Chapter 2
Genomic Designing for Abiotic Stress Tolerant in Potato

Jagesh Kumar Tiwari, G. Vanishree, Virupaksh U. Patil, Tanuja Buckseth, Som Dutt, Dalamu, and Rajesh K. Singh

Abstract Potato is an important food crop of the world. Besides other factors, potato crop suffers from various abiotic stresses such as heat, drought, salinity, nutrients and frost. Of these, heat is the most important followed by drought stress for climate change point of view, while improving nutrient use efficiency in also essential to save environment and reduce cost of production. Salinity is another problem under irrigated condition, while frost is an issue of temperate climate. Overall, these environmental factors affect crop growth and ultimately reduce tuber yield. Potato is rich in diverse gene pool, however, a little has been utilized for its genetic improvement. With the increasing genomics resources like potato genome sequences, numerous functional genomics sequence data at multiple time points of various plant tissues, high-density potato maps and molecular markers particularly single nucleotide polymorphism (SNP) linked to the trait of interest, there is possibility of rapid potato breeding for tolerance to above abiotic stresses. This chapter highlights on the abiotic stress related information in potato on the genetic resources, genetic diversity, classical genetics and breeding, molecular mapping (linkage and association), gene cloning, marker assisted selection, genome sequence, genomics-assisted breeding, genomic selection, functional genomics, genome editing, nanotechnology, bioinformatics and social concerns.

Keywords Abiotic stress · Cold · Drought · Genomics · Heat · Molecular breeding · Potato · Salinity

2.1 Introduction

Potato (*Solanum tuberosum* L.) is the third most important human food in the world after rice and wheat. Potato has high yield potential, high nutritive values and is wholesome food (Chakrabarti et al. 2017). In addition to various other growth affecting factors under a climate change scenario, potato suffers from many

J. K. Tiwari (✉) · G. Vanishree · V. U. Patil · T. Buckseth · S. Dutt · Dalamu · R. K. Singh
ICAR-Central Potato Research Institute, Shimla, Himachal Pradesh 171001, India
e-mail: jagesh.kumar@icar.gov.in

abiotic stresses such as heat, drought, salinity, frost and nutrients (Dahal et al. 2019; Handayani et al. 2019). Cultivated potato crop requires moderate temperatures (about 18–20 °C) for tuberization, while nearly 25 °C is optimum for vegetative growth. Potato cultivation ranges from low to high altitudes and temperate to tropical/subtropical climates with moderate temperatures. In tropical regions having high temperature, potato yields are lesser than temperate or subtropical regions. Although, biotic stresses and poor cultural practices are major factors of low yield in tropical areas, yet abiotic environmental factors like high temperatures combined with limited soil moisture are also important factors. With the rising in global temperature under climate change scenario, heat stress in potato is the most important. Although, potato is an irrigated crop, drought stress is another problem where water is limiting especially African countries. Salinity is problem in high saline soil area, whereas frost is an issue in temperate climates. Moreover, improving nutrient use efficiency of potato is important particularly N fertilizers to save the environment and reduce production cost. Owing to its high productivity, short duration crop and nutritionally superiority, it has the potential to significantly augment the food availability in tropical regions. Variability exists in the potato germplasm and related species for these abiotic stresses. Therefore, top priority is required to be directed for introducing tolerance to abiotic stresses for expansion of potato cultivation in non-traditional areas of tropical and subtropical regions of the world (Demirel et al. 2020). Classical breeding has achieved a lot while developing abiotic stress tolerant varieties but implication of genomics is still limited in potato where genome sequence is available. This chapter highlights genomic designing of potato for abiotic stress tolerance.

2.2 Abiotic Stresses

2.2.1 Heat Stress

Potato is considered primarily a crop for cool and temperate climates. High temperature inhibits crop yield by overall reduction of plant development due to heat stress or by reduced partitioning of assimilates to tubers (Singh et al. 2015). Minimum night temperature plays a crucial role during tuberization in potato and largely determines whether plants will tuberize or not. Tuberization is reduced at night temperatures above 20 °C with complete inhibition of tuberization above 25 °C. The most important effect of high night temperature is on the partitioning of assimilated carbon between leaves and tubers. Exposure of potato plants to high temperature alters the hormonal balance in the plants. As a result most of assimilated carbon is partitioned to the shoots (stems and leaves) and not to the tubers. Heat stress tolerance breeding programs should consider the ability to tuberize at higher night temperature (above 22 °C), low shoot to root ratio at high temperature, and early crop maturity (short duration). Figure 2.1 shows response of plants to high temperature stress in potato.
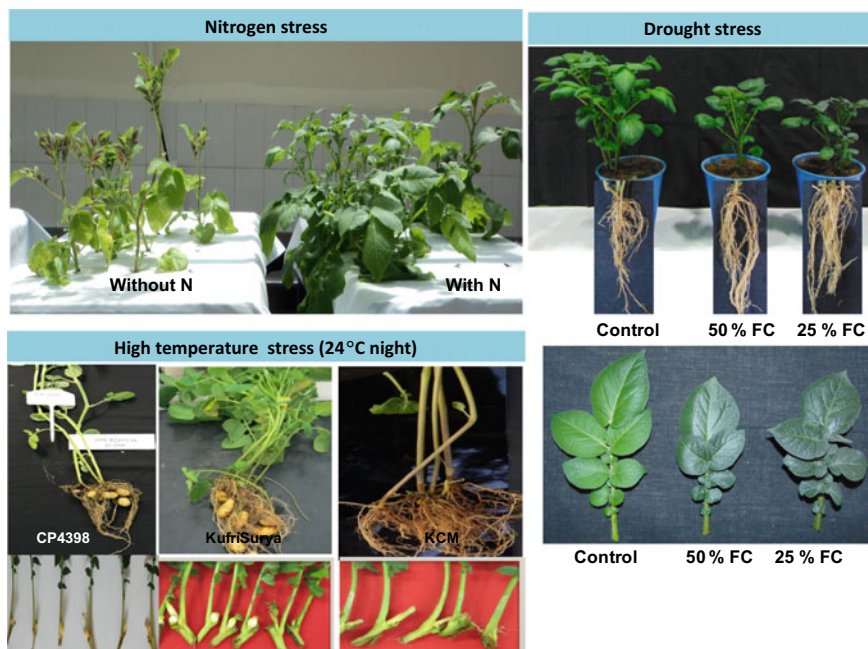
Fig. 2.1 Response of potato plants to different stresses such as nitrogen, high night temperature, and drought under controlled conditions. (FC: Field Capacity; cv. KCM: Kufri Chandramukhi)

2.2.2 Drought Stress

Potato is mostly an irrigated crop in plains and rainfed in hilly regions. Owing to shallow root system it is more prone to drought stress. Drought is an emerging problem of potato production due to availability of irrigation water in the world, which affects tuber yield as well as quality (Monneveux et al. 2013). Drought may occur due to erratic rainfall, inadequate irrigation and lack of water supply. Even with good irrigation water supply, moisture stress may occur because of high transpiration rates especially during high temperature in noon when root system cannot completely meet the water requirements of the plant. Drought may affect potato growth and production by reducing foliage biomass, decreasing photosynthesis rate per unit leaf area, and early crop maturity and shorter vegetative growth period. All stages of crop growth are sensitive to drought. Insufficient water supply in the period between emergence and the beginning of tuber bulking may therefore lead to a reduced growth rate of foliage and ground cover resulting in incomplete plant growth and yields below optimum. Potato plant is highly sensitive to water stress and the decline in photosynthesis is fast and substantial even at relatively low water potentials of –3 to –5 bars. Plants respond to water stress by closing their stomata thus shutting out the supply of CO_2. Tuber traits such as shape, cracking, dry matter content and reducing sugars are highly influenced by the availability of soil moisture during the vegetative

period. Taking into account production conditions and the present yield levels, it is estimated that the average potato yield in the world could be increased by at least 50% if the water supply to the crop could be optimized. Therefore, breeding of drought stress tolerance is now a priority in potato applying genomics approaches. This is important while saving irrigation water to ensure yield and food security under the climate change scenario and growing demand of water. Figure 2.1 shows response of plants to drought stress in potato.

2.2.3   Nutrient Stress and Salinity

Major and minor nutrients are essential for good vegetative growth, yield and quality of potato (Handayani et al. 2019). Among all, nitrogen is the most important nutrient for plant growth and development including potato. Potato is a resource intensive crop and requires high N fertilizer (150–240 kg N/ha) to produce tuber yields (30–50 t/ha) in India. Despite the high cost of N fertilizer, potato crop uptakes nearly 50% of the total N applied and excess lost in the environment. Potato is a shallow rooted crop and mostly irrigated cultivations are practiced on the sandy-loam soils with excessive application of N fertilizers. This practice increases the chance of nitrate leaching, contamination to the groundwater and high cost of cultivation. In addition, improving P and K use efficiency of plants would also save additional costs. Though, field-based approaches have been applied in potato for improving nutrient use efficiency (NUE) for successful tuber production. However, improving N use efficiency of plant is one of the key options to minimize N losses, save the cost of production and improve the environmental quality to achieve sustainable crop yield (Tiwari et al. 2018c, 2020d). Most studies so far focused on agronomic managements for improving NUE in potato. Figure 2.1 shows response of plants to nitrogen stress in potato under aeroponics. In addition, salinity is another issue in highly irrigated conditions. Salinity could be either due to salinity of the soil or irrigation water applied to the crops. Salinity causes nutritional imbalances, restricts plant growth and development, early senescence and severely reduces tuber yield in the semi-arid and arid regions. Variability for salinity stress tolerance exists in potato germplasm, which can be harnessed for development of varieties (Ahmed et al. 2020).

2.2.4   Frost Stress

Frost is the major problem of temperate growing regions of potato. Temperatures below $-2\,^{\circ}\text{C}$ can result into partial or complete loss of the crop. In temperate zones, frosts can occur during spring season when the crop is at initial stage of vegetative growth or during autumn when it is near to maturity. Higher crop losses occur in tropical highlands and subtropical plains where frosts can occur any time during the crop growth period. In India more than 80% of potato is grown during winter in

subtropical plains and the crop is prone to frosts during the months of December and January. Based on the field observations, two types of frosts are often distinguished namely 'White frost' that occurs when there is a decrease in temperature and high relative humidity, and 'Black frost' that occurs under low temperatures and much drier conditions, hence more damaging and severe because plant tissue is darkened immediately. Acclimation or hardening may increase the resistance to frosts in many plants. Exposure of the plants to prolonged low temperature is effective in increasing resistance to frost injury in wild potato species.

2.3 Genetic Resources

Abiotic stresses like heat, drought, nutrients, salinity and frost have significant impact on potato production affecting yield, tuber quality and marketable value. Cultivated potato is autotetraploid with highly heterozygous and a narrow genetic base. The genetic base of the cultivated potato can be expanded by introgressing useful genes from wild *Solanum* species for abiotic stress and others. *Solanum* species evolved under a range of different climatic conditions and they can be found between sea level and 4500 m altitude in temperate environments, humid tropical climates and even in deserts (Hawkes 1990). *Solanum* species offer a vast diversity of traits for breeding. The use of wild species has been suggested as a means to increase tolerance to many stresses including abiotic and thereby improve productivity in a range of crops. Currently large amounts of potato germplasm containing useful alleles are available in gene banks around the world. However, during its evolution and adaptation, potato passed through a genetic bottleneck resulting in a relatively narrow genetic base in the present varieties. Efforts have been made to broaden the genetic base over a century and but still efforts are required to improve abiotic stress tolerance.

Bradeen and Haynes (2011) proposed a potato gene pool concept based on endosperm balance number (EBN), where primary gene pool (5%, 5 species) species are cultivated potato which are 4X (4 EBN), the secondary gene pool (68%, 58 species) is the largest and contains wild relatives which are easy to cross to cultivated potato directly or after ploidy manipulations, and tertiary gene pool (27%, 23 species) species have 1 EBN. These species do not cross directly to cultivated potato but may be introgressed through bridge crosses, embryo rescue or somatic fusion. The potato germplasm resources is composed of 117 wild relatives, four land races, cultivated species and modern cultivars (Spooner et al. 2014). Wild potatoes are distributed from the south-western United States southward to central Chile and Argentina (Spooner et al. 2014). Species richness is greatest in central Mexico and in the Andean highlands. Wild potato species are found in highly diverse habitats including cloud forests, cactus deserts, scrub vegetation, mountain pastures, high grasslands and pine forests. They carry genes for traits that have not been identified in cultivated potato. Designing of crops adapted to unpredictable climatic changes like drought, heat, nutrients, salinity and frost need utilization of available genetic resources. This will facilitate effective use and pre-breeding for introgression

of desired genes from wild species, which have evolved resilience against adverse climatic condition over centuries. In addition to various techniques to utilize wild resources, somatic hybridization has been used widely for introgression of genes from wild species into cultivated potato (Tiwari et al. 2018b). Further, this will help easy access to desired variability, both genetic and allelic for their use in further genetic improvement or designing of cultivars suited to changing climatic conditions.

2.4   Classical Genetics and Traditional Breeding

Earlier potato breeding has mainly focused on yield, quality and biotic stress tolerance, and less attention was driven to breed for abiotic stress tolerance. Present variation in the potato gene pool can be exploited to breed cultivars with tolerance to abiotic stress. There are a large number of potato traits that are regulated by multiple genes, or are quantitative in nature. Most important physiological and agronomic traits are under the control of a very large number of genes, each of which may have relatively low individual effect. The desire to obtain genetic gain in such highly complex and low heritability traits has led some breeders to use progeny tests to identify superior parental combinations. Progeny tests will help to determine the breeding value of the parent for these traits. Phenotypic selection of individuals for highly complex traits has been shown to be less effective than the use of progeny tests for family selection. Progeny testing has been extended to a range of traits in potato breeding (Bradshaw et al. 2009).

Conventional breeding strategies are too slow because they are essentially based on phenotypic selection, involving crosses between tetraploid varieties and advance clones, and then field evaluation and selection. The inherent genetic complexity of potato has made breeding time consuming and often with unpredictable results. Quantitative characters are difficult to maintain intact during breeding process and hence identification of the highly heritable sources and robust screening procedures is important. The selection cycle, from initial crosses to varietal release requires approximately 10 years or sometimes more than 30 years. Moreover, a major obstacle in using wild species in breeding is number of backcrosses required. Three to seven backcrosses are required to transfer a major dominant gene, which may take several generations to reconstitute phenotype of the commercial cultivar (Bradshaw et al. 2006). Erratic nature of the environmental factors and mechanisms involved in stress tolerance make the breeding program difficult. Most of the abiotic stress tolerance traits are polygenic in nature and strongly influenced by the genotype and environment interactions. These traits have low heritability and selection of such traits for tolerance will be less reliable. Careful analysis of environmental constraints together with genetic potential and trait combinations for yield, and simulation modelling are probably the best avenues to improve crop performance. Hence, biotechnology based potato breeding is required.

Efficiency and precision in plant breeding can be enhanced by use of diagnostic DNA-based markers and has been applied to potato breeding. Numerous genetic

mapping experiments have been performed using potato and have identified DNA based markers linked to genes/quantitative trait loci (QTLs) for quality traits and agronomically important traits (D'hoop et al. 2008, 2014). The genome sequence of potato has been completed (844 Mb) and it revealed 39,031 protein coding genes in potato (Potato Genome Sequencing Consortium 2011). This information will be a platform for genetic improvement of potato. Genomic tools like high throughput resequencing, could be used to identify rare alleles and recessive alleles which are gone unnoticed till now and virtually inaccessible to potato breeders. Genotyping by sequencing combined with haplotype characterization has been shown to be an efficient method to characterize allelic variants that influence traits. When valuable alleles are identified and introgressed through hybridization, dense marker data can be used subsequently to efficiently retain the desirable alleles while removing the remaining donor genome. This process will be more effective in diploid inbred germplasm than in heterozygous tetraploids. In the genomics era, germplasm enhancement will increasingly be focused on identifying and introgressing alleles rather than cumbersome phenotyping traits. Alleles will come from a broad pool of genetic resources that includes wild species relatives of potato, landraces, cultivated potato itself, and distantly-related species. Genomics tools will greatly increase the efficiency of introgressing multi-genic traits and will make it possible to identify rare alleles and utilize recessive alleles. Introgression may occur through sexual hybridization, or molecular manipulations, but evaluation of progeny will increasingly involve assessing allelic composition and distribution of parental genomes.

The effect of abiotic stress can be observed visually and such variables can assist in development of cultivars to abiotic stress. To develop heat tolerant crop, leaves related traits like photosynthetic efficiency, haulm growth, tuber initiation and photosynthetic partitioning should be considered. Root architectural traits like root depth, high number of stolon roots, root mass are positively related with drought tolerance. Water use efficiency is another important trait which is correlated with drought stress. Cultivars having tolerance to single abiotic stress have been identified and developed. In India, recently a heat tolerant cultivar Kufri Lima has been released and Kufri Surya is another heat tolerant variety. In Japan, conventional breeding by crossing two commercial potato cultivars, Irish Cobbler and Konafubuki resulted in drought tolerant cultivar, Konyu (Iwama 2008) using high root dry weight as a selection criterion.

2.5 Diversity Analysis

Crop improvement primarily relies on examining the available genetic diversity to identify potential candidate germplasm for further use in research. Cultivars grown in extreme environments have always served as sources of novel genes/alleles/QTLs for improving tolerance traits in cultivated varieties. Screening of several *Solanum* species identified *S. juzepczuckii* and *S. curtilobum* as salt-tolerant (Silva et al. 2001), and three species, *S. juzepczuckii*, *S. acaule* and *S. curtilobum* are frost-resistant

(Martinez et al. 1996). The Andean potato landraces cultivated in cold and dry climatic conditions served as ideal candidates for identifying genes conferring tolerance to drought stress (Ritter et al. 2008; Vasquez-Robinet et al. 2008). Similarly, wild potato germplasm have also been useful in improving drought tolerance traits. The differential response of cultivated as well as wild varieties to salinity stress has identified crucial genes and pathways responsible for salt tolerance (Levy and Veilleux 2007) and water stress (Levy et al. 2013). Genetic diversity reveals wide variability in potato varieties and wild species (Tiwari et al. 2018a, 2019) and exploited in improvement programs.

Primitive forms of cultivated potato and their wild relatives provide rich, unique and diverse sources of genetic variation which could be a source of various traits for potato breeding. This may be because the habitat of these species is highly variable growing at different altitudes from sea level to over 4000 m in an extensive range of temperature, photoperiod, soil and water supplies (Hawkes 1990). Also, the wild genotypes adapted to different ecological conditions ranging from highland, tropics to desert regions. They are equally diverse in morphological traits too. Andes, the center of origin and diversity of the potato represents vast genetic resources. The harsh climate of the Andes is home to the valuable sources of germplasm which can withstand high light, drought and cold stress. They are recognized to be valuable in breeding programs for abiotic stress, environmental tolerance, processing and agronomic traits. The International Potato Centre (CIP), Lima Peru maintains over 600 clones of potato landraces which are found to be variable for agronomically important traits (Cabello et al. 2012).

In Andes, wild relatives are grown near farmers' fields and insect aided cross pollination between tetraploid and wild species is common. Grun (1990) described Andean potato as 'Genetic sponge' as it is capable of absorbing the genes from wild and cultivated relatives from its surroundings and hence cultivated potato is rich in allelic diversity at the centre of origin. So germplasm enhancement programs will help in improvement of the traits like disease resistance, abiotic stress, quality traits and agronomic traits under suboptimal conditions. Unlike disease resistance mechanism of species-specific nature, abiotic stress mechanisms are shared among the species. Hence germplasm enhancement for the abiotic stress mechanisms should be concentrated both in wild species and distantly related plants. Such traits can be introduced to potato through molecular biology approaches viz., transgenics and genome editing tools. Therefore, search in potato germplasm for genes that contribute to salt, drought, heat and cold tolerance in other Solanaceous crop like tomato will help in potato breeding programs.

2.6 Molecular Mapping of Tolerance Genes and QTLs

2.6.1 Linkage Mapping

The cultivated potato (*S. tuberosum* L. ssp. *tuberosum.*) varieties are tetraploid ($2n = 4x = 48$). The complex genetic inheritance (tetrasomic) and high heterozygosity of potato complicate its genetic mapping and therefore diploids were used in the most mapping studies. However, self-incompatability nature of the diploids prevents development of pure lines. Therefore, a number of common mapping approaches based on homozygous lines cannot be applied in potato unlike in other crops. The first potato genetic map was reported in 1988 using tomato RFLP (restriction fragment length poylmorphism) markers (Bonierbale et al. 1988). Two linkage maps were obtained from a cross between a diploid clone of *S. tuberosum* group Phureja and a diploid hybrid line from *S. tuberosum* group Tuberosum × (*S. tuberosum* group Phureja × *S. chacoense*). The alignment of the RFLP loci shows a high level of similarity to the tomato map and the major differences were paracentric inversions on three chromosomes. With advances in molecular markers, many maps have been constructed and used for identifying specific loci, and markers have been used in potato breeding for specific traits.

Simple sequence repeats (SSRs) markers have assisted progress in mapping the potato genome and are valuable for fingerprinting closely related genotypes (Tiwari et al. 2018a, 2019). Amplified fragment length polymorphism (AFLP) markers showed greater merit than multilocus SSRs for fingerprinting in a comparative study of RAPD (random amplified polymporphic DNA), ISSR (inter simple sequence repeat), AFLP, and SSR. These can be made from the expressed sequence tag (EST) database and made more systematical by potato genome information. With further advancement of genome research in plants, many relevant technologies can be applied to potato. DArT (diversity array technology) (Wenzl et al. 2004) benefits potato genetics. Finally, new sets of markers have been applied with advances in the potato genome sequencing. Single nucleotide polymorphism (SNP) has been used widely for marker generation in many species and many markers have been generated also in potatoes. Many quantitative trait loci (QTLs) for yield, agronomic and quality traits have been identified in potato (Bradshaw et al. 2008). The QTL mapping study for the abiotic stress tolerance is at infant stage. Quite a small number of QTLs have been identified for drought stress tolerance (Anithakumari et al. 2012).

Bacterial artificial chromosome (BAC) libraries have become the main vehicle for performing map-based gene cloning and physical mapping in potato. Several BAC libraries were constructed such as cultivated potato and wild species like *S. bulbocastanum* (Song et al. 2000). These libraries represent a potentially useful resource for the study of comparative genome organization and evolution in potato and other Solanaceous crops. A BAC library was used to make the ultra-high-density (UHD) genetic and physical map of potato with 10,000 AFLP loci (van Os et al. 2006). In addition, BAC libraries were used for fluorescence in situ hybridization

(FISH) to develop chromosome-specific cytogenetic DNA markers for chromosome identification in potato (Dong et al. 2000).
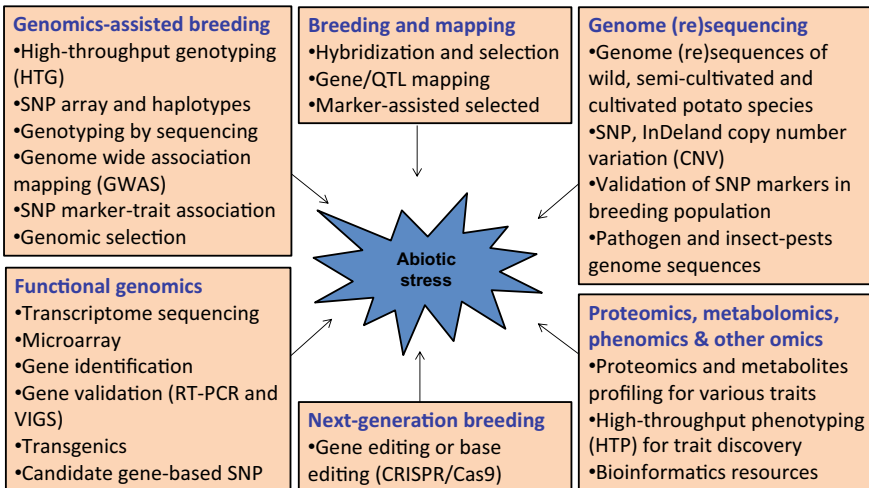
Drought tolerance was surveyed by various physiological parameters such as relative water content, stomatal conductance and chlorophyll fluorescence measurements (Schafleitner et al. 2007). However, these traits show less level of heritability, are controlled by several genes and their epistatic in nature restricts the breeding in potato for drought tolerance. Anithakumari et al. (2011) have identified 23 QTLs (13 QTLs under well-watered conditions, 7 under drought stress condition and 3 recovery QTLs) in a diploid mapping population, and the genes underlying these QTLs were related to root to shoot ratio, plant height, shoot fresh weight, shoot dry weight, fresh root weight, root dry weight, root length, fresh biomass and dry biomass. Interestingly, the study also found the co-localization of SNPs with root to shoot ratio QTL, thus proposing their applicability in MAS for drought tolerance in potato. QTLs associated with root length allow the selection of plants with desirable root characteristics in a non-invasive method as root traits are of immense importance in tolerating drought. In addition, QTLs associated with carbon radioisotope discrimination, chlorophyll content and chlorophyll fluorescence were also identified (Anithakumari et al. 2012), which can serve as good selection criteria since they are easy to measure, fast, and allow little or no sample destruction. Recently, Sharma et al. (2013) have constructed a dense genetic and physical map for diploid backcross progeny of potato using 2469 markers, including SSR, diversity array technology (DArT), and SNPs, and using the same genotypic data of these markers. Khan et al. (2015) constructed maternal and paternal maps to carry out the first QTL study for drought tolerance. The study identified 45 genomic regions associated with nine traits in well-watered and terminal drought treatments and 26 QTLs associated with drought stress. These QTLs will promisingly be used in the breeding of potato for durable tolerance to drought, using conventional as well as genomics-assisted breeding approaches.

2.6.2   Association Mapping

Association mapping, also known as linkage disequilibrium mapping was originally developed to study genetic disorders in humans (D'Hoop et al. 2008). Association mapping is a general approach to detect correlations between genotypic and phenotypic variation in a population based on the property of linkage disequilibrium. As compared to linkage mapping, which requires the use of highly related individuals such as full sibs, association mapping can exploit the properties of more complex populations with various degrees of relatedness. This approach can provide much higher levels of resolution for the genetic dissection of quantitative traits. Association mapping is considered as an alternative approach to the traditional QTL mapping. Association mapping has several advantages over traditional QTL mapping i.e. no need to develop segregating generations, a collection of various cultivars and breeding lines can be utilized for mapping studies and higher mapping population may be

reached with many more meiotic recombination. In view of the advantages and applications of association mapping, it can be applied in potato to develop molecular markers for drought tolerance.

The feasibility of association mapping in tetraploid potato to identify QTLs for agronomically important traits like plant maturity and quality traits (D'Hoop et al. 2008, 2014), allele diversity (Kloosterman et al. 2013), and fry color (Byrne et al. 2020) using diverse potato genotypes. Berdugo-Cely et al. (2017) analyzed 809 andigenum group accessions from the Colombian Central collections (CCC) using SNP markers. They revealed that CCC is a highly diverse germplasm collection genetically and phenotypically and useful to implement association mapping in order to identify genes related to traits of interest and to assist future potato genetic breeding programs. Genome wide association studies (GWAS) are usually applied to large collections of theoretically unrelated individuals, the genetic diversity is supposed to be high and new alleles can be discovered. Furthermore, the high number of ancestral meiosis that occurred in the GWAS population can allow a precise QTL mapping. GWAS are much useful in diverse germplasm which offer new perspectives towards the discovery of new genes and alleles especially for complex traits like abiotic stresses in plants. Figure 2.2 summarizes the use of integrated molecular approaches for abiotic stress management in potato.

Genomics-assisted breeding
•High-throughput genotyping (HTG)
•SNP array and haplotypes
•Genotyping by sequencing
•Genome wide association mapping (GWAS)
•SNP marker-trait association
•Genomic selection

Breeding and mapping
•Hybridization and selection
•Gene/QTL mapping
•Marker-assisted selected

Genome (re)sequencing
•Genome (re)sequences of wild, semi-cultivated and cultivated potato species
•SNP, InDeland copy number variation (CNV)
•Validation of SNP markers in breeding population
•Pathogen and insect-pests genome sequences

Abiotic stress

Functional genomics
•Transcriptome sequencing
•Microarray
•Gene identification
•Gene validation (RT-PCR and VIGS)
•Transgenics
•Candidate gene-based SNP

Next-generation breeding
•Gene editing or base editing (CRISPR/Cas9)

Proteomics, metabolomics, phenomics & other omics
•Proteomics and metabolites profiling for various traits
•High-throughput phenotyping (HTP) for trait discovery
•Bioinformatics resources

Fig. 2.2 Use of integrated molecular approaches for abiotic stress management in potato

2.7   Marker Assisted Selection

Since last two century tremendous progress has been made in molecular biology which provided important tools for plant breeders to accelerate genetic gain. Conventional screening in potato breeding program is performed using field trials, glasshouse trials and laboratory tests in order to identify genotypes with a combination of superior desirable traits. Nearly 40–50 characters are evaluated before development of the new variety. Most of these characters are quantitatively inherited and make the selection less efficient. Among the molecular tools, molecular genetic markers linked to the trait of interest can be utilized for faster development of new varieties. Marker assisted selection can be applied at the seedling stage and it eliminates classification errors due to environmental effects on phenotypic evaluation. It selects the traits early in the breeding program saving several years than the conventional breeding program. It is more effective for the qualitative traits controlled by major genes than the quantitative characters controlled by the minor genes. It can be effectively applied to quantitative characters if QTLs' of large effect contribute to the measured trait. A number of molecular markers have been reported in potato for biotic stress resistance but very negligible in abiotic stress tolerance, and of which only a handful has been employed in practical potato breeding (Barone 2004).

Potato map is one of the most highly saturated maps with different markers and also genome sequencing of potato has provided an ample opportunity for optimal use of DNA analysis for MAS. The most widely used classes of markers in current plant molecular breeding practice are SSRs and SNPs. These markers are generally highly polymorphic, codominant, reliable, relatively simple and cheap to use, and they can also be multiplexed (Collard and Mackill 2008). SNPs are abundant in potato. MAS helps in introgeression of genes from one genotype to another and most genome over 99% can be recovered with only three backcross generations instead six to seven in tradition backcross breeding program. While introgression from the wild species, many undesirable characters will be transferred which are reduced by positive and negative selections. Molecular markers closely linked to the gene controlling the trait of interest can be selected at the early stage without waiting for the specific development stage (positive selection). It reduces time and space and concurrent analysis of more than one trait is also possible. Negative selection can be applied to select the lines exhibiting less wild genome content and linkage drag around the introgressed gene. The availability of molecular markers specific to the donor genome will help to recover the highest recipient genome (Barone 2004).

The increasingly widespread availability of the molecular markers linked to single resistance genes and to QTLs for yield and qualitative traits may offer many new potential applications for the MAS in the production of new commercially available potato cultivars. Genetic marker systems and genome sequencing technology have become more sophisticated and cheaper to apply. Continued improvements in genomics technology and computational analysis, the costs of the MAS are expected to fall still further, permitting its application to a broadened range of traits in potatoes, as in other crops (Slater et al. 2013, 2014). Marker assisted selection for stress

tolerance traits lags far behind MAS for disease resistance genes, because tolerance to theses stresses is often mediated by many interacting genes and factors (Watanabe et al. 2011). Potato responses to drought are mediated by a number of genes including transcription factors (Ambrosone et al. 2011). Anithakumari and coworkers (2011) identified drought resistant genes related to root-shoot ratios which are relatively controlled by few QTLs. These can be many potential candidates for development of linked molecular markers. Responses to drought are highly varied. With availability of complete potato genome sequence, potential development of markers will be facilitated (Ramakrishnan et al. 2015). Similarly many genes were identified for other abiotic stresses viz., cold, heat and salinity stress, but no markers have not been identified for MAS.

2.8 Genomics Assisted Breeding

2.8.1 Genome Sequence

The first reference genome was completed in 2011 using the whole genome shot gun approach by the Potato Genome Sequencing Consortium (Potato Genome Sequencing Consortium 2011). The source for the potato draft genome was DM1-3 516 R44 referred as DM and size of the potato genome is 840 Mb. Two years after the publication of the first reference genome, a new assembly of the DM clone was released with a more accurate arrangement of scaffolds and pseudomolecules (Sharma et al. 2013). This updated assembly of the potato reference genome (version 4.03) was created by integrating linkage data from a segregating diploid potato population derived from the reference sequence clone (DM). Recently an update of the potato reference genome (*S. tuberosum* group Phureja DM1-3 v6.01) has been released (http://solanaceae.plantbiology.msu.edu/pgsc_download.shtml).

   S. commersonii is a wild species carrying important agronomic traits like resistance to root knot nematode, soft rot and blackleg, bacterial and Verticillium wilt, *Potato virus X*, common scab, late blight, and the ability to acclimate to the cold/freezing conditions. In 2015, the draft genome sequence of *S. commersonii* was released using whole genome shot gun approach with smaller genome size (830 Mb) than the potato genome. The smaller size may be due to the lower frequency of the transposable elements (TEs) found relative to the cultivated potato and tomato. The divergence time between cultivated potato and *S. commersonii* was estimated to be approximately 2.3 million years ago (Aversano et al. 2015) and 37,662 genes were predicted from the transcriptome analysis. The wild potato has fewer R genes than the cultivated potato and tomato. A total of 855 genes were differentially expressed in plants acclimated to frost stress and non-acclimated plants. Recently, Kyriakidou (2020) assembled genome of six polyploid potatoes including one Chilean and five Peruvian potato landraces which ranged from triploid to pentaploid (3x to 5x). These polyploid genomes provide a great resource to enhance potato breeding.

2.8.2   Gene Annotations

Structural annotation of the potato (DM) genome assembly was performed by the PGSC and the International Tomato Annotation Group (ITAG) (Potato Genome Sequencing Consortium 2011). The ITAG structural annotation of the potato genome was initially completed to allow for more direct comparisons with the ITAG structural annotation of the tomato genome, as both genomes were annotated using the same computational pipeline. Both structural annotations yielded approximately the same number of predicted genes. The PGSC and ITAG structural annotations have been included within a public online genome browser (http://solanaceae.plantbiology.msu.edu/pgsc_download.shtml). Functional annotation is a main area in which biological information is assigned to genes. Annotation of a large set of ESTs with a combination of similarity searches against protein databases and gene ontology (GO) terms were used to determine important genes under various stress conditions. Similarly, functional annotations have been determined for both potato and tomato defined gene models. A resource has been developed that integrates the PGSC and ITAG functional annotations together allowing researchers to search based on annotation and also visualize associated Pfam domains, and matches to UniRef, Arabidopsis, potato, and tomato proteins. The structural and functional annotation resources provide valuable information that is useful towards advancing potato and other Solanaceae, and increasing the efficiency of potato breeding.

2.8.3   Functional Genomics

The generation of EST collections is a primary source for large-scale gene discovery for many years in several crops including potato. These ESTs were an important source for genes and markers (SNP and SSR) and microarrays. However, now with the availability of whole genome sequence, ESTs are of lesser importance. ESTs generated by pooling across tissues and stress treatments show an importance transcriptome resources in terms of transcription factors, stress response genes, and signal transduction processes in response to abiotic stresses. These sequences in conjunction with RNA-seq reads were instrumental in the annotation of the potato genome assembly.

Functional genomics identifies genes and their associated functions and interactions with gene families at spatial and temporal levels. This requires experimental tools like high throughput gene expression analysis (transcriptomics), bioinformatics, contemporary analysis (metabolomics and proteomics), phenotyping and gene functions techniques. Transcriptomics aims to assess changes in the transcriptomes; proteomics studies the total protein content and metabolomics aims at study of small molecule resulting from the various metabolic processes. Several studies have been conducted for gene expression analysis, proteome analysis and metabolomics (Nakabayashi and Saito 2015). Cheng et al. (2019) analyzed WRKY members of

different Solanaceous crops including tomato, potato and pepper using comparative genomics. Such finding helps to study the evolutionary relationships and development of stress tolerance varieties of potato and tomato.

The aim of the different omics approaches is to acquire comprehensive, integrated understanding of biological processes (Davies et al. 2008) to identify various players for drought and heat stress (Aksoy et al. 2015). The complex and varying nature of the environmental perturbations require a systems biology approach to delineate genes, proteins, and metabolites to properly assess the qualitative and quantitative changes and effects caused by the respective stress condition. However, despite current advancements in the "omics" technology, researchers lack good correlation between the data obtained across different platforms. Changes at the transcript level do not always reflect the alterations at the protein level, which may be majorly attributed to the degradation of transcripts and posttranscriptional regulatory mechanisms. Several studies have offered key insights into gene-level changes and regulatory interplay that occur during specific or combinatorial stress conditions (Bokszczanin et al. 2013). Transcriptome analysis shows numerous genes involved in various abiotic stress tolerance such as heat (Tang et al. 2020), drought (Moon et al. 2018; Chen et al. 2019), salinity (Li et al. 2020) and nitrogen deficiency (Tiwari et al. 2020a, 2020b, 2020c). In parallel, proteomics and metabolomics platforms have also been well-explored to elucidate the underlying complexity of various stress responses (Aghaei et al. 2008).

Proteomics studies are particularly informative which includes quantitative protein profile, protein–protein interaction network and their functions. There is an increasing use of proteomic approach to elucidate the complex relationship between stress tolerance and crop productivity. This information is especially important to identify specific potential biomarkers to come up with smart crop varieties known as proteomic-based marker assisted selection. Proteomics has now become an indispensable tool for high edge development in technology. With advancements in proteomics (mass spectrometry (MS)-based techniques) and quantification methods, proteomics has become a complementary tool for other omics and systems biology approaches (Weckwerth 2011, 2014). Comparative proteome analysis indicates the fine tuning metabolism as a major factor of stress tolerance of potato (Boguszewska-Mankowska et al. 2020).

Metabolomics focuses on the study of dynamics of low molecular compounds which results from the complex metabolic processes in the cell. The intensity of these processes is under the influence of both biotic and abiotic stress factors. Plants synthesize plenty of compounds of various chemical structures. These compounds are generally grouped as those of the primary metabolism which ensure the existence of any living being, and of the secondary metabolism which are characteristic of certain groups of organisms. Recently, gas chromatography-mass spectrometry (GC-MS), high-performance liquid chromatography-mass-spectrometry (HPLC-MS), ultra-performance liquid chromatography tandem mass-spectrometry (UPLC-MS), capillary electrophoresis-mass spectrometry (CE-MS) and nuclear magnetic resonance (NMR) spectroscopy are used to separate and identify metabolites (Hong et al. 2016). Advanced technologies and uniform extraction and detection methods, and available

databases for the identification of various compounds, as well as multivariate statistics methods provide excellent opportunities for metabolomics studies.

2.8.4   Genome Wide Selection

Potato improvement applying next-generation breeding techniques is essential to shorten the breeding cycles. The availability of potato genome sequencing allows discovery of novel genes and markers associated with biotic stress tolerance and yield contributing traits. In order to achieve this, partitioning of genetic variance and genome wide prediction with allele doses is important in tetraploid potato (Endelman et al. 2018). In addition to conventional methods, new techniques have emerged for rapid crop improvement. GWAS and genomic selection (GS) based on high-throughput genotyping (HTG) by sequencing (GBS) and SNP array coupled with high-throughput phenotyping (HTP) and bioinformatics are powerful tools for rapid breeding of new varieties (Uitdewilligen et al. 2013; Vos et al. 2015; Sharma et al. 2018; Caruana et al. 2019). A few genome level studies in potato indicate starch phosphorylation associated SNPs by GWAS analysis using Illumina 22 K SNP potato array (GGP Potato V3) (Khlestkin et al. 2019), GBS for diversity and genomic selection in tetraploid potato (Caruana et al. 2019), HTP for rapid advancement of genetic gain in crop breeding programs (Zhao et al. 2019) and genetic diversity in CIP genebanks using SolCAP 12 K SNP array (Ellis et al. 2018).

Current cultivars continue to be susceptible to a range of biotic and abiotic stresses, and so are capable of significant improvement for a range of complex characters. Conventional potato breeding is relatively slow, laborious and a difficult process to obtain genetic gain. Modern breeding methods and technologies offer great benefit for the enhancement of breeding programs, but still require validation and cost-effectiveness in order to be adopted in practical breeding. In particular, MAS has the ability to select for traits earlier than conventional screening methods. MAS will be more effective for qualitative traits, but will also be valuable for quantitative characters, especially if QTLs of large effect contribute to the measured character, or if a group of markers can be identified that are linked to a group of alleles of smaller effects influencing the trait.

Animal breeding programs have adopted the estimation of breeding values using best linear unbiased prediction (BLUP), to exploit the additive genetic variance. Recently, the use of BLUP estimated breeding values (EBVs) in potato breeding clearly demonstrated the advantage of using EBVs over progeny means in cross-generation prediction of performance, particularly for traits with low heritability (Slater et al. 2014). As the BLUP analysis uses information from all relatives in the analysis, more data is used to calculate the EBV. EBVs represent the additive genetic effect transmitted from parents to progeny. This process enables identification of genotypes based on properties of the population in addition to individual pheno-types, and provides a more accurate selection process for such traits, and potentially enabling breeding from the best progeny much earlier (Slater et al. 2014).

MAS can reduce the duration of the breeding cycle between crossing programmes in order to combine these genes with other desirable traits. A combination of MAS with EBVs for complex traits will result in a significant reduction in the breeding cycle for all measured traits. Consequently, the use of both of these breeding tools will ensure rapid progress in combining traits and improving genetic gain in potato breeding programmes. Therefore, adoption of MAS and EBVs will provide substantial benefits within a multi-trait breeding strategy, and allow the design of superior combinations and their analysis for maximum genetic gain. Previous studies have shown that MAS can be cost effectively applied at the second field generation (Slater et al. 2013), and EBVs can be calculated for more complex traits at the same stage (Slater et al. 2014). These advances will greatly accelerate the breeding cycle, as the combined use of MAS and EBVs can reduce the breeding cycle from over 10 to as few as 4 years, and therefore, accelerate genetic gain relative to conventional breeding methods. At the same time, they will ensure that improvement is made in all measured traits, from those under simple genetic control to those under far more complex control.

2.9 Recent Concepts and Strategies Developed

2.9.1 Genome Editing

Highly heterozygous and tetrasomic inheritance nature of cultivated potato has made research complicated and time-consuming and therefore necessitates gene editing (Andersson et al. 2017). Given that efficient genetic transformation protocol and sequence information are available, potato is a good candidate for gene editing (Butler et al. 2015). There are many challenges that need to be addressed in establishing potato mutants using programmable nucleases. Genome editing which creates novel allelic variants in the genome uses sequence specific nucleases (SSNs) includes old techniques like ZFNs (Zinc Finger Nucleases), TALENs (Transcription Activator-Like Effector Nucleases), and recent CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR- associated proteins (Cas9) system. Among them, CRISPR-Cas9 system is an emerging next-generation breeding technology and an RNA-guided approach to target DNA sequence (now RNA also). Recent reports on the genome editing of major crops of economic importance including potato have shown high efficiency of SSN platforms for site directed precise mutagenesis (indels) of desired gene for resolute modification (Dangol et al. 2019). Owing to limitations with commercialization of genetically modified organism (GMO) in Europe or elsewhere, CRISPR-Cas is the most powerful genome editing tool. CRISPR/Cas9 is accounted for efficient site directed mutagenesis and gene silencing in potato (Tiwari et al. 2020d). In a study, CRISPR/Cas9 was designed with two sgRNAs to target *StALS1* gene (responsible for herbicide resistance) in *Solanum tuberosum* (Butler et al. 2015). Genome editing of the potato *GBSS* gene involved in amylase

synthesis showed CRISPR/Cas9 system would be desirable for novel germplasm development, with targeted gene knockouts without any stable integration of DNA (Andersson et al. 2017). Genome editing in elite germplasm may be pursued for promoting alleles controlling desired traits and missing in the breeding populations.

2.9.2   Nanotechnology

Conventional breeding have met a limited success in improving stress tolerance due to complex traits, low genetic variability and inefficient selection methods. Despite so much progress, crop yield has reached a plateau in most crops including potato. This requires intervention of modern technology like nanotechnology to increase in food production from the available land area through efficient resource utilization while saving environments (Das and Das 2019). Nanotechnology is one of the most promising areas in the era of agricultural biotechnology (Usman et al. 2020). Nanotechnology explores wide area and opens large scope for diverse applications in fields of biotechnology, agricultural sector and health benefits (Cattaneo et al. 2010). Nanoparticles commonly referred to as nano scale particles (NSP's) size between 1 and 100 nm, and having extreme small size nanoparticles (NPs) have unique and diverse physical and chemical properties such as increased reactivity, expanded surface area, flexible pore size and diverse particle morphology (Sanzari et al. 2019). In the current scenario nanoparticles can be a potential to be affectively used as plant growth and development promoters, herbicides, nanopesitcides, nanofertlizers and so many others.

Abiotic stresses such as drought, heat, salinity, cold, nutrient deficits, chemical toxicity or oxidative stress are one of the major causes of crop loss worldwide. The application of nanoparticles or nanodevices causes both positive and negative impacts on various plant growth and development stages. Zinc oxide nanoparticles are involved in growth, flowering and seed productivity in onion (Laware and Raskar 2014). Nanotechnology promises the significant effort subjected to various traits in crops. In potato, Gowayed et al. (2017) suggest that the use of SiO_2-NPs at 50 mg L^{-1} as optimized dose improves plant growth under salinity stress through increased expression of genes and proteins associated with salt stress responses. Under stress conditions, ROS scavenging-related metabolic pathways are triggered, such as shikimate-phenylpropanoid biosynthesis and ascorbate and aldarate metabolism. Such over accumulated ROS in plants under abiotic stress can be scavenged by nanoparticles such as CeO_2, C60 and Fe_2O_3, NPs showed better performance under stress conditions, and further NPs enhance photosynthesis by light harvesting, electron transfer and ROS scavenging capacities (Zhou et al. 2021). Nanomaterials can alleviate the damage resulting from different abiotic stresses through activating process of plant defense system. Due to the properties of nanomaterials they can regulate water uptake by plant tissues, hence promote seed germination and plant growth (Zhou et al. 2021).

2.10 Genetic Engineering

2.10.1 Cisgenesis

Climate change has a tremendous negative impact on agriculture production. Due to increase in population, demand for food is increasing rapidly. Hence there is a need to breed crop varieties with increased yield simultaneously able to cope up abiotic stresses besides diseases and pests which requires effective utilization of the genetic diversity. Traditional crop improvement methods involve genetic crossing, selection of the natural or artificial mutations, somatic hybridization and transgenics for the trait of interest. Genetic crossing from the wild species is time consuming due to crossing barriers, linkage drag and several generations are required to develop a line. Transgenic plants which involve introduction of gene from the different or same species or different kingdom brought considerable concerns about safety and impact on health and environment. Recent progress in pant genome sequencing has facilitated isolation of genes from crossable species and such genes are known as cisgenes. Such genes allow the plant to be modified while remaining plants within the same gene pool. Since, linkage drag is another problem faced by the breeders while transferring genes from wild species into cultivated type and it takes several generations to recover the recurrent parent genome. Using cisgenesis approach, late blight resistance genes were transferred to cultivated potato from wild potato species *S. bulbocastanum* (van der Vossen et al. 2003). It involves only the interested genes transfer along with native promoter, introns and terminators. Genotype and phenotype of the varieties remain unchanged and breeders have higher knowledge of transferred sequences. Cisgenesis can be applied in potato for abiotic stress tolerance as plenty of wild species are available containing number of genes for such traits. Thus cisgenic insertion of additional copies of native genes may provide a new approach to modify plant genomes, expand genetic variance in plant architecture available to breeders and accelerate the transfer of alleles between species which are difficult to cross.

2.10.2 Gene Stacking

The growing population and emerging environment challenges demand the development of more productive crops, more resistant to pests and diseases and tolerant to many stressful threats such as high salt, drought, flood, freezing and adaptation to poor quality agricultural land. In addition, the need to save water in agriculture is compelling the development of crops more efficient in its utilization. Gene stacking refers to introduction of two or more transgenes of agronomic interest in the same plants. Advances in genetic transformation technologies and genome sequencing have facilitated the introduction of multiple genes and characteristics in a single variety using gene stacking strategies. The multi-engineering in plants can make those goals more feasible to achieve. Examples of gene stacked crops are rice,

wheat, canola, cotton, potato, soybean etc. In potato GAANTRY (Gene Assembly in *Agrobacterium* by Nucleic acid Transfer using Recombinase technologY) system was used to develop transgenic plants with stacked transgenes (McCue et al. 2019). Plants with stacked genes now form a significant part of GM crops grown throughout world just like gene pyramiding in breeding. In the future, tolerance to abiotic stresses would be priority in changing climate scenario.

2.11   Bioinformatics

Bioinformatics is an indispensable tool to analyze genome level data generated through high-throughput next-generation sequencing technologies. With the increasing genomics resources and reducing cost, it opens more avenues for the use of bioinformatics in plant research. Genomics and post-genomics research necessarily requires the use of modern bioinformatics tools for genomics, transcriptomics, metabolomics, proteomics and phenomics data analysis and also for repository of enormous amount of data. Enormous amount of nucleotides (DNA and RNA) and protein sequence data are deposited with the international gene banks. The most commonly used resources for data submission are NCBI (National Centre of Biological Information) in USA (http://www.ncbi.nlm.nih.gov), EMBL (European Molecular Biology Laboratory) in Europe (http://www.ebi.ac.uk/embl/), and DDBJ (DNA Data Bank) in Japan (http://www.ddbj.nig.ac.jp). Besides, there are a number of various other bioinformatics tools used for data analysis.

The potato genome sequence, deciphered in 2011 by the Potato Genome Sequence Consortium, is maintained by the SpudDB, Potato Genomics Resources, Michigan State University, USA (http://solanaceae.plantbiology.msu.edu/pgsc_download.shtml). This website describes about the potato genome sequence, annotation, search tools, genes and various other information for breeders and biotechnologist. Recently, this database has been updated with recent version of the potato genome sequence and the genome sequence of M6, a wild potato species (*S. chacoense*). Moreover, a dedicated genomics resource is available for Solanaceous crops i.e. Solanaceae Genomics Network (http://solgenomics.net/). This includes a collection of genetic maps, genomes, and tools. PoMaMo (Potato Maps and More) () provides information about maps, anchor markers, genes and variants. The PlantGDB (http://www.plantgdb.org/StGDB/) database has data on genome, gene model, alignment, gene structure annotation and annotated protein alignment. Importantly, there are several international potato gene banks for germplasm exchange such as the International Potato Centre (CIP) (https://cipotato.org/), Lima, Peru; the US Potato Genebank (NRS) USA; the CGN Potato Collection at the Centre for Genetic Resources, the Netherlands (CGN); and the Commonwealth Potato Collection (CPC) of the Vavilov Institute (VIR). CIP is amongst the largest international potato gene bank in the world, which provides potato germplasm throughout the world. A number of country-specific databases have been developed such as the European Cultivated

Potato Database (ECPD) (https://www.europotato.org/), UK potato variety database (http://varieties.ahdb.org.uk/). Thus, there are several potato-based databases in many countries for research and development.

2.12 Social, Political and Regulatory Issues

The potato industry involves protection of intellectual property rights (IPRs) related to industrial applications, such as processing, product development, and trademarks. Biotechnological inventions are widely covered by patents. Seed-tuber production and quality assurance have a strong association with proprietary technology, such as that on tissue culture, diagnostics, biotechnological products like genes and processes, and technical aspects of breeding may be protected under IPRs. New varieties can be protected by plant variety protection laws under the International Union for the Protection of New Varieties of Plants (UPOV). In the public sector, including state universities, has filed and owns a variety of patents rather than use the UPOV scheme. A patent on genetic markers can be made to allow a diversity of free public users of markers. Genes have been protected under the IPR system, and applications for commercial use often have been hindered due to the difficulty of integrating patent licenses. Financial resources and/or cross-licensing deals need to be considered for IPR coordination. Private–public cooperation could provide an open arena for the utility of IPRs. This will provide flexibility to the potato breeders by allowing free IPR platforms for cultivar development.

CIP has pioneered in the area of participatory research (PR) which includes farmer back to farmer, integrated management of pest and diseases, participatory variety selection, farmer field school, participatory approaches for native potato variety value chains using the participatory market chain analysis, advocacy for PR and policy change, ending with nutrition-related PR in more recent years. The farmer-back-to-farmer model emerged from an interdisciplinary CIP team that included both social and biophysical researchers. Researchers must work with farmers to identify the right problem to solve, interact creatively with them, work on-farm and on-station, and present the results back to farmers for feedback. Further, an agronomist at ICRISAT (International Crops Research Institute for the Semi-Arid Tropics) developed a new method called the mother-and-baby trial design (MBT) for participatory research with farmers. The MBT method, also known as the central/satellite design, involves a complete, replicated trial in a central location in a community, with satellite mini-trials in farmers' fields. The method was widely used at CIMMYT and elsewhere as well. At CIP, Lima the mother-and-baby trial design allowed plant breeders to understand farmers' selection criteria (e.g. large tubers, resistance to frost and to late blight). Farmers in the highland communities participated in assessing CIP's improved clones from a breeding population. This helped not just to select desired traits, but to facilitate registration of new varieties and to disseminate them with farmers, buyers, processors, and consumers.

Adaptation and resilience to climate change requires farmers to adapt faster information. ICTs offer the possibility of connecting a larger number of farmers with sources of information and advice to make decisions to tackle climate change. The essence of PR approaches has been maintained over the years as a mechanism to facilitate the dialogue between scientists and farmers. PR method needs to include a larger number of viewpoints from the innovation and agri-food systems. New ICTs offer the possibility of improving communication, analysis, and decision making systems. World over social scientists, breeders, pathologists, and agronomists have been continuously working together to create influential participatory methods with farmers and different stakeholders with the help of emerging technologies.

2.13   Future Perspectives

Crop designing based on genomics resources is need of the day for potato improvement. The information presented about various abiotic stresses affecting potato growth and development such as genetic resources, genetic diversity, classical genetics and breeding, gene mapping, marker assisted selection, genomics assisted breeding, recent concepts (genome editing and nanotechnology), genetic engineering, bioinformatics tools and social political and regulatory issues. In climate change scenario, vertical expansion through increasing productivity is one of the possible approaches via intervention of genomics resources. This will also help in fast breeding of potato varieties. Introgression of yield enhancing genes/QTLs and early stage selection through genomic selection coupled with stress tolerance would be feasible to enhance productivity. Identification of new genes and markers in wild resources would be required for diverse genetic background for tolerance to various abiotic stresses such as heat, drought, salinity, nutrients and frost. The introgression of genes from wild resources will be important to widen the genetic diversity of the cultivated potato through modern genomics approaches like gene editing or base editing. Given that wild relatives are important source of various tolerance genes to abiotic stresses and therefore integration of genes and use of molecular markers especially genomics-assisted approaches is necessary for expansion of productivity. Furthermore, horizontal expansion of potato cultivation under nontraditional areas particularly tropical regions where high temperature and other environmental factors prevail is necessary to break the yield barriers. The expansion in nontraditional areas would require integrated approach for various abiotic factors along with early stage selection through genomics selection and yield enhancement. Taken together, there is immense potential for horizontal and vertical expansion of potato cultivation through both cultivation in nontraditional areas and productivity enhancement. This is possible through introgression of genes from wild/semi-cultivated/cultivated into the cultivated potato gene pool, reducing breeding cycles via genomic selection, abiotic stress tolerance genes, gene pyramiding, next-generation breeding and yield enhancement.

References

Aghaei K, Ehsanpour AA, Komatsu S (2008) Proteome analysis of potato under salt stress. J Proteome Res 7:4858–4868

Ahmed HAA, Şahin NK, Akdoğan G, Yaman C, Köm D, Uranbey S (2020) Variability in salinity stress tolerance of potato (*Solanum tuberosum* L.) varieties using *in vitro* screening. Ciência e Agrotecnologia 44:e004220

Aksoy E, Demirel U, Öztürk ZN, Çalişkan S, Çalişkan ME (2015) Recent advances in potato genomics, transcriptomics, and transgenics under drought and heat stresses: a review. Turk J Bot 39:920–940

Ambrosone A, Costa A, Martinelli R, Massarelli I, Simone VD et al (2011) Differential gene regulation in potato cells and plants upon abrupt or gradual exposure to water stress. Acta Physiol Plant 33:1157–1171

Andersson M, Turesson H, Nicolia A, Fält AS, Samuelsson M, Hofvander P (2017) Efficient targeted multiallelic mutagenesis in tetraploid potato (*Solanum tuberosum*) by transient CRISPR-Cas9 expression in protoplasts. Plant Cell Rep 36:117–128

Anithakumari AM, Dolstra O, Vosman B, Visser RGF, van der Linden G (2011) In vitro screening and QTL analysis for drought tolerance in diploid potato. Euphytica 181:357–369

Anithakumari AM, Nataraja KN, Visser RG, van der Linden CG (2012) Genetic dissection of drought tolerance and recovery potential by quantitative trait locus mapping of a diploid potato population. Mol Breed 30:1413–1429

Aversano R, Contaldi F, Ercolano MR, Grosso V, Iorizzo M et al (2015) The *Solanum commersonii* genome sequence provides insights into adaptation to stress conditions and genome evolution of wild potato relatives. Plant Cell 27(4):954–968

Barone A (2004) Molecular marker-assisted selection for potato breeding. Amer J Potato Res 81(2):111–117

Berdugo-Cely J, Valbuena RI, Sa´nchez-Betancourt E, Barrero LS, Yockteng R (2017) Genetic diversity and association mapping in the Colombian central collection of *Solanum tuberosum* L. Andigenum group using SNPs markers. PLoS ONE 12(3):e0173039

Boguszewska-Mańkowska D, Gietler M, Nykiel M (2020) Comparative proteomic analysis of drought and high temperature response in roots of two potato cultivars. Plant Growth Regul 92:345–363

Bokszczanin KL et al, Solanaceae Pollen Thermotolerance Initial Training Network (SPOT-ITN) Consortium, Fragkostefanakis S (2013) Perspectives on deciphering mechanisms underlying plant heat stress response and thermotolerance. Front Plant Sci 4:315

Bonierbale MW, Plaisted RL, Tanksley SD (1988) RFLP maps based on a common set of clones reveal modes of chromosomal evolution in potato and tomato. Genetics 120:1095–1103

Bradeen JM, Haynes KG (2011) Introduction to potato. In: Bradeen JM, Kole C (eds) Genetics, genomics and breeding of potato. Science Publishers, Enfield, NH, USA, pp 1–19

Bradshaw JE (2009) Potato breeding at the Scottish plant breeding station and the Scottish crop research institute: 1920–2008. Potato Res 52:141–172

Bradshaw JE, Bryan GJ, Ramsay G (2006) Genetic resources (including wild and cultivated Solanum species) and progress in their utilisation in potato breeding. Potato Res 49:49–65

Bradshaw JE, Hackett CA, Pande B, Waugh R, Bryan GJ (2008) QTL mapping of yield, agronomic and quality traits in tetraploid potato (*Solanum tuberosum* subsp. *tuberosum*). Theor Appl Genet 116:193–211

Butler NM, Atkins PA, Voytas DF, Douches DS (2015) Generation and inheritance of targeted mutations in potato (*Solanum tuberosum* L.) using the CRISPR/Cas system. PLoS ONE 10:e0144591

Byrne S, Meade F, Mesiti F, Griffin D, Kennedy C, Milbourne D (2020) Genome-wide association and genomic prediction for fry color in potato. Agronomy 10:90

Cabello R, Mendiburu F, Bonierbale M, Monneveux P, Roca W, Chujoy E (2012) Large-scale evaluation of potato improved varieties, genetic stocks, and landraces for drought tolerance. Am J Potato Res 89:400–410

Caruana BM, Pembleton LW, Constable F, Rodoni B, Slater AT, Cogan NOI (2019) Validation of genotyping by sequencing using transcriptomics for diversity and application of genomic selection in tetraploid potato. Front Plant Sci 10:670

Cattaneo AG, Gornati R, Sabbioni E, Chiriva-Internati M, Cobos E et al (2010) Nanotechnology and human health: risks and benefits. J Appl Toxicol 30(8):730–744

Chakrabarti SK, Conghua X, Tiwari JK (2017) The potato genome. Springer Nature, Switzerland. p 326

Chen Y, Li C, Yi J, Yang Y, Lei C, Gong M (2019) Transcriptome response to drought, rehydration and re-dehydration in potato. Int J Mol Sci 21(1):159

Collard BCY, Mackill MJ (2008) Marker assisted selection: an approach for precision plant breeding in the twenty first century. Philos Trans R Soc B 363:557–572

D'hoop B, Keizer PC, Paulo MJ, Visser RF, Eeuwijk F, Eck H (2014) Identification of agronomically important QTL in tetraploid potato cultivars using a marker–trait association analysis. Theor Appl Genet 127:731–748

D'hoop BB, Paulo MJ, Mank RA, van Eck HJ, van Eeuwijk FA (2008) Association mapping of quality traits in potato (*Solanum tuberosum* L.). Euphytica 161:47–60

Dahal K, Li X-Q, Tai H, Creelman A, Bizimungu B (2019) Improving potato stress tolerance and tuber yield under a climate change scenario—a current overview. Front Plant Sci 10:563

Dangol SD, Barakate A, Stephens J, Çalıskan ME, Bakhsh A (2019) Genome editing of potato using CRISPR technologies: current development and future prospective. Plant Cell Tiss Org Cult 139:403–416

Das A, Das B (2019) Nanotechnology a potential tool to mitigate abiotic stress in crop plants. In: de Oliveira AB (ed) Abiotic and biotic stress in plants. IntechOpen. https://doi.org/10.5772/intechopen.83562

Davies H, Bryan GJ, Taylor M (2008) Advances in functional genomics and genetic modification of potato. Potato Res 51:283–299

Demirel U, Morris WL, Ducreux LJM, Yavuz C, Asim A et al (2020) Physiological, biochemical, and transcriptional responses to single and combined abiotic stress in stress-tolerant and stress-sensitive potato genotypes. Front Plant Sci 11:169

Dong F, Song J, Naess SK, Helgeson JP, Gebhardt C, Jiang J (2000) Development and applications of a set of chromosome-specific cytogenetic DNA markers in potato. Theor Appl Genet 101(7):1001–1007

Ellis D, Chavez O, Coombs J, Soto J, Gomez R et al (2018) Genetic identity in genebanks: application of the SolCAP 12K SNP array in fingerprinting and diversity analysis in the global in trust potato collection. Genome 61:523–537

Endelman JB, Carley CAS, Bethke PC, Coombs JJ, Clough ME et al (2018) Genetic variance partitioning and genome-wide prediction with allele dosage information in autotetraploid potato. Genetics 209(1):77–87

Gowayed SMH, Hassan A-Z, Ehab M (2017) Improving the salinity tolerance in potato (*Solanum tuberosum*) by exogenous application of silicon dioxide nanoparticles. Intl J Agr Biol 19:183–192

Grun P (1990) The evolution of cultivated potatoes. Econ Bot 44:39–55

Handayani T, Gilani SA, Watanabe KN (2019) Climatic changes and potatoes: how can we cope with the abiotic stresses? Breed Sci 69(4):545–563

Hawkes JG (1990) The potato: evolution, biodiversity, and genetic resources. Press, Washington, DC, Smithsonian Inst

Hong J, Yang L, Zhang D, Shi J (2016) Plant metabolomics: an indispensable system biology tool for plant science. Intl J Mol Sci 17(6):767. http://solanaceae.plantbiology.msu.edu/pgsc_download.shtml. Accessed on 2nd Jan 2021

Iwama K (2008) Physiology of the potato: new insight into root sys-tem and repercussions for crop management. Potato Res 51:333–353

Khan MA, Munive S, Bonierbale M (2015) Early generation in vitro assay to identify potato populations and clones tolerant to heat. Plant Cell Tiss Org Cult 121:45–52

Khlestkin VK, Rozanova IV, Efimov VM, Khlestkina EK (2019) Starch phosphorylation associated SNPs found by genome-wide association studies in the potato (*Solanum tuberosum* L.). BMC Genetics 20(Suppl 1):29

Kloosterman B, Abelenda JA, Gomez MMC, Oortwijn M, de Boer JM et al (2013) Naturally occurring allele diversity allows potato cultivation in northern latitudes. Nature 495:246–250

Kyriakidou M, Anglin NL, Ellis D, Tai HH, Strömvik MV (2020) Genome assembly of six polyploid potato genomes. Sci Data 7(1):88

Laware SL, Raskar S (2014) Influence of zinc oxide nanoparticles on growth, flowering and seed productivity in onion. Intl J Curr Microbiol App Sci 3(7):874–881

Levy D, Coleman WK, Veilleux RE (2013) Adaptation of potato to water shortage: irrigation management and enhancement of tolerance to drought and salinity. Amer J Potato Res 90:186–206

Levy D, Veilleux RE (2007) Adaptation of potato to high temperatures and salinity-a review. Amer J Potato Res 84:487–506

Li Q, Qin Y, Hu X, Li G, Ding H, Xiong X, Wang W (2020) Transcriptome analysis uncovers the gene expression profile of salt-stressed potato (*Solanum tuberosum* L.). Sci Rep 10(1):5411

Martinez CA, Maestri M, Lani EG (1996) In vitro salt tolerance and proline accumulation in Andean potato (*Solanum* spp.) differing in frost resistance. Plant Sci 116:177–184

McCue KF, Gardner E, Chan R, Thilmony R, Thomson J (2019) Transgene stacking in potato using the GAANTRY system. BMC Res Notes 12:457

Monneveux P, Ramírez DA, Pino MT (2013) Drought tolerance in potato (*S. tuberosum* L.): can we learn from drought tolerance research in cereals? Plant Sci 205–206:76–86

Moon KB, Ahn DJ, Park JS, Jung WY, Cho HS, Kim HR, Jeon JH, Park YI, Kim HS (2018) Transcriptome profiling and characterization of drought-tolerant potato plant (*Solanum tuberosum* L.). Mol Cells 41(11):979–992

Nakabayashi R, Saito K (2015) Integrated metabolomics for abiotic stress responses in plants. Curr Opin Plant Biol 24:10–16

Potato Genome Sequencing Consortium (2011) Genome sequence and analysis of the tuber crop potato. Nature 475:189–195

Ramakrishnan AP, Ritland CE, Sevillano RHB, Riseman A (2015) Review of potato molecular markers to enhance trait selection. Amer J Potato Res 92:455–472

Ritter E, Barandalla L, López R, de Galarreta JIR (2008) Exploitation of exotic, cultivated Solanum germplasm for breeding and commercial purposes. Potato Res 51(3–4):301–311

Sanzari I, Leone A, Ambrosone A (2019) Nanotechnology in plant science: to make a long story short. Front Bioeng Biotechnol 7:120

Schafleitner R, Rosales ROG, Gaudin A, Aliaga CAA, Martinez GN et al (2007) Capturing candidate drought tolerance traits in two native Andean potato clones by transcription profiling of field grown plants under water stress. Plant Physiol Biochem 45(9):673–690

Sharma SK, Bolser D, de Boer J, Sønderkær M, Amoros W et al (2013) Construction of reference chromosome-scale pseudomolecules for potato: integrating the potato genome with genetic and physical maps. G3 (Bethesda) 3(11):2031–47

Sharma SK, MacKenzie K, McLean K, Dale F, Daniels S, Bryan GJ (2018) Linkage disequilibrium and evaluation of genome-wide association mapping models in tetraploid potato. G3 (Bethesda) 8(10):3185–3202

Silva JAB, Otoni WC, Martinez CA, Dias LM, Silva MAP (2001) Micro-tuberization of Andean potato species (*Solanum* spp.) as affected by salinity. Sci Hort 89:91–101

Singh A, Siddappa S, Bhardwaj V, Singh B, Kumar D, Singh BP (2015) Expression profiling of potato cultivars with contrasting tuberization at elevated temperature using microarray analysis. Plant Physiol Biochem 97:108–116

Slater AT, Cogan NOI, Forster JW (2013) Cost analysis of the application of marker-assisted selection in potato breeding. Mol Breed 32:299–310

Slater AT, Wilson GM, Cogan NOI, Forster JW, Hayes BJ (2014) Improving the analysis of low heritability complex traits for enhanced genetic gain in potato. Theor Appl Genet 127:809–820

Song J, Dong F, Jiang J (2000) Construction of a bacterial artificial chromosome (BAC) library for potato molecular cytogenetics research. Genome 43(1):199–204

Spooner DM, Ghislain M, Simon R, Jansky SH, Gavrilenko T (2014) Systematics, diversity, genetics, and evolution of wild and cultivated potatoes. Bot Rev 80:283–383

Tang R, Gupta SK, Niu S, Li XQ, Yang Q et al (2020) Transcriptome analysis of heat stress response genes in potato leaves. Mol Biol Rep 47(6):4311–4321

Tiwari JK, Ali N, Devi S, Kumar V, Zinta R, Chakrabarti SK (2018a) Development of microsatellite markers set for identification of Indian potato varieties. Sci Hort 231:22–30

Tiwari JK, Devi S, Ali N, Luthra SK, Kumar V et al (2018b) Progress in somatic hybridization research in potato during the past 40 years. Plant Cell Tiss Org Cult 132:225–238

Tiwari JK, Plett D, Garnett T, Chakrabarti SK, Singh RK (2018c) Integrated genomics, physiology and breeding approaches for improving nitrogen use efficiency in potato: translating knowledge from other crops. Funct Plant Biol 45:587–605

Tiwari JK, Ali S, Devi S, Zinta R, Kumar V, Chakrabarti SK (2019) Analysis of allelic variation in wild potato (*Solanum*) species by simple sequence repeat (SSR) markers. 3 Biotech 9:262

Tiwari JK, Buckseth T, Devi S, Varshney S, Sahu S et al (2020a) Physiological and genome-wide RNA-sequencing analyses identify candidate genes in a nitrogen-use efficient potato cv. Kufri Gaurav. Plant Physiol Biochem 154:171–183

Tiwari JK, Buckseth T, Singh RK, Kumar M, Kant S (2020b) Prospects of improving nitrogen use efficiency in potato: lessons from transgenics to genome editing strategies in plants. Front Plant Sci 11:597481

Tiwari JK, Buckseth T, Zinta R, Saraswati A, Singh RK et al (2020c) Genome-wide identification and characterization of microRNAs by small RNA sequencing for low nitrogen stress in potato. PLoS ONE 15(5):e0233076

Tiwari JK, Buckseth T, Zinta R, Saraswati A, Singh RK et al (2020d) Transcriptome analysis of potato shoots, roots and stolons under nitrogen stress. Sci Rep 10:1152

Uitdewilligen JG, Wolters AM, D'hoop BB, Borm TJ, Visser RG, van Eck HJ (2013) A next-generation sequencing method for genotyping-by-sequencing of highly heterozygous autotetraploid potato. PLoS ONE 8(5):e62355

Usman M, Farooq M, Wakeel A, Nawaz A, Cheema SA et al (2020) Nanotechnology in agriculture: current status, challenges and future opportunities. Sci Total Environ 721(15):137778

van der Vossen EAG, Sikkema A, Hekkert BL, Gros J, Stevens P et al (2003) An ancient *R* gene from the wild potato species *Solanum bulbocastanum* confers broad-spectrum resistance to *Phytophthora infestans* in cultivated potato and tomato. Plant J 36:867–882

van Os H, Andrzejewski S, Bakker E, Barrena I, Bryan GL et al (2006) Construction of a 10,000-marker ultradense genetic recombination map of potato: providing a framework for accelerated gene isolation and a genome wide physical map. Genetics 173:1075–1087

Vasquez-Robinet C, Mane SP, Ulanov AV, Watkinson JI, Stromberg VK et al (2008) Physiological and molecular adaptations to drought in Andean potato genotypes. J Exp Bot 59:2109–2123

Vos PG, Uitdewilligen JG, Voorrips RE, Visser RG, van Eck HJ (2015) Development and analysis of a 20K SNP array for potato (*Solanum tuberosum*): an insight into the breeding history. Theor Appl Genet 128(12):2387–2401

Watanabe KN, Kikuchi A, Shimazaki T, Asahina M (2011) Salt and drought stress tolerances in transgenic potatoes and wild species. Potato Res 54(4):319–324

Weckwerth W (2011) Green systems biology–from single genomes, proteomes and metabolomes to ecosystems research and biotechnology. J Proteome 75:284–305

Weckwerth W, Wienkoop S, Hoehenwarter W, Egelhofer V, Sun X (2014) From proteomics to systems biology: MAPA, MASS WESTERN, PROMEX, and COVAIN as a user-oriented platform. Methods Mol Biol 1072:15–27

Wenzl P, Carling J, Kudrna D, Jaccoud D, Huttner E et al (2004) Diversity arrays technology (DArT) for whole-genome profiling of barley. Proc Natl Acad Sci USA 101:9915–9920

Zhao C, Zhang Y, Du J, Guo X, Wen W et al (2019) Crop phenomics: current status and perspectives. Front Plant Sci 10:714

Zhou P, Adeel M, Shakoor N, Guo M, Hao Y et al (2021) Application of nanoparticles alleviates heavy metals stress and promotes plant growth: an overview. Nanomaterials 11:26