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Evaluation of Local West Bengal Potato Cultivars for Late Blight Resistance and Characterization of the Gene that Confers Resistance

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ABSTRACT

A total of 14 local potato cultivars were screened against late blight resistance under field conditions with no artificial inoculation. All the cultivars were genotyped with three Microsatellite markers (SSRs) linked to the genes governing late blight resistance viz., Rpi - blb1, Rpi - bt1, and R3a. The aim of the investigation was to identify late blight resistant cultivars from some local potato cultivars so that these resistant cultivars can be utilized in genetic enhancement programs as resistant varieties are an important component of disease management. Two cultivars namely, Deshi-1 and Deshi-2 had moderate resistance towards the late blight in field condition. The rest of the twelve cultivars were expressed susceptible reactions. Through genotyping a late blight resistant gene was identified and characterized in those cultivars showing resistant disease reaction as they expressed band for the R3a gene based primer.

Key words: *Field screening, Genotyping, Late blight resistance, Local cultivars, Potato*

Introduction

Potato is the world's first important solanaceous vegetable as well as the non grain food crop (Zhang *et al.* 2017) and non fat food with energy density similar to pulses. Potatoes are notably important for daily fiber, protein, vitamin, and mineral requirements of human diet (Freedman and Keast, 2011). Consumption of potatoes and potato components has been linked to a reduction in cardiometabolic risk in various studies (McGill *et al.*, 2013) and promote surfeit (Holt *et al.*, 1995; Geliebter *et al.*, 2013; Akilen *et al.*, 2016). Potatoes include a modest amount of resistant starch (RS), which acts as a prebiotic by promoting the growth of healthy gut bacteria (Higgins, 2004; Brit, 2013). However, high glycemic index (GI) of potato may lead to type 2 diabetes and weight gain upon their consumption. How-

ever, observational studies have provided only limited evidence for this deleterious influence on health (Halton *et al.* 2006; Mozaffarian *et al.* 2011).

Major biotic constraints of potato cultivation worldwide are Late blight followed by viruses and cyst nematodes (Seenivasan 2017). The oomycete fungus *Phytophthora infestans (*Mont.) de Bary causes potato late blight disease (LB). This disease has an indelible impression in the history of Ireland i.e. during 1845-1849 the Great Irish Potato Famine caused millions of deaths and huge emigration from Ireland to the New World (Bourke 1993). The two mating forms of *Phytophthora infestans,* A1 and A2, are required for sexual reproduction (Kirk 2009). As demonstrated by several surveys both the mating types (A1 and A2) are present in several countries viz. India, China, Indonesia, Korea, Japan, Thailand and Pakistan (Ahmad *et al.*, 2002; Ghimire *et al.* 2003; Gotoh *et al*., 2005). In epidemic conditions, LB of the potato results in yield loss and may reach up to 95%. As per availability of resources several strategies have been employed to manage LB of potato such as chemical control, host resistance, biological control and cultural control. Host resistance is always a better option than the fungicides, since it is inexpensive and environmentally friendly.

Breeders are mining for durable LB resistance genes to protect from global threat arriving by highly aggressive pathotypes of *P. infestans* (Cooke *et al.,* 2012). Plant breeders have increasingly relied on wild species of Solanum L. section Petota for persistent LB resistance genes in their quest to develop potato cultivars with long-term resistance to LB (Œliwka *et al.,* 2013; Jo *et al.* 2016). Enriching the potato genome through pyramiding R genes in it is an effective approach employed for getting durable resistance against late blight disease. In the present study, fourteen potato cultivars were screened against late blight to identify desirable cultivars for potato improvement.

Materials and Methods

Plant material and experimental design

Fourteen local potato cultivars of West Bengal were screened against the late blight disease using RCBD during the *rabi* season (2018-19) at Agricultural Research Farm ($26^0$2325N, $89^0$2321 E, 44 msl), Regional Research station (RRS), Terai Zone, Pundibari, Cooch Behar, West Bengal, India.

Phenotypic screening for LB resistance

Fourteen potato cultivars of were screened against late blight disease. No pesticides were used in order to achieve maximal disease pressure. The Progression of the disease was recorded based on visual symptoms at two days intervals after the first appearance of the disease by following Henfling modified disease estimation scale (Henfling, 1979). A heat map was constructed with the help of percent infection. The Area under disease progress curve (AUDPC) was computed by following Shanner & Finney (1977). The relative area under disease progress curve (rAUDPC) was also computed.

Genotyping for LB resistance

The genomic DNA was extracted from all the genotypes using the method developed by Mandal *et al.*, (2016). Three R gene primers were selected namely Rpi-blb1, Rpi-bt1 (Chen *et al*., 2017) and an EST-derived primer (designed using Primer 3.0 (Koressar and Remm, 2007)) of the R3a gene for screening against late blight resistance gene. PCR amplification was performed in a total of 25 µl of reaction volume that contained 2 µl of gDNA, 0.2 µl of 5 U/µl Taq DNA polymerase (Xcelris.), 2.5 µl of 10X PCR Buffer, 1 µl of 2.5 mM dNTPs mixture, 1 µl of each primer (100 ng/µl) and 16.8 µl of HPLC grade water. The PCR amplification conditions were as follows: 5 min of initial denaturation at 94 °C, followed by 35 cycles of denaturation at 94 ^{0}C for 1 min, annealing at 55-65 °C for 30 s, and extension at 72 °C for 1 min with a final extension at 72 °C for 5 min and a 4°C holding temperature. PCR products were detected using 3% agarose gel and Gel photographs for bands of amplified DNA were captured through Gel Doc System. Sequencing was done by Xcelris Pvt. Ltd. Service centre and got the accession number from DDBJ.

Results

For phenotypic screening regular monitoring was done from the date of the onset of disease to record the severity of the disease based on the symptom development of late blight. Disease development was recorded on keen observation and categorization of cultivars was done. Where 2 cultivars namely Deshi-1 and Deshi-2, exhibited moderate resistance, while the rest of all shown susceptible. By utilizing the percent infection, a heat map was constructed

(**Figure 1**). Heat map revealed that those two cultivars (Deshi-1 & Deshi-2) were less susceptible to the late blight disease.

In view of further investigation disease severity

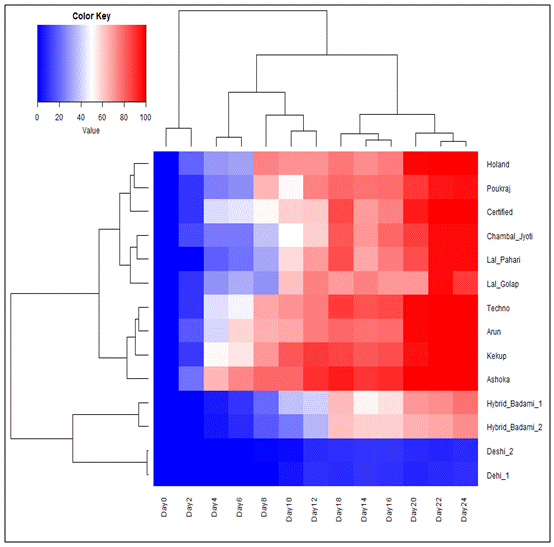

Fig. 1. Heat map based on percent disease incidence with 2 days interval

was converted into the AUDPC and rAUDPC were calculated (**Table 1**). Minimum AUDPC was recorded for Deshi-1 (170.81) followed by Deshi-2 (171.96). Whereas cultivar Ashok exhibited the maximum (1848.5) AUDPC, followed by Kekup (1710.41) and Techno (1633.17) respectively. Deshi-1 & Deshi-2 were having moderate field level resistance to the late blight disease as the values of

Table 1. Total AUDPC and relative AUDPC of 14 potato cultivars

Genotype	AUDPC	rAUDPC
Dehi- 1	170.81	7.76
Deshi- 2	171.96	7.82
Certified	1475.00	67.05
Kekup	1710.41	77.75
Hybrid Badami -1	861.00	39.14
Hybrid Badami-2	820.50	37.30
Holand	1520.33	69.11
Techno	1633.17	74.23
Lal Pahari	1319.00	59.95
Lal Golap	1318.33	59.92
Ashok	1848.50	84.02
Arun	1606.17	73.01
Poukraj	1432.33	65.11
Chambal Jyoti	1336.83	60.77

AUDPC ranges between 150.10 and 375. Rests of the genotypes were susceptible as their AUDPC was greater than 375 (Lal *et al.*, 2016). Among the tested cultivars, rAUDPC was less for Deshi-1 (7.76) and Deshi-2 (7.82) against highly susceptible cultivar Ashok (84.02).

Among the three primers used for molecular screening (Table 2), two primers namely BLB1 and BT1, did not show any amplified DNA fragment in the gel electrophoresis results for all the 14 genotypes. But one primer, i.e. R3a showed an amplified band of DNA with approximately 800bp size for the genotypes Deshi-1 and Deshi-2 (**Figure 2 and Table 3**). After confirmation of the single fragment appearance PCR product of Deshi-1 was sequenced using pyro-sequencing technology.

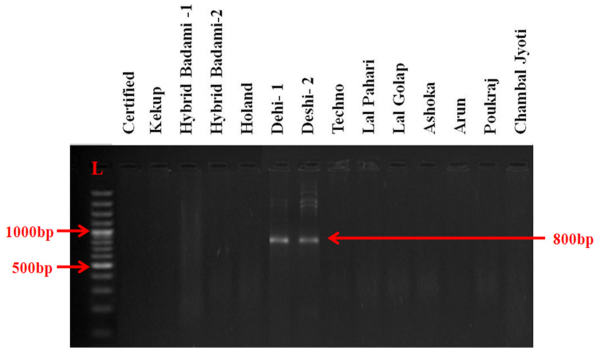

Fig. 2. Amplified product of R3a gene based primer. L is a DNA ladder.

A nucleotide homology search was done by taking sequences into consideration, against the EST datasets of selected potato species available at National Centre for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov) using BLASTn algorithm (Altschul *et al.*, 1990). A match was considered significant at E-value ≤ 1×10^{-5}. The similarity of sequence was considered as significant at E-value ≤ 1×10^{-08}. BLAST results for the sequence of the putative gene had shown significant homology with genes of known function in LB disease resistance which are available in the public database. The homology search and function classification revealed that upregulated the sequences were the result of potato-*P. infestance* interaction seems to function in cellular metabolism and development, biotic/abiotic stress responses, transcription and signal transduction responses. The sequence was deposited to the DNA Data Bank of Japan (DDBJ) i.e. a gene bank database. The sequence of putative gene had the greater

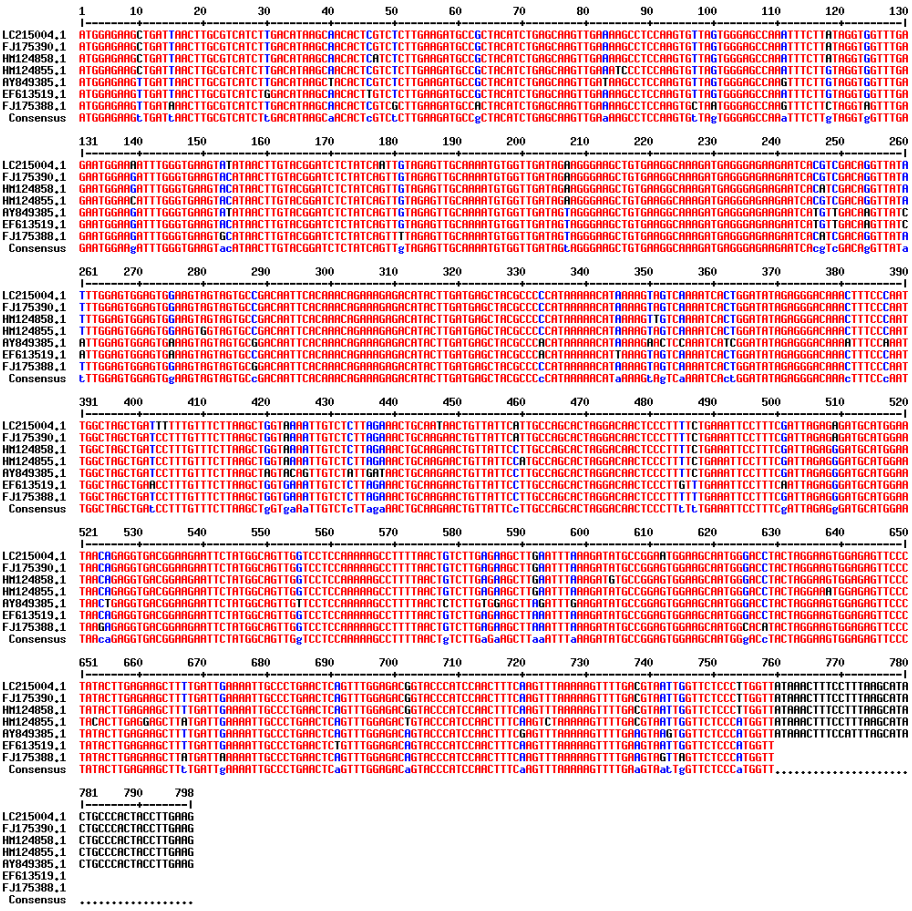

Figure 3: Multiple sequence alignment of identified putative gene with other similar gene

Table 2. SSR markers identified in the vicinity of R genes for screening

Name of the R gene	Primer name	Primers	Annealing Temperature (°C)
Rpi - blb1	BLB1	**F:** AACCTGTATGGCAGTGGCATG **R:** GTCAGAAAAGGGCACTCGTG	58
Rpi - bt1	BT1	**F:**CTACATGGCTGTCATTCACT **R:**CATAGGGCAACATTTAATCTC	53
R3a	R3a	**F:** ATGGAGAAGCTGATTAACTT **R:** CTTCAAGGTAGTGGGCAGTA	59

than 99% identity with previously reported late blight resistance gene. The multiple alignment and result of homology search were presented in the Figure 3 and Table 4.

Discussion

Experimental location was hotspot for the potato late blight disease (Hembram *et al.,* 2018). Therefore no artificial inoculation was done to develop the disease. Scoring of the disease based on foliage destruction is a common method of field assessment for LB resistance. In our study disease development was recorded using Henfling modified disease estimation scale (Henfling, 1979). Categorization of genotypes was done for disease reaction on the basis of AUDPC in accordance with Lal *et al.* (2018). In natural epiphytotic conditions the late blight disease might be caused by complex races of the pathogen (*P. infestance*). As a result, foliage of most of the cultivars was destroyed by the disease before the end of the growing season under field condition in our

Table 3. Summary molecular screening of 14 potato cultivars for three R gene based markers

Cultivars	Primers		
	BLB1	BT1	R3a
Dehi- 1	-	-	+
Deshi- 2	-	-	+
Certified	-	-	-
Kekup	-	-	-
Hybrid Badami -1	-	-	-
Hybrid Badami-2	-	-	-
Holand	-	-	-
Techno	-	-	-
Lal Pahari	-	-	-
Lal Golap	-	-	-
Ashok	-	-	-
Arun	-	-	-
Poukraj	-	-	-
Chambal Jyoti	-	-	-

Note: '-' Indicates absence and '+' Indicates presence of band of PCR product

Table 4. Similarity among the different resistance genes with identified gene

S. N.	Accession	Sequence Description	Query Cover	E Value	Percent Identity
1	FJ175390	Putative Late blight Resistance gene	100%	0.0	99.12%
2	FJ175389	Putative Late blight Resistance gene	100%	0.0	99.12%
3	AY849385	R3a Late blight Resistance gene	100%	0.0	99.12%
4	FJ175386	Putative Late blight Resistance gene	100%	0.0	98.62%

study. Only two cultivars Deshi-1 and Deshi-2 had moderate foliar late blight resistance.

Field resistance was corroborated by molecular screening due to the appearance of the amplified band of DNA for the same cultivars (Deshi-1 and Deshi-2) when amplified by the EST-derived primer of the R3a gene. *R3a* and *R3b* are two closely linked genes of the locus *R3* (on chromosome XI of *S. tuberosum* L.) with different pathogen resistance specificities (Li *et al.,* 2011). The resistance expressed by the cultivars Deshi-1 and Deshi-2 might be due to the presence of the locus R3 or R3a gene alone. The presence and expression of the R3a gene in cultivars Deshi-1 and Deshi-2 may not be sufficient to exhibit the resistance reaction towards all the races of the pathogen prevailing in the locality. So they exhibited moderate resistance at field level under natural epiphytotic conditions.

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References

Ahmad, I.S.B and Mirza, J.I. 2002. Distribution of Mating Types of *Phytophthorainfestans*in Pakistan. In: Lizarraga C, eds. *Late Blight: Managing the Global Threat, Proceedings of the Global Initiative on Late Blight Conference, 11-13 July*. pp 129-30. Hamburg, Germany, International Potato Center, Lima, Peru.

Akilen, R.D., N. Neljoomanesh, S. Hunschede, C.E. Smith, M.U. Arshad, Kubant, R. and Anderson, G.H. 2016. The effects of potatoes and other carbohydrate side dishes consumed with meat on food intake, glycemia and satiety response in children. *Nutr Diabetes.* **6**: e195.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. and Lipman, D.J. 1990. Basic local alignment search tool. *Journal of Molecular Biology*. 215(3): 403-410.

Black, W. 1970. The nature and inheritance of field resis-

tance to late blight *(Phytophthora infestans)* in potatoes. *American Potato Journal.* 47 : 279-288.

Bourke, A. 1993. 'The Visitation of God'? The potato and the great Irish famine. Dublin, Ireland, Lilliput Press, Ltd.

Brit, D.F. 2013. Resistant starch: Promise for improving health. *Advances in Nutrition.* **4**:587–601.

Chen, S., Borza, T., Byun, B., Coffin, R., Coffin, J., Peter, R. and Wang-Pruski, G. 2017. DNA markers for selection of late blight resistant potato breeding lines. *American Journal of Plant Sciences.* **8**: 1197-1209

Cooke, D.E.L., Cano, L.M., Raffaele, S., Bain, R.A., Cooke, L.R., Etherington, G.J., Deahl, K.L., Farrer, R.A., Gilroy, E.M., Goss, E.M., Grunwald, N.J., Hein, I., MacLean, D., McNicol, J.W. and Randall, E. 2012. Genome analyses of an aggressive and invasive lineage of the Irish Potato Famine pathogen, *PLoS Pathog.* **8:** p.e1002940. doi 10.1371/journal.ppa

Freedman, M.R. and Keast, D.R. 2011. White potatoes, including French fries, contribute shortfall nutrients to children's and adolescents' diets. *Nutrition Research.* **31**: 270–277.

Geliebter, A., M. Lee, M. Abdillahi and Jones, J. 2013. Satiety following intake of potatoes and other carbohydrate test meals. *Annals of Nutrition & Metabolism.* **62**: 37–43.

Ghimire, S.R., Hyde, K.D., Hodgkiss, I.J., Shaw, D.S. and Liew, E.C.Y. 2003. Variations in the *Phytophthora infestans* population in Nepal as revealed by nuclear and mitochondrial DNA polymorphisms. *Phytopathology.* **93**: 236-243.

Gotoh, K., Akino, S., Maeda, A., Kondo, N., Naito, S., Kato, M., and Ogoshi, A. 2005. Characterization of some Asian isolates of *Phytophthora infestans*. *Plant Pathology.* 54: 733-739.

Halton, T.L.,W.C.Willett, S. Liu, J.E. Manson, M.J. Stampfer and F.B. Hu. 2006. Potato and French fry consumption and risk of type 2 diabetes in women. *The American Journal of Clinical Nutrition.* **83**:284–290.

Hembram, S., Patsa, R., Baskey, S. and Hansda, M. 2018. Effect of Weather Indices on Late Blight of Potato and Its Management in Northern District of West Bengal, India. *Int. J. Curr. Microbiol. App. Sci.* *7*(9):1737-1743.

Henfling, W. 1979. Late blight of potato. Technical information bulletin. International Potato Centre, Lima Peru. pp. 13.

Higgins, J.A. 2004. Resistant starch: Metabolic effects and potential health 539 benefits. *Journal of AOAC International.* **87**: 761–768.

Holt, S.H.A., J.C. Brand-Miller, P. Petroz, and E. Farmakalidis. 1995. Satiety index of common foods. *European Journal of Clinical Nutrition.* 49: 675–690.

Jo, R.R., Zhu, S., Bai, Y., Hutten, R.C.B., Kessel, G.J.T., Vleeshouwers, V.G.A.A., Jacobsen, E., Visser, R.G.F. and Vossen, J.H. 2016. Problematic crops. 1. Potatoes: towards sustainable potato late blight resistance by cisgenic*R* gene pyramiding. In: *Plant Pathogen Resistance Biotechnology*, Collinge, D.B., Ed., New York: Wiley, pp. 171–191.

Kirk, W. 2009. Potato Late Blight Alert for the Midwest. Field Crop Advisory Team Alert Current News Articles.

Koressaar, T. and Remm, M. 2007. Enhancements and modifications of primer design program Primer. *Bioinformatics.* **23**: 1289-1291.

Lal, M. 2016. Fungal foliar diseases of potato and their management (Course on disease and pest management in potato complied and produced by Sanjeev Sharma, Vinay Sagar, Ravinder Kumar, Sridhar Jandrajupalli, Dhiraj Kumar Singh) pp 22-27. ICAR-CPRI Shimla, India.

Li, G., Huang, S., Guo, X., Li, Y., Yang, Y., Guo, Z. and Vossen, J. H. 2011. Cloning and characterization of R3b; members of the R3 superfamily of late blight resistance genes show sequence and functional divergence. *Molecular Plant-Microbe Interactions*. 24(10): 1132-1142.

Malcolmson, J.F. 1976. Assessment of field resistance to blight *(Phytophthora infestans)* in potatoes. *Transactions of the British Mycological Society.* **67**:321-325.

Mandal. R., Nag. S., Tarafdar, J. and Mitra, S. 2016. A comparison of efficiency parameters of SSR markers and genetic diversity analysis in *Amorphophallus paeoniifolius* (Dennst.) *Nicolson. Braz. Arch. Boil. Technol.* **59**: 1-7.

McGill, C.R., A.C. Kurilich, and J. Davignon. 2013. The role of potatoes and potato components in cardiometabolic health: A review. *Annals of Medicine.* **45**: 467–473.

Mozaffarian, D., Hao, T., Rimm, E.B., Willett, W.C. and Hu, E.B. 2011. Changes in diet and lifestyle and long-term weight gain in women and men. *The New England Journal of Medicine.* **364**: 2392–2404.

Seenivasan, N. 2017. Status of potato cyst nematodes, *Globodera*spp infection on potato at Kodaikanal hills of Tamil Nadu, India and yield loss estimation. *JEZS.* 5: 268-272.

Shanner, G. and Finney, R. E. 1977. The effect of nitrogen fertilization on the expression of slow mildewing resistance in knox wheat. *Phytopathology.* 67: 1057-1066.

Œliwka, J. and Zimnoch-Guzowska, E. 2013. , Resistance to late blight in potato. In : *Translational Genomics for Crop Breeding*, vol. 1: *Biotic Stress*, Varshney, R.K. and Tuberosa, R., Eds., Wiley-Blackwell, pp. 221–240.

Zhang, H., Xu, F., Wu, Y., Hu, H. and Dai, X. 2017. Progress of potato staple food research and industry development in China. *Journal of Integrative Agriculture.* 16(12): 2924–2932.