant difference from conventional propagation techniques is provided by plant tissue culture, which ensures the large-scale production of planting material that is true-to-type. Since somaclonal variations exist, evaluating the genetic stability of the regenerated plants is crucial (Bhattacharrya *et al*., 3). With the advancement in genomics research, molecular markers have emerged as an efficient tool in detecting clonal variation as they are not influenced by environmental factors unlike morpho-biochemical markers. The emergence of gene-targeted Start Codon Targeted (SCoT) markers, which have been shown to be effective in the past, offers a promising method for evaluating genetic fidelity, fingerprinting, and quantitative trait loci (QTL) mapping. SCoT markers were developed based on the short conserved region flanking the start codon (ATG) in plant genes. These markers are reproducible in nature, requires no sequence information and are associated with functional

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genes (Collard and Mackill, 7; Sharma *et al*., 17). Consequently, the current study's objective was to develop a productive *in vitro* regeneration strategy for G6 rootstock, using a range of growth regulator concentrations and combinations. Additionally, it sought to use SCoT markers to determine the molecular genetic consistency of the regenerated G6 plants, guaranteeing the availability of true-to-type quality planting material for commercial gain.

MATERIALS AND METHODS

Axillary and apical buds of the G6 plant were acquired from the Dr Yaswant Singh Parmar University of Horticulture and Forestry's experimental area in the Fruit Science, Department in Nauni, Solan, Himachal Pradesh (India), and used for establishment of *in vitro* shoot cultures. The buds underwent a 20 min. treatment with 0.1% carbendazim (Bavistin BASF), a 10 min. treatment with sodium hypochlorite (5%) and three to four washings in autoclaved distilled water. The medium was autoclaved for 15-20 min at 15 pounds per inch^2 after the pH was adjusted to 5.75 ± 0.5. The cultures were maintained in 40 W white fluorescent tubes (Philips, India) with 50–60 µmol $m^{-1}s^{-1}$ of 16-h photoperiod and amounts of light. The temperature was kept up at 25 ± 2°C. Sterilized buds were grown in MS medium using different levels and mixtures (Murashige and Skoog, 15) of growth regulators, including BA, TDZ, KIN, and others, to encourage shoot proliferation. Bud burst and percent proliferation rate were recorded at an interval of three days.

Healthy formed shoots were placed in MS medium with BA, TDZ, GA_3, IBA, and KIN to multiply the shoots. The most effective mix of growth regulators for multiplying shoots was found by determining the average shoot length and rate of multiplication after four weeks. Routine sub-culturing was carried out and observations were recorded.

Four weeks into the shoot culture, a single-step and two-step procedure was employed to induce roots (Thakur *et al*., 19). On half strength MS medium, containing different concentrations of IBA, NAA, and IAA, shoots were grown in a single stage. The process for the shoots involved two steps: They were moved to a half-strength level on basal media and then incubated in light conditions after being held in different concentrations of auxins, raised with half-strength liquid MS medium for 48 h under dark conditions. For root induction, 3.75 g/L of agar-agar was employed as a solidifying agent. Observations were recorded for rooting percentage, average root length and days taken for rooting after 3-4 weeks of culture. Shoots with roots were taken out of the culture containers, given a gentle rinse under running water to get rid of any agar residue and then given a 30-min treatment with 0.5% carbendazim. Plants were hardened in little plastic pots that were filled with FYM, soil and sand (1:1:1). The plantlets were kept at a high relative humidity by being covered with glass jars and weekly growth observations were made. The plantlets were moved to larger pots filled with garden soil once they got hardened, which took four to five weeks.

The method outlined by (Doyle and Doyle, 8) with minor modifications was used to extract total genomic DNA from leaves of field grown G6 rootstock (mother plants), as well as *in vitro* raised plantlets. RNase was used to treat DNA samples to get rid of RNA impurities. Utilizing an Eppendorf BioSpectrometer UV/VIS spectrophotometer, the quantity of extracted DNA was measured and its quality was evaluated on an ethidium bromide-stained 0.8 per cent agarose gel. Thermal cycling was used to amplify DNA (Bio-RAD) using thirty-six SCoT primers with varying concentrations of (i) template DNA, (ii) *Taq* buffer, (iii) *Taq* DNA polymerase (iv) dNTPs, (iv) Mg^{2+} ions and, (v) Bovine serum albumin (BSA) to optimize the reaction mixture. For the PCR reactions, a 15 µL aliquot was used, which contained 0.03 U/µL of *Taq* DNA polymerase, 0.25 mM dNTPs, 1X PCR buffer containing 80 ng/µL of template DNA, 6 ng/µL of BSA, 10 pmol primer (Eurofins, US), and 1.5 mM $MgCl_2$. The DNA amplification process consisted of 38 cycles: denaturation (94°C for 40 s), and annealing for 1 min at a temperature based on the primer's Tm ± 5°C, extension (at 72°C for one min) and ultimate extension (at 72°C for thirteen min) after three min at 94°C.

Utilising a 1.5% (w/v) agarose gel with 3 µL/100 mL ethidium bromide, the DNA products were examined. Six times the loading dye, or 3.5 µL, was added to each PCR tube, and 10 µL of material was loaded along with a DNA ladder to measure the size of the amplified products. UV trans-illuminator was used to see the amplified products, and a gel documentation system (Biovis) was used to capture pictures.

Images from gels created using SCoT primers were transformed into binary matrix and assessed in terms of genetic relatedness. DNA fragments were scored on a Microsoft Excel sheet, with '0' denoting the lack of a band and '1' denoting its presence. Each primer's polymorphic information content (PIC) was determined using the methodology outlined by Anderson *et al*. (2) and primer's differentiation ability was assessed by resolving power (Rp). The similarity matrix of binary data was found using the Euclidean

distance technique and Jaccard's coefficient using the software packages NTSYSpc-2.2. UPGMA was used to generate a dendrogram and perform cluster examination (Rohlf, 16).

The data for each of the components were analysed using design (CRD) (Cochran and Cox, 6; Gomez and Gomez, 10). The analysis of variance approach was utilised for the CRD statistical analysis, which was predicated on mean values per treatment. A statistical analysis was carried by utilizing IBM SPSS software (Statistical Package for the Social Sciences), version 25.0 (SPSS Inc. for Windows, Chicago, Illinois, USA).

RESULTS AND DISCUSSION

Prunus rootstocks are traditionally propagated through seeds, cuttings or grafting which lead to genetic variations, impacting orchard uniformity. G6, a newly developed cherry rootstock, faces challenges in clonal propagation through conventional methods, particularly in rooting. *In vitro* propagation offers an alternative to overcome these challenges, as they can rapidly produce large number of genetically identical and disease-free plants for commercial production (Vujovic *et al*., 20).

Direct organogenesis was seen following the establishment of surface-sterilized buds in culture tubes on MS medium enhanced with varying dosages of GA_3, KIN, TDZ, and BA. Adding 0.3 mg/L GA_3 and 0.5 mg/L BA, the MS medium exhibited maximum rate of bud proliferation (83.33%) after 12-day period (Table 1; Fig. 1). Previously, (Aghaye *et al*., 1) and (Bošnjak *et al*., 4) used different cytokinins *viz*., BA, TDZ, and KIN for axillary shoot proliferation in G6, and observed the maximum rate of proliferation on media containing 1 mg/L BA. However, (Buyukdemirci, 5) found that the optimal medium for shoot proliferation was MS medium supplemented with 0.5 mg/L BA, 0.01 mg/L IBA, and 0.1 mg/L GA_3. The maximum *in vitro* establishment of explants (70%) for the G5 cherry on MS medium, was obtained using 0.5 mg/L GA_3 and 0.5 mg/L BA (Thakur *et al*.,19; Sharma *et al*. (18).

After being grown using many combinations of growth regulators in MS medium for multiplication, shoots showed vigorous proliferation. The present study revealed variations in the shoot multiplication rates across the tested growth regulator combinations wherein, highest rate (1:6) was observed after being developed on MS medium with 0.1 mg/L IBA, 0.7

Table 1. Effect of different concentration and combination of plant growth regulators on *in vitro* establishment in Gisela 6.

Sl. No.	MS medium fortified with GR (mg/L)				Days taken for bud burst	Percent bud proliferation
	BA	TDZ	KIN	GA_3		
1	0.5	-	-	0.3	11-12	83.33 (65.92) *
2	0.5	-	-	0.5	12-14	70.00 (56.76)
3	0.5	-	-	1	14-16	53.33 (46.89)
4	1	-	-	0.3	14-18	60.00 (50.75)
5	1	-	-	0.5	16-18	63.33 (52.71)
6	1	-	-	1	16-18	56.66 (48.81)
7	-	0.5	-	0.3	19-21	13.33 (21.4)
8	-	0.5	-	0.5	18-21	13.33 (21.4)
9	-	0.5	-	1	19-21	10.00 (18.42)
10	-	1	-	0.3	20-22	10.00 (18.42)
11	-	-	0.5	0.3	18-22	30.00 (33.19)
12	-	-	0.5	0.5	18-21	36.67 (37.25)
13	-	-	0.5	1	20-24	26.66 (31.07)
14	-	-	1	0.3	19-23	20.00 (26.55)
15	-	-	1	0.5	17-20	33.33 (35.24)
16	-	-	1	1	19-23	16.67 (24.08)
$CD_{(0.05)}$						1.79 (1.25)
SE± (m)						0.62 (0.43)

*The values in the parenthesis are arc sine transformed values

Fig. 1. *In vitro* establishment from axillary buds (a), sprouted buds (b), shoot proliferation on MS medium + 0.5 mg/L BA + 0.3 mg/L GA_3 (shoot induction medium) (c), transfer of shoot on MS medium + 0.7 mg/L BA + 0.7 mg/L GA_3 + 0.1 mg/L IBA (shoot multiplication medium) (d), multiplied shoots (e), transfer of shoots to half strength liquid MS medium + 0.7 mg/L IBA (f), transfer of shoots from liquid medium to half-strength basal MS medium for root induction (g), regenerated plantlets (h), primary hardening (i), hardened plantlets after 2 weeks (j), and field transfer after 4 months (k).

mg/L BA, and 0.7 mg/L GA_3 added as supplements, following the 4th sub-culture (Table 2; Fig. 1). The average shoot length of the regenerated shoots was 3.17 cm at its highest. According to reports, the growth regulators like BA, GA_3, and IBA were often employed to propagate cherry rootstocks (Bosnjak *et al*., 4). Maximum shoot multiplication (5:5) on MS medium enhanced with 3.0 mg/L GA_3 and 0.75 mg/L BA has been reported in a *P. cerasus* (Krasinskaya and Kukharchik, 13). As per Hosseinpour *et al*. (11), the maximum number of shoots were appeared in the MS medium after adding 0.7 mg/L of BA only.

Well-developed shoots underwent rooting using both a single-step and a two-step method (Thakur *et al.,* 19). In single-step procedure, the shoots were directly placed in a half-strength MS medium enhanced with auxins at varying concentrations (IBA, NAA, and IAA), to induce root formation. Among these, IBA (0.08 mg/mL) tended to show the highest rooting (21.32%) after 35 days (Table 3). When cultivating shoots in liquid MS medium at half-strength and supplemented with 0.8 mg/L IBA for 48 hours in the dark, followed by further culturing on half strength basal MS medium and incubated under light conditions, the maximum rooting (90.45%) was observed in the two-step rooting procedure (Table 3; Fig. 1). Similar to present findings Bosnjak *et al.* (4) achieved maximum rooting in cherry rootstock, when shoots were kept in a solution of 80 mg/L IBA for 20 h after which they were moved to a hormone-free

Table 2. Effect of different concentration and combination of plant growth regulators on *in vitro* shoot multiplication in Gisela 6.

MS (Basal medium) + GR (mg/L)					Multiplication rate	Average shoot length (cm)
BA	TDZ	GA_3	IBA	KIN		
-	-	-	-	-	1:1	1.68f *
0.50	-	0.50	-	-	1:3	0.87i
1.00	-	1.00	-	-	1:2	1.18h
0.50	-	-	0.10	-	1:3	1.42g
0.50	-	-	0.20	-	1:4	1.92d
1.00	-	-	0.10	-	1:1	2.15c
1.00	-	-	0.20	-	1:2	2.1c
-	0.50	0.50	-	-	1:4	0.64j
-	0.50	0.30	-	-	1:5	1.17h
-	1.00	1.00	-	-	1:5	0.72j
-	0.50	-	0.10	-	1:3	1.14h
-	1.00	-	0.20	-	1:2	1.68f
0.30	-	0.20	-	-	1:5	2.11c
0.70	-	**0.70**	**0.10**	-	**1:6**	**3.17a**
0.30	-	0.30	0.1	-	1:4	1.82e
0.30	-	0.20	0.10	-	1:5	2.07c
-	0.50	0.30	0.10	-	1:3	1.65f
-	0.70	0.50	0.10	-	1:3	1.12h
0.50	-	0.30	0.10	-	1:1	1.71f
0.50	-	0.30	-	-	1:2	0.67j
0.50	-	0.20	-	-	1:4	1.42g
0.25	-	-	0.10	0.25	1:5	1.92d
0.50	-	-	0.10	0.50	1:3	2.16c
0.30	-	0.50	0.10	-	1:5	2.92b
$CD_{(0.05)}$						0.09
SE± (m)						0.03

*Means followed by the same letters are not significantly different from each other (p = 0.05) determined by Duncan's Multiple Range Test (DMRT)

Table 3. Effect of different concentrations of auxins on *in vitro* rooting of Gisela 6 cherry rootstock.

Growth regulator	Concentration (mg/L)	Single-step					Double-step				
		Days taken for root initiation	Days taken for full rooting	Per cent rooting	Average root length (cm)	Average No. of roots per shoot	Days taken for root initiation	Days taken for full rooting	Per cent rooting	Average root length (cm)	Average No. of roots per shoot
IBA	0.10	23.00	39.00	7.17 (2.86)	3.1d	2.78b	21.00	38.00	40.00 (39.21)	4.92de	2.25f
	0.30	23.00	38.00	10.52 (3.39)	3.5cd	2.3cd	22.00	37.00	10.56 (18.95)	5.62c	2.4d
	0.50	22.00	37.00	14.62 (3.95)	3.1d	1.56g	21.00	36.00	22.14 (28.05)	5.82bc	2.9b
	0.80	23.00	37.00	21.32 (4.72)	5.6a	3.4a	20.00	35.00	90.45 (72.02)	7.02a	4.01a
	1.00	21.00	38.00	19.52 (4.53)	4.2b	2.45c	21.00	37.00	25.12 (30.06)	4.92de	2.14g
NAA	0.10	32.00	41.00	10.17 (3.34)	3.74c	2.44c	30.00	40.00	10.51 (18.9)	4.92de	2.3ef
	0.30	29.00	42.00	8.13 (3.02)	3.42cd	1.12h	28.00	41.00	20.41 (26.84)	5.12d	1.2k
	0.50	28.00	41.00	12.33 (3.65)	3.14d	1h	26.00	40.00	30.52 (33.52)	3.92fg	1.4j
	0.80	28.00	40.00	10.24 (3.35)	3.78bc	1.89ef	27.00	39.00	20.43 (26.86)	6.02b	2.35de
	1.00	27.00	41.00	7.00 (2.82)	3.71c	1.75fg	27.00	40.00	10.00 (18.43)	4.82e	1.7h
IAA	0.10	26.00	42.00	6.25 (2.69)	3.67c	1.55g	26.00	41.00	20.00 (26.55)	3.72g	1.6i
	0.30	27.00	42.00	8.11 (3.02)	3.87bc	2.12de	26.00	41.00	20.41 (26.85)	4.92de	2.56c
	0.50	25.00	41.00	15.53 (4.07)	3.57cd	1h	23.00	40.00	10.22 (18.63)	5.62c	1.22k
	0.80	25.00	40.00	10.24 (3.35)	3.78bc	1.89ef	23.00	39.00	10.27 (18.68)	4.92de	2.36de
	1.00	24.00	40.00	4.65 (2.38)	3.54cd	1h	24.00	39.00	8.35 (16.79)	4.02f	1l
$CD_{(0.05)}$				0.52 (0.06)	0.13	0.07			1.54 (1.06)	0.23	0.10
SE± (m)				0.18 (0.02)	0.05	0.02			0.41 (0.35)	0.08	0.03

Means followed by the same letters are not significantly different from each other (p = 0.05) determined by Duncan's Multiple Range Test (DMRT). Rooting % data was transformed using Arc Sin transformation.

medium. In the past, out of all the auxin combinations and concentrations tested, the medium containing 1-2 mg/L of IBA added, half-strength MS medium exhibited the highest rooting percentage (up to 100%), while NAA produced the lowest rooting (35%) in cherry rootstocks (Vujovic *et al*., 20; Aghaye *et al*., 1). The gradual hardening of rooted plantlets was conducted to facilitate their acclimatization to outdoor environmental conditions. Well-developed, rooted plantlets were taken out of an agar-gelled media after three weeks, and then placed in pots with soil, sand, and FYM (1:1:1). With a 60% survival rate, these

potted plantlets were kept in the glasshouse at 25ºC temp. and 90% RH. The plantlets that made it were moved to an open field setting (Fig. 1).

A significant limitation in plant tissue culture arises due to somaclonal variations, resulting in genetic and phenotypic differences that contribute to the instability of clones. Molecular markers, revealing genome banding patterns, provide insights into clonal variations in micro-propagated plants, potentially originating from chromosomal rearrangements, DNA methylation, and mutations (Lukens and Zhan, 14). Evaluating genetic fidelity through gene-targeted SCoT markers has emerged as an effective method for detecting somaclonal variations in tissue culture-propagated plants in the past (Bhattacharyya *et al.,* 3).

The genetic similarity between mother plants produced in the field and *in vitro* raised G6 plants was assessed using gene-targeted SCoT primers. For the study, a total of 36 primers were utilised, out of which, DNA amplification was shown by thirty-one primers (Table 4). A total of 103 amplicons were scored out, of which the highest scorable bands was exhibited by S35 primer, generating 7 monomorphic bands. Highest PIC value for SCoT markers was found to be 0.28 for S11 and S35 primers and Rp value ranged between 12 (S20) to 84 (S1). For the S11 primer, the maximum EMR and MI values were 2.25 and 0.63, respectively (Table 4; Fig. 2), demonstrating the marker's equal distribution and applicability for evaluating genetic integrity. Dendrogram utilizing NTSYS software grouped mother plants and *in vitro* grown G6 plants into two groups based on Jaccard's similarity coefficient, wherein high similarity coefficient (0.94-1.00) was observed between them (Table 5). Mother plants and tissue culture propagated plants exhibited 94% similarity with each other and different replicates of tissue culture propagated plantlets showed 96-100% similarity among them (Table 3; Fig. 3), thus demonstrating that micropropagation may be used to produce planting material that is true to type. These results demonstrate that SCoT markers may be applied to identify very subtle genetic differences, which makes them useful for fidelity evaluation and varietal identification in tissue culture-propagated plants.

In the present study, *in vitro* regeneration protocol for G6 cherry rootstock was developed, aimed to address the requirement for a steady supply of superior planting material. By showing a high degree of resemblance with the mother plants, the application of SCoT markers confirmed the genetic stability of tissue-culture produced plants.

Table 4. Data obtained from SCoT primers used in the study for genetic stability analysis.

Primer Code	Sequence (5'-3')	Length (bp)	Tm (ºC)	GC (%)	Scorable bands	Size (bp)	Monomorphic band(s)	No. of alleles	Rp	PIC	EMR	MI
S1	CAACAATGGCTACCACCA	18	53.69	50.00	7	300-1500	7	42	84	0.00	0.00	0.00
S2	CAACAATGGCTACCACCC	18	55.97	55.56	2	500-700	1	10	20	0.22	0.50	0.11
S3	CAACAATGGCTACCACCG	18	55.97	55.56	3	700-900	3	18	36	0.00	0.00	0.00
S4	CAACAATGGCTACCACCT	18	53.69	50.00	5	500-1400	5	30	60	0.00	0.00	0.00
S5	CAACAATGGCTACCACGA	18	53.69	50.00	5	200-850	3	22	44	0.11	0.80	0.09
S6	CAACAATGGCTACCACGC	18	55.97	55.97	4	400-1185	4	24	48	0.00	0.00	0.00
S7	CAACAATGGCTACCACGG	18	55.97	55.97	2	750-1185	2	12	24	0.00	0.00	0.00
S8	CAACAATGGCTACCACGT	18	53.69	53.69	3	500-1000	3	18	36	0.00	0.00	0.00
S9	CAACAATGGCTACCAGCA	18	53.69	53.69	3	300-1000	3	18	36	0.00	0.00	0.00
S10	CAACAATGGCTACCAGCC	18	55.97	55.97	2	600-900	2	12	24	0.00	0.00	0.00
S11	AAGCAATGGCTACCACCA	18	53.69	53.69	4	400-700	1	17	34	0.28	2.25	0.63
S12	ACGACATGGCGACCAACG	18	58.24	61.11	2	600-1000	2	12	24	0.00	0.00	0.00

Contd...

Table 4 contd...

Primer Code	Sequence (5'-3')	Length (bp)	Tm (ºC)	GC (%)	Scorable bands	Size (bp)	Monomorphic band(s)	No. of alleles	Rp	PIC	EMR	MI
S13	ACGACATGGCGACCATCG	18	58.24	61.11	-	-	-	-	-	-	-	-
S14	ACGACATGGCGACCACGC	18	60.52	66.67	2	700-800	2	12	24	0.00	0.00	0.00
S15	ACGACATGGCGACCGCGA	18	60.52	66.67	3	600-1200	3	18	36	0.00	0.00	0.00
S16	ACCATGGCTACCACCGAC	18	58.24	61.11	4	600-1100	2	22	44	0.11	1.00	0.11
S17	ACCATGGCTACCACCGAG	18	58.24	61.11	2	600-1000	2	12	24	0.00	0.00	0.00
S18	ACCATGGCTACCACCGCC	18	60.52	66.67	2	700-800	2	12	24	0.00	0.00	0.00
S19	ACCATGGCTACCACCGGC	18	60.52	66.67	4	400-1100	4	24	48	0.00	0.00	0.00
S20	ACCATGGCTACCACCGCG	18	60.52	66.67	1	500	1	6	12	0.00	0.00	0.00
S21	ACGACATGGCGACCCACA	18	58.24	61.11	-	-	-	-	-	-	-	-
S22	AACCATGGCTACCACCAC	18	55.97	55.56	4	350-650	4	24	48	0.00	0.00	0.00
S23	CACCATGGCTACCACCAG	18	58.24	61.11	4	1185-2000	4	24	48	0.00	0.00	0.00
S24	CACCATGGCTACCACCAT	18	55.97	55.56	-	-	-	-	-	-	-	-
S25	ACCATGGCTACCACCGGG	18	60.52	66.67	3	800-1500	3	18	36	0.00	0.00	0.00
S26	ACCATGGCTACCACCGTC	18	58.24	61.11	3	400-700	3	18	36	0.00	0.00	0.00
S27	ACCATGGCTACCACCGTG	18	58.24	61.11	3	800-1190	3	18	36	0.00	0.00	0.00
S28	CCATGGCTACCACCGCCA	18	60.52	66.67	2	450-700	2	12	24	0.00	0.00	0.00
S29	CCATGGCTACCACCGGCC	18	62.8	72.22	-	-	-	-	-	-	-	-
S30	CCATGGCTACCACCGGCG	18	60.52	66.67	2	450-550	2	12	24	0.00	0.00	0.00
S31	CCATGGCTACCACCGCCT	18	60.52	66.67	2	350-800	2	12	24	0.00	0.00	0.00
S32	CCATGGCTACCACCGCAC	18	60.52	66.67	-	-	-	-	-	-	-	-
S33	CCATGGCTACCACCGCAG	18	60.52	66.67	3	300-800	3	18	36	0.00	0.00	0.00
S34	ACCATGGCTACCACCGCA	18	58.24	61.11	6	400-1185	6	36	72	0.00	0.00	0.00
S35	CATGGCTACCACCGGCCC	18	62.8	72.22	8	350-2100	7	40	80	0.28	0.13	0.03
S36	GCAACAATGGCTACCACC	18	55.97	55.56	3	350-650	3	18	36	0.00	0.00	0.00

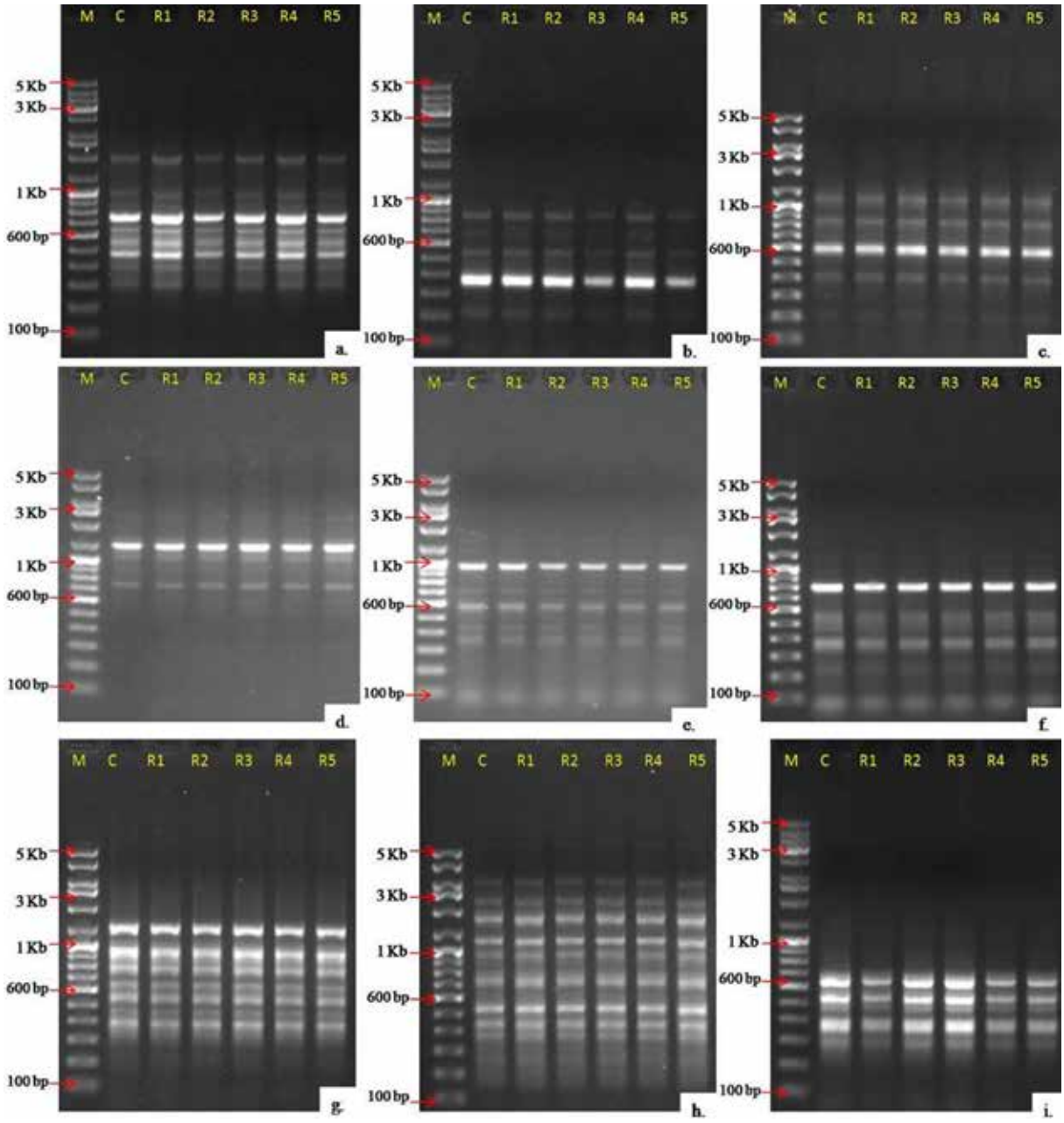

Fig. 2. SCoT banding profile generated using primers S1 (a); S5 (b); S6 (c); S7 (d); S12 (e); S31 (f); S34 (g); S35 (h), and S36 (i). (M: Ladder (100 bp-5Kb); C: Control (mother plants); R1, R2, R3, R4, R5: tissue culture propagated plants).

Table 5. Similarity matrix obtained using Jaccard's coefficient for SCoT primers in Gisela 6 rootstock.

Similarity coefficient	Control	R1	R2	R3	R4	R5
Control	1.0000					
R1	0.9608	1.0000				
R2	0.9612	0.9804	1.0000			
R3	0.9417	0.9802	0.9804	1.0000		
R4	0.9515	0.9706	0.9902	0.9706	1.0000	
R5	0.9417	0.9608	0.9804	0.9802	0.9901	1.0000

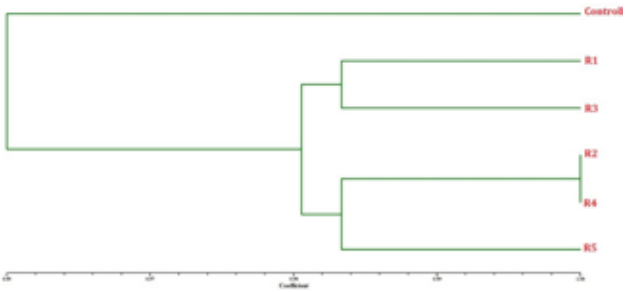

Fig. 3. SCoT marker derived dendrogram based on Jaccard's similarity coefficient.

True-to-type planting material is produced using the specified technique when coupled with genetic stability testing, significantly contributing towards the advancement of cherry rootstock propagation for commercial gains.

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AUTHORS' CONTRIBUTION

MSR and MT were involved in designing tissue culture and molecular experiments. MSR, VKD and MT executed tissue culture experiments. MSR and PS contributed in molecular analysis. PS and MT contributed in writing and editing of the manuscript.

DECLARATION

All authors declare that they have no conflict of interest.

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