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RESEARCH ARTICLE

In vitro Callogenesis and Detection of Somaclonal Variations in *Plantago ovata* L.

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Abstract

Planago ovata L. is an economically important species in the monotypic genus *Plantago*. It is a short-stemmed annual herb. The seed husk of this plant is commonly called psyllium or isabgol which is important in pharmaceutical formulation and food industry. In this study, callus induction was optimized using different explants of *Plantago ovata*. Callus DNA was utilized to access the somaclonal variations using the Random Amplification of Polymorphic DNA (RAPD) markers. The maximum callus growth was observed in Murashige and Skoog (MS) medium containing 4 mg L^{-1} 2,4-D concentration for shoots, 0.5 mg L^{-1} for seeds and 2 mg L^{-1} for roots. Moreover, the effect of culture age was considered in assessing genetic variability. Maximum genetic variability was observed in the DNA samples of callus at the concentration of 2 mg L^{-1} 2,4-D for all explants (roots, shoots, and seeds). Cluster analysis was performed based on 1) similarity coefficient between samples and 2) molecular data using the Numerical Taxonomy and Multivariate Analysis System (NTSYS) PC version 2.01; similarity index was generated by similarity for Quantitative Data (SIMQUAL). Our study indicated that Random Amplified Polymorphic DNAs can successfully be used to explore polymorphism among callus samples at different hormonal concentrations. This study can be useful for the production of callus from *Plantago ovata* and estimation of genetic variations due to tissue culture conditions. Evaluation of genetic variations can display novel features and manipulate genetic bottlenecks in *Plantago ovata*. New genetic variations in somaclones can bring vital insight for plant improvement.

Key words: genetic variations, micropropagation, polymorphism, RAPDs

Introduction

Plantago ovata is a small annual herb growing on temperate, sandy regions located between 26° and 36° N latitudes. The seed husks are commonly called *Psyllium* or Isabgol in Hindi (Dhar et al. 2005). It has a mucilage component and harboring a unique importance in pharmaceutical industries, it is most commonly used as an adjuvant in various pharmacological formulations because of its suspension, emulsifying, and sustainable properties in different drug doses (Shirsand et al. 2009). *P. ovata* is also commonly used in Pakistan, Bangladesh, and India for the treatment of various ailments like metabolic syndrome, irritable bowel syndrome (Galisteo et al. 2009), diarrhoea, adjustment of faecal consistency among patients with colostomies, catarrhal conditions, enitourinary tract infections, anti-inflammation of intestinal membrane, reduction of plasma low-density lipoprotein (LDL) cholesterol, triglycerides, insulin, and can oxidize LDL (Solà et al. 2010). In addition to its medicinal uses, it has its place in dyeing, calico printing, setting lotions, and in the food industry.

Various studies have been carried out to widen the genetic makeup of *Plantago* including the induction of somaclonal variations and different environmental stresses (Li et al. 2005; Pramanik et al. 1995). One of the methods for widen-

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ing the genetic bases of the crop is to induce somaclonal variability through *in vitro* culture. As a first step in this direction, Barna and Wakhlu (1988) reported micropropagation in *Plantago* species. Wakhlu and Barna (1989) developed a protocol for callus initiation from hypocotyl explants of *Plantago* species. In the same year in another report, Barna and Wakhlu (1989) regenerated plants from callus derived from root cultures using MS medium supplemented with IAA (indole acetic acid) and kinetin. Somaclonal variation has been related to growth regulators, cultivar variability, age of cultivars in culture, ploidy level, explant source, and other culture conditions (Skirvin et al. 1994). The presence of certain chemicals like 2,4-D (2,4 dicholorophenoxy acetic acid) also enhances the rate of these variations. Larkin and Scowcroft (1981) suggested that somaclonal variation is a useful source of novel variation for plant improvement. For the detection of somaclonal variations, RAPD markers have extensively been used in different studies for different plant species (Soniya et al. 2001).

Outcrossing, morphological characteristics, seed shattering, cultivation on broader scale, and environmental limitations are the major confronting challenges of *P. ovata* aside from its narrow genetic base (lack of variability), high heterochromatin and small chromosome size, low chiasmata frequency, and low recombination index. Keeping in view the significance and challenges regarding *Plantago* species, our group is working on exploring different aspects of this medicinally important plant. In this context, antioxidative potential (Mahmood et al. 2011a) and genetic diversity (Saeed et al. 2011) studies on *Plantago* species were carried out and data has recently been published. For the evaluation of antioxidant activity, the selected species were *P. ovata*, *P. lanceolata*, and *P. mexicana*). It was observed that antioxidant activity (by ascorbate assay and catalase assay) *P. ovata* and *P. lanceolata* showed antioxidant potential by both assays while *P. mexicana* showed no activity for catalase assay. The data suggested that different *Plantago* species can display varied antioxidant activity potential for different assays (Mahmood et al. 2011a). Furthermore, *rps*14 gene-based phylogenetic analysis was carried out for three *Plantago* species (*P. ovata*, *P. lanceolata*, and *P. mexicana*). In this study, polymerase chain reaction-restricted fragments length polymorphism (PCR-RFLP) technique was applied to study genetic composition and diversity in terms of genetic similarities and differences among *Plantago* species. The data revealed only 14% polymorphism indicating high genetic conservation among the studied *Plantago* species (Saeed et al. 2011). Presently, a study was designed for the development of *in vitro* callus induction system from different explants of *P. ovata*. Effect of different hormonal combinations and concentrations on micropropagation and callogenesis was analyzed and optimized. The calli generated in response to different concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) were subjected to the assessment of somaclonal variations by RAPD markers.

Materials and Methods

Plating of *P. ovata* seeds

Seeds of *P. ovata* were procured from the seed bank of the NARC (National Agricultural Research Centre), Islamabad, Pakistan and stored at 4ºC. After surface sterilization, seeds were plated on MS basal medium (4.43 g L^{-1}) supplemented with 30% sucrose, 2 mg L^{-1} gibberelic acid (GA_3) and 2 mg L^{-1} gellan gum powder. After seed plating, germination of seeds was monitored from time to time.

Nutrient media and culturing conditions

For callus culture, MS medium 4.43 g L^{-1} was used and supplemented with different concentrations of 2,4-D (1, 2, 4, 8, 12, and 16 mg L^{-1}) for optimization of callus growth conditions. Shoots and roots explants were cut into 3 - 4 mm pieces. The callus culturing was done in Petri plates under optimized conditions. The rate of callus growth, color, and texture were recorded regularly. The full callus growth was obtained after 33 - 35 days of culturing. The quantitative measurement of callus growth was estimated in terms of percentage of callus and degree of callus growth. The test tubes cultures were kept under 16 h of photoperiod, 55 ± 5% of relative humidity at 25 - 26 ºC, and the light intensity was maintained at 1,000 lux.

Assessment of somaclonal variations

To detect the somaclonal genetic variations, RAPD markers were used in order to find the effect of different hormonal concentrations on *in vitro*-propagated callus genotype. RAPD is used to trace the genetic variations in micropropagated plants. The type of explants used for micropropagation can also contribute to genetic variations.

DNA extraction

Genomic DNA from *in vitro*-generated shoots, roots (from seeds) and callus of *P. ovata* derived from seeds, shoots, and roots were isolated by CTAB method (Richards 1997). All the DNA samples were stored at -20ºC till further use.

Optimization of polymerase chain reaction (PCR) conditions

Ten primers of OPC (Gene link) series (OPC1-10) were tested for amplification of DNA. Only four primers of OPC series gave amplification profiles. The sequence of the primers used is shown in Table 1. Different PCR conditions were used for the optimization of PCR amplification. However, the best suitable conditions were as follows: 35 PCR cycles comprised of initial denaturation at 94ºC for 30 s, annealing at 40ºC for 1 min, and extension at 72ºC for 2 min. Final cycle was same except extension for 7 min at 72ºC. After PCR, contents were held at 4ºC till use. Amplification reaction was prepared in 25 µL reaction mixture containing 25 - 50 ng μL^{-1} DNA, 25 pmol primer, 12.5 µL 2 X PCR master mix (Fermentas), and 10.5 µL of PCR

Table 1. PCR primers with 10 bp nucleotide length were used for detection of somaclonal variations in *Plantago ovata* L.

Sr. No.	Primer Code	Primer Sequence
1	OPC1	TTCGAGCCAG
2	OPC2	GTGAGGCGTC
3	OPC3	GGGGGTCTTT
4	OPC4	CCGCATCTAC
5	OPC5	GATGACCGCC
6	OPC6	GAACGGACTC
7	OPC7	GTCCCGACGA
8	OPC8	TGGACCGGTG
9	OPC9	CTCACCCTCC
10	OPC10	TGTCTGGGTG

water (Fermentas) by using thermal cycler (Labnet MultiGene). The amplified products were run on agarose gel for data scoring.

Data scoring and analysis

Data was recorded from gels and the presence of a particular band was denoted by "1" while the absence is labelled with "0". In this way, all amplified profiles from the primers used were scored and integrated together to form a data matrix for estimation of genetic distance between the samples. The phylogenetic cluster analysis was performed with Sequential Agglomerative Hierarchical Nested (SAHN), based on molecular data using Numerical Taxonomy and Multivariate Analysis System (NTSYS) PC version 2.01 (Rohalf 2005). The data was generated on the basis of similarity coefficient values generated for our samples analyzed through Simqual which is a subprogram of NTSYS-pc software.

Results

In this study, tissue culture condition for *P. ovata* was optimized by using 2,4-D growth hormone. Callus obtained from tissue culture optimization was subjected to assessment of somaclonal variations.

Seed germination on MS media

The seed germination frequency was 90% in the presence of GA_3 (2 mg L^{-1}) in ½ strength MS medium. Cultures were checked from time to time to monitor the growth response. The seeds developed into plantlets after 23 - 24 days after plating.

Relative potential of various explants for callus formation

To determine the effect of explant used on callus induction, different explants (seeds, shoots, and roots) were cultured on MS media supplemented with different concentrations of 2,4-D (0.5, 1, 2, 4, 8, 12, and 16 mg L^{-1}) for callus induction. There were wide ranges of callus observed on different concentrations of 2,4-D. The color varied from creamy white to yellowish green while some callus were classified as fragile and compact (Table 2). Moreover, the mature seed-derived callus was creamy white to creamy in color while shoot-derived callus was yellowish green in color and fragile. The nature of mature seed-derived callus is compact but fragile and tend to be very dry with increasing concentration of 2,4-D (Table 2). The difference in callus texture may be a result of a difference in hormonal concentrations. It was observed that different explants responded differently towards callogenesis under different hormonal concentrations. It was also observed that the different concentrations of 2,4-D have an effect on the different responses to the time required for callus induction and to the size of callus. It was observed that 0.5 mg L^{-1} of 2,4-D for seeds (78.57%), 1 mg L^{-1} for shoots (75%), and 2 mg L^{-1} for roots (76.92%) have produced the highest percentage of callus (Fig. 1). It was also observed that using root as an explant, callus formation can be achieved at 2 mg L^{-1} 2,4-D. Overall, it was seen that an

Table 2. Effect of 2,4-D on callus formation from different explants (for each treatment 15 explants were used)

Conc. of 2,4-D (mg L^{-1})	Explant source	Callus initiation observed (Days)	Callus culture period (Days)	Weekly Response				Texture3	Color
				1st week	2nd week	3rd week	4th week		
0.5	Seed	3 - 4	30 - 34	+	+++	++++	+++++	Fragile/compact	Creamy white
0.5	Shoot	4 - 5	26 - 29	+	++	+++	++++	Fragile	Yellowish green
1	Seed	3 - 5	27 - 31	+	++	+++	++++	Fragile/compact	Creamy white
1	Shoot	3 - 4	28 - 30	+	+++	+++	+++	Fragile	Yellowish green
2	Seed	3 - 5	29 - 31	+	++	++	++	Fragile/compact	Creamy white
2	Shoot	4 - 5	28 - 30	+	++	+++	+++	Fragile	Yellowish green
2	Root	4 - 6	30 - 34	+	+++	++++	+++++	Compact	Creamy
4	Seed	3 - 5	29 - 32	+	++	+++	+++	Fragile	Creamy white
4	Shoot	3 - 4	28 - 32	+	+++	++++	+++++	Fragile	Yellowish green
8	Seed	5 - 6	28 - 30	+	+	++	++	Fragile/compact	Creamy white
8	Shoot	4 - 6	30 - 32	+	+	++	+++	Fragile	Yellowish green
12	Seed	4 - 5	31 - 33	+	++	++	++	Fragile/compact	Creamy white
12	Shoot	5 - 6	31 - 34	+	+	+	+	Fragile	Yellowish green
16	Seed	4 - 5	30 - 33	+	+	++	++	Fragile/compact	Creamy white
16	Shoot	4 - 6	31 - 34	+	+	+	++	Fragile	Yellowish green

+: Very small size callus, ++: Small size callus, +++: Medium size callus, ++++: Large size callus.

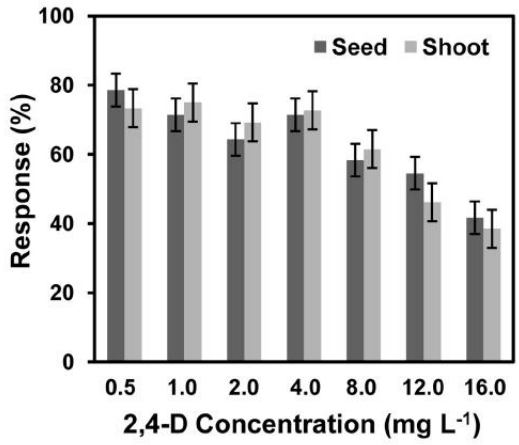

Fig. 1. Percent response of seed and root of *Plantago ovata* at different 2,4-D concentrations.

Table 3. Total number of bands produced by 4 OPC primers

Sr. No.	Primers	MMB*	PMB*	TNB*	Polymorphism (%)
1	OPC 1	17	15	32	46.87
2	OPC 2	17	5	22	22.72
3	OPC 3	17	7	24	29.16
4	OPC 4	17	0	17	0
Total		68	27	95	28.42

* MMB: monomorphic bands, PMB: polymorphic bands, TNB: total number of bands

increase in the concentration of 2,4-D beyond 4 mg L^{-1} has a negative impact on callus induction and there was a decrease in callus growth with an increase in concentration of 2,4-D and the lowest rate of callus induction was observed at 16 mg L^{-1} of 2,4-D (Fig. 1).

RAPD profiling

A total of 95 bands with band size ranging from 250 – 1,100 bp were produced using the selected primers. Among the 95 bands, 68 (71.57%) were monomorphic while 27 (28.42%) were polymorphic. The highest number of bands was produced by OPC 1 while the lowest number of bands was produced by OPC 4 (Table 3). Maximum polymorphism was observed in OPC 1 which contained 15 polymorphic bands and no polymorphism was observed in OPC 4.

Cluster analysis based on amplified bands produced by all primers

Similarity indices were developed on the basis of amplified products of four RAPD primers in 17 samples of *P. ovata* containing 15 callus-derived DNA samples and two samples of *in vitro*-generated shoots and root DNA (Table 4). The range of genetic similarity values were 0.27 to 1.00 with the mean of 0.63. The lowest similarity value was present between samples 1 and 5. However, the highest level of similarity was present in sample 7 (shoot-derived callus DNA from 2 mg L^{-1} of 2,4-D), sample 10 (seed-derived callus DNA from 8 mg L^{-1} of 2,4-D), and sample 13 (shoot-derived callus DNA from 12 mg L^{-1} of 2,4-D) and showed 100% similarity among each other. NTSYS cluster analysis of all primers revealed three major clusters in the cladogram (Fig. 2). Data from four primers of OPC series were analyzed and samples had shown 42% divergence.

Cluster 1 formed a group (G1) which had two samples 1 and 9 containing seeds and shoot-derived callus DNA from 0.5 and 4 mg L^{-1} of 2,4-D representing 66% similarity. Cluster 2 was further sub-divided in 2 sub-clusters (Sc 1 and 2). Sc 1 formed G2 having three samples 2, 3, and 4 (shoot-derived callus DNA on 0.5 mg L^{-1} of 2,4-D, seed and shoot-derived callus DNA on 1 mg L^{-1} of 2,4-D, respectively) indicating 75.5% similarity level. However, Sc2 was further subdivided into four groups G3, G4, G5, and G6. Among these groups, G3 had two samples, 6 and 14 (root-derived callus DNA at 2 mg L^{-1} of 2,4-D and shoot-derived callus DNA at 16 mg L^{-1} of 2,4-D) showing 78% similarity (Fig. 2). The

Table 4. Genetic similarity coefficients of *Plantago ovata* (callus-derived DNA and *in vitro*-generated shoot and root DNA) based on elucidation distance

	1[a]	2[b]	3[c]	4[d]	5[e]	6[f]	7[g]	8[h]	9[i]	10[j]	11[k]	12[l]	13[m]	14[n]	15[o]	16[p]	17[q]
1[a]	1																
2[b]	0.67	1															
3[c]	0.73	0.93	1														
4[d]	0.73	0.93	0.87	1													
5[e]	0.27	0.6	0.53	0.53	1												
6[f]	0.73	0.8	0.87	0.87	0.53	1											
7[g]	0.53	0.87	0.8	0.8	0.73	0.8	1										
8[h]	0.47	0.8	0.73	0.73	0.8	0.73	0.93	1									
9[i]	0.67	0.47	0.53	0.53	0.33	0.67	0.6	0.53	1								
10[j]	0.53	0.87	0.8	0.8	0.73	0.8	1	0.93	0.6	1							
11[k]	0.6	0.8	0.73	0.87	0.67	0.87	0.93	0.87	0.67	0.93	1						
12[l]	0.4	0.6	0.67	0.53	0.87	0.67	0.73	0.8	0.47	0.73	0.67	1					
13[m]	0.53	0.87	0.8	0.8	0.73	0.8	1	0.93	0.6	1	0.93	0.73	1				
14[n]	0.67	0.73	0.8	0.8	0.6	0.93	0.87	0.8	0.73	0.87	0.93	0.73	0.87	1			
15[o]	0.6	0.8	0.87	0.73	0.67	0.87	0.93	0.87	0.67	0.93	0.87	0.8	0.93	0.93	1		
16[p]	0.6	0.8	0.73	0.87	0.67	0.87	0.93	0.87	0.67	0.93	1	0.67	0.93	0.93	0.87	1	
17[q]	0.6	0.8	0.87	0.73	0.67	0.87	0.93	0.87	0.67	0.93	0.87	0.8	0.93	0.93	1	0.87	1

[a] 0.5 mg L^{-1} seeds derived callus DNA, [b] 0.5 mg L^{-1} shoots derived callus DNA, [c] 1 mg L^{-1} seeds derived callus DNA, [d] 1 mg L^{-1} shoots derived callus DNA, [e] 2 mg L^{-1} seeds, derived callus DNA, [f] 2 mg L^{-1} roots derived callus DNA, [g] 2 mg L^{-1} shoots derived callus DNA, [h] 4 mg L^{-1} seeds derived callus DNA, [i] 4 mg L^{-1} shoots derived callus DNA, [j] 8 mg L^{-1} seeds derived callus DNA, [k] 8 mg L^{-1} shoots, derived callus DNA, [l] 12 mg L^{-1} seeds derived callus DNA, [m] 12 mg L^{-1} shoots derived callus DNA, [n] 16 mg L^{-1} seeds derived callus DNA, [o] 16 mg L^{-1} shoots derived callus DNA, [p] *in vitro* generated shoots DNA, [q] *in vitro* generated roots DNA.

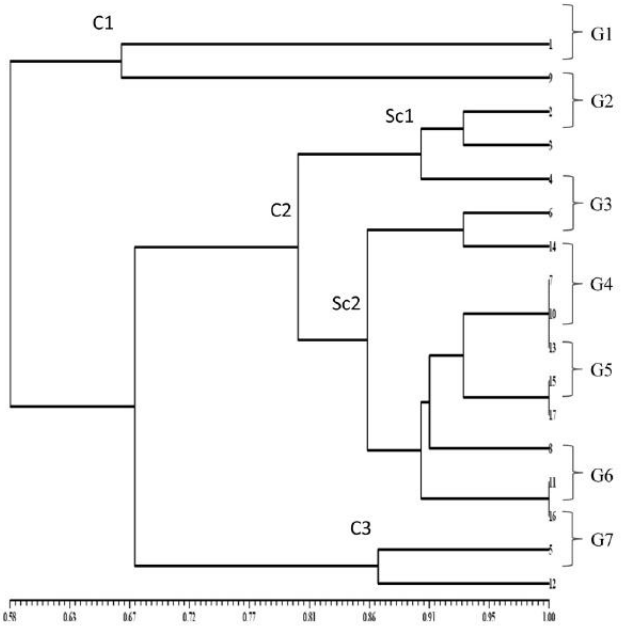

Fig. 2. Cluster analysis of amplified bands produced by all primers. C1: Cluster 1; C2: Cluster 2; C3: Sc1: Sub cluster 1; Sc2: Sub-cluster 2; G1: Group 1; G2: Group 2; G3: Group 3; G4: Group 4; G 5: Group 5; G6: Group 6; G7: Group 7. 1: seed-derived callus DNA at 0.5 mg L^{-1} 2,4-D; 2: shoot-derived callus DNA at 0.5 mg L^{-1} 2,4-D; 3: seed-derived callus DNA at 1 mg L^{-1} 2,4-D; 4: shoot-derived callus DNA at 1 mg L^{-1} 2,4-D; 5: seed-derived callus DNA at 2 mg L^{-1} 2,4-D; 6: root-derived callus DNA at 2 mg L^{-1} 2,4-D; 7: shoot-derived callus DNA at 2 mg L^{-1} 2,4-D; 8: seed-derived callus DNA at 4 mg L^{-1} 2,4-D; 9: shoot-derived callus DNA at 4 mg L^{-1} 2,4-D; 10: seed-derived callus DNA at 8 mg L^{-1} 2,4-D; 11: shoot-derived callus DNA at 8 mg L^{-1} 2,4-D; 12: seed-derived callus DNA at 12 mg L^{-1} 2,4-D; 13: shoot-derived callus DNA at 12 mg L^{-1} 2,4-D; 14: seed-derived callus DNA at 16 mg L^{-1} 2,4-D; 15: shoot-derived callus DNA at 16 mg L^{-1} 2,4-D; 16: *in vitro*-generated shoot DNA, 17: *in vitro*-generated root DNA.

group G4 had three samples 7, 16, and 13 (callus-derived shoots and direct seed-generated shoots at 2 mg L^{-1} of 2,4-D and callus-derived DNA at 12 mg L^{-1} of 2,4-D) indicating 93% similarity. Two samples 15 and 17 (root-derived callus DNA at 16 mg L^{-1} of 2,4-D and *in vitro* generated root DNA) represented the G5 and showed 89% similarity (Fig. 2). The last group of Sc2, G6 had two samples 5 and 12 (seed-derived callus DNA at 2 and 12 mg L^{-1} of 2,4-D). The similarity level in G6 was 86%. Finally, in the cluster 3 there are two samples 5 and 12 (seed-derived callus DNA at 2 mg L^{-1} of 2,4-D and seed-derived callus DNA at 12 mg L^{-1} of 2,4-D) making G7 the last group of the whole tree.

Discussion

In our study it has been observed that GA_3 is effective in overcoming dormancy and causing rapid germination of seeds. High seed germination frequency (90%) was optimized and obtained with 2 mg L^{-1} of GA_3. Earlier, McNeil and Duran (1992) studied the effect of KNO_3, gibberellin and temperature on germination of *P. ovata* and demonstrated that percentage of seed germination was raised by increasing the concentrations of GA_3.

Callus induction

Callus is a coherent but unorganized and amorphous tissue, formed by the vigorous division of the plant cells. Callogenesis is considered to be a significant feature of indirect organogenesis and essential for research on biologically active molecules in medicinal plant species (Abbasi 2000). Earlier in some reports, callogenesis was initiated from shoot buds or hypocotyls of *P. ovata* on MS media with different concentrations of kinetin and 2,4-D (Wakhlu and Barna 1988,1989). In the present study, auxin (2,4-D) was used in different concentrations for callus induction in *P. ovata*. It has been reported that callus induction and growth has consistently shown that callus generation potential varied with explant origin, their growth rate, texture, and color. Explant from seeds and shoots had a higher potential for callus formation while explant from roots were less efficient for callus induction (Li et al. 1995). In a recent study on *in vitro* culturing and callogenesis of *Solanum tuberosum*, it was illustrated that the use of different explants and variations in hormonal concentrations affect variously on callus development and micropropagation. In this analysis RAPD technique was applied in evaluating genetic variations that occurred in potato somaclones (Munir et al. 2011).

Selection of molecular markers for the detection of somaclonal variations

Somaclonal variations are important with respect to crop improvement especially in genetically narrow plants. These variations can be a novel source of genetic variation. Callus undergoes re-differentiation and de-differentiation and remains under stress in *in vitro* conditions resulting in the increase in genome plasticity and restructure. For RAPD analysis, reliable RAPD data can be generated following a standard protocol, replication of amplification reaction, and conservative criterion of band selection (Belaj et al. 2003). The RAPD technique is quick (Colombo et al. 1998), most effective (Fungang et al. 2003), and has the ability to perform analysis without need for prior sequencing of the genome (Huff et al. 1993). There are many reports regarding the use of RAPD markers with optimized conditions/protocols and consistent RAPD profiles (Mahmood et al. 2010a, 2010b, 2011b, 2011c, 2011d; Nazar and Mahmood 2011; Shinwari et al. 2011; Zeb et al. 2011). In another study, RAPD technique was applied for the estimation of genetic variations among five *Terminalia* L. species by utilizing 31 random primers (Deshmukh et al. 2009). In another related study, RAPD marker technique was used to assess genetic diversity for five cultivars of *P. ovata* (Das née Pal and Raychaudhuri 2003). Ten primers from OPC series (OPC1 - 10) were selected for the present study to explore the somaclonal variations in the calli produced by different concentrations of 2,4-D. In our study, 27 polymorphic bands were generated. The overall polymorphism detected was 28.42% indicating genetic variations in somaclones. There was a difference in the intensity of polymorphic bands produced by different

primers. Similarly, it was reported by Munir et al. (2011) that each primer anneals at different intensities to a specific DNA sequence and there may exist several unknown copies of specific target DNA sequence per genome that instigate variance in banding potencies.

P. ovata is important with respect to medicinal values besides it is a model herbal plant. India is the major exporter of *P. ovata* and due to its unique importance in pharmacy and food industries, consumers extensively exploited it. *In vitro* tissue culture techniques are well known to be an important tool in the induction of variation leading to the development of new plant genotypes (Kuksova et al. 1997; Larkin and Scowcroft 1981). This source of variability is considered as a useful tool for geneticists and plant breeders (Amzad et al. 2003). Earlier, Pramanik et al. (1995) developed callus from hypocotyl of *P. ovata* in MS basal medium with different concentrations of 2,4-D/kinetin and NAA/BAP and subsequently regenerated the shootlets. Later on, Li et al. (2005) *in vitro* cultured the *Plantago major* by using MS medium supplemented with 0.2 mg L^{-1} of IAA and 1.0 mg L^{-1} of TDZ and further carried out RAPD analysis to detect the somaclonal variations. Jin et al. (2008) applied RAPD markers for revealing somaclonal variations in cotton (*Gossypium hirsutumii*) and their results indicated that the hormonal combination of 2,4-D and kinetin generated high genetic variability. RAPD has been widely used for the identification of phylogenetic relationships and rational designing of breeding programs (Powell et al. 1996). The banding patterns generated using RADP markers remains the same irrespective of the developmental stage of the plant (Swenson et al. 1995). This feature of RAPD loci has made these markers very useful for identifying lineages for conservation when other methods fail to detect variations or resolve relationships (Esselman et al. 2000). Somaclonal variation could be used to uncover new traits and manipulate genetic bottlenecks in *P. ovata*.

Conclusions

P. ovata also called isabgol has vital importance in pharmaceutical industry and much less attention was paid to improve it as it is genetically narrow plant species. In the current study, protocols were optimized for *in vitro* callogenesis under different concentrations of auxin (2,4-D). The calli DNA was used to assess the somaclonal variation with the help of RAPD markers. Among RAPD primers used, OPC1 have shown maximum genetic variability and it was from callus DNA derived from MS media supplemented with 2 mg L^{-1} of 2,4-D.

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