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Chloroplast Genome Sequencing and QTL Analysis of Heat Tolerant and Late Blight Resistant Potato Varieties

In Bangladesh, potato accounts for about 53% of the total edible vegetables. It has a great demand throughout the year, but its production is concentrated during the months of January to March. Biotic and abiotic stresses like soil salinity in the coastal region, high temperature, devastating diseases like late blight, etc. seriously affect potato production. Recently, the Tuber Crops Research Center (TCRC) of BARI has developed a few high-yielding potato varieties like BARI Alu-46 and BARI Alu-53 (late blight resistant), BARI Alu-72 (heat and salt tolerant) and BARI Alu-73 (heat tolerant). So far, genomic analysis of these varieties has not been done. Molecular profiling sequence based phylogenetic analysis and QTL maps are still lacking in biotic and abiotic stress tolerant tetraploid potato varieties in Bangladesh. Genomic studies using the chloroplast gene are very important. Moreover, use of molecular markers is of basic importance for the efficient exploration of a plant genome and to dissect quantitative traits. Recent efforts have proven their potential in resolving phylogenetic relationships at different taxonomic levels and understanding structural and functional evolution by using the whole chloroplast genome sequences. To date, complete cytoplasmic DNAs (cpDNA) of more than 300 plants have been sequenced. (This KGF sponsored project studied the genetic aspects of tolerance of potato to abiotic stresses like salinity and heat and biotic stress caused by late blight. Chloroplast genomes of TCRC developed late blight disease resistant and heat tolerant potato were sequenced to understand the genetic and phylogenetic relations among the varieties. Chloroplast genes associated with the late blight disease resistance and heat tolerance were studied and genetic linkage maps developed. Recently the chloroplast genome of the potato cultivar Desiree has been sequenced which was used in this study as reference data.

Fig. 1. Late blight disease of potato

Methodology

Stress tolerant potato (*Solanum tuberosum*) varieties such as, heat tolerant (BARI Alu-72, 73), late blight resistant (BARI Alu-46, 53, 77) and susceptible (BARI Alu-7, 8, 13 and 25) varieties were used in this study. Genomic DNA, chloroplast (cp) and cpDNA were isolated from BARI

Alu-7, 25, 46, 53, 72, 73 and 77 at the Molecular Biology Lab, TCRC, BARI, Gazipur from fresh young leaves following modified high salt method. True Seq Nano DNA Kit (Illumina San Diego, United States) was used to assemble the library after DNA fragmentation. The *genomic DNA of five Solanum tuberosum* species was sequenced on 150 bp paired ends on an Illumina NovaSeq 6000 by Macrogen Inc. Korea. Trimmomatic v 0.36 was used for raw data processing, and the resulting clean data were used for assembly and post-analysis. Fastqc v0.11.5 was used to evaluate the quality of the data visually. SPAdes was used to assemble the clean data, and the complete cp genome sequence was obtained after gap closing. Prokka was used to annotate the cp genomes and predict the rRNA/tRNA of BARI Alu-46, BARI Alu-53, BARI Alu-72, BARI Alu-73, and BARI Alu-77 with EggNOG v5.0. Circular cp genome maps were drawn using the Organellar Genome DRAW (OGDRAW) program.

Bi-parental populations developed by crossing (heat tolerant varieties×susceptible varieties and late blight resistant varieties×susceptible varieties) for QTL mapping (Fig. 2). Seventeen progenies were developed through crossing F_1 populations were phenotypically evaluated for late blight resistance and heat tolerance at the Regional Agricultural Research Station (RARS), BARI, Rangpur, OFRD Reginal Station (RS), BARI, Rangpur, Breeder Seed Production Center (BSPC), Debiganj and TCRC, Gazipur. Finally the heat tolerant F_1 populations of heat tolerant and late blight resistant genotypes were phenotypically evaluated at OFRD RS, BARI, Shyampur, Rajshahi and BSPC, Debiganj, respectively during 2021-22. Promising genotypes were selected for varietal development in future. DNA was isolated from the fresh leaves of F_1 populations using a DNeasy Plant MiniKit (Qiagen, USA) for genotyping and map QTL development.

Fig. 2. F_1 population development at TCRC, Gazipur and BSPC, Debiganj

True potato seeds (TPS) produced were used to produce tubers at BSPC. TPS were planted first in the pots and then transferred to the nethouse for multiplication of tubers. Tubers were used for phenotyping at BSPC, Debiganj and DNA was isolated for late blight resistance and heat tolerant gene map. An appropriate agronomic management was followed to grow tubers in the net house and harvested at maturity stage.

Results and Outputs

Approximately 2GB of data for each Cp genome was obtained with a 150 bp read length. The chloroplast genome sequences of the 5 varieties ranged from 176,021 bp (BARI Alu 46) to 176,301 bp (BARI Alu 77) (Table 1). The same typical quadripartite structure was developed in the 5 Cp genomes. Two inverted repeat (IR) regions (44,606 - 44,999 bp) were separated by a large single-copy (LSC) region (85,738-85,986 bp) and a small single-copy (SSC) region (690-717 bp) (Table-1). The variation of the IR/LSC and IR/SSC borders was considered to be the primary mechanism causing the length differences of angiosperm Cp genomes. The GC content ranged from 36.81% to 35.77% for the 5 Cp genomes (Table 1). The five genomes contain total number of genes ranging from 152 (BARI Alu 77) to 161 (BARI Alu 53) and protein-coding genes ranging from 118 (BARI Alu 77) to 127 (BARI Alu 53) (Table 1). Each of the variety also contain 30 tRNA and 4 rRNA (Table 1). Two IR regions (44,606 - 44,965 bp) were separated by an LSC region (85,738-85,986 bp) and an SSC region (690-717 bp). The guanine and cytosine (GC) bases content ranged from 35.77% to 36.81% for the five Cp genomes (Table 1). Chloroplast genome maps were developed for the 5 potato varieties.

Table 1. Summary of the sequence data of heat tolerant and late blight resistant potato varieties

Species	Gene No.	Protein coding genes no.	tRNA gene no.	rRNA genes no.	Cp genome length (bp)	LSC length (bp)	IRa length (bp)	SSC length (bp)	IRb length (bp)	GC (%)
BARI Alu-46	154	120	30	4	176,021	85,738	44,965	700	44,618	36.35
BARI Alu-53	161	127	30	4	176,060	85,738	44,999	717	44,606	36.59
BARI Alu-72	153	119	30	4	176,041	85,738	44,971	690	44,642	35.77
BARI Alu-73	155	121	30	4	176,028	85,738	44,979	694	44,617	36.81
BARI Alu-77	152	118	30	4	176,301	85,986	44,996	702	44,617	36.63

Sequence Read Archive (SRA) accession numbers on sequence data and NCBI accession numbers of the potato varieties BARI Alu 46, BARI Alu 53, BARI Alu 72. BARI Alu 73 and BARI Alu 77 were received which will be accessible by all commencing December 31, 2024.

Phylogenetic relationship have been studied using SSR marker among the susceptible (BARI Alu 7, 8, 25), heat tolerant (BARI Alu 72 and 73) and late blight resistant (BAR Alu 46, 53, 77) potato varieties. No genetic variation between the varieties BARI Alu 7 and 8 was seen, they have 100% genetic similarities except skin color. Also similar genetic make-up was note for the varieties BARI Alu 25 and 53. The potato varieties BARI Alu 46, 72, 73 and BARI Alu-77 are genetically different from other varieties. The varieties BARI Alu 46, 53, 72 and 73 originated from USA and BARI Alu 7, 8, 25,77 from Europe.

Out of the F_1 populations developed from heat tolerant x heat sensitive varieties and late blight resistant x susceptible varieties, 29 promising lines were identified of which 14 were late blight resistant and 15 heat tolerant. A few heat tolerant and late blight resistant potato varieties are expected to be developed from these promising lines.

Two markers linked to heat tolerance (STM1019, STG0001) and four markers (STM1004, STM1008, STM0025, STM0025, STM2013, STM1049_2 linked to late blight resistance were developed.

From QTL analysis, the total map length and average interval of 371 cM and 20.6 cM, 374 cM and 14.96 cM, 294 cM and 19.6 cM, 151 cM and 10.6 cM were found in BARI Alu 25, BARI Alu 53, BARI Alu 63, and BARI Alu 73, respectively.

Expected Impact

Intact chloroplast (cp), cpDNA isolation protocol, cpDNA sequence data and genome maps of five potato varieties will serve as a useful information source for plant scientists, biotechnologists, teachers and biology students to develop their next generation sequence (NGS) research projects. A total of 29 advanced potato lines tolerant to heat and late blight from QTL derived materials were developed which can be used to develop marker assistance based heat tolerant and late blight resistant potato varieties in the future. Heat linked and late blight disease linked markers will help scientists advance generations through marker assisted breeding.

Recommendations

- Utilization of chloroplast genome sequence information for improvement of marker assisted breeding for heat tolerant and late blight resistant potato variety development
- Chloroplast genetic engineering program may be enhanced
- Nuclear genome assembly, gene prediction, variant calling and annotation needed for enhancing cross breeding and mutation breeding
- Research grants needs to continue for basic research on potato improvement utilizing the findings of this study.

This Technical Bulletin has been prepared on the basis of technical information available from a completed BKGET-KGF Funded Project, the details of which are given below:

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