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Genome specific primers: A tool for genetic profiling of potato species

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ABSTRACT

Genome sequencing of the *Solanum phureja* (a diploid species of potato) has provided new insights to understand the complete genome functioning of the *Solanum* species. The present study was conducted to understand the genetic relatedness among different potato species. A set of twenty genotypes, including wild and cultivated, possessing agonomically important traits were analyzed for genetic diversity using the genome specific markers. Twenty randomly selected BAC end sequences were used for primer designing and screening. Expectedly, the commercial potato cultivars along with their progenitors used in the present study were clustered. The wild species viz., *S. albicans, S. sleumeri* and *S. demissum* clustered separately in the dendrogram indicating that these are genetically unrelated with the rest of the species. Result of the principal component analysis (PCA) was consistent with the clustering pattern of the dendrogram. The generation of the genetic profiling of the potato species using the genome specific markers will help in documentation of ownership and protection of intellectual property rights along with the presently used RAPD, AFLP and SSR profiles.

Key words: *Solanum sp*., genome, primers, diploid, tetraploid, PCA

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INTRODUCTION

The cultivated potato, *Solanum tuberosum* L. (2n=2x=24) is a member of an economically important family Solanaceae with more than 3000 species. Cultivated potato has a narrow genetic base owing to the fact, that present day cultivars evolved from a narrow genepool introduced from few germplasm sources of South American centre of origin to the new world and Asia during the western colonial rule. The potato genome consists of 12 chromosomes and has a (haploid) length of approximately 844 million base pairs, making it a medium-sized plant genome. The genome of potato has been sequenced by international consortium of 26 institutes from 13 countries as part of a larger initiative called the 'Potato Genome Sequencing Consortium' (Xu *et al.,* 2011). Along with whole genome sequence other genomic resources for potato have been developed including Expressed Sequence Tag (ESTs) (Rensink *et al.,* 2005), Bacterial Artificial Chromosome (BAC) clone, phasmid libraries (Chen *et al.,* 2004), microarray platforms (Kloosterman *et al.,* 2005) and a dense genetic map (Van *et al.,* 2006). These resources have been utilized in studies on potato physiology, development, responses to abiotic and biotic stress, polyploidy, comparative genomics as well as enhancement of genetic maps (Kloosterman *et al.,* 2005; Rensink *et al.,* 2005). Many studies have shown relationship among different species using comparative genome mapping (Paterson *et al.,* 2000), reconstruction of ancestral genomes (Blanchette *et al.,* 2004), phylogenetic studies (Rokas *et al.,* 2003), deciphering patterns of natural selection on coding regions (Bustamante *et al.,* 2005), and predictions of common gene function across species (Doganlar *et al.,* 2002) in potato. While the cultivated species have been bred for these diverse agronomic traits, genome sequence analysis has indicated that these species share, to a large extent, not only genes (Xu *et al.,* 2011) but also gene order (synteny) between their genomes (De Jong *et al.,* 2004; Xu *et al.,* 2011). While major classes of repetitive sequences are conserved among some Solanaceae species (Ganal *et al.,* 1988; Schweizer *et al.,* 1993), lineage-specific repetitive sequences have been reported, suggesting that divergence of this fraction of the genome has occurred through evolution. The availability of the whole genome sequence data would provide opportunities for exploring DNA-level diversity among the members of a crop species and its relationship to phenotypic diversity. Now the potato genome is sequenced, the next important question for the researchers would be utilization of this genome information to study various agronomically important genes present in different wild species and to get them into the cultivated tetrapliod potato.

Would the sequenced diploid potato (*S. phureja*) data help in studying the other species? If yes, to what extent? How far different potato species are inter-related? Which species of potato are near relative and which are distant relative? To know the answers for these questions a genetic profiling study was conducted using genome specific markers and 20 different potato genotypes including wild, semi-cultivated and cultivated possessing agronomically important traits.

MATERIALS AND METHODS

Plant material

Twenty potato (*Solanum spp.*) genotypes including wild, semi-cultivated and cultivated species were evaluated. Tubers of 20 potato genotypes (Table 1) including few commercial cultivars and an advanced hybrid which possess agronomically important traits were grown under the poly-house conditions in earthen pots during summer, 2010.

DNA extraction and quantification

Total Genomic DNA from the tender leaves of each potato plant was isolated using Sigma kit (GeneElute™ Plant Genomic DNA Miniprep kit) and genomic DNA was quantified using the spectrophototmeter (UV-1700 PharmaSpec, Shimadzu) and quality was checked both by A260/A280 ratio and by gel-electrophoresis. Working stock of 300 ng/ul was made for using in PCR reactions.

Primer designing

Randomly 20 BACs were selected from the Potato BAC end sequence library, 10 each from DM (Double Monoploid, *S. Phureja,* DM1-3 516 R44) and RH (*S. tuberosum,* RH89-039-16) BAC end libraries from the PGSC site (www.secure.potatogenomics.com). The genomic sequence from each BAC end sequence was used to design both forward and reverse primer set using Primer3 software (http://frodo.wi.mit.edu/primer3/). The primers were designed to get amplicon size ranging from 350 bp to 700 bp, with 24 to 30 nucleotide length, having high annealing temperature (Tm) and > 50% GC content in the amplicons to ensure high specificity while amplification. These primer sequences were synthesized from M/S Lab India (IDT) (Table 2).

PCR conditions

All PCR reactions were carried out with total reaction volume of 20 µl containing 1 µl (300 ng) of genomic DNA along with 200 µM dNTPs, 0.2 µM both forward and reverse primer, 2 units Taq polymerase (AmpliTaq, Applied Biosystems) and 1.5 mM Mg^{2+} was used in the PCR reaction of 35 cycles in thermocycler (Gene Amp PCR system 9700

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Table 1: Selected genotypes (*Solanum* species) and their importance

S. No.	Species (Clone No.)	Importance	Selected References
1.	*S. alandiae* (SS 1764-01)	Low cold induced sweetening	Bhardwaj *et al.,* 2011
2.	*S. albicans* (SS 1763-18)	Low cold induced sweetening	Bhardwaj *et al.,* 2011
3.	*S. andigena* (JEX/A-85) *S. andigena* (JEX/A-1038)	Black dot resistance Cyst nematode resistance Late blight resistancePVX resistance	Nadav *et al.,* 2010 Gabriel *et al.,* 2007
4.	*S. balbocastinum* (SS 1721)	Late blight resistance	Song *et al.,* 2003
5.	*S. brevicaule* (SS 1794-07)	Low cold induced sweetening. Late blight resistance	Bhardwaj *et al.,* 2011
6.	*S. cardiophyllum* (SS 1825-10)	Late blight resistance	Bradshaw and Ramsay, 2005
7.	*S. chacoense* (SS 660-16)	Leptin rich, Colarado beetle resistant, PVX and PVY resistant	Susannah *et al.,* 2009; Bradshaw and Ramsay, 2005
8.	*S. demissum* (SS 1850-4)	Cytoplasmic sterility. Late blight resistance	Bradshaw and Ramsay, 2005
9.	*S. jamesi* (SS 1652-09)	Low cold induced sweetening	Luthra *et al.,* 2009
10.	*S. mona* (SS1659-02)	Not studied	-
11.	*S. sleumeri* (SS 1301-04)	Not studied	-
12.	*S. sparsipilum* (SS 1724-2)	Late blight resistance. Root knot nematode resistance Cyst nematode resistance	Finbarr *et al.,* 2010; Kouassi *et al.,* 2005 Katella *et al.,* 2007
13.	*S. spegazzinii* (SS 1725-84)	Cyst nematode resistance. Late blight resistance	Bradshaw and Ramsay, 2005; Paal *et al.,* 2004
14.	*S. tuberosum* ssp. *tuberosum*		
14.1	Atlantic	First exotic high yielding variety	CPRI bulletin
14.2	Kufri Chipsona-III	High yielding, Good processing quality, Late blight tolerant	CPRI bulletin
14.3	Kufri Pukhraj	High yielding, Early blight tolerant and moderately tolerant to late blight	CPRI bulletin
14.4	Phulwa	Parentage of K. Deva and K. Sheetman, K. Safed, K. Chandramukhi	CPRI bulletin
14.5	Kufri Badshah	High yielding, Early and Late blight tolerant	CPRI bulletin
14.6	MP/97-1008	Used as parent in breeding programmme	CPRI bulletin

Table 2: List of genome primers used in the study

Sl No.	Primer	Sequence (5'—> 3')	Amplicon size (bp)
1.	RH 1	F: GCCTGAGCAATCTGCACAGGTC R: CTGGCAAACGACGTCGGCCAAGT	515
2.	RH 2	F: TGATGGTGGTCCAAACCATGGAGA R: TTGCTGGAAATAGGCCTGCT	358
3.	RH 3	F: GAGGAGTGCCTGCTGGAGCT R: TGAGCCAATCCTTTTGGTGAGC	428
4.	RH 4	F: TGAGTTGACCTTCCTTGGTTGTCG R: GAACTCAATGTTGATCGGGTTCTG	164
5.	RH 5	F: CTTTAGGCCTTGGAACCCTT R: GTTTGCGAGCTATTGCATAGGAGC	216
6.	RH 6	F: GTGAGGGGTTTATACCTTGCCACC R: GGGGCCATGACATTCTACCGGCTC	480
7.	RH 7	F: ATCGAGGGGTTCATCCGAATCTCC R: ACACACCGAACCCTACTTGACCCT	448
8.	RH 8	F: TTGGGGAGCACAATTGCTTCCACC R: GATCGGATTCCTTGGTTCGCAGA	256
9.	RH 9	F: TTTCGCAAGGATCGTCGTGGTA R: AGCATGCTCGACACCCGAAC	478
10.	RH 10	F: AGGCTGTTGATGCTCACACT R: GGAAGGCCAATATTGTAGCGGATG	512
11.	DM 1	F: TGAACTGCTCCAAGCAACTG R: ATTCCAACGCACATGCCACA	356
12.	DM 2	F: ACTACAGGGATGAACCCAATCC R: CTACGACACAATCAAACCCGA	367
13.	DM 3	F: ACGACTCGGTAACAGATCGCTT R: TGGGGATTCTTGATTGGTGCTG	260
14.	DM 4	F: GAGGCTTACCTCAGCCACTG R: GAAGTTACCCAAGGGCAGGT	597
15.	DM 5	F: TGGGGATCTTCACTAGTCTGCGA R: GCTTATGCCAGGATTGCGAATGC	245
16.	DM 6	F: TCCTCAAGGCAGTATGGTGCTACG R: ATGACTGGCTCACTAGGTTGCTCA	441
17.	DM 7	F: TTTCAGCGGCGGGCTTACCT R: GATCCTTGCGCCGATTCTCG	519
18.	DM 8	F: TGTCCAACCCGTACGGCCAAAG R: ACCGGTCACAACATCCAGAGAGCT	141
19.	DM 9	F: CACGCATCGATAGGGAACCCA R: AGCTTCGACGAGCTGGGACGT	430
20.	DM 10	F: GCTTGCTCGAGAGAGAGAGAGGTCGT R: CCGCATGAAGACTAGCCTATGGCT	163

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from Applied Biosystems) by using the following temperature sequence: 94 °C for 1 min, 35.5 °C for 1 min and 72°C for 2 min. Cycles were preceded by denaturation for 5 min at 94 °C followed by a final extension at 72 °C for 10 min. The PCR product was run on 2.5% agarose gel with TAE as tank buffer and stained with ethidium bromide. Gel image was captured and stored in a computer using Fluor-S*TM* Multimager (Bio-Rad) (Fig. 1). Manual scoring was done for the presence or absence of the amplicons.

Data Analysis

DNA amplification with each primer was assayed twice. DNA fragment profiles were scored in a binary fashion with 0 indicating absence and 1 indicating presence of a band. A similarity matrix on the basis of band sharing was calculated from the binary data using Dice coefficient (Nei & Li, 1979). Similarities were graphically expressed using the group average agglomerative clustering to generate dendograms. The analysis was done using the software package NTSYS-pc (version 2.02) (Rohlf, 1998). Principal Component Analysis (PCA) from the binary data was performed to graphically summarize associations among the lines. Analysis was done through a batch file following the software package NTSYS-pc.

RESULTS AND DISCUSSION

The importance of divergence in the improvement of crop has been reported both in self and cross-pollinated crops

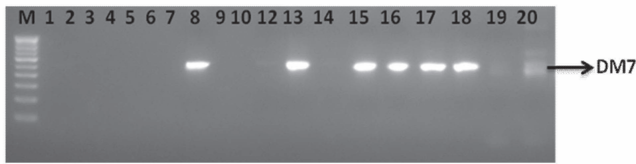

Fig. 1: Genome specific marker DM7 screening with 2.5% agarose (M-100bp ladder, 1-20 potato genotypes as mentioned in the table 1)

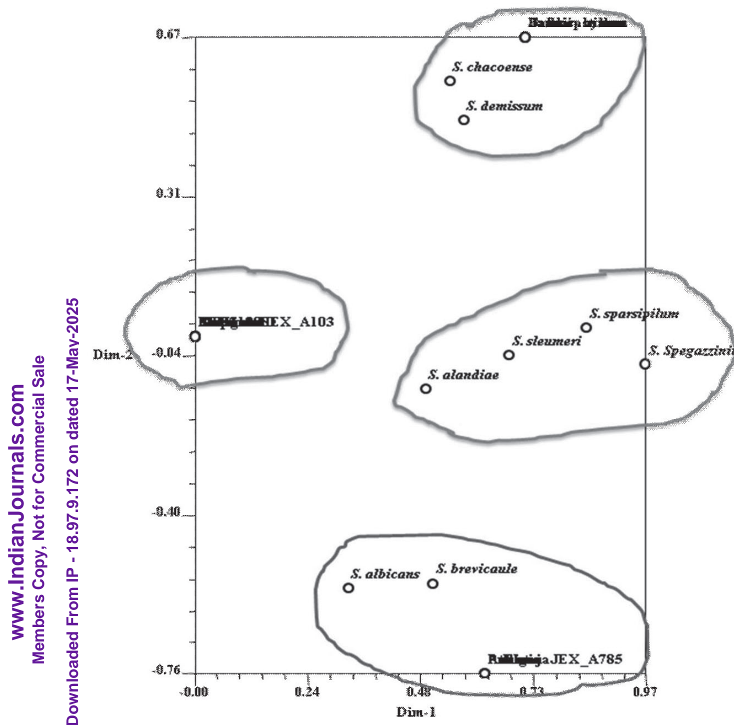

Fig. 2: Principal coordinate analysis (PCA) obtained by genome specific markers

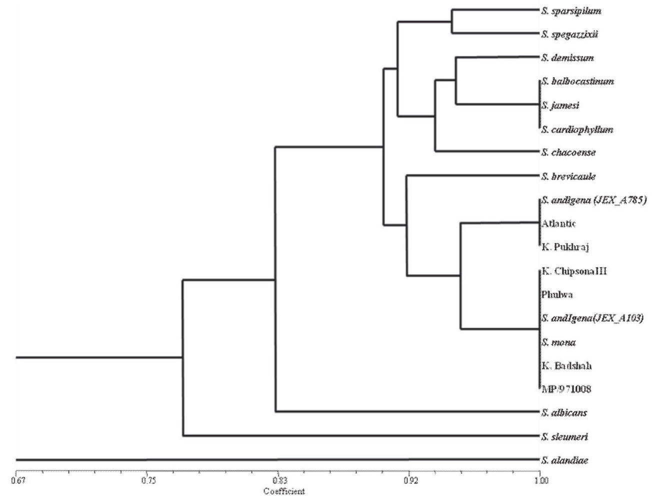

Fig. 3: Dendrogram depicting the phylogenetic relationship among potato genotypes

(Griffing and Lindsstromm, 1954; Gaur *et al.,* 1978). In the present study, 20 different potato genotypes including cultivated, semi-cultivated and wild species were compared using genome specific markers to study the genetic relatedness between the species. Coefficients of similarity were calculated according to Nei and Li (1979) and the matrix of similarity was analysed by the Unweighted Pair-Group Method (UPGMA). The associations among the species and cultivars were characterized by a multivariate technique, principal component analysis (PCA). The Principal Coordinate Analysis, where the first two principal components accounted for 76.1% of the variance, was able to separate the different groups. The PCA results (Fig. 2) indicated that all genotypes can be grouped into four major clusters. Besides, a dendrogram was constructed based on agglomerative clustering (Fig. 3) which also showed four major clusters confirming the PCA results. Clusters I, II and III contain one genotype each suggesting that these three wild species viz. *S. albicans*, *S. sleumeri* and *S. demissum* are un-related with other studied species. Cluster IV was the biggest cluster which further divided into four sub-groups consisting of both cultivars and wild species. In the pedigree of world potato cultivars *S. demissum* and *S. andigena* has been used to the maximum extent (Bradshaw *et al.,* 2006). Two commercial cultivars viz. Atlantic and Kufri Pukhraj closely clustered with *S. andigena* (JEX-A-785), a neo-tuberosum species which is one of the common parents in hybridization programme of these cultivars. Cultivars, Kufri Chipsona-III, Kufri Badshah, Phulwa and an advanced hybrid MP/97-1008 also closely clustered with *S. andigena* (JEX-A-103). In all these potato cultivars different *S. andigena* clones have been used as one of the progenitor in their breeding programmes and therefore the results suggest that these genome specific primers are able to distinguish among wild and cultivated species. Interestingly, *S. jamesi*, which is largely under-studied species, closely clustered with wild species *S. bulbocastanum*, *S. cardiophyllum* and *S. demissum*. This indicated that *S. demissum* is closely related to these three diploid, 1EBN species which are not easily crossable with cultivated *S. tuberosum.* It also largely indicates that all the agronomically important traits present in these three 1EBN species might have been taken care by *S. demissum* in the traditional potato breeding programmes. This study also gives an important clue that closely related wild species viz., *S. cardiophyllum*, *S. bulbocastanum*, *S. jamesi, S. sparsipilum* and *S. spegazzinii* share a common genetic pool of many agronomically important genes. It is also evident from the study that broad genetic diversity is present in unexploited wild species which can be further utilized for

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crop improvement programmes to widen the genetic base of the commercial potatoes (Gopal and Oyama, 2005). The narrow genetic base among commercial cultivars in the study is expected as the markers are genome specific, besides, few markers and low sample size used in the present study. This is in contrast to the wide genetic base of Indian potato cultivars estimated using random amplified polymorphic DNA (RAPD) analysis (Chakrabarti *et al.*, 1999; Chakrabarti *et al*., 2001; Pattanayak *et al.*, 2002). This may be due to nonspecific amplification, lower annealing temperature, smaller primer size, involvement of entire genomic DNA in RAPD amplification. However, results obtained in the present study were in conformity with those obtained by Chimote *et al.* (2004), using simple sequence repeats (SSRs) for studying the diversity among the Indian potato cultivars. The genetic diversity revealed by genome specific markers may not present the actual genetic relationship among the various wild/cultivated *Solanum* species studied as similarity coefficient is based on presence or absence of a amplicon and the potato genotype having an allele in single, double, triple or quadruple doses will be considered same for estimation of similarity value. Therefore, there is a need to estimate allele dosage also. The present results offer an avenue of some closely related wild species with *S. tuberosum* for widening the genetic base of the future potato cultivars.

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This is the first report of using the genome specific primers in potato. Study indicates that use of these markers can clearly distinguish different cultivated, semi-cultivated and wild species. Generation of the genetic profiles using the genome specific primers helps in documentation of ownership and protection of intellectual property rights along with other molecular marker systems (RAPD, AFLP, and SSR). The present study also clearly elucidated the genetic relatedness among the genotypes, which helps in selection of the diverse genotypes for genetic improvement of potato using unexploited wild species. It will also facilitate the construction of library of introgression lines (IL's) of the wild species in genetic background of cultivated potato which in turn would help in genetic study of the agronomically important traits found in the wild potato species.

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