



Molecular characterization of heat tolerance in eggplant (*Solanum melongena*) lines using SSR markers

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ABSTRACT

High temperature is one of the most important environmental stresses causing heavy reduction in yield of eggplant (*Solanum melongena* L.) in India. Therefore, development of heat tolerant eggplant variety is an important breeding objective. The present investigation was conducted to assess genetic variation among 62 eggplant lines including heat tolerant and susceptible lines in order to identify novel heat tolerant lines in changing climate in India. We employed 15 simple sequence repeat (SSR) markers to study polymorphisms among eggplant lines obtained from eggplant breeding program. The observations were analyzed using Power Marker version 3.5. The range of amplified number of bands was from 2 to 3 in each of 15 markers. The allele frequency was ranged from 0.5 to 0.7931 with an average of 2.067 alleles per locus. Among the 15 polymorphic SSR markers, 67% markers had PIC value higher than 0.3 with heterozygosity of individual loci was ranged from 0.00 to 0.1667. The marker emf01C03 had higher number of allele (3) and the marker emd03C01 had maximum PIC value and gene diversity. The NJ dendrogram obtained by 15 SSR markers divides 62 lines into 7 clusters where the cluster VII comprised of most heat tolerant lines (DBL-21, Guhala Chatua Local). The amplification pattern of heat tolerant lines also differ which implies different genetic architecture of heat tolerant lines. The research work will be very useful to combine these diverse sources of genetic variability to develop heat tolerant variety (s) in eggplant.

Key words: Eggplant, Heat tolerance, SSR, Variability

Solanum, one of the largest angiosperm genera of Solanaceae family comprises around 1500 species distributed throughout the world. Among these species, eggplant (*Solanum melongena* L.) is one of the most important economic crops. It has centre of diversity between India and Indonesia (Vavilov 1951). Due to its wide range of available diversity it is known as king of vegetables in India (Daunay 2008). The initial record of eggplant was found at the beginning of Christian era as mentioned in Sanskrit documents (Bhaduri 1951). Southeast Asia, North-East India and South-East China are the regions where eggplants are domesticated near about 2000 years ago (Sekara *et al.* 2007). It is known for its diversity according to Bailey (1947) and is classified into three varietal groups including var. *esculentum* (intermediate sized fruits), var. *serpentinum* group (long fruited varieties), and var. *depressum* (small fruited). It has medicinal properties particularly the white

types have positive effects on hyperglycemic risk factors, diabetics and hypertension and also have moderate antioxidant activity, inhibitory action against glucosidase which is related to glucose absorption in intestine (Saha *et al.* 2016; Kandimalla *et al.* 2015).

The average production and productivity is 12.4 million tonnes and 18.78 t/ha, respectively (NHB 2017). Ideal temperature for its growth is 20-30°C. But during summer months, when temperature goes above 35°C, it affects the growth and subsequently yield (Dhatt and Kaur 2017). *Kharif* season is the main growing season for brinjal cultivation in North India where fruiting occurs during September-December. Brinjal can be grown as off-season crop during summer season in Northern plains which can fetch premium price in the market. The optimum temperature requirement for fruit set in brinjal is 18-21°C and there has many number of cultivars available to grow in autumn-winter season (Nath *et al.* 2008) but when day and night temperature exceeds 35°C, there is drastic reduction in fruit set, drying of stigmatic fluid, pollen degeneration, low germination of pollen and lack of fertilization with lower fruit weight (Kalloo *et al.* 1990, Ansari *et al.* 2011). Crop improvement becomes possible because of wider genetic resources which maintain yield across different environment. It is possible to use this available genetic diversity only for identification of trait specific germplasm and further its

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molecular characterization. High polymorphic molecular marker techniques can be used to identify similarity between varieties and parents can be identified for use in breeding programmes (Terzopoulos and Bebeli 2008). The most important advantages of molecular markers are high use efficiency, less laborious and faster in use (Li-wang *et al.* 2007). Molecular markers are the powerful tools to evaluate genetic diversity and it is not influenced by environment unlike morphological and physiological traits. Molecular markers were being used in breeding for heat tolerance in rice (Cao *et al.* 2002, Zhu *et al.* 2005, Zhao *et al.* 2006). Field screening and selection of superior genotypes for heat stress becomes difficult which can be overcome by molecular markers application. Hence the aim of this study is to identify polymorphism among the available genotypes of eggplant and characterize those using molecular markers.

MATERIALS AND METHODS

The study was conducted at Division of Vegetable Science, ICAR- Indian Agricultural Research Institute, New Delhi, India. The institute is situated at altitude of 228.6 m above the MSL. The latitudinal and longitudinal location of the institute is 28°40'N and 77°12'E respectively that is it lies in the northern hemisphere of the country. Generally temperature during summer months exceeds 40°C and peak during May and June, but the mean annual temperature being 24°C. Delhi has highly distinguished summer season and winter season which leads to temporal variation. The plant materials used for this study include 62 genotypes of brinjal including released varieties, land races, breeding lines, etc. The details of plant materials and their sources are given in Table 1. These materials were grown in the field during summer (March-June) and in *kharif* (July to October) season.

Table 1 List of eggplant lines used in the present study

Genotype	Source
Pusa Ankur	IARI, Delhi
Pusa Anupam	IARI, Delhi
Pusa Bindu	IARI, Delhi
DBL-02	IARI, Delhi
Pusa Purple Cluster	IARI, Delhi
Pusa Purple Long	IARI, Delhi
Pusa Purple Round	IARI, Delhi
DBL-21	IARI, Delhi
Pusa Upkar	IARI, Delhi
Pusa Uttam	IARI, Delhi
Pusa Hara Baingan 1	IARI, Delhi
Pusa Safed Baingan 1	IARI, Delhi
DBGL-164	IARI, Delhi
DBGL-225-2-5-17	IARI, Delhi
DBWL-22-1-11	IARI, Delhi
DBWL-50-7-14	IARI, Delhi

Cond.

Table 1 (Concluded)

Genotype	Source
DBWR-190-44	IARI, Delhi
DBGR-32	IARI, Delhi
DBL-100-1-10	IARI, Delhi
DBL-09	IARI, Delhi
DBL-17	IARI, Delhi
DBPR-23	IARI, Delhi
DBPR-43	IARI, Delhi
DBL-60	IARI, Delhi
DBR-92	IARI, Delhi
DBSR-94	IARI, Delhi
DBGR-131	IARI, Delhi
DBL-160	IARI, Delhi
DBL-128	IARI, Delhi
DBR-184	IARI, Delhi
Pusa Bhairav	IARI, Delhi
DBL-08	IARI, Delhi
DBR-32	IARI, Delhi
DBR-203	IARI, Delhi
DBGR-181	IARI, Delhi
129-5	IARI, Delhi
IC -112991	NBPGR, Delhi
IC-112992	NBPGR, Delhi
Swarnamani Black	RCER, Ranchi, Jharkhand
Khashi Sandesh	IIVR, Varanasi
Pant Rituraj	GBUA&T, Pantnagar, Uttarakhand
Pant Samrat	GBUA&T, Pantnagar, Uttarakhand
Punjab Barsati	PAU, Ludhiana, Punjab
Punjab Sadabahar	PAU, Ludhiana, Punjab
Guhala Chatua Local	Odisha
Keonjhar Local	Odisha
BB-7	Odisha
Kushpada Local	Odisha
Arka Nidhi	IIHR, Bengaluru, Karnataka
Arka Neelkanth	IIHR, Bengaluru, Karnataka
Arka Keshav	IIHR, Bengaluru, Karnataka
NDB-25	NDUAT, Faizabad, Uttar Pradesh
190-10-12	Panipat
Panipat Gole	Panipat, Haryana
Br-112	HAU, Hisar, Haryana
Manjari Gota	MPKV, Rahuri, Maharashtra
Bangar Begoon	West Bengal
Nabanita (Lukri Begoon)	West Bengal
Kalo Solia	West Bengal
DEB- 3709	West Bengal
Debjuri Hajari	West Bengal
Boulder	West Bengal

In case of summer season crop, sowing was done in end of February and the seedlings were transplanted in end of March. In *kharif* season sowing was done on mid-June and the seedlings were transplanted in the main field in mid-July. For molecular analysis leaves are taken from plant of *kharif* season. In each genotype 20 plants were grown in both the season in Randomized Block Design (RBD) with 3 replications at spacing of 75 × 75 cm. For DNA analysis, terminal leaf samples are collected from every 62 genotypes. Extraction of total genomic DNA of all the genotypes was done with the help of DNA extraction kit. The extracted DNA is quantified with the help of 0.8% agarose gel while Hind III-cut λ DNA is used as a standard. Extract of DNA is diluted to working concentration of 20 ng/μl for undergoing PCR reaction. Initially SSR primers are diluted to the concentration of 15 μM which is again diluted to 5ppm of working concentration. SSR marker was amplified in 20μl volume with 50ng genomic DNA, PCR buffer of 2μl, 0.2 μl of Taq DNA polymerase, 0.5 μl of DNTP, Forward and reverse primer each of 0.5 μl, further total volume is made into 20 μl with double distilled water. Selected primers were amplified in PCR. Following conditions are set during PCR amplification one cycle at 94°C for 5 min; 10 cycles of 94°C for 0.5 min, 65–55°C decreasing by 1°C per cycle for 1 min, and 72°C for 1 min; 30 cycles of 94°C for 0.5 min, 55°C for 1 min, and 72°C for 1 min; and a final cycle of 72°C for 10 min (Nunome *et al.* 2009). These PCR products were resolved by 3% agarose gel by running gel electrophoresis then ethidium bromide was used for staining gel which was observed using gel documentation system. 100bp ladder was used to detect the band size. Further data analysis is done using Power Marker V 3.25 software which was used in calculating index of gene diversity, PIC (polymorphic information content) and allele number. Method used by Nei (1973) was used in calculating genetic distance which was used to calculate similarity tree using unweighted

pair-group method of mathematical averages (UPGMA) as implemented in MEGA.V4 (Tamura *et al.* 2004).

RESULTS AND DISCUSSION

A total of 15 SSR (Simple sequence repeats) was used for molecular diversity assessment among 62 eggplants and the details of forward and reverse primers along with their sequence are presented in Table 2. The SSR marker was proved to be immense and robust tool for capturing the genetic diversity and to determine their genetic relationships between and among the *Solanum* species group (Munoz-Falcon *et al.* 2009). The 15 SSR markers are amplified with scorable polymorphic alleles. The amplification profile for marker emf21N03 is presented in Fig 1. The range of amplified number of bands was 2 to 3 in each of 15 markers. Total 31 alleles, with mean of 2.067 alleles per locus were amplified in 62 eggplant genotypes which is lower than Verma *et al.* (2012) and Tiwari (2001). Among 62 genotypes heterozygosity of individual loci ranged from 0.00 to 0.1667. The major allele frequency ranged between 0.5 to 0.7931 (Table 3). Primer emf01C03 was found with higher number of alleles (3), and for rest 14 primers the number of alleles was 2. The gene diversity in the markers ranges from 0.3347 (emf01C03) to 0.5 (emd03C01), with mean value of 0.4779. The PIC value for 15 SSR markers in the present study varied from 0.2897 (emf01C03) to 0.3750 (emd03C01), with an average PIC value of 0.3636. The marker emd03C01 was found with maximum PIC value and gene diversity. The average PIC value (0.36) indicated that the loci displayed intermediate level of polymorphism as reported by Ge *et al.* (2013). This result is almost similar to those observed by Nunome *et al.* (2009) and Munoz Falcon *et al.* (2011). Polymorphism can be measured using average PIC value which is found to be ideal index for its measurement. PIC values more than 0.50 indicate high polymorphism, values between 0.25 and 0.50 indicated

Table 2 List of the SSR markers and their sequences used in the present study

Marker name	Forward primer	Reverse primer
emb01M15	GCAAGGCTCAAAGTCACAAGTCAA	GGCTCTGCCCCTAACATCTACAAA
emh11I06	ATTTCAAACCGTTCTCTGCTCTT	GTTTGACAATCATCAAGGCTCCTC
emf21N03	ACCAGAGGAGCAAAGGGAAAAAT	GTTTACGCTACTGGACCAAACCAAC
emd03C01	ACGGGAGTTGTTTGTGGAAGTCC	GTTTCAAATTTTGGGTCGTGACA
eme05B09	ATGAAAACTCCACTCTACTCTACT	GTTTGCTAACGTACGCCTCAATTGC
em135 ^a	ATCCTGTTGCTGCTCATTTTCCTC	AGGAGGATCCAAGAGGTTTGTGA
emg21G24	ATAAATCCACCAGACCAGCAAAAC	GTTTCAGTTATCCCCCTTCTGTTCCCTC
emf01O01	AGGAATTGGATTTCCTACTCATACG	GTTTGGAAGATGAGATTCTTTCTTGA
emf21K08	ATCAATGACACCCAAAACCCATTT	GTTTGAAAACCCAATACAAATCCGA
emf01O04	ATCCGTTGATACTAGCCGTTGCCT	GTTTCACTTCTTGGTCCATTGTTTCA
emf01J09	ATAGCACCCACACTAAACCTTGGG	GTTTCACTTCTTGGTCCATTGTTTCA
emk03O04	ATGATTTGGGCGAGCCACTTTTGTGTA	GTTTGGAACCAACTAACTTAGGGCA
emg21I17	ACAACATTCTAAGGGCCTTCACG	GTTTGGGCATATTTGGCACTTGTGAAT
emf01C03	AGTCCACCATGAGTGAGTGAGTGA	GTTTACGTGTTGGGCCTCCAAATATC
emh11O61	ATTGTGTCGATGAGATTTGGTCA	GTTTAGCTACGTTGGTTTGGTGCTGAA

Table 3 Primers, major allele frequency, allele no, gene diversity, heterozygosity, PIC values of 15 markers

Marker	Major allele frequency	Allele No	Gene diversity	Heterozygosity	PIC
emb01M15	0.6441	2.0000	0.4585	0.1356	0.3534
emh11I06	0.5565	2.0000	0.4936	0.0484	0.3718
emf21N03	0.5410	2.0000	0.4966	0.0000	0.3733
emd03C01	0.5000	2.0000	0.5000	0.0667	0.3750
eme05B09	0.5726	2.0000	0.4895	0.1129	0.3697
em135 ^a	0.5806	2.0000	0.4870	0.0323	0.3684
emg21G24	0.5508	2.0000	0.4948	0.0508	0.3724
emf01O01	0.5161	2.0000	0.4995	0.0645	0.3747
emf21K08	0.6066	2.0000	0.4773	0.0656	0.3634
emf01O04	0.5333	2.0000	0.4978	0.0000	0.3739
emf01J09	0.6000	2.0000	0.4800	0.1667	0.3648
emk03O04	0.5403	2.0000	0.4967	0.0161	0.3734
emg21I17	0.5574	2.0000	0.4934	0.0656	0.3717
emf01C03	0.7931	3.0000	0.3347	0.0000	0.2891
emh11O61	0.6230	2.0000	0.4698	0.0984	0.3594
Mean	0.5810	2.0667	0.4779	0.0616	0.3636

intermediate polymorphism between loci and less than 0.25 indicated loci of low polymorphism (Ge *et al.* 2013). The results revealed that little genetic polymorphism among the genotypes and existence of a narrow gene pool. Solanaceous crops found to have narrow genetic base which was earlier reported by Nunome *et al.* (2003) and Stägel *et al.* (2008) due to its autogamous nature.

Different studies depicted genetic diversity in eggplant. High level of genetic diversities was seen in Chinese accession ($He=0.494$) and for Sri Lankan accession ($He=0.540$) (Hurtado *et al.* 2012). The Nei's genetic

diversity of different eggplant groups from Spain varied from 0.20 to 0.44 (Munoz-Falcon *et al.* 2011). Genetic diversity at considerable extent was observed between all the accessions studied. However, in the present study, the genetic diversity was less than the report of Hurtado *et al.* (2012). The differences between the present study and the previous report may be due to the difference in sample size used and markers. The very low genetic diversity was observed in present investigation. This might be due to very low level of outcrossing in self-pollinated crop like brinjal and other possibilities like inbreeding depression, higher mutation rate, and the presence of heterozygotes (Gowda 1981).

The NJ dendrogram obtained by 15 SSR divides 62 genotypes into 7 clusters (Fig 2). The cluster I predominantly consisted of 13 genotypes. The cluster II contains a total of 9 genotypes which mainly comprised 3 susceptible genotypes DBGR-131, DBPR-23, DBGL-225-2-5-17 (5 susceptible and 5 tolerant genotypes are found using morphological characterization). The cluster V contains the genotypes like Kushpada Local, IC-112991, Pant Samrat, Pusa Bindu, NDB-25 which includes only one tolerant genotype Pant Samrat. The cluster VII contains 4 genotypes like DBL-21, Punjab Sadabahar, Guhala Chatua Local, DBWL-22-1-11 and most of the tolerant genotypes belong to this group Table 4. Use of RAPD and SSR markers to characterize heat tolerant tomato lines were used previously (Comlekcioglu *et al.* 2010). In our study we use SSR markers which are more robust as compared to RAPD and SRAP markers. Among various genetic markers, SSR are the most desirable because they are crop specific, though their genome coverage is less and little genetic polymorphism was found among the genotypes due to

Table 4 Grouping of brinjal lines based on NJ dendrogram

Cluster	Genotype
I	Pusa Uttam, Pusa Upkar, DBR-92, Pusa Purple Cluster, Debjuri Hajari, Arka Nidhi, DBR-32, BB-7, Khashi Sandesh, 190-10-12, DBGR-181, IC-112992, DEB-3709
II	Punjab Barsati, DBR-184, DBL-128, DBR-203, Pant Rituraj, Pusa Hara Baingan 1, DBGR-131, DBPR-23, DBGL-225-2-5-17
III	DBGR-32, Arka Neelkanth, Pusa Purple Long, Pusa Purple Round, DBWR-190-44, DBL-09, Boulder, Keonjhar Local, Br-112, Panipat Gole, DBL-08, DBL-100-1-10, Bangar Begoon
IV	White oblong, DBL-160, Kalo Solia, G-17, Pusa Anupam, Pusa Safed Baingan 1, Manjari Gota, DBL-02, Arka Keshav, Nabnitha (Lukri Begoon), Pusa Ankur, DBSR-94, DBPR-43.
V	Kushpada Local, IC-112991, Pant Samrat, Pusa Bindu, NDB-25.
VI	Swarnamani Black, DBGL-164, 129-5
VII	DBL-21, Punjab Sadabahar, Guhala Chatua Local, DDWL-22-1-1-11.

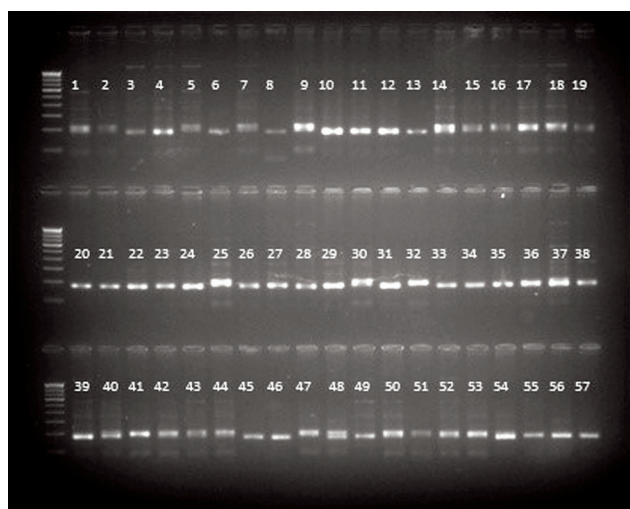


Fig 1 The representative gel image among the 57 brinjal lines of marker emf21N03.

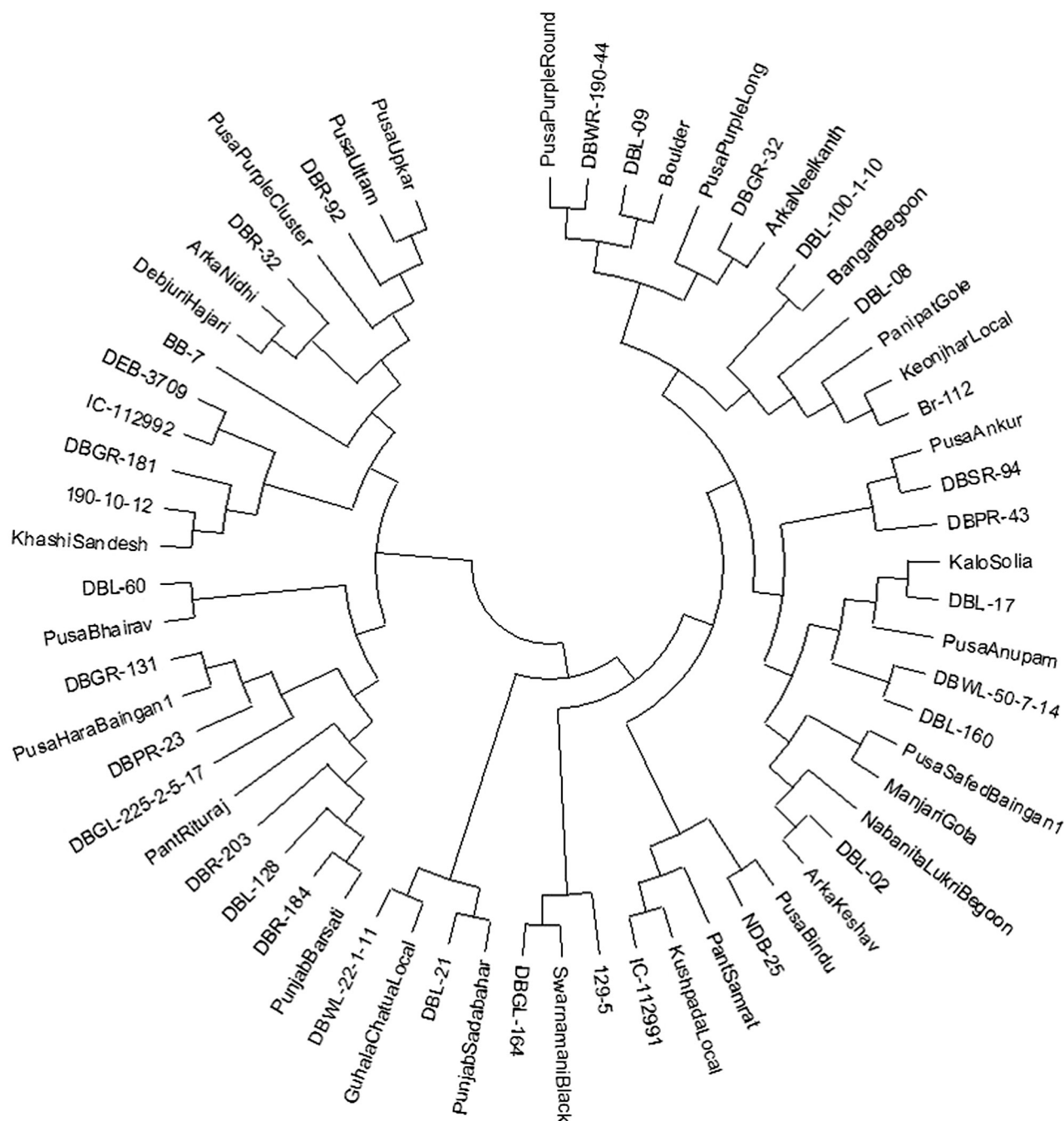


Fig 2 NJ clustering of 62 brinjal lines based on 15 polymorphic SSR markers.

existence of a narrow gene pool. Morphological markers, although most important as they cover more genome and are the result of expressed genes and their interactions, are highly influenced by environment. However, previous knowledge about the genetic diversity structure within the collected genotypes may provide immense value in order to make decisions on breeding strategies which in turn can be used in both current and future breeding programmes. In order to utilize the genetic variability for crop improvement, both morphological and SSR markers are more effective in studying the diversity among genotypes. In current study the genetic base was narrower in the genotypes taken for

study this may be due to genotypes taken mostly include cultivated genotypes and brinjal being self-pollinated crop. The idea about genetic diversity using molecular technology proves to be immense aid apart from morphological and other physiological traits which can be effectively used in heat stress tolerance breeding.

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