Indian J. Plant Genet. Resour. 32(1): 85-92 (2019)
DOI 10.5958/0976-1926.2019.00012.3

RESEARCH ARTICLE

Molecular Characterization of Oilseed *Brassica* Reference Set for Stem Rot Disease

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(Received: 21 August 2017; Revised: 19 April 2018; Accepted: 12 July 2018)

Present study was conducted on oilseed brassica reference set covering resistance and susceptible genotypes (41 *Brassica juncea*, two *Brassica carinata*, one *Sinapis alba*) to gain the information on extent of genetic diversity using 30 polymorphic Simple Sequence Repeat (SSR) markers. The dendrogram obtained by the UPGMA analysis of 82 polymorphic loci amplified using 30 polymorphic SSR markers, resulted in definite groupings among the reference set. The polymorphic information content (PIC) values varied from 0.23 to 0.67 with an average of 0.53. The highest PIC value was observed for marker named BRMS-054 making it most informative marker. The polymorphic SSR markers identified in the present study can be used for characterizing *Brassica* cultivars, constructing database, tagging genes/QTL governing stem rot tolerance and ultimately in identifying markers for the disease.

Key Words: Brassica, Genetic diversity, Polymorphic information content (PIC), SSR markers, *Sclerotinia* stem rot

Introduction

Rapeseed-mustard group of crops are major source of edible oil, vegetables and condiments. The genus Brassica belongs to the family *Brassicaceae* which include 338 genera and 3,709 species (Warwick *et al.*, 2006). Rapeseed-mustard group of crops comprise seven cultivated Oilseed Brassicas namely, *Brassica napus* L., *B. rapa* L. ssp. yellow sarson, *B. rapa* L. ssp. *brown sarson* and *B. rapa* L. ssp. *toria*, *B. juncea* (L.) Czern. & Coss., *B. carinata* Braun. and *B. nigra* (L.) Koch. The other economically important crop under the family *Brassicaceae* is *Sinapis alba*, which is cultivated as oilseed and fodder crops, particularly on marginal agricultural locations with poor soils or sub-optimal climatic conditions (Duhoon and Koppar, 1998). Modern plant breeding techniques that include intensive selection over prolonged period have drastically reduced the genetic diversity in these crops resulting into increase in the vulnerability of crops to pests and also reduced their ability to respond to changes in climate or agricultural practice (Cowling, 2007; Ananga *et al.*, 2008). However, reduction in genetic diversity caused by this intensive selection can be counterbalanced by introgression of genes from novel germplasm that include exotic lines, wild species and relatives of the crop (Miflin, 2000). These germplasm can be used as valuable source of gene for improvement of crop *Brassicas*. Therefore, attempts have become extremely essential to analyze possible genetic diversity among different species of *Brassicas*.

The analysis of DNA sequence variation is of major importance in genetic studies. In this context, molecular markers are useful tools, and these have greatly enhanced the genetic analysis of crop plants in recent years (Varshney *et al.*, 2005). Molecular marker approach is widely preferred for genetic variability assessment as compared to biochemical and morphological approaches due to their more précised efficiency, reliability and inert behavior to environmental conditions (Mishra *et al.*, 2011). Among the various molecular marker techniques available, simple sequence repeats (SSRs) have been the marker of choice in modern plant breeding because of their reproducibility, multi-allelic nature, co-dominant inheritance, relative abundance and good genome coverage (Gupta and Varshney, 2000).

The present study was undertaken to gain the information on extent of genetic diversity on reference set of oilseed *brassica* covering resistance and susceptible genotypes against Sclerotinia stem rot infestation. Sclerotinia stem rot of rapeseed-mustard, caused by

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the fungus *Sclerotinia sclerotiorum*, is one of the most significant yield-limiting factor (up to 80 percent) and least controllable diseases in oilseed *Brassica* worldwide (Mehta *et al.*, 2010).

Materials and Methods

The present investigation was conducted on reference set of oilseed *brassica* covering resistance and susceptible genotypes (41 *Brassica juncea*, 2 *Brassica carinata*, 1 *Sinapis alba*) to gain information on extent of genetic diversity. The genotypes were selected on the basis of their relative tolerance/susceptibility to stem rot infestation caused by *Sclerotinia sclerotiorum* (Table 1). These 44 genotypes were planted in two rows of

Table 1. Disease Reaction for Sclerotinia stem rot and pedigree information of *Brassica* genotypes

S.No.	Genotypes Name	Origin/Pedigree	Mean Disease Incidence (%)	Disease Reaction
1	NRCYS-5-2	Selection from YS 09/04	35.05	Susceptible
2	EC-597312(JM 6004) (YS)	Australian germplasm	5.6	Moderately Resistant
3	EC-597314(JM 6009)	Australian germplasm	5.0	Moderately Resistant
4	EC-597315(JM 6010)	Australian germplasm	5.9	Moderately Resistant
5	EC-597316(JM 6011)	Australian germplasm	8.3	Moderately Resistant
6	EC-597317(JM 6012)	Australian germplasm	2.3	Resistant
7	EC-597319(JM 6014) (YS)	Australian germplasm	21.4	Moderately Susceptible
8	EC-597320(JM 6015)	Australian germplasm	12.1	Moderately Susceptible
9	EC-597321(JM 6018)	Australian germplasm	18.7	Moderately Susceptible
10	EC-597325(JM 6026) (YS)	Australian germplasm	7.1	Moderately Resistant
11	EC-597328	Chinese germplasm	16.3	Moderately Susceptible
12	EC-597331	Chinese germplasm	9.1	Moderately Resistant
13	EC-597333	Chinese germplasm	11.4	Moderately Susceptible
14	EC-597334	Chinese germplasm	12.1	Moderately Susceptible
15	EC-597341	Chinese germplasm	25.8	Susceptible
16	EC-597343	Chinese germplasm	15.2	Moderately Susceptible
17	EC-597344	Chinese germplasm	18.6	Moderately Susceptible
18	EC-597340	Chinese germplasm	22.9	Moderately Susceptible
19	EC-552573	Australian germplasm	30.6	Susceptible
20	EC-552576	Australian germplasm	22.3	Moderately Susceptible
21	EC-552578	Australian germplasm	37.2	Susceptible
22	EC-552581	Australian germplasm	22.5	Moderately Susceptible
23	EC-552583	Australian germplasm	13.1	Moderately Susceptible
24	EC-552584	Australian germplasm	17.7	Moderately Susceptible
25	RH (0E) 0801	Not available	16.5	Moderately Susceptible
26	RH (0E) 0901	Selection from exotic material	30.9	Susceptible
27	RH (0E) 0902	Selection from exotic material	16.4	Moderately Susceptible
28	RH (0E) 0903	RH(OE)0205 × JN018	19.9	Moderately Susceptible
29	PUSA KRISHMA	Pusa Barani × ZEM 1	21.9	Moderately Susceptible
30	NUDBYJ-10	Eh 1 × MS 21	8.8	Moderately Resistant
31	LES-46	SEJ 8 × LET 18	4.8	Resistant
32	PUSA MUSTARD-21	Pusa bold × ZEM 2	8.3	Moderately Resistant
33	LES-47	Pusa Agarni × LET 14	17.9	Moderately Susceptible
34	RLC-2	QM 4 × Pusa bold	6.9	Moderately Resistant
35	ZEM-1	Australian low erucic line	16.9	Moderately Susceptible
36	ZEM-2	Australian low erucic line	11.5	Moderately Susceptible
37	DRMR-150-35	RH 819 × Pusa Bold	18.6	Moderately Susceptible
38	*Sinapis alba*	*Brassica* species	0	Immune
39	PUSA SWARNIM	HC 4 × Early mutant	1.2	Resistant
40	KIRAN	Selection from HC 5	1.2	Resistant
41	RH-749	RH 781 × RH 9617	12.8	Moderately Susceptible
42	ROHINI	Selection from natural population of Varuna	29.15	Susceptible
43	NAV GOLD	Bio 902 × BM 185-11	10.05	Moderately Susceptible
44	RH-406	RH 9608 × RH 30	9.45	Moderately Resistant

5 m length during *rabi* 2016-17. All the recommended approaches were followed for raising a good crop. The disease pressure was created artificially by inoculating the stems using mycelial suspension of *Sclerotinia sclerotiorum*. The disease scoring was done on the basis of percentage incidence of the disease.

DNA Isolation, Purification and Quantification

For DNA isolation fresh and young leaves (2 g) from healthy plants were collected from each variety. Total genomic DNA was isolated by using CTAB method of Saghai-Maroof *et al.* (1984) with slight modifications. For estimating the quantity and quality of DNA, the isolated DNA samples were loaded on a 0.8% (w/v) agarose gel. Quantification of DNA on gel requires loading a sample DNA of known concentration. DNA samples with high concentration were further diluted in TE buffer to a working concentration of 20 ng/µl and were stored at -20ºC for further use in PCR analysis.

PCR Assay

A total of 110 SSR markers were used to evaluate 44 reference set to assess the genetic diversity among them. PCR amplification was performed in a total volume of 10 µl containing 20 ng template DNA, 20 ng of each primer, 10x standard buffer containing 2.5 mM $MgCl_2$, 200 µM of each dNTP and 1.0 U of Taq DNA polymerase. PCR reactions were carried out in an Eppendorf thermocycler. PCR mastercycler was programmed at 94°C for 5 min for initial denaturation followed by 35 cycles of 45 s denaturation at 94°C, 50 s annealing at 58°C-62ºC and 1 min extension at 72°C with a final extension of 72°C for 7 min. PCR-amplified fragments were electrophoretically separated on 3.5% agarose gel containing 0.01% ethidium bromide, prepared in 1×TAE buffer (Tris-Acetic acid-EDTA). Allele sizes were predicted by comparing with 50 bp DNA ladder (Thermo Scientific). The gel was run for 3 hrs at 80 V. After electrophoresis, the amplification products were visualized in a gel documentation system. (IG/LHR, Syngene, UK).

SSR Analysis and Data Scoring

Amplified fragments of variable sizes were considered as different allele. SSR scoring of gel was done qualitatively from gel photographs by analyzing the amplified fragment. The primers which did not produce amplification were repeated at least two times before discarding them. The clear, instantly recognizable amplicons which migrated to variable distances within the gel were considered as polymorphic, whereas the amplicons which migrated same distance within gel were considered as monomorphic. The binary matrix of 0 (absence) and 1 (presence) for each SSR marker was compiled for amplified products. To compute Jaccard's similarity coefficient (Jaccard, 1908) the binary data matrix was entered into NTSYS-pc version 2.02e programme and analyzed using SIMQUAL. The similarity matrix thus obtained was used in cluster analysis using an unweighted pair-group method with arithmetic averages (UPGMA) given by Sneath and Sokal, 1973. The SHAN clustering algorithm was used to generate dendogram displaying relationships among 44 genotypes. Polymorphic information content (PIC) values for each SSR were calculated using the formula as recommended by Anderson *et al.* (1993).

$$PICj = 1 - \sum_{i=1}^{n} Pij^2$$

where, i = the ith allele of the jth marker,
n = the number of alleles at the jth marker and
p = allele frequency.

Inoculum Preparation and Inoculation of Sclerotinia sclerotiorum

The *S. sclerotiorum* BHP isolate was collected from infected accession available in ICAR-Directorate of Rapeseed Mustard Research, Bharatpur, India. A solo isolate of S. sclerotiorum was taken and sterilization procedure was followed as suggested by Clarkson *et al.* (2003). The potato dextrose broth was used to place half of the sclerotium under sterilized condition (laminar air flow). Subcultures were maintained on PDA at temperature of 25°C and 12 h light/ dark condition under fluorescence. The autoclaved *Sesbania* leaves in glass jar were used to mass multiply the pathogen. The multiplied pathogens were mixed with FYM in soil prior to sowing. The mycelial suspension of the pathogen was prepared in the laboratory on potato dextrose broth. These suspensions were sprayed by the help of automizer at 45 days after sowing. The plants were further inoculated at 60 days of sowing with the pathogen growing on potato dextrose broth and the stem of the plants were tied with the help of parafilm (Li *et al.*, 2007).

Disease Scoring

$$\text{Disease incidence} = \frac{\text{Sum of total of numerical ratings}}{\text{No. of plants examined} \times \text{maximum grade}} \times 100$$

The disease incidence was calculated by using following formula (Phool Chand and Dinesh Rai, 2009). According to intensity of disease, the disease reaction is decided as mentioned below (Table 2)

Table 2. Disease reaction categories with respect to disease intensity

Score	Disease reaction/Grade	Disease intensity (%)
0	Highly Resistant	0
1	Resistant	1-4
2	Moderately Resistant	5-9
3	Moderately Susceptible	10-24
4	Susceptible	25-49
5	Highly Susceptible	>50

Results and Discussion

Disease Data Analysis

The mean disease incidence ranged from 0 to 37.2 percent. Ten out of 44 genotypes had a high disease incidence (>20%), and 34 genotypes had a low disease incidence (<20%). Minimum lesion size was found to be zero whereas the maximum lesion size was recorded 37.8 cm. Stem diameter of 44 genotypes lies in the range of 1.42 cm to 2.46 cm. On the basis of disease severity it was concluded that genotype *Sinapis alba* is highly resistant to stem rot whereas four genotypes namely EC-597317, LES-46, Pusa Swarnim and Kiran showed resistance reaction with disease incidence level ranging from 1.2% to 4.8% as shown in Table 2. Ten genotypes were recorded as moderately resistant with disease incidence ranging from 5.0% to 9.45%, whereas 23 were found to be moderately susceptible according to the grading, disease incidence vary from 10.05% to 22.9%. Maximum disease severity (37.2%) was found among genotype namely EC-552578, whereas none of the genotypes were found to be highly susceptible to Sclerotinia stem rot disease.

SSR Marker Analysis

Among the 110 SSR primers used for polymorphism study, a total of 80 out of 110 SSR markers gave clear amplicons among all the 44 genotypes. A total of 30 SSRs were detected to be polymorphic. All the 30 polymorphic SSR reported in the study provided clear, well-marked and unambiguous amplified fragments which were further taken for genetic diversity evaluation, while rest of the 80 markers did not generate polymorphic banding pattern. The polymorphism percentage revealed in present study is 27.27 percent which is very less as compared with 93.70 percent as reported by Vinu *et al.* (2013) in different set of 44 Indian mustard genotypes. Similarly, Cheng *et al.* (2009) reported 73.4 percent polymorphism in genetic diversity analysis among six *Brassica* genotypes. In present investigation, a total of 82 alleles were amplified from 30 polymorphic markers having 1 to 4 alleles per locus with an average of 2.73 alleles/marker. It is complementary with the results (2.7 allele per locus) as reported by Zhou *et al.* (2006) and Kumawat *et al.* (2015). The average number of allele reported in the present investigation was however, found lesser as reported by 3.53 allele per locus (Thakur *et al.*, 2015), 5.29 allele per marker (Cunmin *et al.*, 2012), 6.6 allele/marker (Yao *et al.*, 2012). The fragment size of amplicons varied from 100 bp to 340 bp.

PIC value is often used to measure the discriminatory power and informativeness of a genetic marker. As suggested by Peng and Lapitan (2005), PIC Value is an important parameter for selecting SSR for germplasm evaluation and gene tagging. Before initiating any research related to genetic diversity assessment by using SSR markers, it is compulsory to select most informative SSRs and this is possible only by estimating PIC value of each SSR marker to be used in the study (Macaulay *et al.*, 2001, Masi *et al.*, 2003, Candida *et al.*, 2006). In this study, the PIC value found to lie between 0.23 (D12) to 0.67 (BRMS-054) with an average of 0.53. The average PIC Value estimated is comparable to 0.57 as calculated by Park J *et al.* (2013) and El-Esawi *et al.* (2016). PIC value observed with marker BRMS-054 was the highest (0.667) indicating it as the most informative and representative marker (Fig. 2). Among all the 110 SSRs used in the study, most of them generated lesser number of bands and lower PIC value which may be due to the reason that these markers are not specific to the species of the genotypes under study. Out of 30 polymorphic markers a total of fifteen markers showed >0.5 PIC value indicates the discriminatory potential and usefulness of these markers for differentiating 44 genotypes under study (Table 3).

Similarity Coefficient and Genetic Relationship Analysis

Jaccard similarity coefficient was found to vary from 0.33 to 0.93 with an average of 0.61. The highest value (92.6%) for genetic similarity was found between EC597316 and EC597317 followed by 89% between EC597315 and EC597316 and also between EC597315 and EC597317. Similar range of genetic variation

Table 3. Detail of PCR amplified product with 30 SSR primers

S. No.	Marker	Forward primer	Reverse primer	Tm (°C)	Range of Amplified product	No.of Polymorphic Bands	PIC Value
1	BRMS-054	GAATCTCTGCAAGAAACAAATG	TTCCTCAGCATCAAGTAACCTC	58	200-250	3	0.667
2	cnu_m621a	GCAGAAGCCTGAGAGTCTGG	AACAAGGCTGAATGCTACCG	55	150-250	3	0.666
3	BJSSR-181	AAACCGGCCTAGCACTCTTC	GGCTTCTCTTCTTGGAGCGT	56	200-300	2	0.660
4	EJU1	GGTGAAAGAGGAAGATTGGT	AGGAGATACAGTTGAAGGGTC	55	180-200	2	0.657
5	BJSSR-473	TCAACATGCTGCTCCTGC	TGGCTCAGGAAGAACAAG	55	200-250	3	0.656
6	BJSSR-125	ACGACGGAGGAGAAACCAAC	CGGACGATACTGAAGGCGAA	55	230-270	3	0.655
7	Ra2-E04	ACACACAACAAACAGCTCGC	AACATCAAACCTCTCGACGG	57	180-300	2	0.654
8	C09	AGCATCAATCTTTTGCTCTGC	TGCACACAAACTCCTTCTCC	55	180-250	3	0.639
9	BJSSR-184	CGTCGATAGCTTCCTCCACC	CTCCCGTGGTCTCTTGTTCC	54	180-230	2	0.624
10	cnu_m593a	TAAGGCAAATTGTTGGGCAT	CCATCTCTCCTTGTCCTCCA	55	300-340	3	0.616
11	Ni2AO7	GGAACCCAACAAGTGAGTCC	AGAGCTTGAGACACATAACACC	55	200-250	3	0.614
12	B02	CGCTGCAATTATACGAAAGC	CCTCATGCTCTCCAAAGACC	55	180-200	3	0.613
13	Ni3-B07	GGAGAAGAGGAAGAAGAAGCC	CGACTTCTAGAGGAACCCCC	55	100-170	3	0.594
14	cnu_m597a	TTGAACCCACGAAAACTTCC	AGAAGGGAGAGAGGTCAGGC	55	180-230	3	0.577
15	PW186	GACGGTGACGACCAATCAGAGCA	GCGTTCGCCATAGACGAGTCCAA	55	120-140	3	0.559
16	E05	CTCGTCTCAGGGATTATGTCG	CAGACAGAGGATAGACCGAACC	58	100-170	3	0.544
17	BRMS-043	GCGATGTTTTTTCTTCAGTGTC	TTAATCCCTACCCACAATTTCC	53	280-320	2	0.524
18	F01	CGTATGTAGAGAGAGAGAGAGAGAGAG	AGAACCGTTGAGGTGCTGTC	58	200-300	3	0.499
19	nia_m042a	CCCATCCCTCCTTGATGATA	GATGGGCATTTCACGACAC	54	250-280	2	0.499
20	ENA28	GGAGTCCGAGCGTTATGAAT	CTTCATCGACCCACCTTGTT	54	150-170	2	0.498
21	Ra3-D02B	CACAGGAAACCGTGGCTAGA	AACCCAACCTCAACGTCTTG	55	350-400	2	0.485
22	cnu_m585a	TTTATCAGTCCGGTTTTGCC	GATGCTCTGAGACACCCAAA	53	100-130	2	0.48
23	cnu_m602a	CATCCACAAGTCCACCAGTC	CGCATTAGACCCTAAAAACCG	56	200-230	2	0.456
24	A09	AAAGGGCGAAGAAGCAGC	TTTCTTCCATTTGACCGACC	53	150-250	2	0.418
25	cnu_m587a	CATCATTGGCTTTGGGAGTT	CGACTGGGAAAGAAAAACACA	52	180-240	2	0.405
26	Ni2AO1	TGCTGCTACAGACAGTGTTGG	AAAGGCTACACACTCATGAAACC	56	230-250	2	0.389
27	ENA20	GATGGAGGAAGAAGACAAGAC	TCTGAACTACCAAAGCCAAC	55	130-150	2	0.357
28	BRMS-033	GCGGAAACGAACACTCCTCCCATGT	CCTCCTTGTGCTTTCCCTGGAGACG	64	150-250	2	0.356
29	nia_m066a	TTGGGAAGAGAGTTGGGTGA	TCGTTTCATATGGGCCTTGT	53	250-300	3	0.256
30	D12	GAGATGAGGATTTGCTTTTGC	ACAGTATGAGAGAGAGAGAGAGAG	55	140-170	2	0.225

has also been reported by Gohel and Mehta (2014), Vinu *et al.* (2013) in Indian mustard. Whereas, the least similarity coefficient was observed in between EC 597321 and ZEM-1 which represents high genetic diversity among these two genotypes. The dendrogram obtained by the UPGMA analysis of 82 polymorphic loci amplified by 30 polymorphic SSR markers resulted in definitive groupings among the studied genotypes, which is being partitioned into two major groups i.e. cluster I and cluster II with a similarity coefficient of 0.53 in the UPGMA dendogram (Fig. 1). The cluster I is further divided into two sub-clusters i.e. cluster I A and cluster I B. The sub-cluster I A and I B showed the genetic similarity of 56%. The sub-cluster I A consists of solitary genotype "NRCYS-5-2" which is found to be a susceptible genotype for stem rot disease in the present study, whereas the cluster I B consists of 37 genotypes. Maximum number of genotypes (30 out of 37) in cluster 1B showed moderately susceptible to moderately resistant disease reaction. Cluster II consists of total 6 genotypes namely LES-47, ZEM-1, ZEM-2, RLC-2, Pusa Swarnim and Kiran. Both the *B. carinata* genotypes viz. Pusa Swarnim and Kiran fall in same cluster and exhibit the genetic similarity of 59% .These genotypes show resistant reaction. However, other genotypes of cluster II are moderately susceptible. Australian low erucic acid

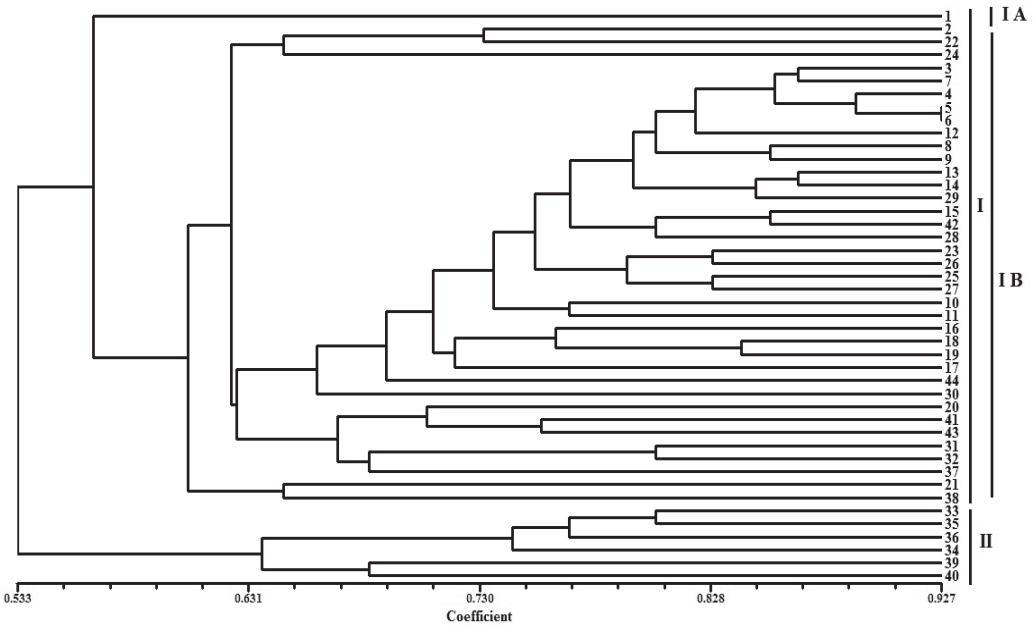

Fig. 1. UPGMA dendrogram showing genetic relationships among 44 genotypes based on SSR markers 1- NRCYS-5-2, 2- EC-597312(JM 6004) (YS), 3- EC-597314(JM 6009), 4- EC-597315(JM 6010), 5-EC-597316(JM 6011), 6- EC-597317(JM 6012), 7- EC-597319(JM 6014) (YS), 8- EC-597320(JM 6015), 9- EC-597321(JM 6018), 10- EC-597325(JM 6026) (YS), 11- EC 597328, 12- EC 597331, 13- EC 597333, 14- EC 597334, 15-EC 597341, 16- EC 597343, 17- EC 597344, 18-EC 597340, 19-EC 552573, 20- EC 552576, 21- EC 552578, 22- EC 552581, 23- EC 552583, 24- EC 552584, 25- RH (0E) 0801, 26- RH (0E) 0901, 27- RH (0E) 0902, 28- RH (0E) 0903, 29- PUSA KRISHMA, 30- NUDBYJ-10, 31- LES-46, 32- PUSA MUSTARD-21, 33- LES-47, 34- RLC-2, 35- ZEM-1, 36- ZEM-2, 37- DRMR-150-35, 38- *Sinapis alba*, 39- PUSA SWARNIM, 40- KIRAN, 41- RH-749, 42- ROHINI, 43- NAV GOLD 44-RH-406

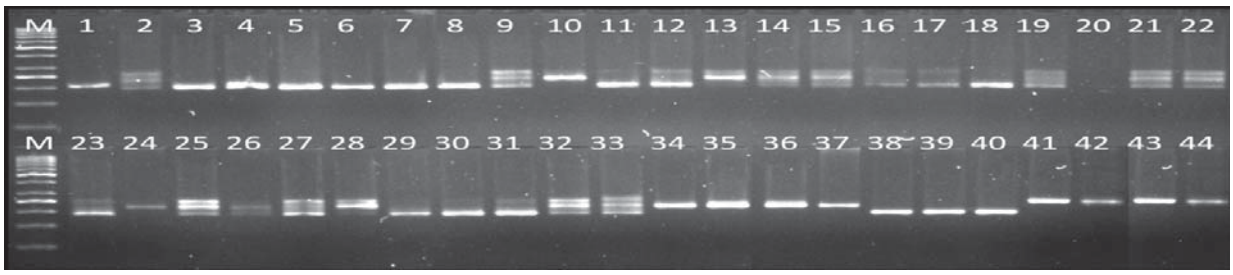

Fig. 2. Polymorphic-SSR marker (BRMS-054) profile of 44 oilseed mustard genotypes
Lane M- 50 bp ladder 1- NRCYS-5-2, 2- EC-597312(JM 6004) (YS), 3- EC-597314(JM 6009), 4- EC-597315(JM 6010), 5-EC-597316(JM 6011), 6- EC-597317(JM 6012), 7- EC-597319(JM 6014) (YS), 8- EC-597320(JM 6015), 9- EC-597321(JM 6018), 10- EC-597325(JM 6026) (YS), 11- EC 597328, 12- EC 597331, 13- EC 597333, 14- EC 597334, 15-EC 597341, 16- EC 597343, 17- EC 597344, 18-EC 597340, 19-EC 552573, 20- EC 552576, 21- EC 552578, 22- EC 552581, 23- EC 552583, 24- EC 552584, 25- RH (0E) 0801, 26- RH (0E) 0901, 27- RH (0E) 0902, 28- RH (0E) 0903, 29- PUSA KRISHMA, 30- NUDBYJ-10, 31- LES-46, 32- PUSA MUSTARD-21, 33- LES-47, 34- RLC-2, 35- ZEM-1, 36- ZEM-2, 37- DRMR-150-35, 38- *Sinapis alba*, 39- PUSA SWARNIM, 40- KIRAN, 41- RH-749, 42- ROHINI, 43- NAV GOLD, 44-RH-406

line i.e. ZEM-1 and ZEM-2 clustered together in same cluster with a genetic similarity of 60%. All Chinese genotypes were grouped in same cluster i.e. cluster I B and most of them are moderately susceptible to the stem rot disease. This shows the effectiveness of SSR markers in identifying the close pedigree relationship in breeding material and can be used in background selection of recurrent parent genome. In present investigation, the clustering of 44 genotypes based on SSR polymorphisms corresponded well to their known origin/pedigree data as well as their response to the Sclerotinia stem rot disease. The polymorphic SSR markers identified in the present study can be used for characterizing *Brassica* cultivars, constructing database, tagging genes/QTL governing

stem rot tolerance and ultimately in identifying markers for the disease.

Acknowledgements

Authors wish to acknowledge financial support received from ICAR under Incentivizing Research in Agriculture (IRA Scheme) and facilities provided by Director, DRMR for execution of this experiment.

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