Polymorphism analysis in advanced mutant population of oat (*Avena sativa* L.) using ISSR markers

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Abstract Present investigation was carried out to evaluate genetic diversity among 38 M6 population of oat cv. JO-1. To validate the observed morpho-physiological variations, these lines were analyzed with 21 ISSR primers. A total of 132 loci were amplified by these 21 ISSR markers and 116 loci were found to be polymorphic (87.87 %). The genetic similarity coefficient values among 39 oat genotypes based on ISSR analysis ranged from 0.305 to 0.957. The cluster analysis divided the oat genotypes into two groups. Mutants JMO 81 and JMO 82 were found to be most divergent, hence can be used as parents in breeding program for the development of superior cultivars.

Keywords *Avena sativa* · Polymorphism · Oat · ISSR · Mutant population

Introduction

Oats is an annual winter cereal plant and is known for its quality fodder and nutritious grains with high lysine and protein content. Differing from other cereal grains such as wheat and barley, oat is rich in the antioxidants α -tocotrienol, α -tocopherol, and avenanthramides, as well as total dietary fiber including the soluble fiber β -glucan etc. (Oliver et al. 2010). During past few years, with the advent of intensified dairy

industry in our country, oat have attracted the attention of breeders for its improvement due to its nutritious fodder for livestock and its grains as animal feed with high net energy gains. Some progress has been made and few reports are available on molecular diversity analysis of oat genotypes in India (Ruwali et al. 2013).

The study of genetic diversity is very important for breeding programmes especially in elucidating the genetic variability available to breeders (Govindaraj et al. 2015). Spontaneous and induced mutations have been extensively useful for generating genetic variability. Genetic diversity and associated population structure depends largely on historical patterns of deliberate and passive efforts to create improved crop varieties (Garris et al. 2005; Chao et al. 2007). Genetic analysis in mutant populations has been carried out by several investigators using different markers viz. RAPD in sugarcane clone NIA-98 (Khan et al. 2010), rhododendron (Atak et al. 2011), ISSR markers in soybean (Mudibu et al. 2011) and microsatellite markers in banana (Miri et al. 2014). The objective of this study was to assess the polymorphism in M6 population of oat using ISSR markers.

Materials and methods

In the present investigation oat cv. JO-1 was used as control because, it is a released variety and have broad leaves with higher fodder yield. Dry seeds of oat cv. JO-1 treated with 150, 200, 300 and 400 Gy doses of gamma rays at Radiation Facility of JNKVV, Jabalpur were planted to establish M1 generation of mutants. Progenies of these mutants were advanced up to M6 generation. The plants of advanced M6 population of oat were raised in the research field of All India Coordinated Research Project on Fodder Oat, Jawaharlal Nehru Agricultural University, Jabalpur and

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planted with two replications in randomized block design. Morphological observations on total green fodder yield, days to harvest, leaf per plant, leaf area, leaf color (light, medium and dark green), number of awns, description of awn (single, double, straight, absent), culm base hairiness (present, absent), regeneration capacity (present, absent) and plant canopy (spread, compact, semi compact) were recorded to analyze phenotypic variability using five plants in each replication. For molecular characterization 38 mutants and cultivar JO-1 (control) were selected for their variability in phenotypic traits and higher fodder yields (Table 1).

DNA extraction and PCR analysis

Genomic DNA was extracted from young leaves of the 38 mutants and the control, non irradiated plants, using the protocol developed by Doyle and Doyle (1990). The quantity and quality of the DNA was estimated by comparative analysis of the samples on 0.8 % agarose gels stained with ethidium bromide. The samples were diluted in ultrapure water to a final concentration of 10 ng/μl. A total of 21 ISSR markers were used for polymorphism analysis among selected mutant progenies. The ISSR markers consisted of UBC primer set developed at University of British Columbia, Vancouver, Canada and a set of 14 ISSR primers (Table 1) published by Tanhuanpaa et al. (2006) were included. For amplification, 20 μl reaction containing 1×PCR buffer, 1.5 mM MgCl₂, 100 μM of each dNTPs (dATP, dTTP, dGTP, dCTP), 0.4 μM of each primer, 50 ng of genomic DNA and 1 Unit of *Taq* DNA polymerase (Fermentas) were used. Amplifications were carried out in the Thermo Hybaid thermocycler with the following amplification steps: 94 °C for 4 min followed by 45 cycles of 94 °C for 1 min, 50 °C for 1 min, 72 °C for 2 min, with final extension of 72 °C for 7 min. The amplified products were separated by 1.5 % gel electrophoresis. Amplification repeated thrice to ensure repeatability of primers.

Scoring and data analysis

The data for ISSR analysis were scored from digital photographs of ethidium bromide stained agarose gels from Syngene Multi Genius Bio Imaging System. DNA bands were considered to be similar if they occurred exactly at the corresponding position on the electrophoresis gel. Band size was determined by comparing with standard DNA ladder. An ISSR band presence (1)/absence (0) profile was recorded into a matrix. This matrix was subjected to genetic similarity analysis using Jaccard's coefficient, clustering was done using SAHN and dendrogram was constructed by unweighted paired group method using arithmetic averages (UPGMA). All computations were carried out using NTSYS-pc 2.0 software program (Rohlf 2000). The PIC value for each locus was

calculated using formula (Roldan-Ruiz et al. 2000); $PIC_i = \frac{2fi}{(1 - fi)}$, Where PIC_i is the polymorphic information content of the locus *i*, *fi* is the frequency of the amplified fragments and $1 - fi$ is the frequency of non amplified fragments. The frequency was calculated as the ratio between the number of amplified fragments at each locus and the total number of accessions. The PIC values of each primer were calculated using the average of the loci of each primer.

Results and discussion

Morphological analysis

Results from the analysis of variance are presented in Table 2. Significant effects of mutation persisted in M6 population. These results indicate differential responses of individuals in M6 population with significant variability for total green fodder yield (TGFY), days to harvest (DTH), leaf per plant (LP) and leaf area (LA) revealing effectiveness of mutagenic treatment.

Molecular analysis

In plant breeding, genetic variation is essential for the creation of plants with superior traits. Mutation is a powerful technique, which may well produce desired variation (Hautea et al. 2004). Mutagenesis alters the normal biological composition of an organism and the true genetic changes are desirable. Ionizing radiations and chemical mutagens have been extensively used in mutation breeding in different crops. Mutations may be recessive or dominant but the former is more common although these do not express phenotypically unless two recessive genes come together as homozygotes (Edmé et al. 2005). This expression requires one or more generations of recombination of two or more similar recessives for the phenotypic expression in the population. Several unusual features like high frequency of mutations and reversions, non-random mutations, segregation, distortions, unstable variants in advanced generations, somatic mutations, multiple character mutations, multiple alternate forms, homozygous mutations and mutation out bursts and are difficult to explain through conventional mutation theory (Gowda et al. 1996).

This study was conducted to determine the extent of genetic diversity among M6 and non- irradiated control JO-1 oat cultivar based on the ISSR technique. Initially, 55 ISSR primers were screened using two mutants and one parent to check the amplification and reproducibility of the primers. A total of 21 primers successfully amplified sharp resolution and had di and tri nucleotide repeat motifs. Among 21 ISSR primers, 10 had di-nucleotide and 11 had tri-nucleotide repeats. The size of the amplified markers ranged from 100 bp to 3500 bp. Maximum number of bands i.e. 11 were scored for

Table 1 Parent and individuals of mutant population (M_6) of oat used in present study

Sl.	Genotypes	TGFY	DTH	L/P	LA	LC	N&NA	BH	RC	AC
1.	JO-1(P)	23.05	102.00	6.95	123.34	LG	Ab	Ab	N	L
2.	JMO-7	32.27	99.00	7.40	121.26	LG	Ab	Ab	N	L
3.	JMO-11	35.95	103.67	7.90	106.47	MG	Ab	Ab	Y	L
4.	JMO-12	37.35	103.00	8.70	124.04	LG	Ab	Ab	N	L
5.	JMO-14	34.08	106.00	8.00	137.23	LG	Ab	Ab	N	L
6.	JMO-23	31.95	109.00	8.40	131.32	MG	Ab	Ab	N	L
7.	JMO-24	26.48	108.67	8.30	108.90	LG	Ab	Ab	N	L
8.	JMO-29	28.05	105.00	8.60	125.20	LG	Ab	Ab	N	L
9.	JMO-41	27.38	103.50	8.70	120.73	MG	Ab	Ab	Y	L
10.	JMO-44	30.73	104.00	8.70	114.25	LG	Ab	Ab	N	L
11.	JMO-49	31.30	100.00	8.60	128.24	MG	Ab	Ab	Y	C
12.	JMO-52	41.35	101.50	8.00	120.98	LG	Ab	Ab	N	L
13.	JMO-54	30.98	101.00	7.00	94.55	DG	S&S	Ab	N	L
14.	JMO-60	31.28	107.00	7.70	131.14	LG	Ab	Ab	N	SC
15.	JMO-63	29.33	106.00	8.20	116.49	MG	Ab	Ab	N	L
16.	JMO-65	24.53	105.00	8.60	129.10	MG	Ab	Ab	Y	L
17.	JMO-66	27.45	108.00	8.50	113.81	LG	Ab	Ab	N	L
18.	JMO-75	27.23	107.00	8.10	129.62	MG	Ab	Ab	N	SC
19.	JMO-79	32.78	109.00	8.10	135.67	LG	Ab	Ab	N	L
20.	JMO-81	30.60	103.00	8.20	146.43	LG	Ab	Ab	N	L
21.	JMO-82	34.50	101.00	8.60	129.19	MG	Ab	Ab	N	L
22.	JMO-93	27.03	101.50	7.75	103.72	MG	Ab	Ab	Y	L
23.	JMO-95	24.38	104.00	8.23	125.23	DG	Ab	Ab	N	L
24.	JMO-101	31.50	105.00	8.70	139.29	LG	Ab	Ab	N	L
25.	JMO-111	30.08	102.50	8.00	114.43	LG	Ab	Ab	N	L
26.	JMO-117	30.00	99.50	7.90	117.63	LG	Ab	Ab	N	L
27.	JMO-121	38.18	100.00	8.10	127.23	MG	Ab	Ab	N	L
28.	JMO-123	31.75	103.00	7.90	112.50	MG	Ab	Ab	N	L
29.	JMO-122	34.48	102.00	7.40	123.93	MG	Ab	Ab	N	C
30.	JMO-139	27.83	104.00	7.40	120.88	DG	Ab	Ab	N	SC
31.	JMO-144	30.60	98.00	7.10	116.73	MG	Ab	A	N	L
32.	JMO-159	27.00	106.00	7.50	113.27	MG	Db	P	N	C
33.	JMO-215	35.78	105.00	6.45	118.63	MG	Ab	Ab	N	L
34.	JMO-214	29.84	104.00	6.50	108.06	DG	Ab	Ab	N	L
35.	JMO-243	34.97	104.00	7.10	117.72	MG	Ab	Ab	N	C
36.	JMO-248	39.45	104.00	6.50	124.34	MG	Ab	Ab	N	C
37.	JMO-249	37.19	102.00	6.40	129.42	MG	Ab	Ab	N	C
38.	JMO-187	34.83	106.00	7.15	110.64	DG	Ab	Ab	N	C
39.	JMO-448	37.90	103.80	7.50	104.10	MG	Ab	Ab	N	L

TGFY total green fodder yield, DTH day to harvest, L/P leaf per plant, LA leaf area, LC leaf colour (LG light green, MG medium green, DG dark green), N&NA number and nature of awn (SS single straight, Db double, Ab absent), BH basal hairiness (P present, Ab absent), RC regeneration capacity (P present, Ab absent), AC axis compactness (L loose, C compact, SC semi compact)

primer UBC811 and ISSR20, while minimum number of band i.e. 2 were obtained with primer UBC851 and ISSR10 (Table 3). These 21 ISSR primers amplified a total of 132 ISSR marker loci. Banding pattern of primers UBC836 (Fig. 1a) and ISSR14 (Fig. 1b) are illustrated. Out of total 132 loci, 116 were found polymorphic (87.87 %) across all

the mutants. The percentage of polymorphism ranged from 22.22 % for primer ISSR21 to 100 % for many primers. This high polymorphism indicates variation at the DNA level within radiation-induced mutants from JO-1 after treatment with gamma rays resulted in a high frequency of mutants. Average numbers of bands per primer was 6.28, while average

Table 2 ANOVAs of morphological traits in mutant oat genotypes

S.O.V	df	TGFY	LA	L/P	DTH
Replication	2	165.86	708.81	7.22	42.50
Genotype	38	153**	2455.09**	8.43**	26.84**
Error	76	53.59	1685.09	1.62	7.97
CV%	—	12.37	24.93	9.49	22.11

TGFY total green fodder yield, DTH day to harvest, L/P leaf per plant, LA leaf area

**Significant at 1 % probability levels

number of polymorphic bands per primer was 5.52 (Table 3). The range of similarity coefficient varied from 0.305 to 0.9574, which indicated the genetic distance among the *A. sativa* mutants. In this study, high PIC value of 0.36 for primer ISSR7 and low PIC value of 0.11 for primer ISSR21, with an average value of PIC per primer 0.15 was obtained.

Based on electrophoretic banding pattern of ISSR primers, pair wise genetic similarity among 38 *Avena* mutants and one parent for polymorphism and genetic diversity analysis were

estimated and a dendrogram was generated by Unweighted Pair Group Method (UPGMA) sub programme of “NTSYS-PC” (Rohlf 2000).

The cluster analysis grouped oat mutants into two groups, the first major group consisted 38 mutants and parent JO-1 grouped separately into second. Among mutants, JMO-82 separates from rest of the mutants, followed by 3 sub-clusters of three (JMO-448, JMO-159, JMO-95), four (JMO-249, JMO-215, JMO-122, JMO-7) and two (JMO-49, JMO-11) mutants. The first group further divided into two sub-groups one major with 36 mutants namely JMO-12, JMO-14, JMO-139, JMO-144, JMO-243, JMO-248, JMO-111, JMO-117, JMO-121, JMO-187, JMO-93, JMO-214, JMO-101, JMO-60, JMO-63, JMO-23, JMO-24, JMO-29, JMO-41, JMO-44, JMO-52, JMO-54, JMO-66, JMO-75, JMO-79, JMO-65, JMO-123 and then JMO 81 from the rest. Highest genetic similarity (95.74 %) was shown between JMO-75 and JMO-66 followed by 93.75 % among JMO-54, JMO-75 and JMO 66, these all mutants grouped closely in same cluster (Fig. 2). The minor subgroup was consisted only one mutant JMO-82. Among all the mutants studied, JMO-81 and JMO-82 were found to be the most diverged from each other. In terms of morphological traits, JMO-81 exhibited maximum

Table 3 Sequence and polymorphic profile of ISSR primers used in the study

S1.	Code	Sequences (5'-3')	Band sizes (bp)	TB	PB	PP	PIC
1	UBC 811	(GA)8C	100–2000	11	11	100.00	0.34
2	UBC 836	(AG)8YA	125–2500	7	3	42.85	0.16
3	UBC 842	(GA)8YG	100–2000	8	5	62.50	0.21
4	UBC 851	(GT)8YG	100–2500	2	2	100.00	0.31
5	UBC 855	(AC)8YT	100–3000	8	8	100.00	0.29
6	UBC 889	(AC)7DBD	150–3500	5	5	100.00	0.32
7	UBC 890	(GT)7VHV	100–1500	6	6	100.00	0.30
8	ISSR 1	(ACC)6C	120–2500	8	8	100.00	0.28
9	ISSR 3	(ACC)6T	100–2800	7	7	100.00	0.20
10	ISSR 4	(AGC)6C	225–3000	6	6	100.00	0.31
11	ISSR 5	(AGC)6G	125–3500	7	7	100.00	0.22
12	ISSR 6	(AGC)6T	100–1500	6	6	100.00	0.32
13	ISSR 7	(AC)9T	350–3200	4	4	100.00	0.36
14	ISSR 9	(CAC)7A	100–2500	4	4	100.00	0.34
15	ISSR 10	(CAC)7T	250–3400	2	1	50.00	0.13
16	ISSR 11	(CTC)6A	250–2500	4	4	100.00	0.30
17	ISSR 14	(GCT)6C	200–3500	8	7	87.50	0.22
18	ISSR 18	(TCG)6G	300–3200	6	6	100.00	0.32
19	ISSR 20	(TGC)5C	100–2500	11	11	100.00	0.30
20	ISSR 21	(TG)8C	125–2200	9	2	22.22	0.11
21	ISSR 24	(AC)9C	150–3500	3	3	100.00	0.20
Total				132	116	—	—
Average				6.28	5.52	87.87	0.15

Where, Y = (C, T), B = (C, G, T), D = (A, G, T), H = (A, C, T), V = (A, C, G)

TB total bands, PB polymorphic bands, PP percentage polymorphism, PIC polymorphism information content

Fig. 1 Banding pattern generated among 38 mutants with parent (Table 1) **a** UBC836 and **b** ISSR14

leaf area. Leaf area per se plays an important role in total biomass production of the crop/fodder crop and add to the fodder quality as well. Hence, JMO-81 may be used in breeding programme to develop cultivars with higher biomass.

During this pioneer attempt to use ISSR to evaluate the genetic variability in mutant oat, 13 primers namely UBC 811, UBC 842, UBC 855, UBC 889, UBC 890, ISSR 1, ISSR 4, ISSR 5, ISSR 6, ISSR 14, ISSR 18, ISSR 20 and ISSR 21 amplified specific bands for one or two accessions. These primers can be used to differentiate the specific mutants from other mutant genotypes. In a similar study with M3 population of Oat, De Souza et al. (2005) found UBC primers

811, 854 and 855 specific to genomic regions related to different characters. In case of M2 population of rice cultivar Nemat, similar results were observed with 30 RAPD primers in 15 mutant genotypes (Babaei et al. 2010).

Conclusions

The genetic relationship among different mutant genotypes of *A. sativa* was analyzed using ISSR marker systems, which are effective and reliable tools for this type of analysis. The results obtained from both morphological and molecular analyses

Fig. 2 Dendrogram showing genetic relationship among M_6 mutant population of oat derived from JO-1

show that more point mutations were generated by irradiation with gamma rays. The results represent significant steps that could provide a sound basis for the successful application of mutation and molecular marker techniques to the improvement of the oat in the India.

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