Genomics in Management and Genetic Enhancement of Potato Germplasm

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Abstract

The systematic characterization and utilization of naturally occurring genetic variation in the plant genetic resources have become an important approach in plant genome research and breeding. The development of molecular techniques now allows a more accurate analysis of a large collections of potato germplasm. The rapid progress in high-throughput technology such as next-generation sequencing (NGS) offers an exciting tool for novel gene discovery involved in phenotypic traits expression. In the years to come, genomics, transcriptomics and other 'omics' technologies will play a key role in potato improvement. The discovery and high-throughput screening of single nucleotide polymorphism (SNP), the presence/absence of allelic variations in diverse germplasm collections will give a detailed insight into the origin, domestication and available trait-relevant variations in the polyploid crops such as potato. In the process, novel approaches and possibilities for marker/genomics-assisted potato breeding are facilitated. This chapter highlights the use of potato genome sequence in management and the genetic enhancement of the potato through its characterization and identification of novel gene/QTL/allele followed by their applications in potato improvement with agricultural relevance.

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8.1 Introduction

Potato is the fourth most important food crop of the world, after rice, wheat and maize with record global annual production of 381.68 million tonnes (MT) from a 19.09 million hectare area in 2014. China is the largest potato producer, followed by India (95.51 and 46.39 MT, respectively). However, potato yield has been

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erratic across the world during 2000–2014, ranging between 16.3 and 19.98 t/ha, though showing an overall slight increase (FAOSTAT 2014). A summary of worldwide statistics of area, production and productivity of potato is outlined in Table 8.1. Given the pivotal role of the potato, the United Nations declared the year 2008 as 'International Year of the Potato' (IYP). Besides, it is also known as the 'Hidden Treasure of the Andes' and identified as the 'Food for the Future' by the Food and Agriculture Organization. There is an imperative need to use the available vast genetic diversity of potato gene pools for the genetic enhancement of the crop to ensure food and nutritional security in the years to come.

The main cultivated potato *Solanum tuberosum* L. is a tetraploid ($2n = 4x = 48$), is highly heterozygous, suffers acute inbreeding depression, and is susceptible to many pests and diseases. Together, these traits raise problems for potato improvement in classical breeding methods. Therefore, a challenge to the scientific community was to decipher the whole genome sequence of potato that would ultimately augment genomics-assisted potato breeding. To overcome this issue, researchers across the world successfully sequenced the potato genome sequence at an international platform by the Potato Genome Sequence Consortium (PGSC). The PGSC discovered the potato genome (844 Mb) and predicted 39,031 protein-coding genes regulating various growth and development in the potato crop (Xu et al. 2011; http://www.potatogenome.net). The first potato genome sequence data provides a basic platform for the genetic improvement of potato.

Potato belongs to the genus *Solanum* which contains ~2000 species, of which ~235 are tuber-bearing, that varies from $2x$ (73%) to $6x$ (6%) (Hawkes 1990). Potato gene pools include four types of germplasm: (1) cultivated potato (*Solanum tuberosum* subsp. *tuberosum*); (2) native potatoes occurring in the centre of diversity (7–12 species); (3) wild species (180–200 species); and (4) other germplasm or research material, genetic stocks, etc. (FAO 2010). Moreover, cultivated potato (*Solanum tuberosum* L.) has two subspecies; (1) *Solanum tuberosum* subsp. a*ndigena* is adapted to short days and widely distributed in the Andean regions of Venezuela and northern Argentina; and (2) *Solanum tuberosum* subsp. *tuberosum* is adapted to long days and occurs in southern Chile. In South America, the native primitive cultivated potato species are *S. juzepczukii* ($3x$),

Table 8.1 Statistics of area, production and productivity of potato in the world

SN	Country	Production (million tonnes)	Area (million ha)	Yield (tonne/ha)
1.	China	95.51	5.64	16.92
2.	India	46.39	2.02	22.92
3.	Russian Federation	31.50	2.10	14.99
4.	USA	20.05	0.42	47.15
5.	Germany	11.60	0.24	47.41
6.	Ukraine	23.69	1.34	17.64
7.	Poland	7.68	0.27	27.76
8.	France	8.08	0.16	47.97
9.	Netherlands	7.10	0.15	45.66
10.	Bangladesh	8.95	0.46	19.38
11.	United Kingdom	5.91	0.14	41.92
12.	Iran	4.71	0.15	29.67
13.	Australia	1.17	0.03	39.70
World total		381.68	19.09	19.98

Source FAOSTAT (2014)

S. chaucha (3*x*), *S. stenotonum* (2*x*), *S. ajanhuiri* (2*x*), *S. goniocalyx* (2*x*), *S. curtilobum* (5*x*), *S. phureja* (2*x*) and *S. tuberosum* subsp. *andigena* (4*x*) (Hawkes 1990).

Conservation and utilization of crop genetic diversity are essential to improve the productivity, sustainability, and nutritional quality in the changing climates, pests, pathogens, and consumer demands. Global potato gene banks exist to conserve the diversity of the cultivated and the wild *Solanum* species. Farmers in the crop's centre of origin and diversity still maintain hundreds of native potatoes and thereby actively contribute to the ongoing in situ conservation and evolution of the cultivated potato. Globally, about 98,000 accessions can be found ex situ (in vitro conservation), 80% of which are maintained in 30 key gene bank collections. Accessions are conserved as botanical seeds or vegetatively as tubers and in vitro plantlets. Latin American collections contain many native cultivars and wild relatives and the collections in Europe and North America contain modern cultivars and breeding materials, as well as wild relatives (FAO 2010). The largest collection of the potato germplasm is maintained by the International Potato Centre (CIP), in Lima in Peru, followed by other gene banks such as the Dutch-German Potato Collection, the Centre for Genetic Resources (CGN), The Netherlands; the Commonwealth Potato Collection, Dundee, Scotland, United Kingdom; the NRSP-6, United States Potato Genebank, USA, and many others.

Solanum germplasm represents a huge diverse gene pool source, however, only a very small amount of the available biodiversity has been exploited in the potato breeding. Attempts to document and characterize in situ collections of *Solanum* species diversity are needed as a baseline for future research. However, breeders prefer to use well-adapted germplasm or research materials of cultivated potato with interesting traits. Therefore, tapping the rich genetic diversity in a *Solanum* species and their wild relatives is a prerequisite for potato improvement in the future. Hence, modern biotechnological tools must be developed and exploited to accelerate breeding by accessing available genetic diversity stored in the gene banks around the world.

Recent developments in the potato genome sequencing and high-throughput molecular marker technologies have great potential for genetic enhancement of potato germplasm. Plant genomics researchers have readily embraced bioinformatics and molecular approaches to generate genome, transcriptome and epigenome datasets for crop species, though ploidy and heterozygosity together have been a challenge. The ability to generate de novo transcriptome assemblies provides an alternative approach to bypass these complex genomes and to access the target genes (Hamilton and Buell 2012). Moreover, at present, omics approaches such as transcriptomics and metabolomics are used the world over not only for basic research to understand the relationships between important traits and metabolism but also to develop the next generation of breeding strategies of crop plants. In this chapter, we highlight the impact of the potato genome sequence data on management and genetic enhancement of potato using next-generation sequencing technologies and other genomics-based novel approaches.

8.2 Use of Genomics in Gene Bank Management and Genetic Diversity Analysis of *Solanum* Germplasm

Only a small fraction of the naturally occurring genetic diversity available in the worldwide gene banks has been explored to date, but now it is expected to rapidly increase with the advent of high-throughput genotyping and sequencing technologies. It is imperative that the systematic genotyping of gene bank accessions will be more effective to study potato crop biodiversity. Large-scale genotyping and targeted re-sequencing have the potential to significantly advance the conservation, characterization, and utilization of the potato genetic resources. This approach of germplasm exploration will discover the genetic potential of underutilized resources,

examine genome-wide natural variation and make predictions about novel genes/alleles linked with phenotypes. Genomics information would also enable germplasm curators to monitor how their germplasm is being used in research and breeding.

The size of germplasm collections continues to increase, often through the uncontrolled duplication of materials in different gene banks. As their size increases, there is increasing pressure to reduce the size of collections by eliminating 'duplicate samples' to reduce redundancy. One objective of a gene bank is to support the broadening of the diversity in commercial crop varieties through providing ready access to novel and useful genetic variation. Until recently, amplified fragment length polymorphism (AFLP) or simple sequence repeat (SSR) were the molecular markers of choice for genotyping of crop genomes. High-throughput genotyping and sequencing can be used to quantify levels of genetic variation among individuals within an accession as well as between accessions (McCouch et al. 2012). Moreover, due to the amenability of systematic development and quick detection, single nucleotide polymorphism (SNP) molecular markers are abundant and distributed enough in a whole genome to distinguish individuals in a population. SNP markers are increasingly being applied to study genetic diversity in germplasm collections of thousands of accessions. Researchers at the International Rice Research Institute (IRRI) have used 384-SNP assays based on the rice genome for the classification of rice genotypes (Thomson et al. 2012). The development of an Infinium 8.3 K SNP Potato Array of the Solanaceae Coordinated Agricultural Project (SolCAP) (http://solcap.msu.edu) has shown its relevance in the characterization of a core collection. The potato core collection of 48 genotypes, representing a collection of 350 tetraploid potatoes, was defined in order to maximize the allelic diversity by SolCAP SNP array (Esnault et al. 2012). The recent discovery of 20 K SNP array allows population structure analysis, breeding uses and many other biotechnological applications in potato improvement (Vos et al. 2015). SNP markers are useful in the detection of the genetic integrity of crop plants conserved in gene banks. The germplasm of 20 maize landrace accessions was investigated for genetic integrity using 1150 SNPs and 235 SNP haplotypes (Wen et al. 2011). Many of the germplasm accessions have been established as association panels for linkage disequilibrium (LD) mapping to provide linkage between phenotypic and genotypic data. As potato is a clonally propagated crop, the maintenance of great numbers of germplasm accessions make comprehensive and impractical to maintain accurate descriptions of in vitro plants. So in the clonal regeneration, one of the most crucial concerns of curators is to retain genetic stability of in vitro propagating material using molecular markers (Tiwari et al. 2013a, 2013d). Second, to retain most of the genetic diversity of the original collection, a core collection of the Andigena potato has been constructed based on morpho-agronomic traits and genotyped by 24 SSR markers for their efficient exploitation of genetic resources (Tiwari et al. 2013e). Robust molecular markers linked to disease resistance have been developed and validated for distinctness, uniformity and stability (DUS) testing in tomato (Arens et al. 2010). A set of SNP markers has been designed and applied for cultivar identification in *Capsicum* (Jung et al. 2010). Thus, different marker type of choice and number based on genome sequence data could be used on a collection of *Solanum* germplasm for the molecular characterization of interspecific somatic hybrids (Chandel et al. 2015; Sarkar et al. 2011; Tiwari et al. 2010), cytoplasm type/organelle genome analysis (Tiwari et al. 2014, 2016), genetic diversity (Tiwari et al. 2013b, 2013c, 2013e), to evaluate the breeding potential of somatic hybrids (Luthra et al. 2016), for population structure and many more studies. An overview of late blight-resistant wild potato species is shown in Fig. 8.1. A dendrogram showing the SSR markers-based molecular diversity among the wild potato species, based on the Jaccard similarity coefficient by the UPGMA method is depicted in Fig. 8.2. Further, genetic enhancement of potato germplasm through somatic hybridization has been achieved (discussed in Chap. 13 in this volume) and

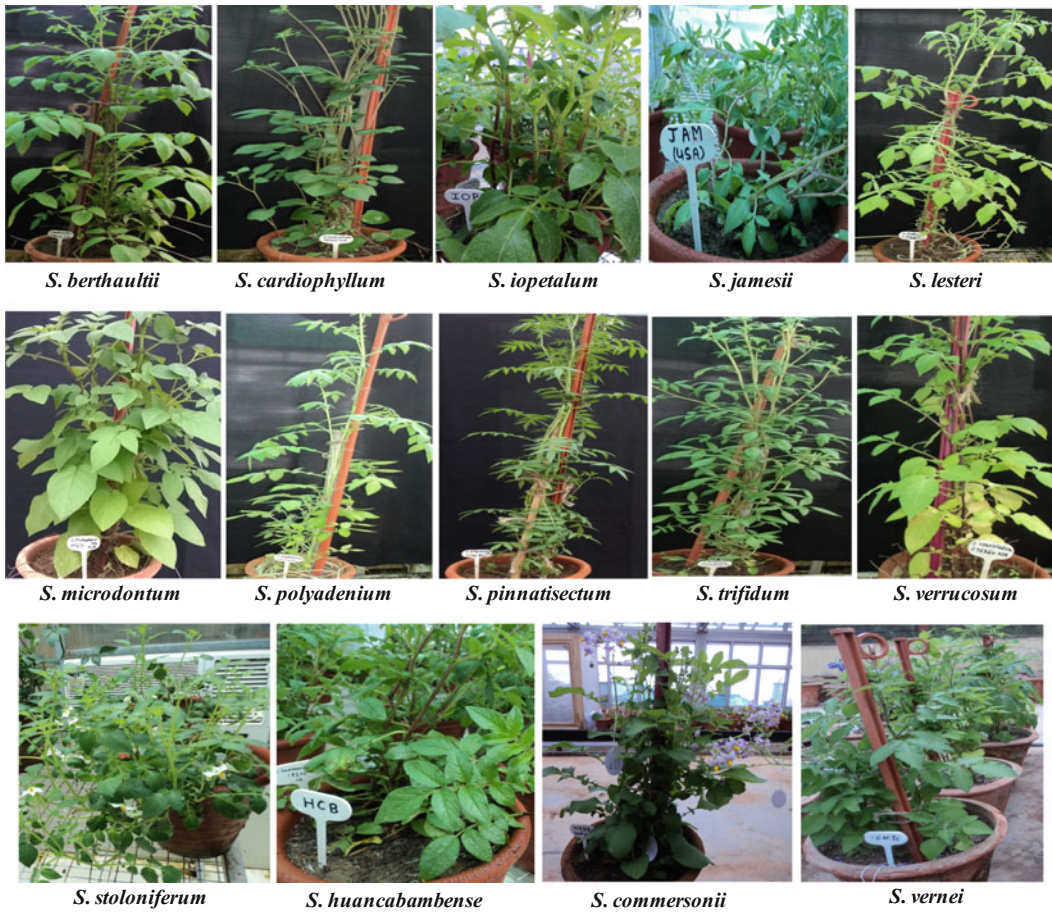

Fig. 8.1 An overview of late blight-resistant potato wild species except *S. commersonii* and *S. vernei*

net-house views of a few somatic hybrids are shown in Figs. 8.3 and 8.4.

Large-scale genomics experiments now are getting underway to characterize wild and landrace resources. Intensive genetic and phenotypic characterization of genetic resources by high-throughput sequencing techniques will increasingly become important. Potato gene banks have to prepare for enriching the genomic era by developing new strategies and novel information tools to assess the genetic diversity of their collections. This effort involves identifying combinations of alleles and regions of the genome responsible for variation in elite genetic backgrounds of interest. The integration of genomic data into gene bank documentation systems and its combination with taxonomic, phenotypic and ecological data will usher in a new era in the potato genetic resources. From the determination of phenotypic traits to the application of NGS to whole genomes, this process will have great impact on both conservation of *Solanum* germplasm and its utilization in potato breeding (Kalian and Graner 2012).

8.3 Genome-Wide Characterization, Mapping, Gene and SNP Discovery in *Solanum* Germplasm Using NGS Technology

The most promising potential to increase the use of potato germplasm genes is the advances in the field of genomics. While introgression was not easily detectable with the genetic tools earlier,

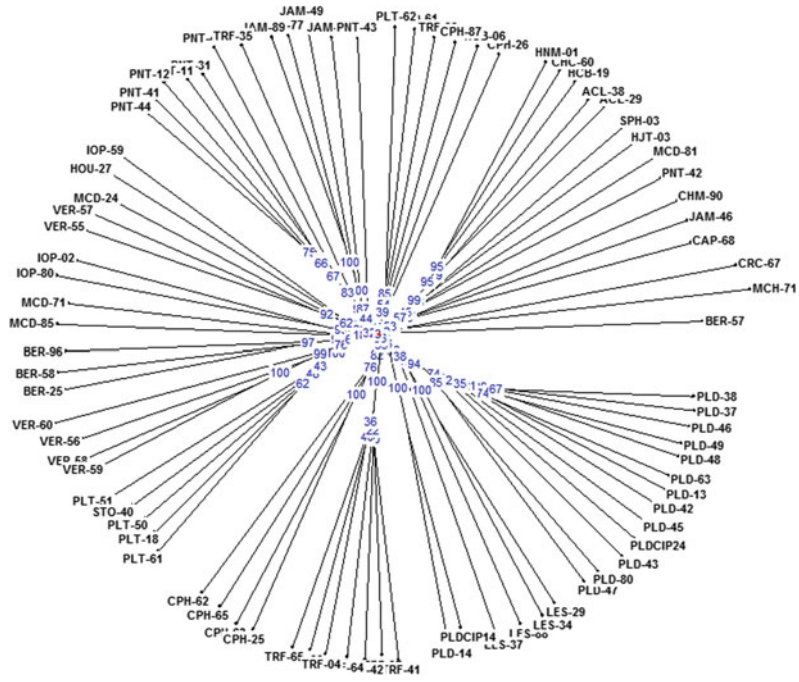

Fig. 8.2 Dendrogram showing SSR markers-based molecular diversity among the wild potato species, constructed based on the Jaccard similarity coefficient by the UPGMA method

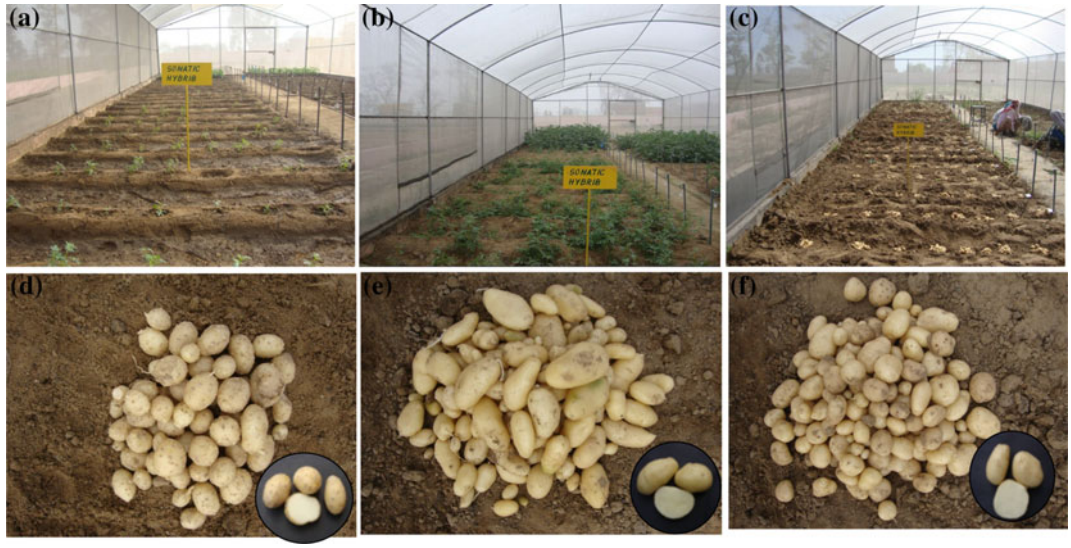

Fig. 8.3 Net-house view of potato somatic hybrids C-13 (+) *S. pinnatisectum* grown under the sub-tropical plains of Modipuram, Uttar Pradesh, India (**a**, **b**, **c**); *S. pinnatisecturm* (**d**), dihaploid C-13 (**e**), and somatic hybrid P-8 (**f**)

the recent discovery of molecular markers and next-generation sequencing (NGS) technologies has helped in isolating beneficial genes which are difficult to detect based on phenotypes. NGS technologies have enormous power and potential to access the complex polyploid genomes of

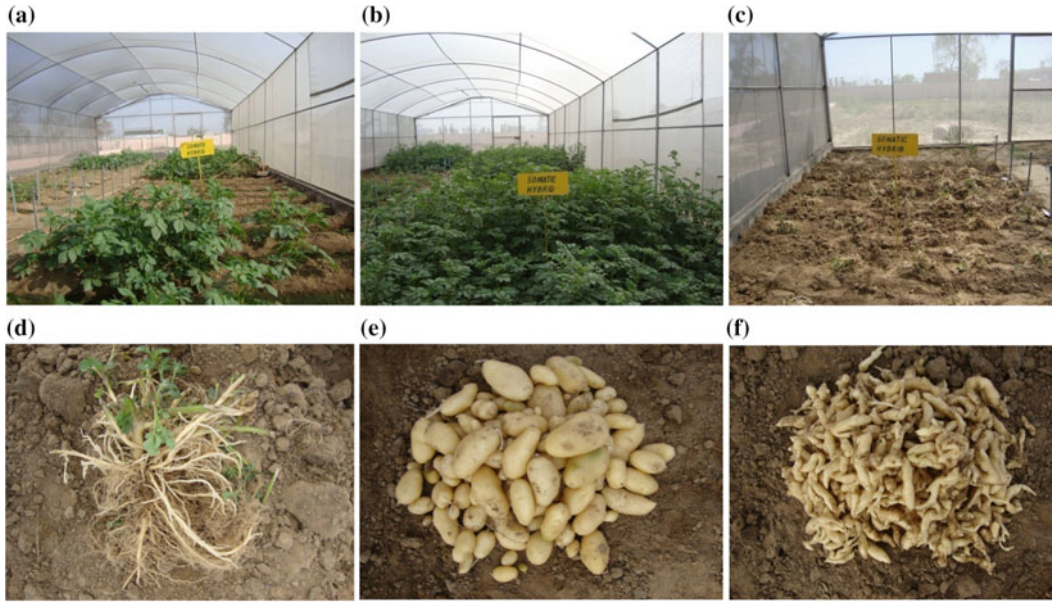

Fig. 8.4 Net-house view of potato somatic hybrids, C-13 (+) *S. etuberosum* grown under the sub-tropical Modipuram conditions. Net-house view (**a**, **b**, **c**); *S. etuberosum* (**d**), C-13 (**e**) and somatic hybrid Etb6-2 (**f**)

many crops that offer high density markers. Tracking of genetic variation has become so efficient and precise that thousands of genes can be identified within large gene bank collections. Using NGS technologies, it is possible to re-sequence candidate genes, entire transcriptomes or entire plant genomes more efficiently and economically than ever before. Advances in sequencing technologies will allow for whole-genome re-sequencing of hundreds of individuals. Discovery and screening of genome-wide SNPs are no longer a bottleneck even in polyploidy genomes, opening the way for the application of high-resolution association genetics and genomic selection approaches to crop improvement (Edwards et al. 2013). In this way, information on thousands of genes can be harnessed to analyse genetic diversity, perform linkage mapping, identify individual genes and determine their functional diversity. A gene underlying a major effect quantitative trait locus for plant maturity and initiation of tuber development has been discovered recently by exploring naturally occurring allele diversity that allows potato cultivation in northern latitudes (Kloosterman et al. 2013).

8.3.1 Allele Mining

Allele mining is a research field aimed at identifying such allelic variation for a known gene within germplasm collections for breeder uses that have been left behind during the domestication of the process. These resources stored in gene banks remain unexplored due to lack of efficient strategies to detect target alleles. Germplasm collections may be screened for allelic variation to identify genes of known function and their DNA sequence. The most effective strategies to detect allelic richness at a given locus are determined through the DNA sequence. Allele mining facilitates the discovery of novel resistance genes that can be used in breeding programmes. In allele mining studies, allelic variation is analysed for the identified genes whose function and basic DNA sequence are known and whose map position in the genome will generally have been determined. Sequencing of candidate genes has been applied to study phylogenetic and evolutionary process of crop species. Allele mining in *Solanum* species identified conserved homologous types of *Rpi-blb1* in *Solanum stoloniferum* (Wang et al. 2008) and

many other wild *Solanum* species (Tiwari et al. 2015a). The primary targets of allele mining efforts are the loci of agronomic importance. The germplasm utilized for allele mining should contain maximum allelic variation at the loci of interest, in the smallest possible number of samples (Reeves et al. 2012). Using an allele mining approach in potato, Pankin et al. (2011) analysed wild *Solanum* species for homologues of *RB/Rpi-blb1* gene conferring durable late blight resistance. A cluster analysis based on the Neighbor-Joining coefficient using the UPGMA method between DNA sequences of the 17 new resistance gene analogues (RGAs) isolated from the wild potato species and the reference sequences of late blight resistance genes is shown in Fig. 8.5. In our study, selected late blight-resistant wild species *S. berthaultii*, *S. cardiophyllum*, *S. iopetalum*, *S. jamesii*, *S. lesteri*, *S. microdontum*, *S. pinnatisectum*, *S. polyadenium*, *S. polytrichon*, *S. trifidum* and *S. verrucosum* were PCR amplified using gene-specific primers, gel eluted, cloned and sequenced analyzed for homology of potato R genes in the wild species. The potato R genes (*Rpi-pta1*, *Rpi-edn1.1*, EDNR2GH7, *Rpi-hjt1.1*, SNKR2GH5, *Rpi-snk1.1*, *Rpi-blb1*/RB, *Rpi-vnt1.1*, *Rpi-bt1*, *Rpi-sto1*, *Rpi-blb2*, RGA1, RGA3 and RGA4) were used for allele mining in these wild species. The successfully isolated and

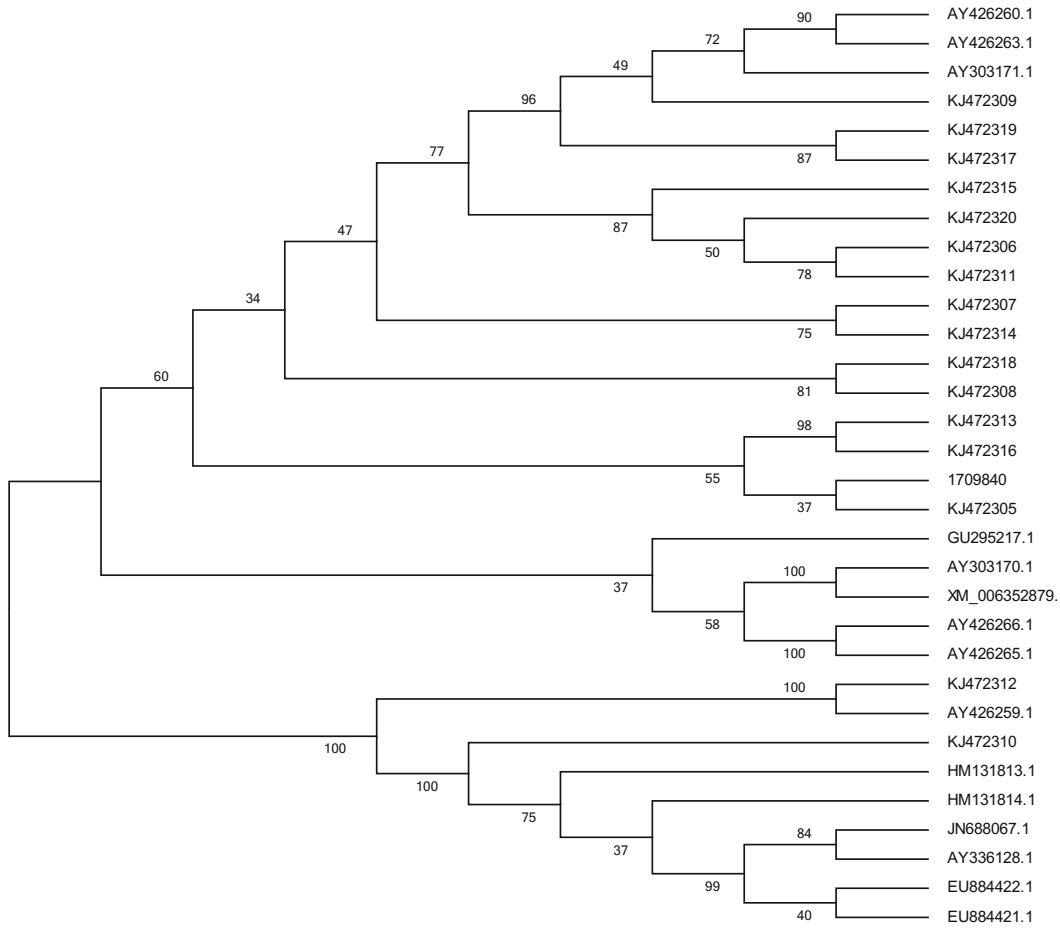

Fig. 8.5 A cluster analysis based on the Neighbor-Joining coefficient by UPGMA method between DNA sequences of the 17 new resistance gene analogues (RGAs) isolated from the wild potato species and the reference sequences of late blight resistance genes

Table 8.2 Summary of R gene homologues isolated from the wild potato species

SN	Isolated R gene homologues	Wild species	Potato R gene (NCBI Acc. No.)
1.	PTA1_CPH62	*S. cardiophyllum*	*Rpi-pta1* (EU884422.1)
2.	PTA1_MCD24	*S. microdontum*	*Rpi-pta1* (EU884422.1)
3.	EDN1.1_BER57	*S. berthaultii*	Rpi-edn1.1 (GU563963.1)
4.	EDNR2_TRF22	*S. trifidum*	EDNR 2GH7 (GU563968.1)
5.	EDNR2-VER55	*S. verrucosum*	EDNR 2GH7 (GU563968.1)
6.	SNK1.1_JAM07	*S. jamesii*	*Rpi-snk1.1* (GU563975.1)
7.	SNKR2_IOP59	*S. iopetalum*	SNKR2GH5 (GU563977.1)
8.	SNKR2_LES34	*S. lesteri*	SNKR2GH5 (GU563977.1)

sequenced genes were: (1) *Rpi-pta1* (in *S. cardiophyllum* and *S. microdontum*); (2) *Rpi-edn1.1* (in *S. berthaultii*); (3) EDNR2GH7 (in *S. trifidum* and *S. verrucosum*); (4) SNKR2GH5 (in *S. iopetalum* and *S. lesteri*); and (5) *Rpi-snk1.1* (in *S. jamesii*). The isolated homologous R genes from different wild species are summarized in Table 8.2 and wild species are shown in Fig. 8.1. There are various novel approaches for SNP discovery/allele mining in potato germplasm that will accelerate genomics-assisted potato improvement.

8.3.2 Development of High-Density Potato SNP Arrays and Genome-Wide Characterization

The potato genome sequence data, and the reducing costs of NGS technologies, open great opportunities for using genomic tools to investigate allelic variation in wild relatives to accelerate the potato breeding. Gene bank collections have been established worldwide in order to conserve the genetic diversity of the germplasm, including cultivated, semi-cultivated and wild species. Recent advances in genomics and molecular marker technologies particularly next-generation sequencing technologies provide novel tools to assess the genetic diversity of such collections and permit genome-wide sets of SNP markers to be generated. Currently, SNPs that result from a change in a single nucleotide position represent the most abundant type of genetic polymorphism in plant genomes and are the most preferred molecular marker today for genome-wide characterization and high-throughput automation. This will allow an understanding of the genetic basis of traits so that more rapid improvement can occur over the next century of breeding.

SNP genotyping arrays have been useful in many applications that require a large number of molecular markers, such as high-density genetic mapping, genome-wide association studies (GWAS), and genomic selection. The discovery of the Infinium SolCAP 8.3 K SNP potato array (http://solcap.msu.edu) which covers the whole potato genome-wide sets of markers has revolutionized the characterization of *Solanaceous* species. In order to characterize the genetic diversity, potato germplasm has been genotyped using the SolCAP SNP array and genotypic differentiation was observed within the germplasm, including processing clones, wild species, genetic stocks and cultivated potatoes. A high degree of concordance between the linkage maps and the pseudomolecules demonstrates the quality of the potato genome sequence and the functionality of the Infinium SolCAP SNP array. The broad genome coverage of the SolCAP SNP array compared to other marker sets will enable numerous downstream applications (Felcher et al. 2012). The discovery of a large number of SNPs in elite north American potato germplasm showed the importance of the quality of the potato genome sequence (Hamilton et al. 2011).

The SNPs will enable high-throughput genotyping of germplasm and populations, which in turn will enable more efficient marker-assisted breeding efforts in potato. Due to a huge genetic diversity among potato germplasm population, genomics of wild potatoes using genome-wide SNP arrays aim to establish patterns of nucleotide polymorphism and divergence between several closely related, morphologically distinct wild species. Based on a large number of SNPs, population-genetic analyses can assess the relative importance of nucleotide diversity and interspecific divergence. Genotyping of a panel of 350 diverse tetraploid potato cultivars using the SolCAP SNP array showed some insights into the different genetic aspects of potato such as genetic diversity, population structure, or linkage disequilibrium (LD). It revealed the potential of genome-wide association mapping in tetraploid potato using high-density genotyping to assess marker-trait associations (Sharma et al. 2012). Like the population genomics of wild tomatoes, the use of the potato genome sequence data is equally important for studying potato genetic resources. Ganal et al. (2011) have established a large maize SNP array for its use in diversity analysis and high density linkage mapping. Moreover, use of potato genome sequence reflects genome-wide analysis of plastome sequence variation and the development of plastidial CAPS markers in common potato and related *Solanum* species (Gargano et al. 2012).

8.3.3 High-Throughput Genotyping and Genome-Wide Mapping for Elite QTLs and Alleles

Sequencing of the potato genome has opened up new vistas for potato genetics and breeding. Due to the genetic complexities posed by the tetrasomic inheritance, most potato genetic studies are carried out at the diploid level. This causes some difficulties in translating and applying the derived results at the tetraploid level in cultivated potato. For practical reasons, the molecular dissection of traits at the tetraploid level and across a wider germplasm using a high-throughput molecular marker platform is highly desirable. Therefore, association mapping is gaining importance in studying the genetics of natural variation and greater allelic diversity in polyploidy crop species. In association genetic studies no prior information about the genes of interest is available, but associations between genetic markers and the considered traits are simply derived from observational research. Association genetics focus on the identification of correlations between phenotypic traits and genetic markers with the aim of identifying and locating the underlying genes in the genome. In addition, the huge volume of information generated through Genotyping-by-Sequencing (GBS) has been exploited to understand domestication, for the construction of maps, high-resolution analyses of genetic diversity, population structure and linkage disequilibrium, high-throughput discovery of genes and alleles, and whole genome selection for complex traits. The breeding process, gene content variation, and low diversity regions have also been revealed by large-scale re-sequencing. The GBS is a high-throughput and low-cost genotyping platform originally developed for highly inbred maize and sorghum populations. This type of approach can reduce the problems associated with ascertainment bias typically encountered with other genotyping platforms (http://www.igd.cornell.edu/index.cfm/page/projects/GBS.htm).

Whole genome association (WGA) mapping of European potato varieties using the SolCAP SNP array for bruising resistance identified SNPs linked to the trait, thereby discovering new candidate genes (Stich et al. 2013). Genome-wide association mapping in elite CIP potato clones of advanced breeding population B3 for late blight resistance showed some quantitative trait loci (QTLs) linked to the trait. A set of 103 clones were genotyped using the SolCAP SNP array-identified QTL that explains a significant amount of variation for late blight resistance. The associated SNP marker could prove useful in molecular breeding for late blight resistance (Lindqvist-Kreuze et al. 2014).

A phureja-tuberosum diploid potato cross-segregating for several traits was genotyped with the SolCAP SNP array and identified several QTLs linked with large effect QTLs. Thus, the identified candidate genes in the potato genome that are used to identify informative markers are linked to commercially valuable traits, in conjunction with an ongoing association mapping analysis of 300 tetraploid cultivars. Later a dense SNP-based linkage map of a diploid potato population was generated and major QTLs for tuber shape and eye depth on chromosomes 2 and 10 were identified (Prashar et al. 2014). Using genomics approaches, a panel of 224 tetraploid potato cultivars was analysed with 384 SNP array and identified five genotypic classes and trait associations for flesh colour and eye depth (Voorrips et al. 2010). Draffehn et al. (2010) uncovered a very high natural allelic variation in a set of five potato invertase genes which are involved in cold-induced sweetening of tubers by cDNA sequencing and SNP genotyping. The associations found between specific invertase alleles and chip quality, tuber starch content and starch yield will facilitate the selection of superior potato genotypes in breeding programmes. Comparative next-generation mapping of the *Phytophthora infestans* resistance gene *Rpi-dlc2* in a European accession of *Solanum dulcamara* showed pyramiding of *Rpi-dlc2* and *Rpi-dlc1* significantly increased resistance, compared with only one of the genes (Goals et al. 2013). High-throughput genotyping of tomato and its wild relatives applying 5528 SNP array revealed tomato breeding strategies in the genomics era. Genotyping allows the evaluation at the level of heterozygosity and introgressions among commercial varieties using diagnostic markers (Víquez-Zamora et al. 2013). Numerous researchers have conducted high-throughput genome-wide SNP genotyping and discovered the SNP/allele/gene in many crops like sorghum (Morris et al. 2013), or mungbean (Van et al. 2013) to name a few. Hackett et al. (2013) studied linkage analysis and QTL mapping using the SolCAP SNP array in a tetraploid potato mapping population and overall 3839 of the 5378 polymorphic SNPs can be assigned to putative genetic locations. QTL analysis of drought tolerance in a diploid mapping population with 499 SNP markers discovered SNPs in public EST databases using QualitySNP software with the Illumina GoldenGate assay (Anithakumari et al. 2012). A diploid population comprised of 138 F_1 hybrids was mapped for after-cooking darkening using simple sequence repeat and high-resolution melting (HRM) markers (Koeyer et al. 2010).

8.3.4 Next-Generation Re-Sequencing and Comparative Genomics in Wild Genomes

To implement genomics into plant breeding requires a comprehensive knowledge of the genetic variation present in the crop germplasm. The accessibility of the high quality reference potato genome is an important tool for the analysis of wild and semi-cultivated potato species. Efforts by the PGSC resulted in a genome sequence of high quality that can be used as a reference for re-sequencing of additional wild, cultivated, semi-cultivated species, lines, and cultivars for genetic diversity, allele mining and gene discovery in potato germplasm. Recently, *Solanum commersonii* is a wild potato species native to Central and South America and exhibits tolerance to both biotic and abiotic stresses. This *Solanum commersonii* has been sequenced and several genes have been identified particularly for freezing tolerance (Aversano et al. 2015). We at the Indian Council of Agricultural Research-Central Potato Research Institute, Shimla, Himachal Pradesh, in India have sequenced the whole genome of a *Solanum tuberosum* dihaploid 'C-13' derived from tetraploid potato variety Kufri Chipsona-2. It revealed 98.68% assembly and annotated 42642 genes (98.68%) with the potato reference genome (unpublished) and the image of androgenic dihaploid potato clones is shown in Fig. 8.6. Further, comparative genomics focuses on the integration of genome information derived from different species with the aim of obtaining more insight into the genetic

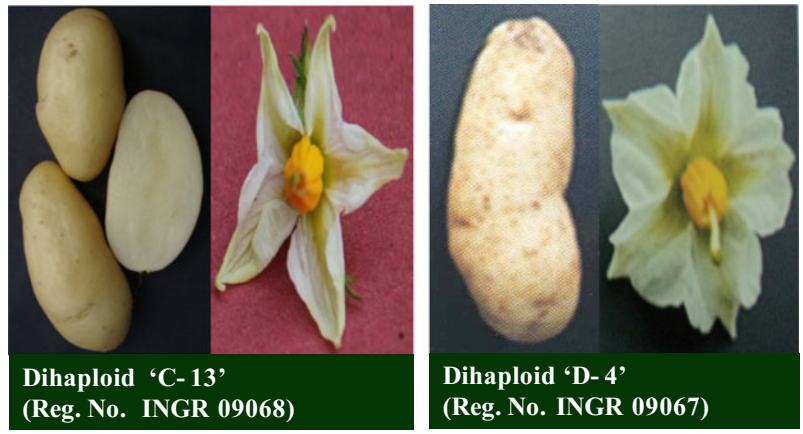

Fig. 8.6 Androgenic *Solanum tuberosum* dihaplpoids 'C-13' and 'D4' were developed from the potato variety Kufri Chipsona-2 and TPS population JTH/C107, respectively

organization of traits through the identification of conserved mechanisms. Together with the availability of an increasing number of genome sequences, including those of known genes, the conservation of gene sequences and their functions among species have been investigated and are used to further develop the knowledge obtained from previous genetic linkage maps for different species. Comparative genomics of a wild tomato species *Solanum galapagense*, endemic to the Galapagos Islands, was analysed to understand the genomic differences among the wild and cultivated species particularly for high salt tolerance, pathogen susceptibility, and morphological variation (Strickler et al. 2015).

In order to characterize the distribution and quality of SNPs in the tomato genome, eight cultivated tomato inbred lines were re-sequenced with the Illumina technology to a 25–40x genome coverage in order to fully investigate SNP variation in cultivated tomato material on a genome-wide scale. Based on the sequencing data, ~1 million SNPs were identified in single copy sequences of the tomato genome (Ganal et al. 2012). Many re-sequencing initiatives are currently under way that will reap the potato genome sequence information to help elucidate a variety of processes, such as plant disease resistance, development, and domestication. At the SOL Genomics Network (SGN), sequencing a tomato accession from a breeding programme exhibiting unique resistance, and from a wild relative of the tomato, *S. galapagense* has given insight into the whole genome diversity present in *Solanum*. This set of sequenced accessions contains an underexploited wealth of genetic variation, and will therefore offer a useful gene pool to cope with existing and new breeding challenges (Mueller et al. 2012). Re-sequencing of 120 tomato lines representing a broad allelic diversity for the red fruit cluster, identified ~6 million SNPs and other variations. Further, a linkage disequilibrium study identified a set of 200 K SNPs that may be useful for genetic mapping and association studies. The data provided a valuable resource to facilitate tomato genetics and breeding (Huang et al. 2012). Nonetheless, this approach will provide further insight into the organization and dynamics of the potato genome-based study. Developing efficient methods to analyse and compare genomes, or perform re-sequencing is essential to the many routine applications on a large scale in the near future, such as characterization of germplasm used in breeding.

8.3.5 Sequence-Based Transcriptomics (RNA-Seq), Microarray and Other 'Omics' Technologies

Utilization of the natural genetic variation in traditional breeding programs remains a major challenge in crop plants. Identification and access

to allelic variation that affects the phenotype are of the utmost importance for the use of genetic resources, particularly in variety development. Considering the huge number of germplasm collections that are collected worldwide in the potato gene banks, these are deemed a wealth of huge allelic variants. So, the challenge is how to reveal this variation. Recent advances in these technologies have permitted the dissection of the genetics of complex traits in crop species. The development of high-throughput 'omics' technologies, such as genomics, transcriptomics, metabolomics, proteomics and others has brought the potential of population-wide data collection. In the last few years, functional transcriptomics has been advanced by both microarray technology as well as high-throughput sequencing of cDNA (RNA-seq). Transcriptomics represents a resource for targeting specific genes underlying phenotypic traits and can serve, in the absence of a full genome, as a reference for RNAseq digital gene expression profiling. In the past decades, microarrays (DNA chip technology) have been used extensively to quantify the transcripts/mRNA corresponding to the target genes. Microarray is a high-throughput screening technique based on the hybridization between oligonucleotide probes (genomic DNA or cDNA) and either DNA or mRNA. Certainly, the microarray technology has achieved its technical limits and is incresingly being complemented by high-throughput next-generation sequencing technologies. Hence, more recently RNA-seq has emerged as an alternative powerful tool to study transcriptomes. Unlike microarrays, RNA-seq can evaluate absolute transcript levels of sequenced and unsequenced organisms, detect novel transcripts, reveal sequence variations and many more. As the cost of sequencing decreases, it is believed that the use of RNA-seq for differential expression analysis will be increasingly rapid (Mutz et al. 2013).

Wild species of *Solanum* were used to identify resistance against late blight, the most devastating disease of potato, which could be used to achieve durable resistance in the popular potato varieties. Microarray-based gene expression profiling in a late blight-resistant Indian potato cultivar Kufri Girdhari identified many up-regulated candidate genes upon challenge inoculation by *Phytophthora infestans* (Sundaresha et al. 2014). In other microarrays-based studies, novel genes were identified in leaf tissues of potato somatic hybrids using cDNA microarrays designed on the potato genome sequences for late blight resistance (Singh et al. 2016) and for potato tuberization (Tiwari et al. 2015b). To identify polymorphism in loci that confer resistance to potato late blight, transcriptome sequencing using Illumina GA2 of *S. berthaultii*, *S. venturii* and *S. nigrum* identified polymorphism and genes for late blight resistance. These multiple R genes could be simultaneously introduced in popular varieties in order to achieve durable resistance (Verweij et al. 2010). Novel candidate genes were uncovered in potato host plants for resistance to *Phytophthora infestans* of compatible and incompatible interactions by DeepSAGE (serial analysis of gene expression) transcriptome analysis (Gyetvai et al. 2012). A comparative transcriptome analysis of potato leaf tissue with a Verticillium wilt-resistant clone showed increase expression of genes involved in protein translation and photosynthesis (Tai et al. 2012b). Transcriptomes of tuber derived tissues were analysed and transcripts were identified that set apart the tuber from the rest of the plant tissues to shed light on how this unique organ is functioning at transcript level especially for carbohydrate metabolism (Sønderkær et al. 2010). Genes driving potato tuber initiation and growth were examined based on transcriptional changes using the POCI (Potato Oligo Chip Initiative) array (Kloosterman et al. 2008). De novo DNA sequence-driven bulk segregant analysis for water use efficiency identified polymorphisms on several chromosomes and many candidate genes (Kaminski et al. 2012). Drought tolerance candidate genes were identified in Peruvian native potatoes by RNA-seq analysis. Filtered reads were mapped to the potato genome, a large percentage of these read maps uniquely to the reference genome (Fernandez et al. 2012). Drought-responsive compounds have been identified in potato through combined transcriptomic and targeted metabolite approaches (Evers et al. 2010).

In other crops like tomato, a transcriptomic approach was followed to identify regulatory genes involved in the fruit set of wild and cultivated genotypes (Ruiu et al. 2012). Comparative transcriptomics (RNAseq) revealed the effects of artificial and natural selection patterns in cultivated tomato and five related wild species. Based on sequence differences, 50 genes were detected for positive selection and thousands of shifts in gene-expression level were documented (Koenig et al. 2013). A set of differentially expressed genes was identified in wild tomato *S. pennellii* in response to drought stress by microarray and RNA-seq analysis (Ye et al. 2012). A genome-wide deep-coverage short-read sequencing approach was followed to analyse the transcriptomes of one accession of domesticated tomato and three wild relatives for changes in gene expression, coding sequences, and gene regulation (Sinha et al. 2012).

To investigate the effects of temperature and day-length on potato tuber formation, *a* 60 K-feature potato microarray was used to compare global gene expression profiles in leaves and tubers. Correlations between transcriptomic and metabolomic analysis revealed potential novel regulatory networks (Morris et al. 2012). In potato cv. Désirée, response to cold and salt exposure was investigated at both transcriptomic and proteomic levels in a growth chamber experiment. Cold exposure in potato resulted in a higher number of regulatory genes compared to salt exposure (Legay et al. 2012). Analysis of natural variation of the potato tuber proteome revealed novel candidate genes for tuber bruising. The comparison of 20 potato varieties yielded insight into the high natural variation of tuber protein patterns due to genetic background (Urbany et al. 2012).

A range of volatile and non-volatile metabolites associated with potato flavour and candidate genes associated with the biosynthesis of these molecules have been identified (Taylor 2010). A diploid potato population was screened for potato tuber quality for gene-expression and secondary metabolite content using a microarray and liquid chromatography–mass spectrometry (LC–MS) approach, respectively, and identified several expression and metabolite quantitative trait loci (eQTL and mQTL) (Kloosterman et al. 2010). Metabolomics and transcriptomics for Colorado potato beetle resistance of *S. oplocense* and *S. tuberosum* showed increased expression of protease inhibitors associated with a defence mechanism (Tai et al. 2012a). A combination of metabolic and transcript profiling identified a gene GAME4 that appears to play a key role in biosynthesis of steroidal alkaloid (SA) pathway for solanine in the sprouting potato tubers (Aharoni 2012). To identify the genetic factors underlying variation in primary metabolism in potato, a diploid mapping population derived from crosses between *S. tuberosum* and wild relatives, was profiled using gas chromatography-time of flight-mass spectrometry. In total, 139 polar metabolites were detected, of which 72% detected compounds were metabolite quantitative trait loci (Carreno-Quintero et al. 2012). Variation within candidate genes for phytonutrient acquisition, specifically iron and zinc, acquired from the soil by native Andean potato cultivars, was found over a two-fold range in tubers. Using the potato genome browser, orthologous genes were identified by sequence similarity to candidate genes for phytonutrient acquisition in other crops (Medina et al. 2012).

8.4 Markers/Genomics-Assisted Breeding of Potato Germplasm

The potato breeding programme has distinct pre-breeding and commercial cultivar development phases. Pre-breeding research is focused on developing the capacity to use elite parental materials, by applying molecular tools such as marker-assisted selection (MAS). In recent years ‘breeding by design’ as a concept described by Pele man and van der Voort aims to bring together superior alleles for all genes of agronomic importance from genetic resources. This might be achievable through high-resolution allele detection based on precise mapping of potential parental resources (Gai et al. 2012). Diagnostic DNA-based markers are either

derived directly from polymorphisms in genes with a trait of interest or are in linkage disequilibrium with those genes. They can be used to identify superior genotypes among parents and progeny in precision breeding programmes. Diagnostic markers can be identified by a combination of QTL, candidate gene and association mapping using functional and positional candidate genes as markers. This approach was successfully used to identify loci, which contribute to the natural variation of pathogen resistance or tuber traits in tetraploid breeding populations. Candidate genes associated with field resistance to late blight or tuber quality traits have been developed and currently are being tested for their diagnostic power in marker-assisted selection experiments (Gebhardt et al. 2010).

The efficiency of breeding new resistant cultivars may be improved by the development of resistant parental lines derived from germplasm resources. Parental lines are donors of traits of interest, having the genetic background enriched with genes originating from various *Solanum* species. One of the bottlenecks in the development of potato production is the availability of cultivars with multiple resistance to major pests and pathogens such as the most devastating disease, late blight, caused by *P. infestans* followed by PVY and other viruses, bacterial diseases and cyst nematodes. A genome-wide infection library of *P. infestans* RXLR effectors that include avirulence (AVR) proteins has been created to accelerate cloning and specificity profiling targeted by resistance proteins (R genes) from wild *Solanum* species. This effectoromics strategy has proven effective and complementary to classical breeding approaches. Effector-based resistance breeding will facilitate selection and combining qualitative and quantitative resistance that may lead to a more durable resistance to late blight in potato (Vleeshouwers et al. 2012). Application of MAS in pre-breeding of common potato clones resistant to pathogens has been demonstrated for the genes *Ry-f*$_{sto}$ (PVY resistance), *Rm* (PVM resistance), *Ns*$_{adg}$ (PVS resistance), *Rpi-phu1* (late blight resistance), *H1* (resistance to *Ro1-4* of *Globodera rostochiensis*). The PCR markers linked to respective resistance genes allowed a combination of phenotypic and MAS (Zimnoch-Guzowska and Flis 2012). In order to develop diagnostic markers for MAS for quantitative resistance to late blight in tetraploid potato, SNP close to the QRL were identified by the use of the potato genome sequence (Marhadour et al. 2012). Three PCR-based diagnostic markers (SC895, S1d11 and B11.6) were developed and validated for marker-assisted selection of hypersensitive response (HR) genes for resistance to PVY in the cultivated potato (Szajko et al. 2012). Asano et al. (2012) demonstrated marker-assisted evaluation of potato genotypes for potential resistance to potato cyst nematode pathotypes in Japan without phenotypic evaluation.

Physical mapping and comparative genomics were investigated for the potato cyst nematode resistance locus *H1* at three haplotypes in potato and identified a large cluster consisting of the CC-NB-LRR type of *R* genes (Finkers-Tomczak et al. 2011). Examination of three wild tuber-bearing species *S. vernei*, *S. sparsipilum* and *S. spegazzinii* conferring a high resistance level to *G. pallida* led to identification of QTLs. Further, allele-specific molecular markers for the *S. spegazzinii* GpaV resistance QTL has been proven for marker- assisted breeding (Chauvin et al. 2012). Marker-assisted potato breeding has been advanced rapidly all over the world, for example, in Australia for potato cyst nematode (PCN), tomato spotted wilt virus (TSWV), and potato virus Y (PVY) resistance (Slater et al. 2010); in Poland for viruses (Zimnoch-Guzowska and Flis 2012); in India for PVY and late blight (Tiwari et al. 2012, 2013b, 2013c, 2013f). Wild species *S. microdontum* and *S. kurtzianum*, that represent the extremes for tuber calcium traits, were analysed to understand the genetics of tuber calcium uptake and the potential role of tuber calcium in tuber quality (Palta et al. 2010). Candidate gene markers at loci encoding ADP-glucose pyrophosphorylase and the invertase *Pain-1* were identified and validated for MAS of potato cultivars with improved tuber quality, particularly chip colour, tuber starch content and starch yield (Li et al. 2013).

8.5 Genetic Modification in Potato Germplasm

The use of resistant cultivars in potato breeding programmes is an important tool for disease management. Recent advances in plant molecular genetics have identified several genes for resistance to potato pests and pathogens from within the germplasm pool available to potato breeders. Genetic transformation with resistance genes is expected to enhance durable resistance against pathogens, especially for potato late blight. Functional gene stacking of multiple genes has important implications in achieving more durable resistance against potato late blight (Zhu et al. 2012, 2013). They demonstrated integration of triple R genes (*Rpi-sto1, Rpi-vnt1.1* and *Rpi-blb3*) transformants and the inheritance of resistance against potato late blight. On the contrary, even loss of susceptibility factor/gene (S-gene) is a novel breeding strategy for durable and broad-spectrum resistance in non-host-like resistance crop species. The absence of certain host-factors encoded by plant S-genes enables plants to escape the defence suppression and thus to maintain their non-host status. With the aim of achieving non-host-like durable resistance in crops by disabling plant S-genes, researchers have demonstrated that silencing putative tomato orthologs of the S-genes identified in Arabidopsis resulted in resistance to tomato powdery mildew, indicating that multiple S-genes are conserved among unrelated plant species (Pavan et al. 2010). Besides, T-DNA minicircles for Agrobacterium-mediated delivery of potato genes without vector backbone sequences offer a method to reduce DNA molecules to a simple, well-defined expression cassette for transformation. They offer an important tool for effective delivery of cisgenes, intragenes and transgenes through transformation without the vector backbone sequences, an important limitation of current technology (Jacobs et al. 2010). Moreover, in recent years, concepts like intragenesis and cisgenesis as alternatives to transgenic crops have emerged to meet the concerns of the general public. So to exploit the diverse potato gene pool, both concepts implies use of the species itself or from the closely related species capable of sexual hybridization for crop improvement (Holme et al. 2013).

8.6 Conclusion

The worldwide potato germplasm collection provides excellent genetic resources for the use of wild relatives in addressing global food security in the era of climate change and the emergence of new races of pest pathogens. Germplasm has largely untapped genetic variation for abiotic and biotic stress tolerances, and could greatly expand the available domesticated gene pools in the predicted extremes of climate change (Redden 2013). The international potato gene banks collection is critical in order to collect, conserve, characterize, and utilize the germplasm in a systematic and integrated way to meet future food needs. Current genomic strategies can assist in the introgression of these valuable traits into the domesticated crop gene pools, where they can be better evaluated for crop improvement. Knowledge and tools that can be developed through the use of next-generation sequencing technologies, SNP discovery, transcriptomics, other 'omics' approaches and genetic modification will pave the way forward for the exploitation of an amazing germplasm diversity for greater use of genomic research into potato improvement (Van et al. 2011). Nevertheless, genomics-assisted breeding will play a key role in potato, since it is not only cost-effective and efficient but also amenable to high-throughput automation. Indeed, to break yield barriers in potato, multi-faceted potato breeding efforts like the novel discovery of SNPs/allele/genes from germplasm resources and their deployment in broad marker-assisted selection, the enrichment of the narrow genetic base of modern potato varieties, the stability of QTL analysis and genetic modifications that regulate the phenotypic variation for yield-associated traits will certainly help genomics-assisted potato improvement.

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