Pak. J. Bot., 37(2): 249-262, 2005.

RAPD CHARACTERIZATION OF SOMACLONAL VARIATION IN INDICA BASMATI RICE

SULTANA RASHEED*, TAHIRA FATIMA, TAYYAB HUSNAIN, KHURRAM BASHIR AND SHIEKH RIAZUDDIN

National Center of Excellence in Molecular Biology,
87-W Canal Bank Road, University of the Punjab,
Lahore, Pakistan, 53700

Abstract

Seeds of three cultivars of indica basmati rice viz., B-370, B-2000 and Super basmati were sterilized and incubated for 2, 4, 6 and 8 weeks on callus induction medium. Fifteen interested regenerates were selected on the basis of variation in important morphological characteristics. DNAs from leaves of regenerated plants along with parental controls were isolated and analyzed through PCR using 8 Random Amplified Polymorphic DNA (RAPD) Primers. Four primers viz., S-13, S-19, R-17 and OPX-11 produced detectable range of amplification products and 7 interesting clones with different banding patterns were obtained. Index of genetic variation was calculated and dendrograms were constructed using UPGMA method. Genetic variation up to 45.2, 32.3, 32.4 & 35.3% was recorded after 8 weeks of incubation with primers S-13, S-19, R-17 and OPX-11 respectively. Our study indicates that tissue culture generates a wide range of variation, which is related with incubation time and is cultivar specific. Moreover, RAPDs can successfully be used to explore such polymorphism within and among different cultivars of basmati rice.

Introduction

Tissue culture now is a common way to propagate crop plants of commercial importance. The original expectation was that all plants regenerated from cell or tissue culture has a genetic constitution identical to that of the original one. However it was soon observed that phenotypic variation might be abundant amongst regenerated plants. This variation is called as somaclonal variation and may be defined as genetic and phenotypic variation among clonally propagated plants of a single donor clone (Sunderland, 1973; D' Amato; 1977; Scowcroft, 1985; Sun & Zheng, 1990; Kaeppler *et al*., 1998; Olhoft & Philips, 1999; Kaeppler *et al*., 2000). The mechanism of somaclonal variation involves extensive genomic flux e.g., repeated DNA sequences can rapidly be amplified or reduced in copy number, altered methylation patterns can be inherited, DNA replication is disturbed by altered nucleotide pools, and active genes can be silenced or silenced gene may be activated by mutations in associated non-coding regions (Scowcroft *et al.*, 1985). Epigenetic factors are also involved making it more complex (Kaeppler *et al*., 2000). Somaclonal variation has been related to growth regulators, cultivar variability, cultivars age in culture, ploidy level, explant source and other culture conditions (Skirvin *et al*., 1994). The presence of certain chemicals like 2,4-D also enhances the rate of this variation. Larkin & Scowcroft (1981) suggested that somaclonal variation was a useful source of novel variation for plant improvement.

*Corresponding Author: National Center of Excellence in Molecular Biology, 87-W Canal Bank Road, University of the Punjab, Lahore, Pakistan, 53700
Phone: 92-42-5423942 Fax: 92-42-516455
Email: tayyab@cemb.pu.edu.pa

In Pakistan, somaclonal variation could successfully be used to create crop plants of commercial importance and development of Rachna basmati is an example (Abbas, 2000). Somaclonal variation may be manifested as either somatically or meiotically stable events. Somatically stable mutation is often not transmitted to subsequent generations and the primary regenerates become the end products where as meiotically stable variation could be transferred to progeny (Kaeppler *et al*., 2000). So characterization of this variation with molecular markers is of utmost importance to take full advantage of this variation in plant breeding programs.

With the advent of Polymerase Chain Reaction (PCR) technology, it has made it possible to study the genetic differences in crop plants and animals. DNA fingerprinting, gene mapping and phylogenetic studies have tremendously benefited from PCR. One variation of PCR is the random amplified polymorphic DNA (RAPD), which generates DNA fingerprints with a single synthetic oligo-nucleotide primer (Williams *et al.,* 1990). Although molecular markers such as RFLP: Restriction Fragment Length Polymorphism (Jeffreys *et al.,* 1985) and RAPD: Random Amplified Polymorphic DNA (RAPD) (Rafalski *et al.,* 1991) are capable of producing unique pattern of bands that are being extensively used in rice breeding programs for different purposes (McCouch & Tanksley, 1991).

Gene mapping using RAPD markers has several advantages over RFLPs, since RAPD markers are technically simple, quick to perform with small amount of DNA and do not require radioactive labeling (Michelmore *et al.,* 1991). RAPDs are usually dominant and are inherited in a simple Mendelian fashion. Therefore, they are being preferred over RFLP and are being used for tagging genes (Klein-Lankhorst *et al.,* 1991, Martin *et al.,* 1991, Rafalski *et al.,* 1991; Roanald *et al.,* 1992), for detection of genetic polymorphism in cereals (Devos & Gale, 1992; Tanzarella & Proceddu.1990), for identification of cultivars (Hu & Quirose, 1991) and for fingerprinting of genomes (Nybom *et al.,* 1989, Welsh & McClelland, 1991). These markers were also used to characterize different rice accessions (Fukuoka *et al*., 1992) and cultivars (Munthali *et al.,* 1992; Ashikawa *et al.,* 1992; Yu & Nguyen, 1994). Thus RAPD analysis is a useful tool in determining genetic relationships among rice cultivars.

The present work was undertaken to assess the effect of incubation period and cultivar specificity for somaclonal variation and to utilize RAPD markers for detection of this variation in basmati rice varieties viz., B-370, B-2000 and Super basmati.

Material and Methods

One hundred and ninety eight de-husked mature seeds of B-370, B-2000 and Super basmati were selected and sterilized with 70% ethanol followed by 50% sodium hypochlorite. After sterilization these seeds were cultured on callus induction medium and incubated for 2, 4, 6 and 8 weeks in dark at 26 ± 2^{0}C. Calli were then transferred to regeneration medium and plantlets were obtained through somatic embryogenesis. After 35 days, regenerated plants were directly transferred to the field. These plants were scored for morphological variation (data not shown) and 15 interesting clones from all 3 varieties were selected on the basis of this variation in different morphological characteristics. DNAs were extracted from fresh leaves of interesting clones along with parental varieties by standard method (Dellaporta *et al.,* 1983). DNA concentration and its quality were determined with the help of gel electrophoresis and spectrophotometry.

Genome of 15 interesting clones was explored through RAPD analysis. Eight arbitrary primers belonging to S, R and OP-series were used for DNA amplification. Primers used for the amplification were categorized as:

S-08 5'-TTCAGGGTGG-3'
S-13 5'-GTGGTTCCTG-3'
S-18 5'-CTGGCGAACT-3
S-19 5'-GAGTCAGCAG-3'
R-08 5'-CCCGTTGCCT-3'
R-15 5'-GGACAACGAG-3'
R-17 5'-CCGTACGTAG-3'
OPX-11 5'-GGAGCCTCAG-3'
OPT-17 5'-CCAACGTCGT-3'

Amplification reactions were performed in volumes of 25µl containing 1X PCR buffer (50mM KCl, 1.5 mM $MgCl_2$, 10mM Tris-HCl (pH 8.8) and 1% triton), dNTPs (1 mM each), Taq polymerase (2u), primers 2.5mM, 50 ng genomic DNA and autoclaved double distilled water. PCR conditions were standardized as 35 cycles of denaturation (1 min at 94^{o}C), annealing (1 min at 36^{o}C), and elongation (2 minute at 72^{o}C). Amplification products were subjected to electrophoresis on 1.5% agarose gel in 1XTBE (Trizma base 98mM, Boric acid 9.8 mM & EDTA 25 mM) at a constant current of 80 volts. After staining in 0.5 µg/ml ethidium bromide solution, gels were photographed on a UV trans-illuminator. The genomes were scored for presence or absence of polymorphic bands and intensity of these polymorphic bands. Index of genetic variation was calculated and dendrograms were constructed through UPGMA method by using computer software Mega (Kumar *et al.,* 2001).

Results

Seeds of 3 rice varieties were incubated on callus induction medium for 2, 4, 6 & 8 weeks and then transferred to regeneration medium. Regenerates were kept on MS medium for one month and then transferred to soil. Large number of somaclonal variations, with respect to morphological characters, were observed in the plants from all three varieties (Data not shown).

To explore this variation on DNA level, 15 interesting clones of 3 varieties were selected on the basis of morphological variation and genomic DNA was extracted from these selected clones along with control plants of all three varieties. These DNAs were quantified and 50ng of DNA was finally used for RAPD analysis using 8 different primers. Amplification products were detected through agarose gel electrophoreses (Fig. 1).

Four primers (S-13, S-19, R-17, and OPX-11) produced a range of 2 to 14 amplification products per clone. Seven out of 15 different interesting clones of B-2000, B-370 and Super basmati were further selected along with parental plants and again subjected to RAPD analysis using these four primers. All four primers detected different number of polymorphic bands of different sizes, which were classified as highly intense bands, intense bands and visible bands. The clones were differentiated on the basis of number of bands per clone per primer and intensities of these bands and size of amplification products.

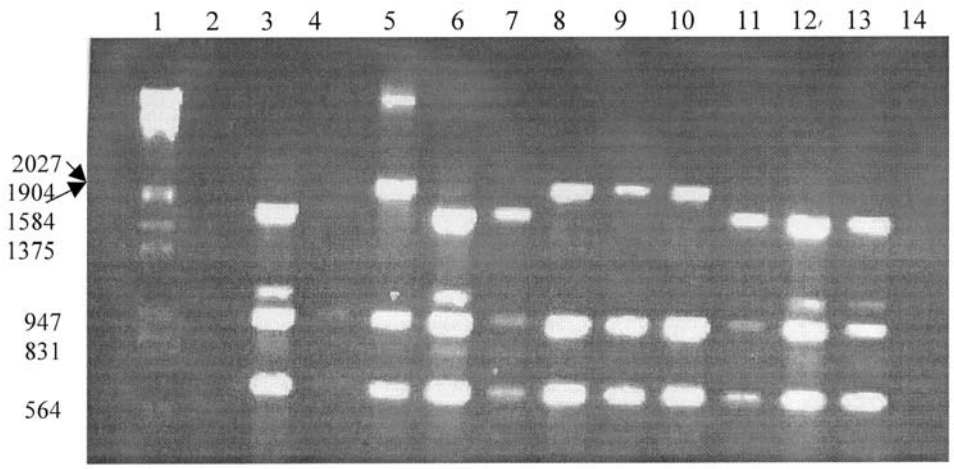
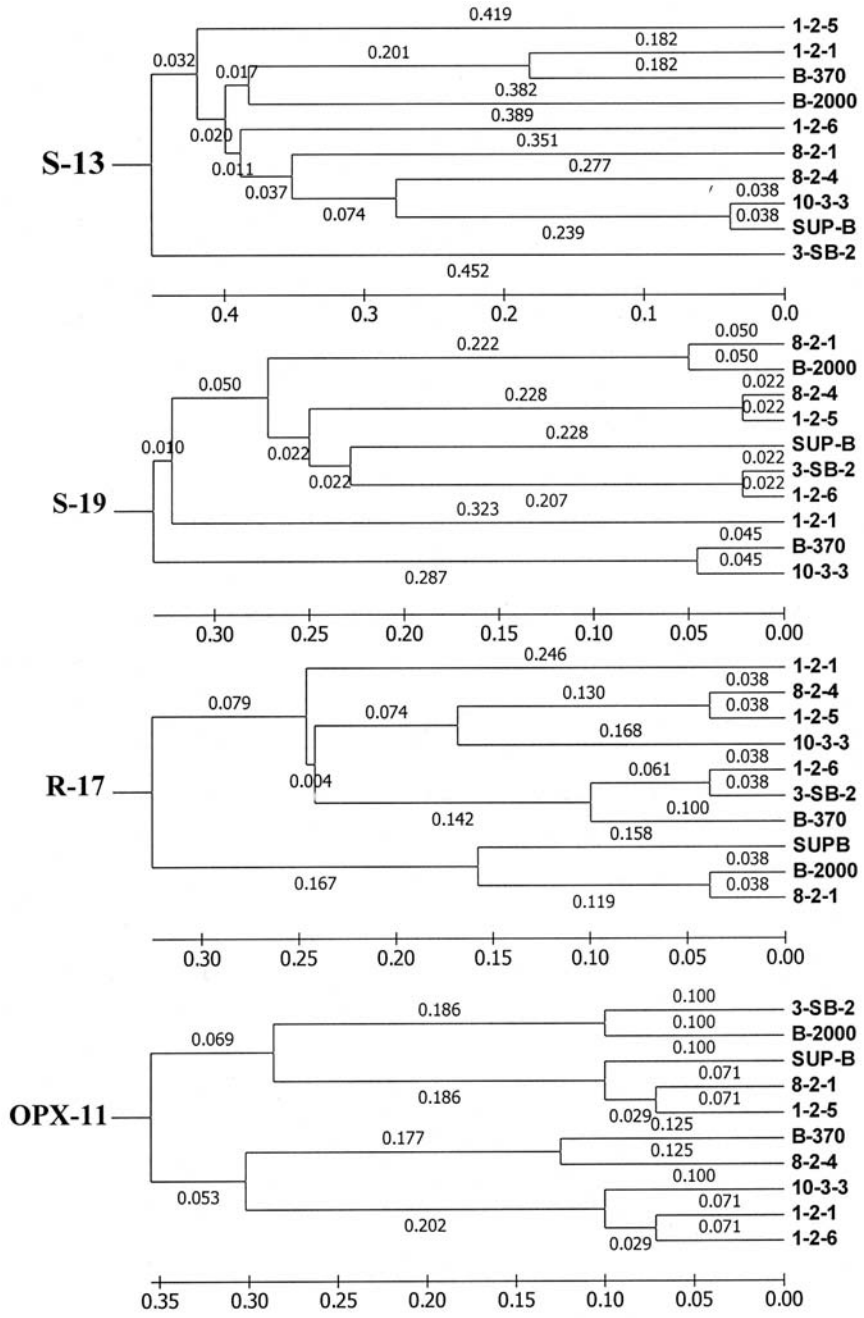


Fig. 1. RAPD analysis of plants varieties B-2000, B-370 and super basmati with S-13 primer:

Lane 1: λHE
Lane 2-4: Ctrl DNA of B-2000, B-370 and Super basmati
Lane 5: Amplicons of 2 weeks Incubated plant of B-2000 (10-2-1)
Lane 6-7: Amplicons of 4 weeks Incubated plants of B-2000 (8-2-1, 8-2-4)
Lane 8-10: Amplicons of 8 weeks Incubated plants of B-2000 (1-2-1,5,6)
Lane 11: Amplicons of 8 weeks Incubated plant of Super Basmati, (3-SB-2)
Lane 12: Amplicons of 4 weeks Incubated plant of Super Basmati, (7-SB-1)
Lane 13: Amplicons of 8 weeks Incubated plant of B-370 (10-3-1)
Lane 14: H_2O control

Maximum variation was observed in B-2000 after 8 weeks of incubation period on callus induction medium. Three interesting clones were repeatedly amplified with 4 primers and the number of bands per primers ranged between 3 to 10 amplification products per clone. Number of very intense bands varied from 0 to 2. While number of intense bands varied from 1 to 6. Two more clones were identified for B-2000 after 4 weeks of incubation and the number of total amplification products ranged between 5 and 12 as compared to 4 to 9 in parental control. For B-2000, primer S-19 produced maximum number of amplification products in clones 8-2-1 & 8-2-4 (12 products per clone) as compared to 9 amplification products in parental control (Table 1). No variation with respect to total number of bands was observed for primer S-13 while variation was observed with respect to number of very intense, intense and visible bands. It could be possible that two or more slightly variable bands might accumulate at one point due to low resolving power of agarose.

Only one somaclone was confirmed in case of Super basmati and B-370. For Super basmati, maximum variation was unrevealed with primer S-13 that produced 14 amplification products and 12 very intense bands for 3-SB-2 as compared to 12 amplification products and 2 very intense bands in parental control. For B-370, total number of amplification products were 10 and 9 for parental control and 10-3-3 respectively, for two primers i.e., S-13 and OPX-11. Number of very intense bands varied between 0 and 2 in all cases (Table 1).



FIg, 2. Dendrogram of different clones
A= Dendrogram of all clones with primer S-13
B= Dendrogram of all clones with primer S-19
C= Dendrogram of all clones with primerR-17
D= Dendrogram of all clones with primer OPX-11

Table 1. Summary of amplified bands with primers S-13, S-19, R-17 and OPX-11.

Varieties	Incubation period	Plant No.	primers	Total bands	Frequency of bands		
					X	+	(+)
B-2000	No Incubation	Ctrl	S-13	5	1.0	1.0	3.0
			S-19	9	0.0	2.0	7.0
			R-17	7	0.0	0.0	7.0
			OPX-11	4	1.0	1.0	2.0
	8 Weeks	1-2-1					
			S-13	5	1.0	2.0	2.0
			S-19	11	2.0	6.0	3.0
			R-17	6	0.0	2.0	4.0
			OPX-11	3	1.0	1.0	1.0
		1-2-5					
			S-13	5	1.0	2.0	2.0
			S-19	11	2.0	6.0	3.0
			R-17	6	0.0	2.0	4.0
			OPX-11	4	1.0	3.0	0.0
		1-2-6					
			S-13	5	1.0	2.0	2.0
			S-19	11	2.0	5.0	4.0
			R-17	6	0.0	2.0	4.0
			OPX-11	3	1.0	1.0	1.0
	4 Weeks	8-2-1					
			S-13	5	1.0	2.0	2.0
			S-19	12	2.0	5.0	5.0
			R-17	7	1.0	2.0	4.0
			OPX-11	6	1.0	2.0	3.0
		8-2-4					
			S-13	5	1.0	1.0	3.0
			S-19	12	2.0	6.0	4.0
			R-17	7	1.0	2.0	4.0
			OPX-11	6	3.0	1.0	2.0
Super Bas.	No Incubation	Ctrl	S-13	12	2.0	6.0	4.0
			S-19	5	0.0	0.0	5.0
			R-17	4	0.0	0.0	4.0
			OPX-11	2	0.0	0.0	2.0
	8 Weeks	3-SB-2	S-13	14	12.0	1.0	1.0
			S-19	6	0.0	1.0	5.0
			R-17	4	0.0	0.0	4.0
			OPX-11	2	0.0	0.0	2.0
B-370	No Incubation	Ctrl					
			S-13	10	1.0	1.0	8.0
			S-19	5	1.0	2.0	2.0
			R-17	6	0.0	3.0	3.0
			OPX-11	10	1.0	4.0	5.0
	2 Weeks	10-3-3					
			S-13	9	1.0	2.0	6.0
			S-19	6	1.0	2.0	3.0
			R-17	7	0.0	0.0	7.0
			OPX-11	9	2.0	6.0	1.0

X= Very Intense band, += intense bands (+) = visible band

These clones were also analyzed for sizes of amplification products. Seventeen different products were observed for primer S-13 ranging between 200 to 1900 bases. Two hundred and 600 band size were present in all clones while 1800 band size was present in 3-SB-2 and Super basmati parental control only. Significant variation was observed for other bands (Table 2). Fifteen products were recorded for primer S-19 ranging between 100 and 2000 bases, 900 base fragment was present in all clones while 500 base fragment was present only in 3-SB-2 and Super basmati parental control. Significant variation was observed for other bands (Table 3).

Table 2. Summary of amplification products with primer S-13.

Band size (Bases)	B-2000	B-370	SUP-B	1-2-1	1-2-5	1-2-6	8-2-1	8-2-4	3-SB-2	10-3-3
200	+	+	+	+	+	+	+	+	+	+
300	–	+	+	–	–	–	–	–	+	+
500	+	–	+	+	+	+	+	+	+	–
600	+	+	+	+	+	+	+	+	+	+
700	–	+	+	–	–	–	–	–	+	+
800	–	–	–	–	–	–	–	–	+	–
947	+	–	+	+	+	+	+	+	+	–
1000	–	+	+	–	–	–	–	–	+	+
1100	–	–	+	–	–	–	–	–	+	–
1200	–	+	+	–	–	–	–	–	+	+
1250	–	–	+	–	–	–	–	–	+	–
1300	–	+	+	–	–	–	–	–	+	+
1400	–	+	–	–	–	–	–	–	–	+
1500	–	+	–	–	–	–	–	–	–	–
1700	–	–	–	–	–	–	–	–	+	–
1800	–	–	+	–	–	–	–	–	+	–
1900	+	+	–	+	+	+	+	+	–	+

Table 3. Summary of amplification products with primer S-19.

Band size (Bases)	B-2000	B-370	SUP-B	1-2-1	1-2-5	1-2-6	8-2-1	8-2-4	3-SB-2	10-3-3
100	–	–	+	–	–	–	–	–	+	–
200	+	–	+	+	+	+	+	+	+	–
300	+	+	–	+	+	+	+	+	–	+
350	–	–	–	+	+	+	+	+	–	–
400	–	+	–	–	–	–	–	–	–	+
500	–	–	+	–	–	–	–	–	+	–
600	+	–	+	+	+	+	+	+	+	–
700	+	–	–	+	+	+	+	+	–	–
900	+	+	+	+	+	+	+	+	+	+
1000	+	–	–	+	+	+	+	+	–	–
1300	+	+	–	+	+	+	+	+	–	+
1500	–	–	–	+	+	+	+	+	–	+
1700	–	–	–	–	–	–	+	+	–	–
1800	+	–	–	+	+	+	+	+	+	–
2000	+	+	–	+	+	+	+	+	–	+

Table 4. Summary of amplification products with primer R-17.

Band size (Bases)	B-2000	B-370	SUP-B	1-2-1	1-2-5	1-2-6	8-2-1	8-2-4	3-SB-2	10-3-3
200	+	+	+	+	+	+	+	+	+	+
450	+	–	–	+	+	+	+	+	–	–
500	+	+	+	+	+	+	+	+	+	+
700	+	–	+	–	–	–	+	+	+	+
900	+	+	+	+	+	+	+	+	+	+
1000	+	+	–	+	+	+	+	+	–	+
1500	+	+	–	+	+	+	+	+	–	+
2800	–	+	–	–	–	–	–	–	–	+

Table 5. Summary of amplification products with primer OPX-11.

Band size (bases)	B-2000	B-370	SUP-B	1-2-1	1-2-5	1-2-6	8-2-1	8-2-4	3-SB-2	10-3-3
200	+	+	–	+	+	+	+	+	–	+
400	–	+	–	–	–	–	–	–	–	+
500	+	+	+	+	+	+	+	+	+	+
700	–	+	–	–	–	–	+	+	–	+
900	–	+	–	–	–	–	–	–	–	+
1000	+	+	–	–	+	–	+	+	–	+
1200	–	+	+	+	+	+	–	–	+	+
1300	–	+	–	–	–	–	+	+	–	+
1400	–	+	–	–	–	–	–	–	–	–
1500	+	+	–	–	–	–	+	+	–	+

Eight different products were observed for primer R-17 ranging between 200 and 2800 bases. Amplification products of 200, 500 and 900 bases were recorded in all clones while variation with respect to other amplification products was observed (Table 4). Ten amplification products ranging between 200 and 1500 bases in size were recorded for primer OPX-11. The fragment of 500 bases was present in all the clones while 1400 base fragment was present in B-370 parental control only. Significant variation was observed in other amplification products (Table 5).

Dendrograms were constructed for all four primers by using Mega software (Kumar *et al*., 2001) through UPGMA method. UPGMA Cluster analysis provided a better resolution of the relationship among somaclones and parental lines. For primer S-13, 45.2% variation was recorded for Super basmati and its clone 3-SB-2 obtained after 8 weeks of incubation, 40% for B-370 and its clone 10-3-3 and 42% variation between B-2000 and its clone 1-2-5 obtained after 8 weeks of incubation period. For primer S-19, 28.8% variation was recorded for Super basmati and its clone 3-SB-2 obtained after 8 weeks of incubation, 4.5% for B-370 and its clone 10-3-3 and 32.3% variation between B-2000 and its clone 1-2-1 obtained after 8 weeks of incubation period. For primer R-17, 32.4% variation was recorded for Super basmati and its clone 3-SB-2 obtained after 8 weeks of incubation, 24.2% for B-370 and its clone 10-3-3 while 32.4% variation between B-2000 and its clone 1-2-1, 1-2-5, 1-2-6 obtained after 8 weeks of incubation and 8-2-4 obtained after 2 weeks incubation. For primer OPX-11, 28.6% variation was recorded for Super basmati and its clone 3-SB-2 obtained after 8 weeks of incubation, 27.3% for B-370 and its clone 10-3-3 and 35.3% variation between B-2000 and its clone 1-2-1 and 1-2-6 obtained after 8 weeks incubation and 8-2-4 obtained after 2 weeks of incubation period.

Discussion

Tissue culture can successfully be used to create significant variation and the phenomenon was supported by a number of reports (Sun & Zheng, 1990; Kaeppler *et al*., 1998; Olhoft & Philips, 1999; Kaeppler *et al*., 2000). A number of factors control the frequency of this variation and the most important were the length of incubation period on callus induction medium and cultivar specificity. As the somaclonal variation might be genetic or somatic so it was important to carefully select the plants, which could successfully transfer this variation to progeny. Conventional methods of progeny studies are time consuming, so there was need to establish methods to carefully select important somaclones in minimum time. Genetic markers like RAPD can successfully serve this purpose.

Fifteen somaclones were selected from plants regenerated after 2, 4, 6 and 8 weeks of incubation on basis of phenotypic variation. Eight different RAPD primers were used to confirm this variation. The polymorphism thus detected was based largely on the presence and/or absence of a characteristic band, intensity of these bands and in some cases variation in the size of the bands. Similar primers were used to detect polymorphism for identification of different genotypes (Farooq *et al.*, 1995; Farooq *et al.*, 1996). In the present study, only 4 out of 8 primers and 7 out of 15 clones produced significant range of variation with respect to the amplified products ranging from 0.5 to 2 Kb in three local varieties. Variation with respect to presence or absence of bands as well as intensity of bands was significant among all clones. Such classification in banding

profiles had been reported by Heun & Helentjaris (1993) in corn and same pattern of characterization was observed by Farooq *et al.,* (1995,) and Farooq *et al.,* (1996). Maximum variation on the basis of number of bands was observed in basmati 2000 after 8 weeks of incubation, which suggested that, the variation was increased with the length of tissue culture time and was genome specific. Several reports suggested the similar results as the changes went on accumulating with the length of culture age and the net change could be of any extent (Skirvin *et al*., 1994). It was also observed that somaclonal variation had no particular behavior and it could produce variation on both sides of parental lines i.e., it might produce additional polymorphic bands or might result in absence of specific bands with respect to parental control. The difference in intensity of different clones might be due to the accumulation of two or more slightly different bands due to low resolving power of agarose, although RAPD technique can potentially detect single base pair mutations or deletions at the level of primer target and also insertions or deletions within the amplified fragments (Williams *et al*., 1990). Present results also indicated that some mutations such as deletion or insertion might occur in the amplified regions and/or that base changes might induce the alteration of primer binding sites.

Dendrograms were constructed with UPGMA method through computer software MEGA (Kumar *et al*., 2001). UPGMA method was valuable tool for the estimating the extent of genetic diversity and was routinely used in investigations involving genetic diversities (Asante & Offei, 2003; Stedje & Ziraba, 2003). Although, the dengrograms gave relationship between different clones, the direction of somaclonal variation was hard to explain. To some extent it was revealed that frequency of this variation was largely affected by the length of incubation period and cultivar specificity. Many other factors including genetic and epigenetic elements and accumulation of genetic changes might also involve (Kaeppler *et al*., 2000). It appeared that the changes at genetic level were abrupt and could not be reproduced successfully with similar tissue culture conditions. It was already documented that the genome of a somaclone could be variant at different location and the changes accumulate in complex characters during callus culture. So a single somaclone might be variant in several traits and in progeny analysis, appeared to assort independently (Skirvin *et al*., 1994**)**. The phenomenon was also supported by a number of reports (Sun & Zheng, 1990; Kaeppler *et al*., 1998; Olhoft & Philips, 1999; Kaeppler *et al*., 2000).

In rice it has been reported that alteration of repeated DNA sequences induced genetic variation during the callus culture (Zehr *et al*., 1987). RAPDs were being used for detection of genetic polymorphism in cereals (Wang *et al*., 1994), gene introgression studies (Durham *et al*., 1994) to characterize different wild and cultivated genomes, salt tolerant and resistant rice species/varieties and genome specific, species specific and cultivar specific RAPD markers can be identified (Farooq *et al*., 1995; Farooq *et. al*., 1996).

The present study however, can serve as an index to investigate the factors involved in frequency of somaclonal variation, use of local varieties in crop improvement studies involving somaclonal variation and to compare the importance of different DNA fragments (markers). It could be inferred from the present study that tissue culture is an important way to create genetic variation in local varieties and identifying clones with RAPD markers can produce more meaningful results and may be helpful to identify clones, which were morphologically superior and physiologically efficient.

Acknowledgement

Authors are grateful to Dr. Shafqat Farooq for kindly providing the sequence of the primers. Authors are also thankful to Dr. Zia-ur-Rahman and Mr. Zafar Saleem for their continuous cooperation throughout the course of the study.

References

Abbas, S.T. 2000. Development of Rachna basmati- a new fine, aromatic rice variety– through biotechnological tools. *Science Vision*., 5(4): 74-84.

Asante, I.K. and S.K. Offei. 2003. RAPD-based genetic diversity study of fifty cassava (*Manihot esculenta* Crantz) genotypes. *Euphy*., 131: 113-119.

Ashikawa, I., S. Fukuoka, T. Inoue, A. Miyao, I. Monna, S. Zhong and T. Sasaki. 1992. Polymorphism analysis of rice genome by PCR. *Rice Genome*, 2: 6-7.

D'Amato, F. 1977. Cytogenetics of differentiation in tissue and cell cultures. In: *Plant Cell, Tissue, and Organ Culture*, (Eds.): J. Reinert and V.P.S. Bajaj. Springer-Verlag, New York, pp. 343–357.

Dellaporta, S.L, J. Wood and J.B. Hicks. 1983. A plant DNA mini preparation. Version II. *Plant Mol. Bio. Reptr*., 1: 19-21.

Devos, K.M. and M.D. Gale.1992. The use of random amplified polymorphic DNA markers in wheat. *Theor. Appl. Genet*., 84: 567-572.

Durham, E.R and S.S. Korban. 1994. Evidence of gene introgression in apple using RAPD markers. *Euphytica*, 79: 109-114.

Farooq, S., T.M. Shah, M. Arif and N. Iqbal. 1995. Utilization of RAPD markers for the identification of cultivated and wild rice species. *Pak. J. Bot*., 27: 127-138.

Farooq, S., T.M. Shah, M. Arif and N. Iqbal. 1996. Inheritance of RAPD markers in the F1 interspecific hybrids of rice. *Pak. J. Bot*., 28: 51-59.

Fukuoka, S., K. Hosaka and O. Kamijima. 1992. Use of Random Amplified Polymorphic DNAs (RAPD) for identification of rice accessions. *Jap. J. Genet*., 67: 243-252.

Heun, M. and T. Helentjaris. 1993. Inheritance of RAPDs in F1 Hybrids of corn. *Theor. Appl. Genet*., 85: 961-968.

Hu, J. and C.F. Quiros. 1991. Identification of broccoli and cauliflower cultivars with RAPD markers. *Plant Cell Reports*, 10: 505-511.

Jeffreys, A.J., V. Wilson and S.L. Thien. 1985. Individual specific "Fingerprints" of human DNA. *Nature*, 316: 76-79.

Kaeppler, S.M., H.F. Kaeppler and Y. Rhee. 2000. Epigenetic aspects of somaclonal variation in plants. *Plant Molecular Biology*, 43: 179-188.

Kaeppler, S.M., R.L. Phillips and P. Olhoft. 1998. Molecular basis of heritable tissue culture-induced variation in plants. In: *Somaclonal variation and induced mutations in crop improvement*. (Ed.): Jain *et al*. Current Plant Science and Biotechnology in Agriculture, 32: 465-484. Kluwer Academic Publishers, Dordrecht, Netherlands.

Klein-Lankhorst, R.M., A. Vermunt, R. Weide, T. Liharska and P. Zabel. 1991. Isolation of molecular markers for tomato (*Lycopersicon esculentum*) using Random Amplified Polymorphic DNA (RAPD). *Theor. Appl. Genet*., 83: 108-114.

Kumar, S., K. Tamura and M. Nei. 2001. *MEGA: Molecular Evolutionary Genetic Analysis*, Ver. 2.1. The Pennsylvania State Univ., Univ. Parks, PA, 168. 2.

Larkin, P.J. and W.R. Scowcroft. 1981. Somaclonal variation: a novel source of variability from cell cultures for plant improvement. *Theor. Appl. Genet*., 60: 197-214.

Martin, G.B., J.G.K. Williams and S.D. Tanksley. 1991. Rapid identification of markers linked to a *Pseudomonas* resistance genes in tomato by using random primers and near-isogenic lines. *Proc. Natl. Acad Sci. USA*., 88: 2336-2340.

McCouch, S.R and S.D. Tanksley. 1991. Development and use of restriction fragment length polymorphism in rice breeding and genetics. *Rice Biotechnology*, pp. 109-133. International Rice Research Institute, Manila, Philippines.

Michelmore, R.W., I. Paran and R.Y. Kesseli. 1991. Identification of markers linked to disease resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating population. *Proc. Natl. Acad. Sci. USA*., 88: 9828-9832.

Munthali, M., B.V. Ford-lloyd and H.J. Newbury. 1992. The random amplification of polymorphic DNA for fingerprinting of plants. *PCR Methods Appl*., 1: 274-276.

Nybom, H., B. Schaal and S.H. Rogstad. 1989. DNA fingerprints can distinguish cultivars of blackberries and raspberries. *Acta Hortica*., 262: 305-310.

Olhoft, P.M. and R.L. Phillips. 1999. Genetic and epigenetic instability in tissue culture and regenerated progenies. In: *Plant Responses to Environmental Stresses: From Phytohormones to Genome Reorganization*. (Ed.): H.R. Lerner, Marcel Dekker, New York, pp. 111-148.

Rafalski, J.A., Tingey, S.V., and Williams, J.G.K. 1991. RAPD markers, A new technology for genetic mapping and plant breeding. *AgBiotech News and Information*, 3: 645-648.

Ronald, P.C., B. Albano, R. Tabien, L. Anenes, K.S. Wu, S. McCouch and S.D. Tanksley. 1992. DNA markers in plant improvement. Genetic and physical analysis of the rice bacterial blight disease resistance locus Xa21. *Mol. Gen. Genet*., 236: 113-120.

Scowcroft, W.R., S.A. Ryan, R.I.S. Brettel and P.J. Larkin. 1985. *Biotechnology in International Agricultural Research*, IRRI, Manila. pp: 99-109.

Skirvin, R.M., K.D. McPheeters and M. Norton. 1994. Sources and frequency of somaclonal variation. *Hort. Sci*., 29: 1232-1237.

Stedje, B. and R.B. Ziaraba. 2003. RAPD variation in *Solanum anguivi* Lam and *S. aethiopicum* L. (*Solanaceae*) in Uganda. *Euphy*., 131: 293-297.

Sun, Z.X. and K.L. Zheng. 1990. Somaclonal variation in rice. In: *Biotechnology in Agriculture and Forestry*, (Ed.): Y.P.S. Bajaj. Vol. 3, Springer-Verlag, Berlin, pp. 288–325.

Sunderland, N. 1973. Nuclear cytology. In: *Plant Tissue and Cell Culture*, (Ed.): H.E. Street. 2nd ed., Blackwell, Oxford, pp. 177–205.

Tanzarella, R.D.O.A. and E. Proceddu.1990. Rapid and efficient detection of genetic polymorphism in wheat through amplification by polymerase chain reaction. *Plant Mol. Biol*., 15: 169-171.

Wang, G., S. Castinglione, J. Zhang, R.Z. FU, J.S. MA, W.B. L1, Y.R. Sun and F. Sala. 1994. Hybrid rice (*Oryza sativa* L.) identification and parentage determination by RAPD fingerprinting. *Plant Cell Reports*, 14: 2-3.

Welsh, M. and M. McCleland. 1991. Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acid Res*., 18: 7213-7218.

Willams, J.G.K., A.R. Kubelik, K.J. Liuak, J.A. Rafalski and S.V. Tingey. 1990. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids. Res*., 18:6531-6535.

Yu, L.X. and H.T. Nguyen. 1994. Genetic variation detected with RAPD markers among upland and lowland rice cultivars (*Oryza sativa* L.). *Theor. Appl. Genet*., 87: 668-672.

Zehr, B.E., M.E. Williams and R.D. Duncan. 1987. Somaclonal variation among the progeny of plants regenerated from callus cultures of seven inbred lines of maize. *Can. J. Bot*., 61: 491–499.

(Received for publication 13 November 2003)