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Identification of lentil genotypes resistant to bruchid (*Callosobruchus chinensis* L.) and selection of diverse parental pair

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ABSTRACT

Bruchid (Callosobruchus chinensis L.) is the most devastating storage pest of the pulses in storage condition. Lentil is one of the important pulse cropsand scanty of literature is available regarding the bruchid resistance in this crop. Climatic condition of West Bengal favours the pulse beetle infestation during storage of pulses. Therefore, we investigated the potential resistant sources among 94 lentil cultivated genotypes by feeding assay against this pest in laboratory condition. Ten high yielding genotypes were screened based on their resistance. Diversity analysis was performed by utilizing 12 phenotypic markers and 12 SSR markers to decipher the best possible parental pair. Based on this study it was found that WBL-77 and Precoz is the best parental pair.

Keywords: Bruchid, diversity, lentil, resistance and SSR

Lentil is the most preferred pulse in West Bengal as the Bengali people consume it as *dal*, almost daily, along with their *vaat i.e.* rice. Although lentil productivity in West Bengal (963 kg ha^{-1}) is higher than that of national average (705 kg ha^{-1}), astonishingly it is produced very less than the actual need. It is one of the most nutritious *rabi* season legume crops due to relatively higher protein content (22-35%), carbohydrates, fibres, and calories than other legumes; it is a rich source of iron, phosphorus, calcium, zinc, carotene. vitamin B, lysine and tryptophan (Muehlbauer *et al*., 1985; Kumar *et al.*, 2015; Anon., 2015). Lentil straw is also used as livestock feed. Lentil seeds possess flatulence causing anti-nutritional factors such as protease inhibitor, lectins, phytohaemoglutins, oligosaccharides which are being minimised during cooking process. Lentil seed coat contains tannin, an anti-nutritional factor, and it could be removed by the de-hulling process. Traditionally lentil seed paste has been used for maintaining healthy skin. Due to low glycemic index, physicians mostly recommend it to the people suffering from obesity, diabetes, and cardiovascular diseases (Srivastava and Vasishtha, 2012). We can say that the lentil carries an important role towards human, animal as well as soil heath improvement by occupying a significant position.

The two most important devastating pulse beetle species are *Callosobruchus chinensis* L. and *C. maculatus* F. (Southgate, 1979). In Indian subcontinent, *C. chinensis* is the most destructive pest for the pulses during storage. The ecological condition of West Bengal favours its infestation. Under optimum storage condition, it can complete four generations in a year (Han and An, 1990). This insect first infests pulse seeds in the field where the adult female lays eggs on young immature pods. After hatching of eggs, larvae bore through the pod wall and feed within the seeds (Southgate, 1979). Male has a longer life span than the female beetle. Longevity of adults for males ranged from 9 to 14 days with average of 11.0±1.87 days and for females was ranged 9 to 12 days with an average of 9.6±1.14 days. It has been found that females laid an average of 78 eggs in its life cycle (Varma *et al.*, 2010). When the crop is harvested and stored, the adult eventually comes out from the seed making a circular hole and simultaneously a secondary infestation is being caused by the emerging adults. Thus the pest can destroy the whole stored seed lot within a time span of 3 to 4 months (Fernandez and Talekar, 1990). The damaged seeds are unsuitable for human consumption and for other commercial and agricultural uses due to presence of an odour. Although fumigation is done to control bruchid but the best and safest way to control it is cultivation of resistant pulses.

The lentil cultivars used in our study (except ILL-8006) were not previously screened against the *C. chinensis* to make any conclusion regarding their resistance. Thus in our present study, we tried to investigate the resistance sources among the existing lentil cultivated genotypes and also recommended the best parental pair for initiating breeding programme in lentil against this devastating storage pest species.

MATERIALS AND METHODS

Bruchid feeding assay

A total of 94 lentil germplasms were collected from ICARDA, NBPGR and IARI, India, Bangladesh and Crop Research Unit (CRU) of Bidhan Chandra Krishi Viswavidyalaya (BCKV), Nadia. The list of the collected germplasms is presented in the table- 1. The bruchid species *C. chinensis* was used for the phenotypic screening of the lentil germplasms mentioned in that

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table. The insects were reared on mung bean seeds keeping in the glass jars of 12 inches height following the procedure of Sarkar and Bhattacharyya (2014). These mungbean seeds are frequently used for making *tadka* curry in India and they show complete susceptibility to bruchid species. The insect rearing glass jars were kept in an incubator at 30°C temperature and 70% relative humidity to maintain stock culture and always fresh mungbean seeds were fed to the newly emerged adults for maintaining favourable environment along with continuous insect supply. Removal of the dead insects as well as damaged seeds was properly done. Fifty seeds were taken from each of the 94 lentil germplasms and were kept into separate small plastic containers with perforated lids. Freshly emerged adults of bruchid were taken out from stock culture and the male and female insects were taken on the basis of their antennae type; the male bruchid are differentiated different from female ones by having pectinate type of antennae whereas females have serrated antennae (Raina, 1970). We released five pairs of freshly emerging adult beetles for laying eggs over the fifty lentil seeds kept in each plastic container following the procedure suggested by Sarkar *et al.* (2011). All the containers were kept in incubator maintaining the above mentioned condition for 20 days. After 20 days, the number of damaged seeds was recorded daily for 60 days so that the bruchid population gets enough time to cover two generations and to infest the susceptible ones. The damaged lentil seed was considered as the susceptible one as an adult insect must had emerged from it after feeding. The undamaged seed was considered as resistant one due to inability of the bruchid larva to feed it and as a result no adult emergence occurred (Sarkar and Bhattacharyya, 2015).

Plant genotypes for diversity analysis

Ten lentil high yielding genotypes *viz*., WBL-77, Subrata, Ranjan, PL-406, Precoz, LR-IC 268239, LR-IC 282863, L-1112-18, LR-IC 560307 and LC-284-1206 were chosen. With a spacing of 25 cm row to row and 10 cm plant to plant, they were grown in AB Block District Seed Farm, Kalyani Simanta of BCKV during *rabi* season (2016-2017) for phenotypic evaluation as well as molecular diversity assessment. Irrigation was given at the time of sowing once, to facilitate seed germination and fertilizer application was done @ 20:40:40 (N: P: K) kg ha^{-1} as basal before sowing.

Morphological markers

Twelve morphological markers of the ten lentil genotypes were taken following guidelines for the conduct of test for Distinctness, Uniformity and Stability (DUS) on lentil (Anon., 2007) and data for the selected genotypes were converted to binary data for analysing genetic diversity among them. Selected twelve characters were either in binary mode or in categorical fashion. Genotypes were scored in 0 and 1 for those characters. Characters which revealed only two alternative forms were considered as binary variable. Characters in categorical fashion were converted to binary mode by considering each category as a separate loci or trait. The twelve characters which were considered are presented in table 1. Data were analyzed and similarity matrix was constructed from binary data with Jaccard's coefficients (Jaccard, 1908) and Dendogram were generated by Unweighted Pair-Group Method of Arithmetic mean (UPGMA) algorithm, using SHAN sub-programme of NTSYS-pc Version 2.11f software (Rohlf, 2000).

DNA markers

Twelve pairs of primers for twelve simple sequence repeat (SSR) markers were selected, on the basis of their high polymorphic information content (PIC) values, from the exiting lentil SSR markers available in the Molecular Biology Laboratory in the department of Genetics and Plant Breeding. The sequence of the forward and reverse primers along with melting temperature (Tm) and PIC value are presented in the table 2. Tender leaves from the full grown field plant were taken for DNA extraction. DNA was extracted following the method described by Bhattacharyya and Mandal (1999). The SSR amplicons were generated by conducting polymerase chain reaction and simultaneously the amplified products were separated by agarose gel electrophoresis as mentioned by Sarkar *et al*. (2011). Presence and absence of band were counted. Then identification of presence and absence of all amplified DNA fragments *i.e*. SSR amplicons in each of the selected 10 lentil germplasms were counted. Score 1 was given for presence of a band and score 0 was given for absence of band. Polymorphic Information Content of each SSR was determined by using the formula given by Powel *et al.* (1996). PIC= 1/ n " 2F (1-F), where n = Number of loci detected and F = Proportion of bands per assay unit.

RESULTS AND DISCUSSION

Screening of lentil grmplasms against bruchid

Our main focus was to choose such diverse parental pairs which compulsorily had one resistant parent preferably a high yielder and another one being high yielding parent, preferably a West Bengal popular cultivar. Hence we picked ten high yielding genotypes *viz*., (1) WBL-77, (2) Subrata, (3) Ranjan, (4) PL-406, (5) Precoz, (6) LR-IC 268239, (7) LR-IC 282863, (8) L-1112-18, (9) LR-IC 560307, and (10) LC-284-1206; they showed 20, 78, 98, 94, 100, 80, 66, 100, 80 and 100 per cent resistance respectively (Table 3). Selected lentil genotypes with their duration and seed size are

Table 1: Morphological characters for DUS testing on lentil

Sl. No.	Characteristics	States
1	Foliage: intensity of green colour	1. Light; 2. Medium; 3. dark
2	Stem: anthocyanin colouration	1. Absent; 2. Present
3	Time of flowering	1. Early (<60 days); 2. Medium (60-80 days); 3. Late(>80 days)
4	Leaf pubescence	1. Present; 2. Absent
5	Leaflet size length	1. Small; 2. Medium; 3. Large
6	Plant growth habit	1. Erect (<30°); 2. Semi-erect (30°-60°); 3. Horizontal(>60°)]
7	Flower colour	1. White; 2. Pink; 3. Blue; 4. Violet; 5. Others
8	Plant height	1. Short (<40 cm); 2. Medium (40-60 cm); 3. long (>60cm)
9	Seed size (weight of 100 seeds)	1. Small (<2.0 g); 2. Medium (2.0-2.5 g); 3. Large (2.6-3.0 g); 4. Very large(>3.0 g)
10	Seed testa colour	1. Green; 2. Grey; 3. Pink; 4. Brown; 5. Black
11	Seed testa mottling	1. Absent; 2. Present
12	Cotyledon colour	1. Yellow; 2. Olive green; 3. Orange

Table 2: List of the SSR primers tested

Sl. No.	Primer name	Forward sequence (5'-3')	Reverse sequence (5'-3')	Tm (°C)	PIC value
1	ALD-15	CAA GCA TGA CGC CTA TGA	CTT CAC TCA CTC AAC TCT C	53	0.7
2	ALD-42	CCG TAA GAA TTA GGT GTC	GGA AAA TAG GTG GAA AG	52	0.75
3	SSR-19	GAC TCA TAC TTT GTT CTT AGC AG	GAA CGG AGC GGT CAC ATT AG	50	0.65
4	SSR-33	CAA GCA TGA CGC CTA TGA AG	CTT TCA CTC ACT CAA CTC TC	55	0.31
5	SSR-59-2	CCA AAT ACT GCA ACA CAC CG	GTT CCC ATC AGG CAG AAG G	55	0.69
6	SSR-66	GGTAGTGGTGAGGAATGAC	GCATCACTGCAACAGACC	58	0.41
7	SSR-107	GCG GCG AGC AAA TAA AT	GGA GAA TAA GAG TGA AAT G	55	0.78
8	SSR-113	CCG TAA GAA TTA GGT GTC	GGA AAA TAG GGT GGA AAG	50	0.67
9	SSR-119	GAA CTC AGT TTC TCA TTG	GAA CAT ATC CAA TTA TCA TC	50	0.71
10	SSR-156	GTA CAT TGA ACA GCA TCA TC	CAA ATG GGC ATG AAA GGA G	55	0.7
11	SSR-213	CAC TCG CAC CTC TTA TG	GAA ATT GTC TCT TAG CAA G	50	0.74
12	SSR-317-2	CAC GTA ACA TCT TGC TTA TG	GTA GCA ATA ATT ACA CCC AC	50	0.77

presented in the table 4. The first four genotypes are the small seeded popular cultivars of West Bengal. Three NBPGR collections *viz.*, LR-IC 268239, LR-IC 282863, and LR-IC 560307 were also selected due to their higher yield as well as small seeded traits. LC-284-1206 is medium sized seeded lentil genotypes. The Precoz and L-1112-18 are large seed producers. Seed size is an economic trait and deserves the attention of geneticists and breeders. It is a special attribute in lentil consumption and trade. The wholesale price of small and large-seeded lentils differs by a large margin depending on the consumer preference and farmers' choice. We could not establish any relation between the seed size and the bruchid resistance in lentil, hence further study is needed. It can be suggested that for developing short duration bruchid resistant lentil genotypes, Subrata (85 days) and LR-IC 268239 (89 days) would be ideal.

In our bruchid feeding assay, female bruchid laid eggs over the surface of each of all the fresh 50 seeds of each of the 94 lentil germplasms and the number of eggs ranged from 1 to 3 in each of the lentil seeds except Precoz and L-1112-18. Astonishingly all the 50 seeds of each of Precoz and L-1112-18 were eggless and during counting, we found 50 undamaged seeds along with ten dead insects, which were nothing but the five insect pairs initially released. For confirmation we repeated the feeding assay only for these two genotypes and found the same result. Hence Precoz and L-1112-18 are being considered as completely as well as extremely resistant to pulse beetle as the insects were unable to lay eggs and they must be one of the parents in all the cases of lentil breeding programme regarding pulse beetle resistance. Precoz and L-1112-18 produce large seed (macrosperma type) hence are not suitable for West Bengal people. Therefore this trait can be easily improved through hybridization of them with any small seeded (microsperma type) lentil genotypes followed by selection. Four popular West Bengal genotypes considered in our study are micorsperma type. Moreover Ranjan and PL-406 showed >90% resistance while Subrata showed 78% resistance (table 3). On the other hand L-1112-18 is an early flowering genotype based

Table 3: Lentil germplasms their source of collection and resistance percentage

Sl. No.	Accession No./ Genotype name	Source	Resistance (%)	Sl. No.	Accession No./ Genotype name	Source	Resistance (%)	Sl. No.	Accession No./ Genotype name	Source	Resistance (%)
1	LR-IC 560297	NBPGR	74	33	LR-IC 266840	NBPGR	92	65	ILL 10971	ICARDA	86
2	LR-IC 560307	NBPGR	80	34	LR-IC 559780	NBPGR	76	66	ILL 10273	ICARDA	98
3	LR-IC 569608	NBPGR	90	35	LR-IC 559666	NBPGR	92	67	ILL 10951	ICARDA	94
4	LR-IC 212676	NBPGR	88	36	LR-IC 524223	NBPGR	94	68	ILL 10803	ICARDA	98
5	LR-IC 559678	NBPGR	92	37	LR-IC 558821	NBPGR	96	69	ILL 6821	ICARDA	96
6	LR-IC 559857	NBPGR	90	38	LR-IC 559738	NBPGR	90	70	ILL 10258	ICARDA	92
7	LR-IC 599831	NBPGR	90	39	LR-IC 560812	NBPGR	96	71	ILL 10103	ICARDA	82
8	LR-IC 560336	NBPGR	98	40	LR-IC 558826	NBPGR	90	72	ILL 10802	ICARDA	96
9	LR-IC 267663	NBPGR	78	41	LR-IC 559659	NBPGR	92	73	ILL-8006	ICARDA	100
10	LR-IC 268343	NBPGR	94	42	LR-IC 560008	NBPGR	96	74	BM-2	Bangladesh	82
11	LR-IC 262847	NBPGR	96	43	LR-IC 201704	NBPGR	82	75	BM-7	Bangladesh	90
12	LR- IC 366117	NBPGR	90	44	LR-IC 559744	NBPGR	84	76	BM-6	Bangladesh	0
13	LR-IC 560148	NBPGR	84	45	LR-IC 267660	NBPGR	98	77	RL-12-178	IARI, India	88
14	LR-IC 268239	NBPGR	80	46	LR-IC 560040	NBPGR	72	78	RL-12-180	IARI, India	20
15	LR-IC 267666	NBPGR	92	47	LR-IC 279686	NBPGR	82	79	WBL-77	CRU	98
16	LR-IC 201703	NBPGR	92	48	LR-IC 560051	NBPGR	92	80	Ranjan	CRU	78
17	LR-IC 208344	NBPGR	40	49	LR-IC 559732	NBPGR	82	81	Subrata	CRU	94
18	LR-IC 560337	NBPGR	80	50	LR-IC 316162	NBPGR	92	82	PL-406	CRU	80
19	LR-IC 311171	NBPGR	94	51	LR-IC 267670	NBPGR	88	83	NDL^{-1}	CRU	86
20	LR-IC 560299	NBPGR	100	52	LR-IC 281600	NBPGR	60	84	PL-639	CRU	96
21	LR-IC 321535	NBPGR	92	53	LR-IC 348094	NBPGR	88	85	L1112-15	CRU	100
22	LR-IC3218081A	NBPGR	98	54	LR-IC 560118	NBPGR	96	86	L1112-18	CRU	92
23	LR-IC 559648	NBPGR	90	55	LR-IC 559876	NBPGR	66	87	L1112-19	CRU	56
24	LR-IC 283423	NBPGR	98	56	LR-IC 559785	NBPGR	84	88	L1112-6	CRU	90
25	LR-IC 560331	NBPGR	96	57	LR-IC 560131	NBPGR	92	89	L1112-13	CRU	94
26	LR-IC 278791	NBPGR	96	58	LR-IC 559890	NBPGR	92	90	LL-147	CRU	84
27	LR-IC 279627	NBPGR	68	59	LR-IC 208322	NBPGR	78	91	L1112-12	CRU	96
28	LR-IC 282863	NBPGR	66	60	LR-IC 560111	NBPGR	100	92	LL 56	CRU	100
29	LR-IC 315962	NBPGR	88	61	LR-IC 560123	NBPGR	92	93	Precoz	CRU	82
30	LR-IC 263285	NBPGR	84	62	LR-IC 560034	NBPGR	98	94	LC-284-1206	CRU	100
31	LR-IC 208352	NBPGR	90	63	LR-IC 559996	NBPGR	98				
32	LR-IC 201700	NBPGR	92	64	LR-IC 559870	NBPGR	74				

on our DUS test but it takes 104 days to mature which is longer than that of the local cultivars selected in this study; it showed extremely resistance against bruchid, *i.e.* 100 per cent resistance as well as no egg was laid over it in our study.

Table 3 shows many in the tested 94 genotypes showing 80 to 100 per cent resistance, but they were not taken into consideration for diversity analysis as we gave the priority to the high yielding lentil genotypes. WBL-77, in spite of showing 80 per cent susceptibility, was chosen only for its extreme popularity among the West Bengal farmers. It is to be noted that BM-6, a genotype from Bangladesh, showed 100 per cent susceptibility to bruchid. Moreover, the ILL-8006 genotype from ICARDA showed susceptibility in the study conducted by Gore *et al*. (2016); but in our study it showed complete resistance against *C. chinensis* (Table 3).

Table 4: Selected ten high yielding lentil genotypes with their duration, seed size

Sl. No.	Genotypes (days)	Duration size	Seed
1	WBL-77	100	Small
2	Subrata	85	Small
3	Ranjan	95	Small
4	PL-406	100	Small
5	LR-IC 268239	89	Small
6	LR-IC 282863	96	Small
7	LR-IC 560307	97	Small
8	LC-284-1206	95	Medium
9	L-1112-18	104	Large
10	Precoz	97	Very large

Diversity analysis on the basis of phenotypic markers

Diversity analysis of ten selected lentil genotypes was performed for selection of most distant parents. Similarity coefficient matrix followed by Dendogram was done following Jaccard's coefficient considering twelve distinct phenotypic characters of lentil previously mentioned in the table 1. According to Dendogram (figure 1), the cultivar WBL-77 has 41 and 47 per cent diversity with Precoz and L-1112-18 respectively. Another popular cultivar of West Bengal Subrata has equal trend where it shows almost 47per cent dissimilarities, which are relatively higher than the remaining genotypes, to both Precoz and L-1112-18. Figure 1 also shows Precoz and Ranjan could be a good pair due to 52per cent dissimilarity between them. PL-406, LR-IC 282863 and LR-IC 560307 could also be good breeding partners to Precoz due to their 41, 53 and 53per cent dissimilarity respectively. Hence from the above findings WBL-77, Subrata, Ranjan, PL-406, LR-IC 282863 and LR-IC 560307 are recommended as one of the diverse parents that could be hybridized with Precoz regarding breeding for bruchid resistance as well as small seed. Similarly L-1112-18 could be s good partner to WBL-77, Subrata and LR-IC 268239 due to 47%, 47% and 41% dissimilarities, respectively.

Diversity analysis on the basis of SSR markers

Twelve pairs of lentil SSR primers were employed to assess the polymorphism between the selected genotypes and they were chosen on the basis of their PIC values (Table 2); the average PIC value was approximately 0.66. Selected SSR primers generated 30 alleles among the selected ten lentil germplasms. The allele number for SSR loci ranged from 1 to 4. The PIC values were calculated that range from 0 to 0.48 with the mean 0.25. Hamwieh *et al.* (2009) reported much higher genetic diversity in lentil accessions using 14 SSRs; moreover average number of allele per loci in this study (2.5) is lower than *Lens* species (2.89) conducted using 31 EST SSRs and 12 genomic SSRs (Dikshit *et al.*, 2015). Polymorphism features of each SSR marker used in our study are presented in the table- 5.

The SSR-19 was monomorphic as its primers amplified 290 bp fragment in each genotype. Both 280 bp and 300 bp fragments were found for the SSR-33 and SSR-119. In case of SSR-33, WBL-77 only generated 280 bp but other nine generated the 300 bp amplicon while the primer pairs for SSR-119 generated only 300 bp fragment in Precoz and 280 bp in the rest nine. In other words it can be said that SSR-33 is WBL-77 specific and SSR-119 is Precoz specific as revealed by our study. Three different alleles were found for the SSR-66, SSR-156 and SSR-59-2 in our study where WBL-77 specific 180 bp amplicon was found for both SSR-66 and SSR-156, but only Precoz specific single fragment of 200 bp was observed for the SSR-59-2. To amplify SSR-213, we found two types of fragments such as 140 bp and 160 bp where 140 bp was specific only for WBL-77 but the 160 bp amplicon was generated in the rest nine genotypes. Hence for hybridization programme of 'WBL-77 X Precoz' these six SSRs will be useful.

Two types of fragments, 270 bp, and 300 bp were produced in case of SSR-113 and its PIC value was also high. In case of another two SSRs namely ALD-15 and ALD-42, two types of fragments, 250 bp and 270 bp, were produced. For the genotype Subrata, the specific amplicon size of ALD-15 was 270 bp that was not found in the rest nine while ALD-42 generated only 250 bp in both Subrata as well as Precoz and 270 bp in the remaining eight genotypes. Four different types of alleles were found in case of SSR-107 and SSR-317-2 among the ten lentil genotypes. It is noted that the 150 bp

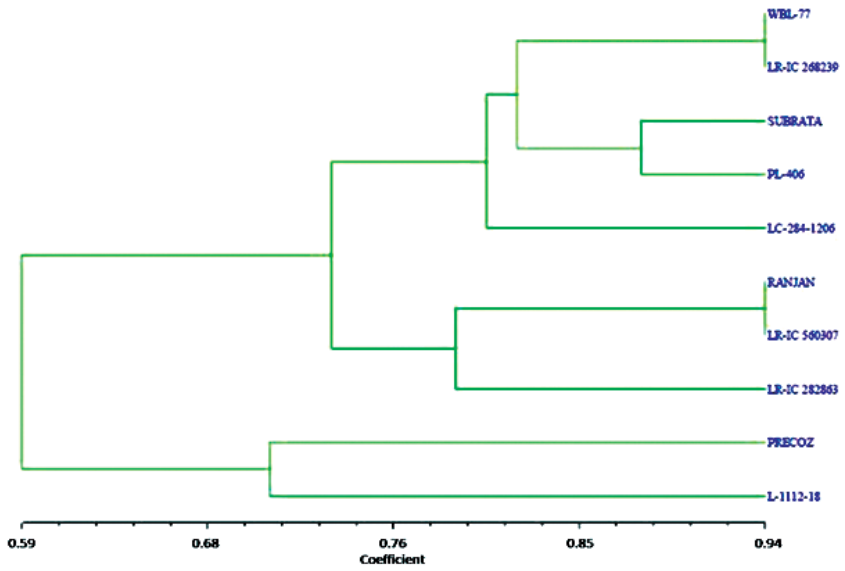

Fig. 1: Dendogram depicting genetic relationship among ten lentil genotypes based on similarity coefficient using phenotypic markers using Jaccard's similarity coefficient

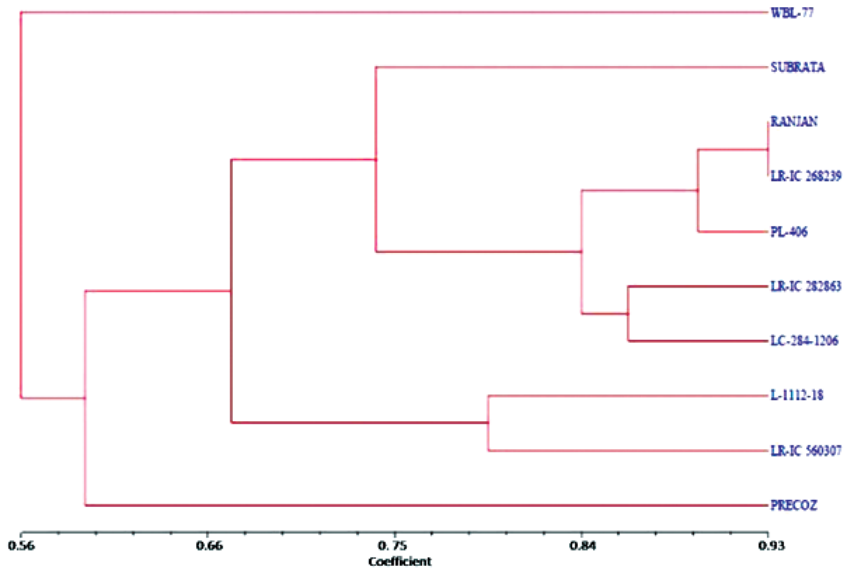

Fig. 2: Dendogram depicting genetic relationship among ten lentil genotypes based on similarity coefficient using twelve SSR markers by using Jaccard's similarity coefficient

amplicon of SSR-107 was only generated in the Precoz. Hence for hybridization programme of 'Subrata X Precoz', SSR ALD-15 as well as SSR-107 will be potential. But ALD-42 cannot be taken in this case. According to the Dendogram (Fig. 2) derived through molecular analysis, we are getting a little different data regarding diversity than that of the phenotypic analysis. The cultivar WBL-77 has maximum diversity of approximately 55% with Precoz than the remaining eight genotypes and previous phenotypic data regarding diversity was 41%; rest eight genotypes showed >50% similarity to Precoz. Subrata and Ranjan both showed almost 66% similarities with Precoz while PL-406 showed 51% similarity with Precoz. Therefore 'WBL-77 X Precoz' is considered as best pair than that of 'Ranjan X Precoz', 'Subrata X Precoz' or 'PL-406 X Precoz' espscially for deriving small seeded bruchid resistant high yielding cultivar suitable for West Bengal condition.

Table 5: Analysis of polymorphic SSR fragments in selected lentil genotypes

SSR MARKERS	Genotypes with the amplicon (bp)										PIC Value
	WBL-77	Subrata	Ranjan	Precoz	PL-406	LR-IC 268239	LR-IC 282863	L-1112-18	LR-IC 560307	LC-284 1206	
ALD-15	250	270	250	250	250	250	250	250	250	250	0.18
ALD-42	270	250	270	250	270	270	270	270	270	270	0.32
SSR-19	290	290	290	290	290	290	290	290	290	290	0
SSR-33	280	300	300	300	300	300	300	300	300	300	0.18
SSR 59-2	100	100	100	200	100	100	100	150	100	100	0.22
SSR 66	180	200	200	200	200	200	200	220	220	200	0.3
SSR-107	180	180	190	150	190	190	180	180	200	180	0.32
SSR-113	300	270	270	300	270	270	270	300	300	270	0.48
SSR-119	280	280	280	300	280	280	280	280	280	280	0.18
SSR-156	180	200	200	200	190	190	200	200	200	190	0.36
SSR-213	140	160	160	160	160	160	160	160	160	160	0.18
SSR 317-2	140	140	140	140	160	140	160	140	180	200	0.29

Previously Sarkar *et al*. (2011) identified one SSR marker (STS br 1) linked to bruchid resistance in mung bean from a wild *sublobata* line; mung bean is another popular legume crop of West Bengal. The same study could be useful for lentil also, but first of all the source of bruchid resistance must be searched out in available lentil germplasms. As there is no information available regarding bruchid resistance among the global lentil genotypes so far, subsequently no lentil SSR linked with bruchid resistance has been identified; our findings will provide necessary information to begin lentil breeding programme in respect to bruchid resistance. The lentil growing states of India like West Bengal, where the climate favours the pulse beetle infestation during storage, will get benefit out of it. We recommend WBL-77 and Precoz as the best parental pair for initiating such programme.

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