Summary
**Phytophthora infestans**, cause of Late blight, the most significant potato crop disease in history, that swept continental Europe, the British Isles and Ireland in the mid-1840s leading to mass starvation and emigration of its inhabitants, still persists to be a problem for potato cultivation world over. Frequent applications of chemical fungicides are necessary to grow potatoes in moist climates to save huge crop losses. Search to find out the source(s) of resistance to late blight began in potato during late 19th century, several resistance genes (Rpi-genes) from wild species have been introduced in to cultivated potato, but the pathogen *P. infestans* forms races that are able to circumvent resistance, conferred by race specific major R-genes of various *Solanum* species. *Solanum demissum*, a common species in central Mexico comprising race specific R1-R11 resistance genes that have been introgressed in to cultivated potato in one of the earliest potato breeding efforts. Primarily, cultivars with R1-R11 were found to be promising, but large scale deployment of such cultivars resulted in breakdown of resistance with the evolution of new matching virulence and eventually loss of durability.

Consequently, the attention was shifted towards general resistance, controlled by many minor genes, which does not exert any directional selection pressure on the fungus. However, this type of resistance is difficult to handle in a backcrossing programme as most of the genes flow only to a low proportion in the progeny and then quickly disappear upon back crossing. Nevertheless, over the years field resistance had become the backbone of late blight resistance-breeding programmes in most of the countries including India.

Since the recent advancement in plant molecular marker technology, most of the R-genes in this set of R1-R11 have been mapped and cloned now; the genetic information gained at the molecular level can now be used to plan efficient and effective resistance breeding programmes. Pyramiding several R-genes in one cultivar/genotype is a convincing viable strategy to increase both durability and level of resistance.
Specific receptors determined by $R$ genes, interact directly or indirectly with $Avr$ effectors and initiates signal transduction pathways, that lead to the hypersensitive reaction and the expression of the disease resistance response. One consequence of this is that, races of the pathogen that contain a mutation in their $Avr$ gene(s) can arise and become virulent. Deploying several $R$-genes in one potato host would increase the selection pressure on the pathogen as the number of unrelated $Avr$ targets would be difficult to overcome and the pathogen would require multiple independent mutations to become virulent. Thus, assembling multiple $R$ genes that detect different $P. Infestans$ $Avr$ effectors may enhance probability of achieving durable and increased level of resistance.

The present investigation was undertaken to study the effect of stacking of functional $R$-genes into single host background through marker assisted selection.

In this study $R$-gene linked markers to late blight were used as an aid, at each step of breeding process i.e. selection of the parents possessing $R$-genes on the basis of linked $R$-genes markers, crossing or hybridization for pyramiding diverse $R$-genes, followed by early generation selection on the basis of markers and finally to compare the phenotypic assessment of the genotypes with stacked gene for enhanced resistance than the parents with single genes. Key steps and the results of research investigation are summarised below.

**7.1 Generation of plant material:** The plant materials used in the present study consisted of One hundred and seventy nine potato genotypes comprising of 77 exotic potato germplasm accessions including a set of 21 late blight differentials, 44 indigenous potato varieties, and 58 advanced potato hybrids available at CPRI national germplasm repository. These were sown in earthen/plastic pots and maintained under glasshouse conditions (day/night temperature 23/18°C, respectively).

**7.2 Genotypic/molecular marker analysis:** Total Genomic DNA was extracted from the potato genotypes (179) including set of late blight
differentials and was PCR amplified with eight selected DNA markers linked to R genes. Out of several markers tested from the toolbox of available markers published worldwide, having tight linkage to different R-genes namely R1, R2, R3a, R3b, R8; markers for only three genes i.e. R1, R2 and R3a were found discriminative in late blight differentials set and subsequently used for genotyping the whole population of 158 genotypes. The results revealed that 17 genotypes possessed R1 gene, 18 genotypes were having R2 gene and 41 genotypes have R3a. Besides 17 genotypes had combinations of either of the genes (R1 & R2), (R1 & R3a) and (R2 & R3a). In total, 93 potato accessions were found to possess either of R-genes. Therefore, 60% of the genotypes under study are the possible source of R-genes imparting resistance to late blight for future breeding programmes.

7.3 Evaluation of parental lines for resistance to P. infestans.
Phenotyping of the screened material was done by detached leaf method. The genotypes were categorized into highly resistant (LA up to 1 cm²), resistant (LA 1.1 to 2.5 cm²), moderately resistant (LA 2.51 to 6.0 cm²) and susceptible (LA >6.0 cm²). Screening of 179 potato cultivars confirmed that the markers of R1, R2, and R3a genes were significantly associated to late blight resistance. Most of the genotypes having multiple R-genes were either resistant or moderately resistant but some of the genotypes MP/99-1189 (R2), B-420 (R3a), HB/83-39 (R3a), VMT14-3 (R3a), MP/97-625 (R2 & R3a) and MP/97-699 (R2 & R3a) were found susceptible to late blight infection, this can be possible since major R-genes codes recognition proteins responsible for detection of the pathogen and only triggers the defense mechanism of other minor genes to become active, the cumulative effect of these minor and major genes finally is responsible for the resistance outcome.

7.4 Statistical analysis: Mann-Whitney-U test was performed in SPSS software for an association between diagnostic marker and the phenotypic data; these were found to be significant at p≤ 0.05. All the four markers i.e. R1-1400/Cos A, R3-1380 and R2-abpt 1 were significantly associated with the resistance phenotype.
7.5 Hybridization among selected resistant parents: For hybridization, fifteen crosses were attempted among the genotypes identified possessing R-genes through markers in this study for combining R1, R2 and R3a genes in single host background. Berries were obtained from five successful crosses i.e. HR 5-2 × K. Himsona, SM/92-338 × K. Girdhari, CP 4045 × CP 1945a, K. Jyoti × CP-4045, CP 4055× K. Kuber. F1 Segregating progenies of 280 seedlings were raised, out of which, 220 plants survived and used for MAS for identification of F1 clones possessing major R- gene combinations for late blight resistance.

7.6 Genotypic analysis of the segregating population: The segregating population (220 genotypes) was analysed using the same set of markers i.e. R1AS, R2abpt1, and R3-1380. The results revealed that this population could be differentiated into four groups: first group was devoid of all the three R genes comprising 30 genotypes, second with single R1, R2, or R3a gene with 70, 57 and 45 genotypes respectively. the third group with stacked R1 and R3a genes comprising of 17 genotypes and the fourth group with only one genotype, where R1 and R2 Genes were de-stacked from one of the parents CP 4045 (R2, R1) and restacked with R3a gene derived from the other parent.

7.7 Phenotypic analysis of the segregating population: Phenotypic evaluation of all the 220 seedlings was done for late blight resistance as described previously using detached leaf test using inoculums dosage 4×10⁴/ml. Marker genotypes were in good agreement with phenotypic assessment for presence or absence for resistance genes in the tested progeny. Further, tubers harvested from 18 clones i.e. the first clonal generation carrying the stacked genes R1 and R3a were once more propagated and were subjected to phenotypic assessment by DLT, with zoospore suspension reduced to 3×10⁴/ml. Parents of the respective crosses SM/92-338 × K. Girdhari and CP 4045 × CP 1945a were also inoculated with same inoculums dosage. Kufri Bahar and Desiree were used as negative controls.
All the clones were highly resistant except for the negative controls. Sizeable difference in the lesion area of the leaves was observed for all the genotypes as compared to the parents used in the cross, expressing the effect of stacking major R-genes in single genotypic background. Clone no C15 was having the least score of 0.19 cm$^2$ with only traces of the infection, showing the most apparent additive behaviour of the pyramided $R1$ and $R3a$ genes.

Resistance score of C13 was 0.67 cm$^2$ which was comparatively very less in comparison to parents of the clone CP 4045 and CP1945a with resistance score 2.84 and 2.43 respectively, which shows an increase in resistance level of the progeny with de-stacking of $R1$ gene (CH-V) and $R2$ (CH-1V) from one of the parent CP4045, and re-stacking of $R1$ gene with $R3a$ (CH-XI), that may have resulted due to occurrence of new recombination event during crossing over and in addition to the original genes i.e. $R3a$, other functional recognition as well as defence response genes might have inherited together and resulted in increased resistance.

There are only few studies demonstrating the use of MAS in enhancing potato late blight resistance through classical breeding programmes. However, recent advances in understanding the molecular basis of genetic resistance against *P. Infestans*, the structural design of $R$-genes, and knowledge of effector biology to identify, characterize, clone and deploy late blight resistance $R$-genes signify notable change in research strategies particularly with reference to pyramiding $R$ genes that could detect multiple *P. Infestans* effectors and thus will provide durable late blight resistance. So far the $R$ genes have been found to be belonging to the CC-NBS-LRR class and there may be ‘n’ number of such $R$ genes available in the vast gene pool available in the semi-cultivated /wild species of potato. It is a matter of time that such genes are identified, cloned and sequenced that will lead to the identification of markers for MAS. This will provide big opportunities for the breeders for practising MAS to introgress these genes into cultivated potatoes using MAS and deploy new resistance sources to combat the new more
virulent races of *Phytophthora infestans*. This approach apparently have an edge today compared to the transgenic approach i.e. transfer of such \( R \) genes by producing transgenics which is being debated across the world.

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