Review of Literature
2.1 Historical perspectives of the potato

The potato crop is not indigenous to India, it originated in the vicinity of Lake Titicaca, high in the Andean highlands of Peru and Bolivia in South America. It was domesticated by hunters and gatherers in the Andes mountain range around that time (8000-6000 BC). Potatoes were taken from South America to Spain’s Canary Islands in the year 1565 and quickly spread to European subcontinent within a span of 40 years, reaching England by 1597. But, once it got the foothold in the European society, it changed everything and the potato enabled and fed the industrial revolution in Europe. The infamous Irish potato famine reminds us about the Europeans, who were depending solely on potatoes, for their daily sustenance during early to mid 19th century. Even the Bavarian war in 19th century was termed as a 'Potato War', because it continued till the potato stock expired.

Potato was brought to India at the southern coast by Portuguese or the Britisher’s during later part of 16th century. Potato cultivation in the country during next 300 years remained constrained, with entire Indian subcontinent contributing only 1% of world’s potato area and production by 1941, mainly due to non availability of indigenous tailored varieties and technologies for growing potato under sub tropical climatic conditions. Importance of potato cultivation was felt and Central Potato Research Institute was established at Patna in 1949, which was later shifted to Shimla in the year 1956, in order to facilitate hybridization work and better production of disease free seed. Commercial Potato varieties known to the western world were secured from wide range of climatic situations and were tried in India, but merely 6% showed good yield level (Pushkarnath, 1976). So, with the shifting of breeding work to hills, varieties suitable for Indian conditions were developed. Today, India ranks among the top three potatoes producing countries of the world. China, India and Russian Federation together contribute about 42% of global potato production. The total potato production in India lately reported to be 42.34 (MT) (FAOSTAT, 2011) with an area of 1.8 million ha dedicated to potato cultivation.
2.2 Economic importance of potato

Potato is the world’s number one non-cereal grain crop with global production reaching a record 374 million tons in 2011 (FAOSTAT, 2011). In a potato tuber, about 80% is water and the rest is dry matter. Starch is the major component of the dry matter accounting for approximately 70% of the total solids. The average raw material composition of a potato tuber is as follows: dry matter (20%), starch (13-16%), total sugars (0-2%), protein (2%), fibre (0.5%), lipids (0.1%), vitamin A (trace/100 g fresh weight), vitamin C (31 mg/100 g fresh weight), minerals (trace), ash (1-1.5%), amylose (22-25%). The potato is a low energy food (97 kcal/100 g fresh weight), because it contains low fat (< 0.1%) and calorie thus potato represents an ideal meal to avoid obesity (USDA data base: http://ndb.nal.usda.gov). It is a highly nutritious, easily digestible, wholesome unique food, because it can be consumed as boiled, baked or processed, all with equal culinary. Production of higher quantity of good quality protein and calories per unit area, per unit time, compared to other food crops is an added advantage of potato. Considering the nutritive value and potential of cultivation, the FAO has rightly identified this crop as ‘food for future’ and declared 2008 as ‘International potato year’.

2.3 Classification of potato

Potato belongs to the family Solanaceae that contains more than 3,000 species. The family is the third most important plant family after cereals and legumes. It also includes other important crops such as tomato, tobacco, eggplant and pepper. Potato has one of the largest related wild species with more than 228 Solanum species documented. The main cultivated germplasm is derived from S. tuberosum Group tuberosum for long day-conditions while S. tuberosum Group andigena is adapted for short-day conditions.

The potato is a starchy, underground tuber known as stolen, which is in fact, a modified stem. The height of potato plant can be as high as 60 cm. The flowers can be white, pink, red, blue, or purple with yellow stamens. Edible part of this crop is tuber itself, shape of a tuber can be oval to round, although shapes can be of intermediate types. It consists of an inner flesh and an outer protective cover known as a skin. Great variation in flesh colour and skin finish is observed.
Depressions on a potato tuber are known as its eyes, that actually are the dormant buds, which give rise to new shoots (white to creamy white or pigmented sprouts) under suitable conditions. The initiation and development of potato in the plant is known as tuberization. Tubers meant for vegetative propagation are known as seed tubers or seed potatoes. Wild Potatoes are generally cross-pollinated mostly by insects, including bumblebees, which carry pollen from other potato plants, but the commercially cultivated potatoes are self-fertilized. Potato plant bear small green fruits, that resemble green cherry tomatoes, each containing up to 300 true botanical seeds known as True Potato Seeds (TPS).

About 90% Potatoes in India are grown under short-day photoperiod (daylight) and cool night temperature during winters in the sub-tropical Indo-Gangetic plains. The potato growing conditions in India are exclusively different from those in temperate potato growing countries of Europe and America. However, temperate potato growing conditions are also available in Indian hills, where the crop is cultivated during summer. But this temperate potato production constitutes only about 8-10 % of the total production. In India, therefore, the potato is regarded as a short-duration crop with an average cropping stand of 90-100 days.

2.4 Genetics and potato breeding

Cultivated Potatoes are self pollinated and vegetatively propagated with a genome size of around 844 MB, which was recently sequenced (Potato Genome Sequencing Consortium, 2011). The ploidy levels vary in potato with different series, based on a haploid number of 12. Most wild tuber-bearing species range from diploid (2n=24) to hexaploid (6n=72) including triploids, tetraploids and pentaploids (Dodds, 1962). The cultivated potato Solanum tuberosum, is an auto-tetraploid, (2n=4x=48) and is believed to have originated as a hybrid between diploid members S. stentotomum and S. sparsipilum with subsequent chromosome doubling (Cribb and Hawkes, 1986).

Successful cultivated potato breeding is tricky and involves tremendous efforts due to tetraploidy and tetrasomic inheritance of the cultivated potato recessive alleles is difficult to track. Alleles occur in four dosages i.e. simplex, duplex, triplex, and quadruplex, instead of two in diploids (homozygous and
heterozygous). Pure lines are not obtained easily due to inbreeding depressions, after repeated selfing and multiple alleles generates a multitude of genotypes, that are difficult to distinguish based on comparing expected and observed genetic segregation ratios (Gebhardt, 2007). Dihaploids having 2n=2x=24 can be obtained from the cultivated tetraploids by parthenogenesis (Hermsen and Verdeniuis, 1973) or by anther culture and microspore culture (Dunwell and Sunderland, 1973; Powell and Uhrlig, 1987).

The available morphological data on potato species have been supplemented with data from cytology, serology, isozymes and several types of DNA data. The cytological data helped in acquiring more insight into the origin and distribution of polyploids in the group (Swaminathan and Howard, 1953), the serological data gave indications of interrelationships among groups of species but difficult to interpret (Lester, 1965) and the isozyme data provided valuable information mainly on diversity of the cultivated forms (Quiros and McHale, 1985; Douches and Quiros, 1988). DNA data are basically in one of the two types: restriction site and primer based data Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism, Simple Sequence Repeat (AFLP) and Simple Sequence Repeats (SSR).

DNA based Molecular markers propose a remarkable promise for potato plant breeding. Marker assisted selection may allow for indirect selection of genes that control traits of interest. Combined with traditional selection techniques, MAS has become a complementary tool in selecting for traits of interest, such as disease resistance and potato quality traits like tuber yield, starch content, starch yield and chip colour are important for industrial uses and food processing of potato (Lili et al., 2013). The chance to select desirable lines based on genotype rather than phenotype, analysing plants at the seedling stage, screening multiple characters, minimizing linkage drag and rapidly recovering a recurrent parent’s genotype can be traced through MAS (Collard and Mackill, 2008).

Potato cultivation is affected mostly by Pests and diseases worldwide, thus durability of disease and pest resistance is a principal goal of most potato breeding schemes. Classical breeding for resistance involves the identification
of resistance sources, which are mostly found in wild relatives of cultivated potato. Since 1990, many of these resistance factors have been located on the potato molecular linkage map using DNA-based markers. They have been mapped either as major genes (\(R\) genes) or as quantitative trait loci (Gebhardt and Valkonen, 2001; Simko, 2002; Tiwari \textit{et al.}, 2013). One of the greatest challenges in potato breeding, which could be supported by MAS, is obtaining potatoes resistant to late blight caused by \textit{Phytophthora infestans} (Mont.) de Bary. This fast-evolving oomycete pathogen is able to recombine sexually in major potato-producing areas, such as Europe (Cooke \textit{et al.}, 2008), and it infects potato foliage as well as tubers.

2.5 Late blight - \textbf{Phytophthora infestans} : (Mont.) De Bary

The genus \textit{phytophthora} belongs to family \textit{Pythiaceae} and class Oomycetes. Potato late blight caused by this fungus like organism., is infamous for having triggered the Great Irish Famine of 1845 to 1847, and ranks as one of the most devastating diseases in human history as it damaged potato crops in Europe leading to mass starvation, immigration from affected countries due to dependency of the poorer working population on potatoes as their source of food and presently continue to cause millions of dollars of loss annually word wide (Haverkort \textit{et al.}, 2008).

2.5.1 \textbf{Origin and diversity of P. Infestans}: The late blight pandemic that included the Irish Great Famine in the nineteenth century was caused by a single \textit{Phytophthora infestans} genotype, which is discrete but narrowly related to the most widespread genotype of the twentieth century. Diversity outside its Mexican center of origin was low until the late 1970s, and one set-up held that a single strain, US-1, had dominated the global population for 150 years; but the recent study by team of international group, suggests that nineteenth century epidemic was caused by a unique genotype, HERB-1, that persisted for over 50 years. HERB-1 is distinct from all examined modern strains, but it is a close relative of US-1, which replaced it outside Mexico in the twentieth century. HERB-1 and US-1 emerged from a metapopulation that was established in the early 1800s outside of the species' center of diversity (Yoshida \textit{et al.}, 2013).
This latest study concludes that the main genomic difference between the HERB-1 and US-1 lineages is the shift in ploidy, from diploid to triploid and even tetraploid. Consequently, if the major selection pressure that led to the substitution of HERB-1 by US-1 was the preface of resistance gene breeding, greater variation at effector genes in polyploid US-1 strains could have contributed to the replacement of HERB-1 soon after $R$ genes from $S. demissum$ and other wild species had been introduced into modern potato germplasm. The population of $P. infestans$ prevailed in the New World, USA and Canada by the US1 type until the 1980s, characterized by the A1 mating type and the Ib mtDNA haplotype (Goodwin et al., 1994b). A2 mating type of $P. infestans$ was first detected in potato fields in central Mexico in 1956 and remained restricted until 1980’s, when it was reported in many European countries (Drenth et al., 1993).

In India, only the A1 mating type existed predominantly until the late 1980s, and the isolates were susceptible to phenylamide fungicides. In India, Singh et al., (1998) and Singh and Shekhawat, (1999) studied the population dynamics of A1 and A2 types in North Western hills. A2 mating type was detected for the first time from Shimla hills in 1990, and observed constant increase in its population thereafter. Oospores formation was demonstrated in inoculated Indian potato fields by Singh et al., (2004). Late blight host resistance due to monogenic resistant genes in earlier Indian varieties (SLB series) which were introgressed from $Solanum demissum$ was broken down, likely due to new aggressive isolates with complex race patterns (Singh et al., 2005). The mating type analysis data recorded between 1996 and 2006 revealed that the A1 mating type prevailed (90%) in the subtropical Indo-Gangetic plains, whereas the A2 mating type was dominant (93%) in temperate highland regions (Chimote et al., 2010).

2.5.2 Disease symptoms: $P. infestans$ favours wet weather conditions with temperature above 20°C (60-80°F), high humidity (70-80%) and recurrent rainfall. The first visible symptom of the disease is the appearance of water soaked, light brown lesions on the leaf blade, within two to three days after infection. When environment conditions become congenial with humid and cloudy weather, these lesions spread fast over the entire leaflet and petiole.
Characteristic lesions are roughest near the edge or tip of the leaf with a water soaked appearance where the dew is retained longest. The lesions turn dark brown after some time, dry and brittle after the infected leaf tissues die. Whitish growth of the fungus can be seen; on the underside of the infected leaves, which becomes more evident under high humidity (heavy dew or rain). The lesions on the stem are regular black strips along the length of the stem, these can remain active even in dry weather conditions (Fig 2.1). Complete crop may become blighted and defoliation occurs within 2-3 weeks if remains unchecked. Potato tubers can be infected before or after the harvest.

**Fig 2.1:** *Phytophthora infestans* infected leaves : (A) Small dark circular to irregularly shaped lesions. (B) White cottony/velvety growth on the undersides of the leaves can be seen during extreme sporulation. (C) Entire leaves /stems may become blighted within days of initial infection. (D) Tuber skin may become discolored and dry granular rot develop under the discolored skin.

2.5.3 **Disease infection cycle of *P. infestans***: The asexual life cycle of *P. infestans* is characterized by alternating phases of hyphal growth, sporulation, sporangia germination, either through zoospore release or direct germination, i.e. germ tube emergence from the sporangium and the restoration of hyphal
growth. The type of germination is governed by environmental conditions, particularly temperature. The sporangia release zoospores at low temperatures 4-12°C but by hyphal outgrowth at higher temperatures 20-27°C (Harrison, 1992). Sporangia are spread by wind or water and enable the movement of *P. infestans* between different host plants. The zoospores released from sporangia are biflagellate and chemotactic, allowing further movement of *P. infestans* on water films found on leaves or soils. Both sporangia and zoospores are short-lived. There is also a sexual cycle, which occurs when isolates of opposite mating type (A1 and A2) meet. In response to hormones, the formation of the sexual spores is elicited called oospores containing diploid nucleus. Oosporan mature to form thick cyst that facilitate them to persist in a viable form for many years, survive in the soil for years. The germination of oospores leads to formation of A1 or A2 that can infect new tubers or stems that come to contact with the soil (Drenth et al., 1995). Pictorial representation of life cycle of *P. infestans* is show in below (Fig. 2.2).

![Fig 2.2 The disease cycle of late blight pathogen Phytophthora infestans (Agrios, 2005).](image-url)
2.5.4 Mechanism of *P. Infestans* for evading Resistance Host: 

*Protein Recognition:* This Oomycete pathogen secretes host translocated RXLR effectors which are pathogen molecules that alter host cell structure and function thereby facilitating infection and/or triggering defence responses which are targeted by resistance (R) proteins from wild Solanum species (Haas *et al.*, 2009; Vleeshouwers *et al.*, 2011). The *P. infestans* genome shows an unusual discontinuous distribution of gene density in which disease effector genes and other virulence factors are localized to repeat-rich and gene-sparse regions of the genome. These repeat rich, gene-sparse regions appear to promote evolutionary plasticity and enhance genetic variation of the subset of genes that determine pathogenicity and host-specificity. *P. infestans* has the capacity to reproduce sexually, a feature that is associated with increased genetic diversity and survival in many parts of the world.

Resistance to *P. infestans* occurs in many tuber-bearing wild Solanum species that belong to the highly diverse section *Petota dumort*, thought to be in central Mexico, from which species migrated southward and evolved in a separate gene pool in South America. *Solanum demissum*, a common hexaploid species in central Mexico, has been used in the earliest potato breeding efforts since the first half of the twentieth century. Eleven *S. demissum* resistance (R) genes designated *R1-R11* are distinguished in a potato differential set by Black and Mastenbroek. Most *Solanum* R genes appear to have co-evolved with *P. infestans* at its center of origin in central Mexico.

2.5.5 *The P. Infestans potato gene-for-gene interaction:* The gene-for-gene relationship was first conceptualised by Harold Henry Flor. She studied the genetics of both the host and parasite and described their integration into one genetic configuration (Flor, 1971). Race specific resistance is characterized by interactions between the products of dominant resistance (R) gene alleles in the host and matching avirulence (Avr) gene alleles in the pathogen called gene-for-gene hypothesis (Fig 2.3). The ‘guard hypothesis’ provides an intriguing conceptual framework for the action of disease effectors and the R-protein complex. It is postulated that specificity of recognition of R-proteins might lie in the hypervariable LRR regions.
Plants producing a specific R gene product are resistant towards a pathogen that produces the corresponding Avr gene product. This is a sophisticated defence mechanism adapted by the host plant against the pathogen to escape the biotic stress (Dangl and Jones, 2001). When R-Avr pair is present in both the host and the pathogen, the results is disease resistance or incompatibility. If either is inactive or absent the results is susceptibility or compatibility. The simplest model that accounts for this genetic interaction requires that R products recognize Avr-dependent signals and trigger the chain of signal-transduction events that culminates, in activation of defence mechanisms and an arrest of pathogen growth (Fig 2.4). The HR generally occurs as a rapid, localized necrosis, a form of programmed cell death. It follows perception by the plant of pathogen signal molecules (elicitors) encoded by avirulence (Avr) genes. Specific receptors, determined by R genes, interact directly or indirectly with elicitors, thus initiating signal transduction pathways that lead to the HR and the appearance of the disease resistance response.
Virulence Target

Susceptible response to favour pathogen growth and development

Host Infection (Disease Development)

(Compatible communication)

R-AVR interaction

Resistance Response (HR) (No Disease)

(Incompatible communication)

Fig 2.4: R-protein present in the resistant plant host, Guard the Virulence target against Avr effector protein in the pathogen.

2.6 Genetics of resistance to Phytophthora infestans:

Majority of disease resistance genes in potato are found in wild and cultivated relatives of potato that have immensely contributed in resistance breeding programmes. Genetic resistance to both wild and cultivated potato species may be of two forms, race specific (Black et al., 1953) that provides vertical resistance is monogenic, and qualitative thus provides narrow specific resistance. The second type of resistance is race non-specific i.e. field or partial resistance provides durable or horizontal resistance (Wastie, 1991), loci associated with such resistance are referred as quantitative resistance loci (QRL). Such type of resistance is polygenic and broad. In both the type of resistances Hypersensitive Response (HR) plays a significant role (Kamoun et. al., 1999a; Veeshouwers et al., 2000). It implies the occurrence of recognition during interaction of P. infestans and host cells. Passive resistance provide indirect protection such as waxy cuticular ‘skin’ layers and preformed antimicrobial compounds involved in the non specific resistance to P. Infestans.

2.6.1 Sources of Resistance: Resistance genes occur in wild potato (Solanum) species mostly originating from the pathogen centre of diversity in Mexico, and have been introduced by breeding into cultivated potato since the beginning of the twentieth century (Hawkes, 1990).
In 1909, Salaman recognised the Mexican wild species *S. demissum* as a source of extreme resistance that could be backcrossed into *S. tuberosum* (Muller and Black, 1953). This hexaploid (2n=6x=72) is most imperative and widely exploited as resistance genes source, since it can be directly introduced in to cultivated tetraploid *S. tuberosum* diploid tuber bearing species like *S. phureja* which can also be used as a bridge; for example cultivar Pentland Ace with resistance gene *R3* resulted from a cross made in 1937, at the Scottish Plant Breeding Station, this cultivar resulted from cross between *S. phureja* and *S. demissum*, and was released in 1951 after only three backcrosses to *S. tuberosum*. Another cultivar, Pentland Del, was introduced in 1963, having resistance genes *R1, R2* and *R3* with two further backcrosses. This cultivar was commercially more successful but then resistant broke down and soon race 4 was the prevalent race of *P. Infestans*.

Nevertheless, introgression of race-specific *R* genes into potato cultivars provided only transient resistance to late blight, as new races rapidly overcame the *R* gene-mediated resistance (Fry and Goodwin, 1992; Wastie, 1991). As a result, many breeders switched to selecting for quantitative field resistance by using races of *P. infestans* compatible with the *R*-genes present in their material or *R*-gene free germplasm was used so that screening could be done with any race (Toxopeus, 1964; Black, 1970; Wastie, 1991; Ortiz, 2001).

Compared with the enormous natural genetic diversity present in the wild relatives of the potato, merely a small number of these species have in fact been used for introgression of resistance traits into cultivars, because the undesirable "wild"traits were also introduced simultaneously with the resistance trait. Several generations of backcrossing and recurrent selection are usually required before suitable cultivars can be obtained from such resources (Gebhardt, 2001). Yet, most genes resistance present in modern potato varieties and breeding materials have been introgressed from closely related tuber-bearing Solanum species. e.g *Solanum bulbocastanum* (2n=2x=24) has valuable traits for potato breeding, but cannot be hybridized directly with *S. tuberosum* cultivars. Both *S. acaule* (2n=4x) and *S. phureja* (2n=2x) were used as bridging specie.
Triploid *S. acaule* × *S. bulbocastanum* progeny (F1) was crossed with *S. phureja*. triple hybrids obtained were tetraploid or nearly so, known as ABPT clone. (Hermesen and Ramanna, 1973) first potato cultivar released carrying genes from this clone was ‘Biogold’.

2.7 Late blight resistant (*R*) genes: Timeline update

Progress in molecular biology during the last two decades has allowed better zooming into chromosomes, subsequently, exact position of most of the genes within the set of *R1-R11* have been assigned. Till date, 21 *R* genes that confer differential resistance specificities to *P. infestans* isolates have been cloned from various Solanum species. A consortium of potato breeders and pathologists established a set of potato *R*-gene differentials that would allow for the detection of discriminate avirulence phenotypes. Eleven *S. demissum* resistance (*R*) genes designated *R1*-R11 are distinguished in a potato differential set (Black *et al.*, 1953; Malcolmson & Black, 1966). The international set of differentials has been maintained clonally and was amended until the late 1960’s (Eide *et al.*, 1959; Black & Malcolmson, 1965; Malcolmson, 1969). The *R* gene class predicted to encode receptors with coiled coil (CC), nucleotide binding site (NBS), and leucine-rich repeat (LRR) domains as explained in detail in the previous section (The *P. Infestans* potato gene-for-gene interaction). Corresponding *Avr* effector genes reside in sparse regions of gene in the *P. infestans*, these vary in size between isolates and are upregulated during early stages of the biotrophic interaction. These encode modular proteins having a signal peptide, an RXLR motif, and a C-terminal effector domain.

2.7.1 Details of major *R*-genes (*S. demissum*): Seven *Rpi* genes responsible for resistance within the set of *R1-R11* have been mapped: *R1* on chromosome 5, *R2* on chromosome 4, *R3a/R3b*, *R6/R7* on chromosome 11 and *R8* on chromosome IX. Further, four *R*-genes within this set have been cloned (*R1, R2, R3a, R3b*). Pathogen oomycete *P. infestans* secretes host-translocated RXLR effectors that include avirulence (*Avr*) proteins, which are targeted by these resistance (*R*) proteins from wild *Solanum* species. Here, we indexed *R-Avr* pairs (*Avr1, Avr2, Avr 3a* and *Avr 4*), characterized till date for *S. demissum*. 

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**R1-Gene**: R1 is located on potato chromosome V (Leonards-Schippers et al., 1992) the region contains quantitative trait loci (QTL) for resistance to late blight and the parasitic root cyst nematode *Globodera pallida* and monogenic resistance to potato virus X have also been mapped to the same region. R1 is the first gene to be cloned (Ballvora et al., 1992) for resistance to late blight by positional cloning with a candidate gene approach. It encodes a protein of 1293 amino acids which belongs to class of CC-NB-LRR. The C-terminal leucine-rich region is very short and consists of only 400 amino acids. The spacing of the leucine residues does not fit the consensus for leucine rich repeats. The molecular mass is of 149.4 kDa and R1 locus is around 10388 bp long. Potato cultivars have been introgressed with R1 but are currently classified as a narrow spectrum resistance gene as the majority of *P. infestans* isolates are virulent on R1 plants.

![R1-High-resolution map of the R1 Region: GP21 and GP179 were used to construct the map. SPUD237 and AFLP1 are converted AFLP markers these are 0.1 cm apart flanking R1. (De Jong et al., 1997; Meksem et al., 1995). R1 Locus is 10388 bp long. DNA (Reproduced from Ballvora et al 2002).](image)

**Avr1**: The Avr1 gene in *P. infestans* which encodes the cognate effectors of R1 was isolated using a positional cloning approach (Van-der Lee et al., 2001, Grunwald and Flier, 2005). Avr1 has a Resistance Host Protein Recognition (RXLR) motif in its N-terminal effector domain (Tyler, 2009). Avr1 resides in a
repeat-rich and expanded region of the *P. infestans* genome and the expression of *Avr1* is highly induced during the biotrophic phase of potato infection.

**R2-gene:** R2 belongs to highly diverse gene Family. It is located at a major late blight Resistance locus on chromosome IV of potato. The R2 family has a leucine-zipper (LZ)-NB-LRR structure proteins and homologs that confer resistance to *P. Infestans*. The size ranges from 844 to 847 amino acids. A number of R2 orthologs conferring resistance against *P. infestans* have been identified: R2, R2-like, Rpi-blb3, Rpi-abpt, Rpi-mcd1.1, Rpi-snk1.1, Rpi-snk1.2, Rpi-eden1.1, Rpi-hjit1.1, Rpihjit1.2, and Rpi-hjit1.3. R2 and R2-like genes were mapped by allele mining approach while Rpi-blb3 and Rpi-abpt by map-based cloning approach. The other R2 ortholog were identified by effectoromics screening of candidate R2 gene homologs (Champour et al., 2010).

The R2 gene homologs confer resistance to *P. Infestans* that have originated from diverse Solanum species like *S. demissum*, *Solanum bulbocastanum*, *Solanum hjertingii*, *Solanum edinense*, *Solanum schenckii*, and *Solanum microdontum*. R2 is a class I fast-evolving R gene. In the case of Rpi-blb3, Rpi-abpt, R2, and R2-like, the LRR domains are nearly identical despite significant polymorphism at the coiled-coil and NBS domains, suggesting conservation of function. In wild *Solanum* genotypes, up to four active R2 homologs are conserved which suggests that R2 is still functional to sustain resistance to late blight in the natural conditions of the host along with other prevalent R genes.

Structural analyses of these R2GHs (R2GH).revealed clear blocks of sequence exchange between paralogs. Broad spectrum resistance can be conferred by resistance gene homologs (R2GH): Rpi-blb3, Rpi-abpt, R2 and R2 like gene were cloned and functional characteristics of these genes were described. Length of R2 gene is nearly 12538 base pairs. Study of these genes reveals a contradictory finding, indicating conservation in the LRR domain but more variation in the NBS domain that may point toward different signaling pathways or additional effectors being recognized by these R proteins (Lokossou et al., 2009).
**Avr2** of *P. infestans* is a member of a highly diverse family having 18 RXLR effectors. PITG_22870 was the gene identified in the genome of *P. Infestans* strain T30-4 (Lokossou, 2009; Champouret, 2010) the PexRD11/ PITG_22870 effector family have 13 candidate effectors with four of these including PEXRD11 and PITG_22870, induce cell death when co-expressed with *R2*, *R2-like*, *Rpi-abpt*, or *Rpi-blb3*. A fifth member of the family has 6 additional *R2* homologs recognition factors. The other PexRD11/PITG_22870 family members are not recognized /mutated proteins or not expressed during infection.

**Fig 2.6:** Integrated genetic linkage map of the different late-blight resistance loci *Rpi-blb3, Rpi-abpt* (Park et al., 2005a), *R2-like* (Park et al., 2005b), and *R2* (Li et al., 1998) on chromosome 4. (Fig. Reproduced from Park et al 2005b).

**R3 gene Complex:** *R3* is a complex locus located on distal part of the short arm of chromosome 11. This is a primitive locus involved in plant innate immunity against oomycete and fungal pathogens. *R3* complex locus has evolved after divergence from corresponding *I2* locus of tomato and has experienced a major expansion in potato without commotion of the flanking regions colinearity.
The locus is a hot spot for number of $R$ genes having functional diversity possibly due to several gene duplications and co-evolved with its pathogen $P_{\text{infestans}}$. Constitutive expression was observed for the $R3a$ gene, and some of its paralogues whose functions remain unknown. Further, Fine-mapping and disease testing with specific $P_{\text{infestans}}$ isolates, allowed the detection of $R3$ locus being composed of two tightly linked $R$ genes, $R3a$ and $R3b$ which are 0.4 cM apart. These genes were separated as a result of recombination and recombinant plants showed different pathogen isolate-recognition specificities (Huang et al., 2004). The genomic region of $R3$ is loaded in functional diversity for $P_{\text{infestans}}$ resistance. High-resolution genetic map of $R3a$ and $R3b$ shows resolution of 0.05-cM. Two cleaved amplified polymorphic sequence (CAPS) markers, cLET5E4 and GP185 spans $R3a$ and two amplified fragment length polymorphism (AFLP) markers, PCAMATA_4 and EATCMAGC_15, across $R3b$, both defining an interval 0.25 cM, both these genes have been cloned. It consists of 3,849 bp (NCBI accession no. AY849382) the gene was cloned by Huang et al., (2005) and $R3b$ gene is about 10,5,207 bp long (NCBI accession version JF900492 EF638452 EF638453 EF638460 HQ437669 HQ437670 JF900492.1 GI: 334902908 (Li et al., 2011).

![Fig 2.7: Combined physical and genetic maps of the $R3a$ and $R3b$ region: Vertical arrows indicate the genetic positions of markers, disease resistance, and figures in between markers indicate the number of recombinants in between the markers. Gray, hatched lines indicate physical positions of markers in the contig. trans and in cis to $R3b$, respectively (Reproduced from Li et al 2011).]
Avr3a: Two alleles of Avr3a have been identified in P. infestans populations, these encode secreted proteins AVR3aK80/l103 (AVR3aKI) and AVR3aE80/M103 (AVR3aEM), the difference in two amino acids was found in their effector domains (Armstrong et al., 2005). The difference intensely affects host response: AVR3aKI triggers effector-triggered immunity by activating the potato resistance protein R3a (Hein et al., 2009, Huang et al., 2005). Both alleles of Avr3a are capable of suppressing the cell death response provoked by the P. infestans-secreted protein INF1. Avr3a suppress immunity when bound to the host E3 ubiquitin ligase CMPG1. Avr3aEM arose from an Avr allele after gene duplication and positive selection. It is found that Avr3a is essential for complete virulence of P. infestans (Bos et. al., 2010).

Avr3b: the genetic studied reveals the matching avirulence in the P. Infestans to R3b. Avr3b was identified by effector PBHR_490 by candidate screening approach (Rietman, 2011). The Avr3b-Avr10-Avr11 locus resides in a sub telomeric region. The study concludes that Avr3b, Avr10 and Avr11 co-localised on linkage group VIII (Vander Lee, et al., 2001; Jiang, et al., 2006).

R4 gene: This resistance gene is fourth dominant gene derived from of S. demissum. It is positioned at long arm of chromosome XI (Van Poppel, 2010; Verzaux, 2010). R4 Gene has not been cloned, thus decoding functional annotation to this gene is still limited. However, R4 gene is linked to a molecular genetic marker sharing sequence homology to the Rx gene, which confers resistance to PVX (Bendahman, 1999). Thus, this suggests that R4 resides in an R gene cluster containing sequences similar to Rx- on chromosome XI. This is also narrow spectrum R gene and is less significant in resistance breeding.

Avr4: Avr4 of P. infestans corresponding to R4 gene in host. it has been cloned using map-based cloning and cDNA-AFLP (Amplified Fragment Length Polymorphism)-based profiling (Guo, 2006; Van Poppel, 2008). Avr4 consists of effector protein consisting of 287 amino acids. The gene is a single copy that resides in an approximately 100-kb expanded repeat-rich region of the P. infestans genome (Haas, 2009). Avr4 is highly polymorphic in P. infestans. Strains virulent races have allelic variants that have deletions leading to early stop codon that result in truncated region with non functional proteins. The
strains that carry a full-length copy Avr4 are avirulent on plants carrying R4. Fascinatingly, nonsense or early termination mutations of Avr4 does not effect virulence of *P. infestans*, that supports evolving of the virulent races with high frequency (Van Poppel, 2008). Hence, Avr4 is dispensable effector that conquers resistance through pseudogenization of the full-length gene. *R* gene proteins that recognize such dispensable effectors cannot provide durable resistance thus not considered as potential targets in resistance breeding programs.

**R6 and R7 genes:** R6 and R7 are mapped to distal arm of chromosome XI as the *R3* (El-Kharbotly *et al.*, 1996). Markers that are linked to *R3* are also linked to *R6* and *R7* markers. RFLP marker loci GP 185 (a) and GP 250 (a) which resides in the vicinity of *R3* locus (El-Kharbotly *et al.*, 1994) were found linked to *R6* and *R7* as well. *R7* was separated by one by one recombination event from marker loci GP 185 (a) and GP 250(a). *R6* was separated from from the same markers by ten recombination events so exact localisation reveals that *R7* was closely linked with these markers whereas, *R6* was separated from the same marker loci by 12 Cm.

![Fig. 2.8: Map position of R6 and R7 genes on potato chromosome XI. Markers GP 185 (a) and GP 250(a) are closely linked to these genes (Fig. reproduced from El Kharbotly et al., 1996).](image-url)
**R8 gene**: R8 gene was reported to be localised on chromosome XI (Huang et al., 2005) along with R3, R10 and R11 complex. However, recent studies suggests otherwise. The genetic mapping of the R8 late blight resistance gene was done from the differential clone MaR8., NBS profiling strategy following marker sequence assessment to the potato and tomato genome draft sequences, now suggests that the R8 gene is located on the long arm of chromosome IX and not on the short arm of chromosome XI (Jo et al., 2011). Gene cluster directed profiling markers CDPSw54 and CDPSw55 flanked the R8 gene at the distal end (1cM). CDPTm21-1, CDPTm21-2 and CDPTm22 flanked the R8 gene on the proximal side (2 cM). Exact localization of R8 gene is essentially required in further studies for sequencing and integrating this gene in breeding programme.

![Diagram of R8 gene localization](image)

**Fig. 2.9**: Positions of NBS profiling markers and R8 gene on chromosome 9. Markers in large font indicate the NBS profiling markers that were linked to R8. Marker TG328 displays an informative SCAR type polymorphism. (Fig. reproduced from Jo et al., 2011).

**R5, R9, R10 and R11 genes**: R5, R9, R10 and R11 are the allelic versions of the R3 complex locus studied by marker analysis and mapping results (Huang et al., 2005). R10 and R11 were mapped in the original R-gene differentials of Black namely, tetraploid clones 3681ad1 and 5008ab6. Mapping them in these clones revealed these to be associated with quantitative and qualitative traits, through their linkage to molecular markers. R10 contrasting to R11, behaves more like a QTL than a major dominant R-gene (Bradshaw, 2006). Further
work has been carried forward and very recent studies have demonstrated high-resolution map of \textit{R10} and the gene was delimited to a genetic interval of 0.26 cM in the map of chromosome 11. The clustering of the qualitative gene \textit{R10} with resistance QTLs could be explained with high field resistances. Thus, it is now concluded that potato \textit{R10} resistance specificity to late blight is conferred by both a single dominant \textit{R} gene and quantitative trait loci (Xu \textit{et al} 2013).

\textbf{Fig. 2.10:} High Resolution genetic map of \textit{R10} gene. Markers and \textit{R10} are shown on the right side. Genetic position is represented by centi Morgon (cM). The centromic side is shown by ‘Cen’ and Telomeric side is shown by ‘Tel’ (Fig. reproduced from Xu \textit{et al} 2013).
<table>
<thead>
<tr>
<th>Year</th>
<th>Genes</th>
<th>CH*</th>
<th>Species of origin</th>
<th>Reference</th>
</tr>
</thead>
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<tr>
<td>2002</td>
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<td>V</td>
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<td>Ballvora et al., 2002</td>
</tr>
<tr>
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<td>RB</td>
<td>VIII</td>
<td>S. bulbocastanum</td>
<td>Song et al., 2003</td>
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<tr>
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<td>Vossen et al., 2003</td>
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<td>XI</td>
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</tr>
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<td>2005</td>
<td>Rpi-b1b2</td>
<td>VI</td>
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<td>VIII</td>
<td>S. stoloniferum</td>
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<td>Li et al., 20011</td>
</tr>
</tbody>
</table>

* Chromosome position identified.
2.8 Pyramiding major potato late blight resistance genes for durable resistance

2.8.1 An overview:

The continuing worldwide attempts to breed different crop varieties for durable resistance so far have had the modest effect. Pyramiding is the accumulation of R-genes in to single host cultivar that is possible by combining different R-genes, defeated \( R_{pi} \) genes or alleles of single gene i.e. the allele dosage.

The effect of pyramiding several genes in to one cultivar has been studied by different groups on diverse crops with varied results. Higher level of resistance was reported in crops like cereal cyst nematodes in wheat when \( CreX \) and \( Cre\ Y \) genes were pyramided (Barloy et al., 2007). Bacterial blight resistance genes in rice have been reported to show increased level of resistance (Huang et al., 1997; Singh et al., 2001; Yoshimura et al., 1995; Zhang et al. 2006). However, the study through marker assisted selection of the brown plant hopper resistance genes \( Bph1 \) and \( Bph2 \) showed resistance level of the pyramided line equivalent to that of the \( Bph1\)-single introgression line (Sharma et al., 2004).

Breeding for resistance in potato through pyramiding started long time, but exact quantification of the resistance effect was not reported. Pentland dell, Escorts were some early potato cultivars developed through such breeding strategies. Effect of allele dosage was studied in \( P.\ infestans \) resistance in duplex \( R3 \) or triplex \( R3 \) genotypes compared to simplex \( R3 \) genotypes but no additive effect was observed due to allele dosage (Toxopeus, 1957). Further \textit{Phytophthora infestans} resistance genes \( RPi-mcd1 \) and \( RPi-ber \) from the wild tuber-bearing potato species \textit{Solanum microdontum} and \textit{S. berthaultii} was studied in a diploid \textit{S. tuberosum} population suggesting an additive effect of stacking these genes, irrespective of the weak or strong effect of individual genes (Tan, 2010). ‘Sarpo Mira’ a potato cultivars containing a natural pyramid of \( R \)-genes with at least five different \( R \) genes such as \( R3a, R3b, R4, \) and \( Rpi-Smira1, \) and \( Rpi-Smira2, \) have also been introduced with enhanced level of resistance (Kim et al. 2011; White and Shaw, 2010).
2.8.2 Biotechnological Approaches for Pyramiding Resistance Genes:

2.8.2.1 *Transgenic or cisgenic*: Several novel breeding approaches may change the present scenario around the globe to develop resistant potato varieties with durable resistance through biotechnological approaches like production of genetically modified potatoes to speed up the solution of the late blight problem either using transgenes or more preferably genetic modification with cisgenes (Jacobsen and Schouten, 2007).

Cisgenes are defined as natural indigenous potato genes or those from crossable species that are or can be used in existing breeding programmes with which potato can make natural crosses. They have their own promoters and terminators in a normal orientation. It is marker-free pyramiding of several resistance genes and their spatial and temporal deployment yielding noble varieties that contain resistant potato genes. The approach involves in potato only the use of cisgenic *R*-genes in the absence of antibiotic resistance genes as selection marker. Cross between resistant species and a susceptible is made and the progeny segregates into two distinct groups of susceptible and resistant clones. The resistant clones must have one (qualitative) resistance gene (*R* gene) responsible for the development of a *Phytophthora* resistance.

The selection marker containing plants are only used to quickly assess the best possible combination of *R* genes, after which these genes are introduced into the desired potato variety by a marker free approach (De Vetten *et al.*, 2003) In this activity, the plantlets or their leaves are subjected to known races of late blight and the resistant ones are also subjected to a molecular technique (PCR) to make sure that the sequenced gene is actually present. The other way around is first PCR-selection followed by testing for resistance. This way no marker gene with e.g., antibiotic (such as kanamycine) resistance is needed to prove the presence of the desired gene. Individual plantlets of the new resistant genotype are then allowed to grow into a plant as the original variety.

In this GM-approach at least 15 years are saved as backcrossing and selection programmes to get rid of the linkage drag. Cisgenesis is being considered as even safer than conventional breeding because it prevents
introduction of genes via linkage drag which could lead to all kinds of unwanted trait that are not required. One recent study with this approach was studied using three broad spectrum potato R genes (Rpi), Rpi-sto1 (Solanum stoloniferum), Rpi-vnt1.1 (S. venturii) and Rpi-b1b3 (S. bulbocastanum) were selected, pooled into a binary vector pBINPLUS and transformed into the susceptible cultivar Desiree (Zhu et al., 2012). Developing cisgenic potato varieties may be a significant way to breach the worldwide public criticism/ban on GM-crops and introduction into the environment of GM-plants and the release of GM-varieties in public domain.

2.8.2.2 Marker-assisted pyramiding of R-Genes: As we know that, Pyramiding is the process of combining several genes together into a single genotype that may be possible through conventional breeding but it is usually not easy to identify the plants containing more than one gene. DNA markers have enormous probability to advance the efficiency and precision of conventional plant breeding via marker-assisted selection (MAS). Marker assisted selection is the practice of using the results of DNA markers linked that are located very close to major genes of interest in the selection of individuals to become parents for the next generations. DNA testing information combined with the phenotypic selections/performance is expected to improve the accuracy of selection and increase the possibility of identifying organisms carrying desirable and undesirable traits at an early stage of development.

The Study undertaken here is an attempt to stack or pyramid R-genes. DNA marker assays are safe and markers for multiple specific genes can be tested with a single DNA sample without phenotyping numerous genes collectively into a single genotype through marker assisted selection. The purpose for this has been the development of ‘durable’ disease resistance since pathogens frequently overcome single-gene host resistance over time due to the emergence of new plant pathogen races. In the past, it has been difficult to pyramid multiple resistance genes because they generally show the same phenotype, necessitating a progeny test to determine which plants possess more than one gene. With linked DNA markers, the number of resistance genes in any plant can be easily determined.

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