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Assessment of molecular diversity of CMS, maintainer and restorer lines of sunflower (*Helianthus annuus* L.) using RAPD, ISSR and SSR markers

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ABSTRACT

The present investigation was conducted to assess genetic diversity in cytoplasmic male sterile (CMS), maintainer and restorer lines of sunflower (Helianthus annuus L.) using RAPD, ISSR and SSR markers. The RAPD and ISSR markers detected, respectively, 67.74 and 91.30 per cent polymorphism across the 20 elite seed parents and restorers. The efficiencies of all three types of oligos were assessed using PIC values. The mean PIC for RAPD, ISSR and SSR were 0.193, 0.234 and 0.289, respectively. All the binary data, scored from RAPD, ISSR and SSR were combined to generate Neighbor-Joining tree which clustered all the genotypes in three major groups. Genotype specific bands were produced by RAPDs and ISSRs.

Keywords: CMS, genetic diversity, *Helianthus annuus*, ISSR, RAPD, SSR

Sunflower is an annual, diploid species (2n= 34), belonging to the family *Compositae* and genus *Helianthus*. The genus *Helianthus* consist 51 species and 19 subspecies *i.e*. 14 annual and 37 perennial with different ploidy level (Seiler, 2007; Schilling and Heiser, 1981). Agriculturally, only two species, *Helianthus tuberosus* and *Helianthus annuus* are used for cultivation as oil seed crop. Worldwide sunflower is fourth important oil seed after soybean, groundnut, and mustard. In India, it is grown under 8.5 lakh ha area with total annual production 4.99 lakh tones during 2011(Anon., 2013).

The narrow genetic base of cultivated sunflower has been broadened by introgression of genes from wild species. This has resulted in continuous improvement of agronomic and economic traits in cultivated sunflower (Thompson *et al.*, 1981; Seiler, 1992; Seiler and Rieseberg, 1997). Non-availability of adequate quantity of good quality seed, particularly of hybrids, non-availability of insect pollinator, partial development of axial flowers, inbreeding depression, self incompatibility, heavy rains hampering, cross-pollination and non/partial adoption of recommended package of practices leads to low productivity and yield. Being a cross-pollinated crop, the loss of purity and vigor is often observed in its hybrids. The assessment of the genetic diversity in crop species is of interest for the conservation of genetic resources, broadening of the genetic base, and practical applications in breeding programs (Sudrik *et al.,* 2014).

Molecular markers are being widely used in plant breeding for evaluating genetic diversity and determining cultivars identity which has helped breeders to trace down the origins and degrees of relatedness of many land races and cultivars. Considering the potential of molecular markers the crop breeders extended their hands to supplement other tools currently exploited in their crop breeding activities.

Random Amplified Polymorphic DNA (RAPD) markers is prevalent as it is quick, easy and requires no prior sequence information (Isac *et al.,* 2003 ; Nandini and Chikkadevaiah, 2005). However, RAPD markers often cannot be consistently reproduced in different laboratories (Rafalski and Tingey, 1993). Inter Simple Sequence Repeat (ISSR) and Simple Sequence Repeat (SSR) markers have been applied to determine genetic diversity and relationships in a number of crop species (Barth *et al.,* 2002; Gomes *et al.,* 2009; Leal *et al.,* 2010; Pei *et al.,* 2010; Srivastava and Gupta, 2008). SSRs are usually co-dominant, reproducible and easily detected by polymerase chain reaction (PCR).

Relying on the importance on molecular markers, the present study was proposed to assess the genetic diversity in elite parents and restorers of sunflower using RAPD, ISSR and SSR markers.

MATERIALS AND METHODS

A set of 20 genotypes (Table 1) comprised of five male sterile parents (A lines), five male sterile maintainer parents (B line) and 10 restorers (R lines) were collected from Oilseed Research Station, Latur (Maharashtra). DNA was isolated using modified CTAB method from 15-20 days old plant (Doyle and Doyle, 1990). Fifteen RAPD (Table 2), 20 ISSR (Table 3) and 5 SSR (Table 4) primers were used for PCR amplification. PCR amplification was carried out in thermocyclers in a

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volume of 25 µl contained 1 µl of genomic DNA (25 ngì l^{-1}), 2.5 µl of 1X PCR buffer, 0.2 mm dNTPs (10 mM), 1.5 mM of 25 mM $MgCl_2$, 1 U *Taq* polymerase (MBT, Fermentas, U.K.) and 1 µl of 10 pmol primer (MWG, Bangalore). PCR reaction was performed using the following PCR profiles: initial denaturation at 94°C for 5 min followed by 45 cycles of 1 min at 94°C, 1 min at 37°C, 2 min at 72°C, and final extension at 72°C for 7 min. In case of ISSR and SSR, PCR profile was same as RAPD except for annealing temperature which ranged from 45°C to 60°C. PCR products were resolved on 1.2 per cent agarose gel. The SSR amplified products were resolved on a 6 per cent non-denaturing polyacrylamide gel at 60 W for 3.0 h using gel electrophoresis system. The gels were stained with modified silver staining procedure.

Each of the amplicon was scored independently for present (1) and absent (0). Data analysis was performed using DARwin 5.0 version (Perrier *et al.,* 2003). The results from all the markers used in the study, were merged to get a combined relationship matrix. The DICE dissimilarity coefficients were calculated using the binary codes of RAPD, ISSR and SSR scoring. Neighbor-Joining weighted analysis based phylogenetic tree was constructed using the DICE dissimilarity matrix. The resulted dissimilarity matrix was used to construct PCoA (Principal Co-ordinate Analysis) plot.

RESULTS AND DISCUSSION

Out of 15 RAPD primers, 7 produced polymorphic banding patterns (Fig.1). The seven most reproducible and polymorphic RAPD primers produced a total of 31 bands across all the 20 genotypes. Out of 31 bands, 21 bands were found polymorphic with 67.74% polymorphism. The amplicon size ranged from 200 bp to 3000 bp. The number of bands per RAPD ranged from 2(OPH-20) to 6 (OPG-04 & OPR-04) with an average of 4.42 bands per primer. Maximum polymorphism (100%) was observed using the primers, OPG-02 and OPG-10. PIC value obtained by RAPD marker ranged from 0.09(OPH-20) to 0.3(OPG-02).

By employing 10 ISSR primers, a total of 92 amplicons were observed across all the genotypes with an average 9.2 bands per primer (Fig. 2). Out of 92, 84 amplicons were found to be polymorphic with 91.30% polymorphism. The maximum (14) amplicons were observed in IS-5 while minimum (3) amplicons in IS-3. Out of 10, 5 ISSRs (IS-2, IS-3, IS-4, IS-5 and IS-10) generated only polymorphic amplicons. The mean PIC value revealed by this marker system was 0.234. Maximum dissimilarity (60%) was depicted between CMS-17B and 7-1A while it was minimum (12.2%) between 234A and 127A. The RAPD revealed 29.5per cent dissimilarity among current set of genotypes which found in congruence with earlier studies conducted on sunflower (Rieseberg *et al.,* 1993; Lawson *et al.,* 1994; Hogtrakul *et al.,* 1997). Two of the RAPD markers generated by OPH-20 found specific for a restorer line (RHA-1-1) and for a CMS line (234A) would be employed in identification of the respective lines for breeding purposes. ISSR markers employed in the study found little more effective than RAPD, and generated 31.5 per cent diversity among current set of genotypes. Except more diversity, ISSRs also generated 18 bands found specific for various sunflower lines used in the study. IS-4 ISSR primer found efficient in producing polymorphism (100%), and also produced highest 5 genotype specific bands.

Out of 5 SSR primers, we detected allelic variations in only 4 SSRs (Fig. 3). The polymorphism information content (PIC) value ranged from 0.2 (ORS-705) to 0.35 (ORS-799) with a mean value of 0.289. Maximum (81.8%) dissimilarity was detected between P-35R and 234A while minimum (0%) dissimilarity was recorded among genotypes *viz.* CMS-17A with 7-1B, J-6 with CMS-2023B, R-271 with CMS-2023B, J-6 and RLW-5 with CMS-2023B, J-6 and R-272-1. This result is similar to the study of Paniego *et al.,* 2002 (3.5) and Hvarleva *et al.,* 2007 (2.67). It is much lower than the mean number of allele per locus reported in other studies for inbred lines of sunflower (Tang and Knapp, 2003 and Yu *et al.,* 2002). However, RAPD and ISSR generated more number of polymorphic bands but SSR have the advantage as co-dominant markers to detect heterozygote from the homozygote.

Principal coordinate analysis (PCoA) for displaying the genetic relationships among the sunflower genotypes was shown in the figure 4. Quadrant I had three genotypes all from CMS line *i.e*. 234A, 127A and CMS-17A. Quadrant II had six genotypes including three maintainer lines (127B, CMS-17B, CMS-2023B) and three restorers (R-272-1, LOC-6443, NDR 7-1B). The Quadrant III had six genotypes with five restorers (P-35R, R-271, RLW-5, MR-1-234B and R-274) and one CMS line *i.e*. CMS 2023A which showed most diversity in this quadrant. In quadrant IV comprised five genotypes, two from CMS lines (17A and B); two from restorers (J-6 and RHA-1-1) and one from maintainer line (234B).

Dendrogram resulting from combined data matrix of RAPD, ISSR and SSR markers comprised three major clusters (Fig. 5) and revealed that all CMS lines with their maintainer were grouped only in two clusters (Cluster I and II) due to similarity of major genomic content as well as agronomic and quantitative traits and origin of genotyped lines except for only one trait *i.e.* male sterility. Cluster I with two sub cluster had eight genotypes. Sub cluster IA comprised six genotypes,

Table 1: List of sunflower genotypes with their distinctive features collected from different sources

Genotypes	Features	#Source
7-1A & B	Monohead, susceptible to downy mildew and necrosis, high seed yield, big head diameter	DOR, Hyderabad
17 A & B	High seed yield, susceptible to downy mildew and necrosis, low oil content	AICRP, Bangalore
127 A & B	Monohead, early, dwarf, high seed feeling percentage, resistance to downy mildew	DOR, Hyderabad
234 A & B	Early, high oil content, susceptible to downy mildew, necrosis and *Alternaria*.	AICRP, Bangalore
2023 A & B	High seed yield, monohead, late	DOR, Hyderabad
P-35R	Monohead, high seed yield	PAU, Ludhiana
RHA-1-1	Multihead, downy mildew resistant, low hull content	ORS, Latur, Maharashtra
MR-I-234 B	Monohead, high seed and oil content, downy mildew resistant	ORS, Latur, Maharashtra
R-272-1	Monohead, high seed yield, tall	ORS, Latur, Maharashtra
LOC-6443	Monohead, high seed yield	ORS, Latur, Maharashtra
RHA-274	Monohead, low hull content	DOR, Hyderabad
NDR-7-1B	Monohead, low hull content, high oil content	ORS, Latur, Maharashtra
R-271	Monohead, high seed yield	ORS, Latur, Maharashtra
J-6	Monohead, downy mildew, necrosis and *Alternaria* resistant (multiple resistance), High seed yield and high oil content	ORS, Latur, Maharashtra
RLW-5	Multihead, white pollen, low oil content, good seed filling %	ORS, Latur, Maharashtra

#AICRP= All India Co-ordinated Research Project, DOR= Directorate of Oil seeds, PAU= Punjab Agricultural University, ORS= Oil Seeds Research Station

Table 2: Polymorphism exhibited by RAPD markers in sunflower

Primers	Sequences (5'- 3')	Total Amplicons	polymorphic amplicons	Percent polymorphism	PIC
OPQ-09	GAACGGACTC	3	2	66.66	0.228
OPG-02	GGCACTGAGG	4	2	50	0.300
OPG-04	AGCGTGTCTG	5	5	100	0.179
OPG-09	CTGACGTCAC	6	3	50	0.134
OPH-20	GGGAGACATC	2	1	50	0.091
OPG-10	TCGTTCCGCA	6	3	50	0.232
OPR-04	CCCGTAGCAC	5	5	100	0.187
Total		31	21		0.193

Table 3: Polymorphism exhibited by ISSR markers in sunflower

ISSRs	Sequences (5′- 3′)	Total Amplicons	polymorphic amplicons	Polymorphism %	PIC
IS-1	AGC AGC AGC AGC AGC GA	10	9	90	0.220
IS-2	AGC AGC AGC AGC AGC GG	8	8	100	0.277
IS-3	AGC AGC AGC AGC AGC GT	3	3	100	0.347
IS-4	AGC AGC AGC AGC AGC GC	13	13	100	0.188
IS-5	AGC AGC AGC AGC AGC AT	14	14	100	0.247
IS-6	CAC ACA CAC ACA CAA C	10	8	80	0.157
IS-7	CAC ACA CAC ACA CAG T	12	10	83.33	0.175
IS-8	CAC ACA CAC ACA CAG C	10	8	80	0.226
IS-9	CAC ACA CAC ACA CAG A	5	4	80	0.287
IS-10	CAC ACA CAC ACA CAA A	7	7	100	0.227
Total		92	84		0.235

Table 4: Amplification pattern of 20 sunflower genotypes evaluated by SSR markers

SSR markers	Forward primer	Reverse primers	Total amplicons	Polymorphic amplicons	Percent polymorphism	PIC
HA 1805	GAAGTTGGGAGGGTTGTTCAAG	CCTCCTGTTGGAACACCAAAT	2	2	100	0.318
ORS 511	TGGCTCAGATTAAGTTCACACAG	CGGGTTGCGAGTAACAGGTA	1	0	0	0.000
ORS 705	TCTGCTTCTATGACACGGTTGT	CACGGTCAACTCCCCTTTTA	5	5	100	0.210
ORS 799	TCCAGCAAGTCAGCAACAAC	ACTCCCTCCCATTCTCGTCT	2	2	100	0.352
		Total	**10**	**9**		**0.220**

(CMS-17 A and B, 127A and B, CMS-2023A and B) in which CMS-17 A and CMS-17B grouped closely with a bootstrap value of 89 per cent. In sub cluster IB, two genotypes (R-272-1 and LOC-6443) were cluster with 61per cent bootstrap value. Cluster II had eight genotypes which were further divided into sub cluster IIA and sub cluster IIB. Sub cluster IIA comprised five genotypes (7-1A and B, 234A and B and RHA-1-1) of which 7-1A and B grouped with 100 per cent bootstrap value while 234A and B both were clustered with a bootstrap value of 97 per cent. RHA-1-1 in this cluster accommodated in separate branch due to variation. Sub cluster IIB had three genotypes viz. R-274, P-35R, and R-271 all from restorer line of which R-274 and P-35R showed high similarity with 70 per cent bootstrap value. Cluster III comprised four genotypes (RLW-5, MR-I-234B, J-6 and NDR-7-1B) all from restorer lines of which RLW-5 and MR-I-234B grouped closely with 64 per cent bootstrap value. A pair wise dissimilarity among all genotype was calculated with the combined molecular data of RAPD, ISSR and SSR markers was presented in the table 5.

PIC values estimate the discriminatory power of a marker. The mean of PIC values for markers used in this study was 0.289 among 20 sunflower genotypes. All of the CMS lines grouped with their respective maintainer with high similarity, and three of CMS lines with their cognate maintainer were divided as different subgroup. However, the regional effect were not observed in the clustering, and two of the CMS lines from DOR, Hyderabad grouped with one CMS line from AICRP, Bangalore. Second group of the cluster also housed two of CMS lines with their maintainers each from Hyderabad and Bangalore. If more number of RAPD and ISRR were added, the categorization of lines would be more interesting. Some inbred lines of CMS with their maintainer were specifically clustered in the same group (group A) might be due to the similar genetic makeup or ancestor relationship. All the restorers were grouped in the sub-cluster II and III. Further, the cluster analysis showed that all restores are distantly related due to geographical origin and genetic makeup.

In conclusion, the present results indicated that male sterile line CMS-17A (RAPD analysis); CMS-2023A (ISSR analysis) and restorer line P-35R (SSR analysis) were more genetically diverse than others, will be utilized for hybrid development program. It was clear from the molecular marker analysis of diverse sunflower germplasm that the relationships among 20 inbred lines of sunflower showed considerable variation and good correlation with the genetic composition among genotypes. Highly diverse genotypes can be successfully employed in hybridization program for the production of improved sunflower hybrids.

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REFERENCES

Anonymous, 2013. FAOSTAT, http://faostat.fao.org.

Barth, S., Melchinger, A. E. and Lubberstedt, T. 2002. Genetic diversity in *Arabidopsis thaliana* L. Heynh. investigated by cleaved amplified polymorphic sequence (CAPS) and inter-simple sequence repeat (ISSR) markers. *Mol. Ecol*., **11**: 495-505.

Doyle, J. J. and Doyle, J. L. 1990. Isolation of plant DNA from fresh tissue. *Focus*, **12**:13-15

Gomes, S., Martins-Lopes, P., Lopes, J. and Guedes-Pinto , H.2009. Assessing genetic diversity in *Olea europaea* L. using ISSR and SSR markers. *Plant Mol. Bio. Rep*., **27**:365-73.

Hongtrakul, V., Huestis, G. M. and Knapp, S. J. 1997. Amplified fragment length polymorphism as a tool for DNA fingerprinting sunflower germplasm: genetic diversity among oilseed inbred lines. *Theor. Appl. Genet*., **95**:400-07.

Hvarleva, T. Z., Bakalova, A., Chepinski, I., Hristova-Cherbadji, M., Hristov, M. and Atanasov, A.2007. Characterization of bulgarian sunflower cultivars and inbred lines with microsatellite markers. *Biotechnol Equip.*, **21**:408-12.

Table 5: Dissimilarity matrix based on RAPDs, ISSRs and SSRs

	127A	127B	234A	234B	7-1A	7-1B	CMS-17A	CMS-17B	CMS-2023A	CMS-2023B	J-6	LOC-6443	MR-I-234B	NDR-7-1B	P-35R	R-271	R-272-1	RHA-1-1	RHA-274
127B	0.18																		
234A	0.20	0.30																	
234B	0.13	0.21	0.16																
7-1A	0.36	0.40	0.47	0.36															
7-1B	0.26	0.26	0.35	0.29	0.33														
CMS-17A	0.28	0.30	0.38	0.32	0.42	0.29													
CMS-17B	0.30	0.35	0.40	0.34	0.46	0.38	0.20												
CMS-2023A	0.29	0.42	0.38	0.35	0.42	0.37	0.35	0.34											
CMS-2023B	0.29	0.38	0.35	0.33	0.41	0.35	0.35	0.31	0.15										
J-6	0.28	0.30	0.38	0.28	0.40	0.33	0.30	0.30	0.31	0.31									
LOC-6443	0.19	0.22	0.28	0.22	0.36	0.19	0.28	0.30	0.28	0.28	0.22								
MR-I-234B	0.30	0.29	0.39	0.29	0.41	0.30	0.35	0.37	0.32	0.30	0.14	0.23							
NDR-7-1B	0.26	0.31	0.36	0.29	0.38	0.24	0.34	0.36	0.35	0.32	0.23	0.25	0.21						
P-35R	0.37	0.36	0.42	0.36	0.35	0.38	0.36	0.33	0.31	0.35	0.31	0.26	0.28	0.37					
R-271	0.34	0.35	0.38	0.31	0.39	0.31	0.39	0.35	0.33	0.32	0.26	0.27	0.22	0.31	0.18				
R-272-1	0.26	0.28	0.29	0.25	0.38	0.26	0.31	0.34	0.31	0.35	0.21	0.14	0.20	0.28	0.29	0.25			
RHA-1-1	0.36	0.40	0.42	0.41	0.37	0.41	0.36	0.38	0.36	0.35	0.32	0.30	0.37	0.38	0.26	0.32	0.34		
RHA-274	0.26	0.27	0.33	0.26	0.38	0.27	0.26	0.28	0.29	0.22	0.25	0.22	0.17	0.26	0.24	0.19	0.23	0.33	
RLW-5	0.25	0.27	0.36	0.26	0.40	0.29	0.34	0.36	0.25	0.28	0.16	0.18	0.11	0.25	0.29	0.28	0.22	0.30	0.22

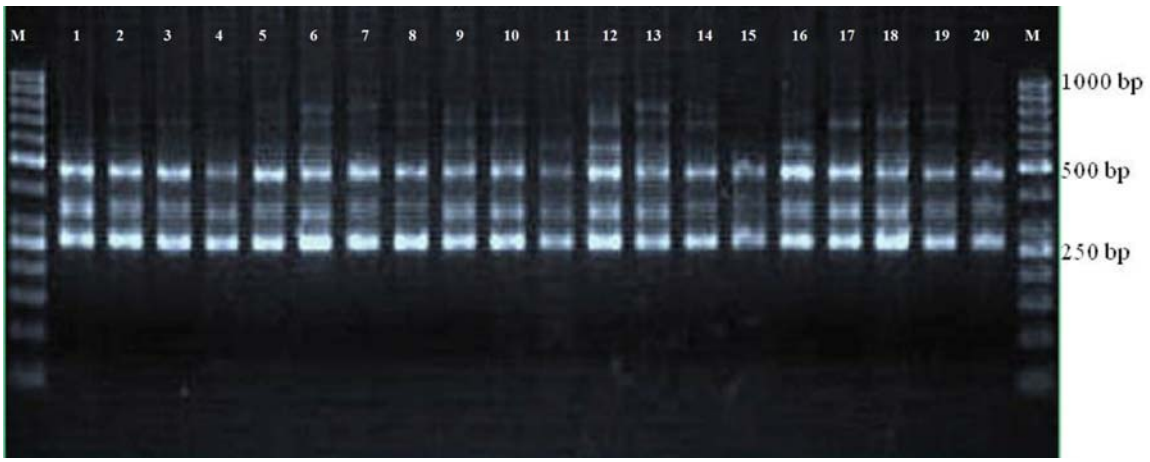

Fig. 1: Molecular profiling of RAPD marker, OPG-04

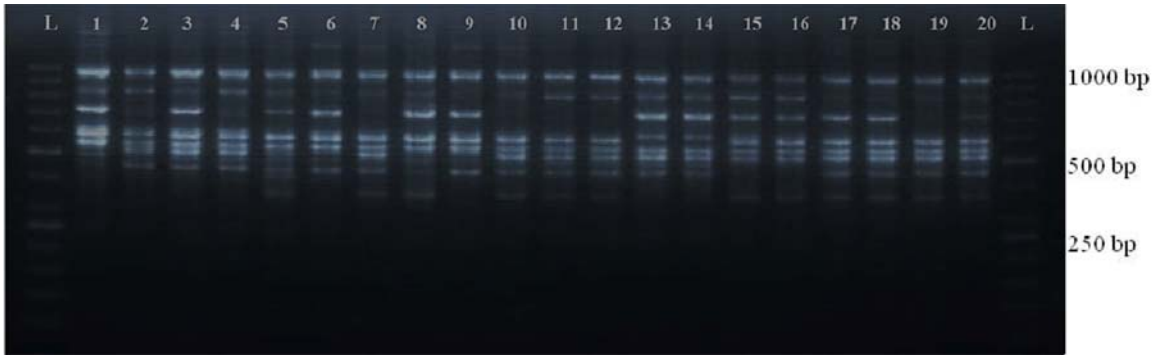

Fig.2 : Molecular profiling of ISSR marker,IS-8

1. RHA-1-1	6. MR-234B	11. 7-1B	16. CMS-17A
2. P-35R	7. R-272-1	12. 7-1A	17. 127B
3. RHA-274	8 J-6	13. CMS-2023B	18. 127A
4. R-271	9. RLW-5	14. CMS-2023A	19. 234B
5. NDR-7-1B	10. LOC-6443	15. CMS-17B	20. 234A

M= 50 bp Ladder

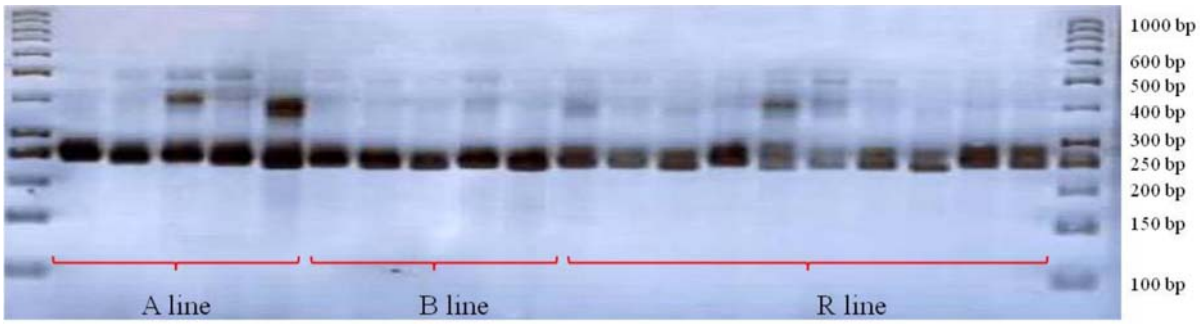

Fig.3: Molecular profiling of SSR marker, HA1805

Isaacs, S. M., Manivannan, N. and Muralidharan, V.2003. Genetic diversity analysis using RAPD marker in inbred lines of sunflower (*Helianthus annuus* L.). *Helia*, **26**:59-66.

Lawson, W. R., Henry, R. J., Kochman, J. K. and Kong, G. A.1994. Genetic diversity in sunflower (*Helianthus annuus* L.) as revealed by random amplified polymorphic DNA analysis. *Aus. J. Agri. Res*., **45**:1319-27.

Leal, A. A., Mangolin, C. A., Amaral Júnior, A. T., Gonçalves, L. S. A., Scapim, C. A., Mott, A. S., Eloi, I B O, Cordovés, V. and Silva, M. F. P.2010. Efficiency of RAPD versus SSR markers for determining genetic diversity among popcorn lines. *Genet. Mol. Res*., **9**:9-18.

Nandini, R. and Chikkadevaiah, A.2005. DNA fingerprinting of sunflower genotypes (*Helianthus annuus* L.). *Helia*, **28**:9-18.

Paniego, N., Echaide, M., Munoz, M., Fernandez, L., Torales, S., Faccio, P., Fuxan. I., Crrera, M., Zandomeni, R., Syarez, E. Y. and Hopp, E.2002. Microsatellite isolation and characterization in sunflower (*Helianthus annuus* L.). *Genome*, **45**:34-43.

Pei Z, Gao J, Chen, Q, Wei, J., Li, Z., Luo ,F., Shi, L., Ding, B. and Sun, S.2010. Genetic diversity of elite sweet sorghum genotypes assessed by SSR markers. *Biol. Pl*., **54**:653-58.

Perrier, X., Flori, A. and Bonnet, F.2003. *Data Analysis Methods, Genetic Diversity Analysis of Cultivated Tropical Plants.* Seguin M and glaszmann J C (Eds), Enfield, Science publisher, Montpellier. pp. 43-76.

Rieseberg, L. H., Choi, H. C., Chan, R. and Spore, C.1993. Genomic map of a diploid hybrid species. *Heredity*, **70**:285-93.

Schilling, E. E. and Heiser Jr, C. B. 1981. Intrageneric classification of *Helianthus* (*Compositae*). *Taxonomy*, **30**:393-03.

Seiler,G.J.2007. Wild annual *Helianthus anomalus* and *H. deserticola* for improving oil content and quality in sunflower. *Ind. Crops Prod*., **25** : 95-100.

Seiler ,G. J.1992. Utilization of wild sunflower species for the improvement of cultivated sunflower. *Field Crop Res*., **30**:195–230.

Seiler, G. J. and Rieseberg, L. H.1997. *Systematics, Origin and Germplasm Resources of the Wild and Domesticated Sunflower*. Schneiter A A (Ed). ASA, CSSA, and ASSA, Madison, Wisconsin. pp. 21-66.

Srivastava ,S. and Gupta, P. S.2008. Inter simple sequence repeat profile as a genetic marker system in sugarcane. *Sugar Tech*, **10** : 48-52.

Sudrik, B. P., Ghodke, M. K., Patil, V. S., Chavan, S. K. and Kesale, N. B.2014. Evaluation and characterisation of sunflower (*Helianthus annuus* L.) germplasm. *J. Crop.Weed*, **10** : 73-76.

Tang, S. and Knapp, S. J.2003. Microsatellites uncover extraordinary diversity in native American land races and wild populations of cultivated sunflower. *Theor. Appl. Genet.*, **106** : 990-1003.

Thompson,T. E., Zimmerman, D. C. and Rogers, C. E.1981. Wild *Helianthus* as a genetic resource. *Field Crop Res*., **4** : 333-43.

Yu, J. K., Mangor, J., Slabaugh, M. B. and Knapp, S. J.2002. Allelic diversity of simple sequence repeats among elite inbred lines of cultivated sunflower. *Genome*, **45** : 652-60.