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ASSESSEMENT OF SOMACLONAL VARIATION IN *IN VITRO* PROPAGATED CORMELS OF GLADIOLUS

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Abstract

In vitro raised cormels of 3.0 to 4.0 mm diameter of 3 commercial grown varieties of gladiolus viz. *Traderhorn, White Friendship* and *Peter Pears* were used to assess percentage of clonal fidelity with each other and with mother cormels using Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeat (ISSR) markers**.** In order to regenerate cormels under *In vitro* conditions, direct organogenesis was followed using cormel sprout as an explant. Cormel sprouts obtained by culturing the whole cormels (0.6 g) in a polar position on MS medium supplemented with BAP (4 mg L^{-1}). Out of the three varieties, *White friendship* obtained more number of shoots (22.07). However, statistically similar number of roots was recorded in *White Friendship* (22.67) and *Peter Pears* (19.60) when MS supplemented with IBA (2 mg L^{-1}). Cormel production was not affected by any variety of the gladiolus using MS medium supplemented with IBA (1 mg L^{-1}) and sucrose (7%). The *In vitro* produced cormels were assessed for their clonal fidelity using RAPD and ISSR markers. The RAPD similarity tendencies among *In vitro* propagated cormels ranged from 80% to 95% in *Traderhorn*, 88% to 95% in *White Friendship* and 80% to 90% in *Peter Pears*. However the similarity tendencies between mother and *In vitro* propagated cormels, on average were 86% in *Traderhorn*, 92% in *White Friendship* and 83% in *Peter Pears*. In comparison, ISSR primers produced higher percentage of similarity matrix than RAPD. The ISSR cluster analysis for genetic similarity between mother and *In vitro* propagated cormels had varied degree of differences detected 90% in *Traderhorn*, 96% in *White Friendship* and 85% in *Peter Pears*. The genetic differences among *In vitro* propagated cormels ranged from 88 to 100% in *Traderhorn*, 94 to 100% in *White Friendship* and 82 to 100% in *Peter Pears*.

Introduction

Gladiolus is a potential cut flower grown throughout the world for their attractive spikes and corm production. Being a cormous plant, it is principally propagated by the natural multiplication of new corms and cormels (Hartman *et al*., 1990; Ziv & Lilien-Kipnis, 1990). However, owing to their low rate of multiplication and to a high percentage of spoilage of corms during storage, there is an insufficient supply of planting material (Singh & Dohare, 1994). *In vitro* propagation, due to high multiplication rate has been recognized as an efficient method for mass and clonal multiplication of elite species of the plant material (Shabbir *et al*., 2009). A number of protocols have been developed for the establishment of *In vitro* propagation of gladiolus (Ziv *et al*., 1970; Grewal, *et al*., 1995; Kumar *et al*., 1999; Boonvanno & Kanchanapoom, 2000; Ziv & Lilien-Kipnis, 2000; Goo *et al*., 2003; Priyakumari & Sheela, 2005; Prasad & Gupta, 2006; Roy *et al*., 2006; Aftab *et al*., 2008). Although few authors have reported *In vitro* corm formation in very few varieties of gladiolus such as in *White flowered variety Pacificia* (Roy *et al*., 2006) *Balady* (Al-Juboory *et al*., 1997), *Golden wave* (Sinha & Roy, 2002), *Kinneret* (Steinitz *et al*., 1991), *Friendship* (Dantu & Bhojwani, 1995) and *Green Bay* (Sen & Sen, 1995). However, according to the review article of Ascough *et al*., (2009) no *In vitro* production of cormels was studied on the varieties viz. *Traderhorn, White Friendship* and *Peter Pears* used in the current study.

The major aspect of the current study was to check clonal fidelity between mother and *In vitro* regenerated propagules as clonal multiplication of any *In vitro* propagated regenerant is the major concern for the horticulturist. It is possible that *In vitro* regenerated propagules exhibit somaclonal variation (Larkin & Scowcroft, 1981). This variation may be caused through pre-existing genetic variation occurred in the explant and the variation induced by the *In vitro* conditions (Skirvin, *et al.,* 1994). This variation is manifested in the form of DNA methylations, chromosome rearrangements and point mutations (Phillips *et al*., 1994). Long duration of *In vitro* culture, alterations in auxin-cytokinin concentrations, explant source and the stress created by *In vitro* environment all together or independently may be responsible to induce somaclonal variation (Modgil *et al*., 2005). Oxidative stress is also produced by *In vitro* culture environment that leads production of free radicals within the cells and ultimately cause DNA damage (Jackson *et al*., 1998).

In order to assess clonal fidelity, *In vitro* regenerated propagules need to be thoroughly checked for their clonal character by using various PCR based molecular techniques. Isozymic analysis provides a cheap and convenient method for detection of clonal variation but leads to ontogenic variations. Polymerase Chain Reaction (PCR) based markers such as Random Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeat (ISSR), Simple Sequence Repeat (SSR) and Restriction Fragment Length Polymorphism (RFLP) are nowadays more reliable for detection of clonal fidelity

over morphological and Isozymic analysis. Whereas RFLP is a non PCR based marker and is not practical to use due to high cost of enzymes used in the methods, radioactive labeling and extensive care. On the other hand, RAPD and ISSR markers are simple and faster, needs only little amount of DNA and no need of radioactivity tests. These markers have been successfully used for the detection of somaclonal variation in various micropropagated plants (Carvalho *et al*., 2004; Martins *et al*., 2004; Ramage *et al*., 2004; Modgil *et al*., 2005). RAPD, in comparison to other molecular markers is relatively easiy to apply (Khatri *et al*., 2009); quite workable and efficient (Williams *et al*., 1990) in closely related species. However, ISSR is highly discriminative, reliable and cost-effective (Pradeep *et al*., 2002).

At present, RAPD (Williams *et al*., 1990) and ISSR (Zietkiewicz *et al*., 1994) have been successfully used for the assessement of clonal fidelity in various plant species such as banana (Ramage *et al*., 2004; Lakshmanan *et al*., 2007), lillium (Varshney *et al*., 2001) ginger (Rout *et al*., 1998) *Robina ambiguna* (Guo *et al*., 2006) and medicinal herb *Swertia chirayita* (Joshi & Dhawan, 2007). As far as my knowledge is concerned, only report of Roy *et al*., (2006) has published on clonal fidelity of *In vitro* regenerated cormels of gladiolus cv. *Pacifica* through isozymes, RAPD and ISSR markers. However, in the present study, the *In vitro* produced cormels of three different cultivars of gladiolus were assessed for their clonal fidelity by using RAPD and ISSR molecular techniques.

Materials and Methods

***In vitro* regeneration of cormels:** In order to regenerate cormels under *In vitro* conditions, cormel sprouts were obtained by culturing the whole cormels (0.6 g) in apolar position (growing point downward and physiological base upward) on MS medium supplemented with BAP (4 mg L^{-1}). Prior to culturing, the cormels were cleaned and sterilized. The outer scale of cormels was removed with surgical blade. The descaled cormels were soaked in tap water for 30 minutes to remove any sticky material present on the cormels following 4-5 washings with distilled water. The explants were then treated with 70% ethanol (v/v) for 15 minutes, 1% sodium hypochlorite for 3-4 minutes, 1% $HgCl_2$ for 1-2 minutes followed by 5-6 rinses in sterile distilled water under a laminar airflow cabinet. MS medium containing standard salts and vitamins, 3% sucrose and 0.8% agar was used and pH of the each medium was adjusted separately to 5.7 prior to addition of Agar. Medium was sterilized in autoclave at temperature of 121^0 C and pressure 15 psi for 20 minutes. They cormels were then cultured in 15 x 2.5 cm tubes each amended with 10 ml of culture medium. The cultures were incubated in a culture room where temperature was maintained at 25-27 ºC under continuous a photoperiod of 16 h light and 8 h dark. The light intensity was fixed at 2500 lux by using white fluorescent tubes in the growth room.

The cormels sprouted downward into medium within four days of inoculation. These cormel sprouts (single from each cormel) gradually underwent swelling at the base along with elongation and were of light green colour. These swelled cormel sprouts (with cormel base) were taken out and re-cultured on the same new fresh medium with growing point upward (polar inoculation) and physiological base downward. Within a week, all these swelled sprouts burst into number of multiple shoots. The cluster of shoots with 1-3 shoots of 3 to 5 cm in length from regenerated cultures were separated from the base of the each explant and cultured on MS basal medium (without PGR). No root initiation was found even after 20-25 days of inoculation. In contrast root initiation was observed on MS medium supplemented with IBA (2 mg L^{-1}) and sucrose (3%) within two weeks of inoculation. After two weeks of root initiation (not fully developed roots), the whole cluster of rooted plantlets were taken out from cultures and equally divided into two halves in such a way that each had both shoots and roots. The divided clusters were cultured on cormel induction medium (MS + IBA (1 mg L^{-1}) + sucrose (7%). Trimming of the roots in few cultures was also followed where it was thought necessary for shifting of the rooted plantlets to the cormel induction medium. New root formation and further development of already existed roots was observed in each explant during the 1st week of culture following cormel induction within 4-7 weeks. The dried stock of *In vitro* produced cormels was harvested after 3 weeks of cormel formation and graded on the basis of their cormel diameter.

The experiment was laid out in Completely Randomized Design (CRD) maintaining twenty cultures in each nutrient medium along with three replications. The data were subjected to statistical analysis of variance by using Statistics and means were compared according to DMR test at 5% level of probability (Steel *et al*., 1997).

Clonal fidelity using RAPD and ISSR molecular markers

Genomic extraction: *In vitro* raised cormels of 3.0 to 4.0 mm diameter from three random cultures and one sample from mother were used to assess percentage of clonal fidelity with each other and with mother cormels of each variety. Each sample was replicated thrice. Genomic DNA was extracted from mother as well as *In vitro* grown cormels using CTAB method with modification (Doyle & Doyle, 1990). DNA quantity was determined by diluting each DNA sample to a uniform concentration of 15ng/µl by adding double distilled deionized water and stored the DNA dilutions at 4°C till further use.

Molecular analysis: The PCR thermal cycler (Eppendorf AG No. 533300839, Germany) was used for the RAPD-PCR amplification, and different concentrations of template DNA, 10X PCR buffer, $MgCl_2$, dNTPs, primer and *Taq* DNA polymerase were used to optimize conditions. The optimized reaction conditions for Random Amplified Polymorphic DNA (RAPD) contained the following components/concentrations: 1.0 U *Taq* DNA polymerase (MBI, Fermentas, Vilnius, Lithuania), 50 mM KCl, 3 mM $MgCl_2$, 25 mM each of dNTPs, 0.2 M decamer primer (Gene Link Company, Hawthorne, NY, USA). Thirty RAPD decamer primers (Table 1) were used to analysed the samples. The DNA amplification protocol was 94°C for 5 min, followed by 40 cycles of 94°C for 1 min, 36°C for 1 min, 72°C for 2 min, and finally 72°C for 10 min. All amplification products were electrophoresed on 1.2% (w/v) agarose gels at 80 V for 2

h, stained with ethidium bromide, visualized in a UV transilluminator at 300 nm and photographed in a gel documentation system (SynGen, Synoptics Ltd., UK).

Table 1. RAPD primers along with their sequences

Sr. No.	Primer Name	Sequence
1.	GL DecamerA-02	TGCCGAGCTG
2.	GL DecamerA-04	AATCGGGCTG
3.	GL DecamerA-05	AGGGGTCTTG
4.	GL DecamerA-13	CAGCACCCAC
5.	GL DecamerA-14	TCTGTGCTGG
6.	GL DecamerA-15	TTCCGAACCC
7.	GL DecamerA-20	GTTGCGATCC
8.	GL DecamerB-10	CTGCTGGGAC
9.	GL DecamerB-14	TCCGCTCTGG
10.	GL DecamerC-05	GATGACCGCC
11.	GL DecamerC-07	GTCCCGACGA
12.	GL DecamerC-19	GTTGCCAGCC
13.	GL DecamerC-20	ACTTCGCCAC
14.	GL Decamer J-01	CCCGGCATAA
15.	GL Decamer J-06	TCGTTCCGCA
16.	GL Decamer I-09	TGGAGAGCAG
17.	GL Decamer J-11	ACTCCTGCGA
18.	GL Decamer J-16	CTGCTTAGGG
19.	GL Decamer J-17	ACGCCAGTTC
20.	GL Decamer J-18	TGGTCGCAGA
21.	GL Decamer K-02	GTCTCCGCAA
22.	GL Decamer K-04	CCGCCCAAAC
23.	GL Decamer K-07	AGCGAGCAAG
24.	GL Decamer K-08	GAACACTGGG
25.	GL Decamer K-12	TGGCCCTCAC
26.	GL Decamer K-14	CCCGCTACAC
27.	GL Decamer K-15	CTCCTGCCAA
28.	GL Decamer K-17	CCCAGCTGTG
29.	GL Decamer K-19	CACAGGCGGA
30.	GL Decamer K-20	GTGTCGCGAG

ISSR primers of different series were custom synthesized from Genelink Co.USA and used in this study. Different concentrations of template DNA along with PCR reaction mixture were used to optimize conditions for ISSR analysis. The optimized reaction conditions contained 2.0 µl DNA template 1.0 U *Taq* DNA polymerase (MBI, Fermentas, Vilnius, Lithuania), 50 mM KCl, 3 mM $MgCl_2$, 100 mM each of dNTPs, 0.2 M ISSR primer (Gene Link Company, Hawthorne, NY, USA). The total reaction was performed in a volume of 20.0 µl. Template DNA was initially denatured at 94°C for 2 min, followed by 40 cycles under the following parameters: denaturation for 30 seconds at 94°C. Annealing temperature for each primer vary according to the base composition of the primer ranges from 44-52^{0}C for 45 seconds is presented in Table 2. The primer extension was carried out for 1 minute and 30 seconds at 72°C. A final incubation for 10 min at 72°C was performed to ensure that the primer extension reaction proceeded to completion.

Table 2. ISSR primers used in the present study

Primer name	Sequence (5' 3')	Annealing temperature (^{o}C)
3ASSR01	$(GA)_8TC$	52
3ASSR08	$(TC)_7GGA$	51
3ASSR20	$(CT)_7AGT$	49
3ASSR26	$(CT)_7GCA$	51
3ASSR30	$(CT)_7GAA$	49
3ASSR40	$(TCT)_5AG$	44
3ASSR42	$(GACA)_4C$	51
3ASSR50	$(CA)_7AGT$	49
3ASSR53	$(AG)_8CA$	52
3ASSR62	$(TG)_7$ ACT	49

Data analysis: Amplified products were scored as present (1) or absent (0) for all clones. Less intensic bands were not scored. Only easily distinguished and clearly visible amplified products were counted. The fingerprints were examined under Ultra Violet Transilluminator and photographed using SyneGene Gel Documentation System. The data generated from the detection of polymorphic fragments were analyzed using popgen32 software (Ver. 1.44) (Yeh *et al*., 2000).

Results

The response for organogenesis varied with the variety (Table 3). Out of three varieties, *White Friendship* got more number of shoots (22.07) on MS medium supplemented with BAP (4 mg L^{-1}) as compared to other varieties. However, *Traderhorn* and *Peter Pears* exhibited statistically similar response for shoot regeneration. In contrast, the highest and statistically similar results for number of roots were observed from *White Friendship* (22.67) and *Peter Pears* (19.60) on MS medium supplemented with IBA (2 mg L^{-1}). In contrast, cormel production among different gladiolus varieties was not affected by MS medium supplemented with IBA (1 mg L^{-1}) and sucrose (7%). Various sizes of cormels produced and grading of cormels was made on the basis of their cormel diameter. More percentage of A (55%) grade cormels produced from *White Friendship* as compared to *Peter Pears* (50%) and *Traderhorn* (30%). However, B grade cormels (55%) were obtained by *Traderhorn*.

Table 3. Organogenesis from cormel sprouts of three different varieties of gladiolus.

	Shoot formation	Root formation	Cormel formation	Grading of *In vitro* produced cormels		
	MS + BAP (4 mg L^{-1})	MS + IBA (2 mg L^{-1})	MS + IBA (1 mg L^{-1}) + Sucrose (7%)	A	B	C
Traderhorn	12.00 B	15.20 B	17.40	30	55	15
White friendship	22.07 A	22.67 A	22.47	55	30	15
Peter pears	16.07 B	19.60 A	16.40	50	30	20
Mean	16.71	19.16	18.76			

A= 2.8-3.2 mm, B = 2.1-2.6 mm, C = 0.8-1.2 mm

Molecular analysis through RAPD and ISSR Markers: The clonal fidelity was checked between mother and *In vitro* regenerated cormels by using PCR-based Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeat (ISSR) molecular markers. Twelve samples per replication of three different varieties viz. *Traderhorn, White Friendship* and *Peter Pears* were analyzed by RAPD using 50 decamer oligonucleotide primers out of which 30 primers produced consistent results and rest of the primers were not selected as they produced weak banding pattern. Primers selected produced distinct, easily detectable bands of variable intensities. The bands reproducible over repeated runs with sufficient intensity to detect presence or absence with confidence were used for fingerprinting. A total of 146 bands were amplified in the RAPD-PCR of 12 cormel genotypes with 30 RAPD primers, out of which 68 bands were polymorphic showing 47% overall polymorphism. The primers produced maximum of 8 bands i.e. GLJ-11 and two primers GLK-7 and GLK14 produced minimum number of bands i.e. 2 with an average of 4.8 bands per primer. The primer GLC-19 produced maximum number of polymorphic bands i.e. 6. The total of 10 primers produced single polymorphic bands.

Molecular Characterization of Traderhorn, White Friendship and Peter Pears with RAPD markers: Multivariate analysis was conducted to generate a similarity matrix using Popgen 32 software, version 1.44 (Yeh *et al*., 2000) based on Nei's Unweighted Paired Group of Arithmetic Means Average (UPGMA) to estimate genetic distance and relatedness of three cormel genotypes. Dendrogram drawn for the genetic distances of *Traderhorn*, *White Friendship* and *Peter Pears* (Figs. 1, 2 and 3). Cluster analysis for RAPD data was performed individually for each variety on the basis of similarity coefficients between mother and *In vitro* propagated cormels (Table 4, 5 and 6). The RAPD similarity matrix among *In vitro* propagated cormels ranged from 80% to 95% in *Traderhorn*, 88% to 95% in *White Friendship* and 80% to 90% in *Peter Pears*. However the similarity tendencies between mother and *In vitro* propagated cormels, on average were 86% in *Traderhorn*, 92% in *White Friendship* and 83% in *Peter Pears*.

Table 4. Similarity matrix of *Traderhorn* with RAPD markers.

	In vivo	*In vitro*	*In vitro*	*In vitro*
In vivo	****	0.9515	0.8058	0.8350
In vitro	0.0498	****	0.8350	0.8641
In vitro	0.2159	0.1804	****	0.9320
In vitro	0.1804	0.1461	0.0704	****

Table 5. Similarity matrix of *White Friendship* with RAPD makers.

	In vivo	*In vitro*	*In vitro*	*In vitro*
In vivo	****	0.9320	0.8932	0.9223
In vitro	0.0704	****	0.8835	0.8932
In vitro	0.1129	0.1239	****	0.9515
In vitro	0.0809	0.1129	0.0498	****

Table 6. Similarity matrix of *Peter Pears* with RAPD makers.

	In vivo	*In vitro*	*In vitro*	*In vitro*
In vivo	****	0.8350	0.8641	0.8058
In vitro	0.1804	****	0.8738	0.8350
In vitro	0.1461	0.1349	****	0.9029
In vitro	0.2159	0.1804	0.1021	****

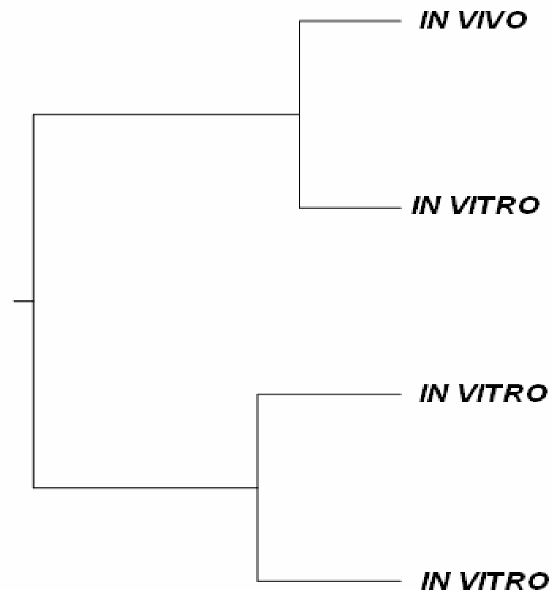

Fig. 1. Cluster analysis of *Traderhorn* with RAPD markers.

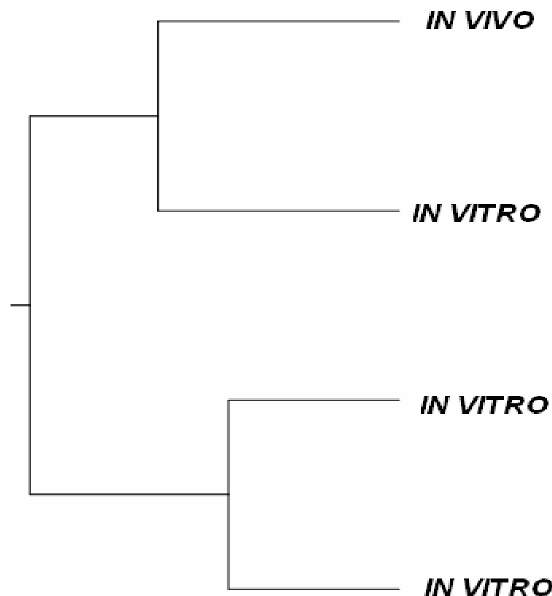

Fig. 2. Cluster analysis of *White Friendship* with RAPD markers.

Molecular characterization of *Traderhorn*, *White Friendship* and *Peter Pears* with ISSR markers: In comparison, ISSR primers produced higher percentage of similarity matrix than RAPD. The Multivariate analysis conducted to generate a similarity matrix using Popgen 32

software, version 1.44 (Yeh *et al*., 2000) based on UPGMA to estimate genetic distance and relatedness of three cormel genotypes (Figs. 4, 5 & 6). The ISSR cluster analysis for genetic similarity between mother and *In vitro* propagated cormels had varied degree of differences detected 90% in *Traderhorn*, 96% in *White Friendship* and 85% in *Peter Pears* (Table 7, 8 and 9). The genetic differences among *In vitro* propagated cormels ranged from 88 to 100% in *Traderhorn*, 94 to 100% in *White Friendship* and 82 to 100% in *Peter Pears*. The *In vitro* sample 2 and 3 had 100% similarity with each other in each variety.

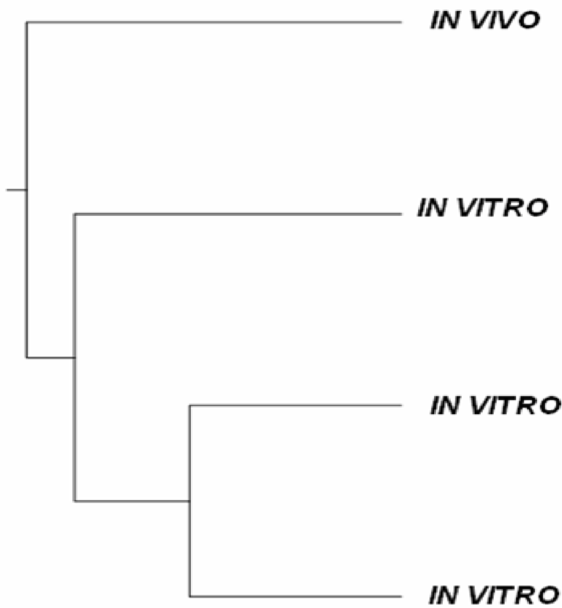

Fig. 3. Cluster analyses of *Peter Pears* with RAPD markers.

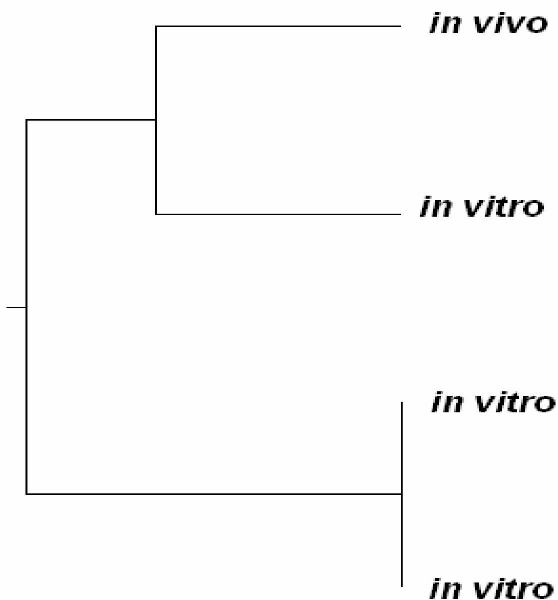

Fig. 4. Cluster analysis of *Ttraderhorn* with ISSR marker.

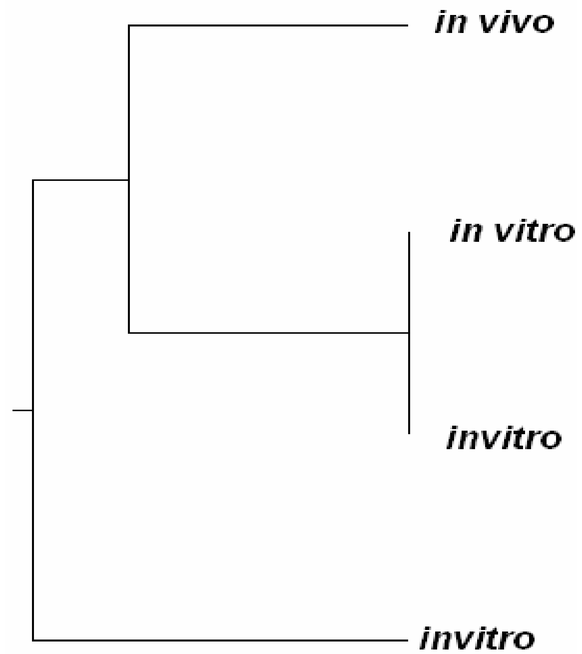

Fig. 5. Cluster analysis of *White Friendship* with ISSR marker.

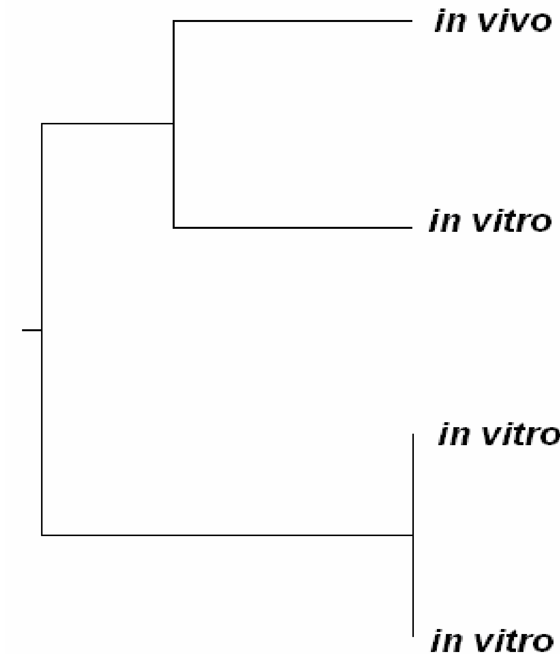

Fig. 6. Cluster analysis of *Peter Pears* with ISSR marker.

Table 7. Similarity matrix of *Traderhorn* with ISSR maker.

	In vivo	*In vitro*	*In vitro*	*In vitro*
In vivo	****	0.9412	0.8824	0.8824
In vitro	0.0606	****	0.9412	0.9412
In vitro	0.1252	0.0606	****	1.0000
In vitro	0.1252	0.0606	0.0000	****

Table 8. Similarity matrix of *White Friendship* with ISSR maker.

	In vivo	*In vitro*	*In vitro*	*In vitro*
In vivo	****	0.9412	0.9706	0.9706
In vitro	0.0606	****	0.9706	0.9706
In vitro	0.0299	0.0299	****	1.0000
In vitro	0.0299	0.0299	0.0000	****

Table 9. Similarity matrix of Peter Pears with ISSR marker.

	In vivo	*In vitro*	*In vitro*	*In vitro*
In vivo	****	0.9118	0.8235	0.8235
In vitro	0.0924	****	0.9118	0.9118
In vitro	0.1942	0.0924	****	1.0000
In vitro	0.1942	0.0924	0.0000	****

Discussion

In vitro propagation techniques by using cormel sprouts proved very applicable method for mass production of cormels. Generally multiplication is usually achieved through excessive shoot proliferation and transfer of rooted plantlets to soil. However, in gladiolus delivery can be made by producing cormels *In vitro*. In both the cases, shoot regeneration is the basic and major step for subsequent regeneration. Different responses of shoot regeneration were recorded from different cultivars. However, higher dose requirement of BAP was recognized as to be genotype dependent (Hussain *et al*., 2001). They studied shoot regeneration of two varieties (white and pink) of gladiolus and reported maximum number of shoots from shoot tip explants at a much higher BAP concentration (3.0 mg L^{-1} and 4.0 mg L^{-1} respectively for the two varieties). Priyakumari and Sheela (2005) obtained maximum number of shoots (4) from *Peach Blossom* by using enhanced release of axillary buds on MS medium supplemented with BAP+NAA (4 + 0.5 mg L^{-1}). Grewal *et al*., (1995) reported more number of multiple shoot primordia from different cultivars in response to BAP. They obtained single shoot per explant on MS medium supplemented with BAP (1 mg L^{-1}) in cultivars viz. *Mayur*, *Sylvia*, *Spic* and *Span*, whereas those cultured on MS medium supplemented with BAP (5 mg L^{-1}) obtained 14-20 shoot primordia within 4 weeks.

Efficient methods for developing roots are equally important for better cormel formation. Root initiation responses also varied from variety to variety although same explant and same nutrient medium was used for each variety. However, *White Friendship* and *Peter Pears* produced statistically same results. Hussain *et al*., (1994) produced extensive root growth from *In vitro* shoots in variety *White Friendship* in response to MS medium supplemented with IBA (2 mg L^{-1}). Priyakumari & Sheela (2005) produced the earliest and longest roots on IBA (2 mg L^{-1}), whereas the highest number of roots (24) was recorded in "*Peach blossom"* on MS medium containing NAA (1 mg L^{-1}). Kumar *et al*., (1999) recorded no or very poor response for root initiation in different cultivars on MS medium containing IBA or NAA. On the other side they reported that sucrose concentration had positive effect on the rooting response and quality of roots formed in "*Her Majesty*" and "*Aldebaran*" varieties. However they could not recorded root initiation from same sucrose concentration in "Bright Eye".

In vitro production of cormels may help to circumvent survival difficulties occurred during acclimatization (Ziv 1979; Steinitz & Yahel, 1982; Sengupta *et al*., 1984) as cormels are storage organs and be stored and planted easily as seed in the soil (Estrada *et al*., 1986; Ziv & Lillien Kipinis, 1990). Cormels are easier to handle and reduced labour cost (Slabbert & Niederwieser, 1999). The cormel induction and number of cormels was greatly affected by increasing levels of sucrose (Mares *et al*., 1985; Dantu & Bhojwani, 1987) which is considered to be stored as starch in the storage tissue of the bulbous plants (Van Aartrijk & Blom-Barnhoorn, 1980). Higher sucrose concentrations have been reported for bulblet formation or size enhancement in many bulbous plants such as tulip (Rice *et al*., 1983; Taeb & Al-Derson, 1990), narcissus (Squires *et al*., 1991), hyacinth (Bach, 1992) and *Lachenalia* (Slabbert & Niederwieser, 1999). In gladiolus cormel formation on higher levels of sucrose was reported by Roy *et al*. (2006) in "*Pacifica*"; Sinha and Roy (2002) in *Golden Wave* and Kumar *et al*., (1999) in "*Her Majesty*", "*Aldebaran*", and "*Bright Eye*" and Steinitz *et al*., (1991) in "*Kinneret*". Sinha and Roy (2002) produced more number of cormels (16.5 per shoot) of different sizes in "*Golden Wave*" on half strength MS medium supplemented with IBA (2 mg L^{-1}) and 6% sucrose. In this study more number of cormels of different size were recorded in each variety on full strength MS medium supplemented with IBA (1 mg L^{-1}) and sucrose (7%).

The assessment of somaclonal variation is a major step for successful *In vitro* propagation for true to type clones. The presence of somaclonal variation by using RAPD and ISSR molecular markers was detected among *In vitro* propagated cormels and had varying degree of variation from mother cormels in each variety of the gladiolus. Our results are contradictory with the results of Roy *et al*., (2006). They used cormel sprouts of White Flowered variety *Pacifica* and reported identical DNA profiles through isozymic, RAPD and ISSR analyses among mother corms, corms derived through tissue culture and field corms after one and two seasons. Bhatia *et al*. (2008) assessed clonal variation among the plants of gerbera regenerated from leaf explant by using ISSR marker. The exact cause is not known. It might be due to explant source or due to mode of regeneration (Goto *et al*., 1998); media composition or culture conditions (Damasco *et al*., 1996) and sub and supra-optimal levels of plant growth regulators (Martin *et al*., 2006). Potter and Jones (1991) reported that undifferentiated tissues are the main cause for creating variation among clones. However, clones regenerated from well organized cultures are genetically stable. Rout *et al*., (1998) and Joshi and Dhawan (2007) reported that the adventitious buds or well developed meristematic tissues had low tendency for genetic variation, whereas, more clonal variation was recorded in regenerants produced through callus phase as compared to those regenerated from embryogenic tissues (Yang *et al*., 1999). Somaclonal variation by using different microsattelite techniques (RAPD, ISSR and SSR) was reported in many other plants other than gladiolus that were micropropagated even from organized cultures (De Laia *et al*., 2000; Devarumath *et al*., 2002) such as shoot buds (Rahman & Rajora, 2001; Bindiya &

Kanwar 2003) and nodal segments (Devarumath *et al*., 2002; Chandrika & Rai, 2009). Bindiya and Kanwar (2003) used 18 micropropagated plants and reported similarity tendencies from 0.86 to 0.96. Rahman and Rajora (2001) reported somaclonal variation among *In vitro* propagated plants of trembling aspen (*Populus tremuloides*) using different genotypes derived from the tissues of well organized vegetative buds.

The somaclonal variation was also reported in many other *In vitro* propagated plants such as *Populus deltoids* (Rani *et al*., 1995), Peach (Hashmi, *et al*., 1997), *Robinia pseudoacacia* (Major *et al*., 1998); *Populus tremuloides* (Rahman & Rajora, 2001). Rani *et al*. (1995) observed somaclonal variation of 26% *in In vitro* cultured samples of *Populus deltoide*. Watanabe *et al*. (1998) reported similarity coefficients range from 84-97% in plants of *Angelica acutiloba*. Similarity tendencies between 12 *In vitro* cultured samples of *Robinia* were also reported from 0.51 to 0.95 (Major *et al*., 1998).

Conclusion

The present study demonstrated that the RAPD and ISSR molecular markers can be successfully used for the detection of clonal fidelity. ISSR proved more authentic as compared to RAPD for assessment of the clonal fidelity and study also confirms that the clonal fidelity of *In vitro* propagated plants cannot always be assured, it can occur even from organized tissues of the plant.

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