

Application of Biotechnology on Potato (*Solanum tuberosum*)

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Abstract

The potato is the most widely grown non-cereal crop and the third most important food crop in the world. Its use in biotechnology for crop enhancement has a long history since it is a species that adapts itself well to cell culture. This analysis starts with a historical overview of potato biotechnology advancements, including disease eradication, widespread hybridization, ploidy manipulation, and cell culture applications. We go through recent advancements and fresh ideas for transferring genes to potatoes. The sole effective method for producing isogenic populations of certain genotypes or varieties is by transformation. This method works particularly well for adding individual genes to current top potato clones with little to no disturbance to their genetic history. Due to the high heterozygosity in the tetraploid potato genome, it is almost difficult to achieve this using traditional breeding since the genetic makeup of potato clones is destroyed during sexual reproduction as a result of allele segregation. The creation of genetic mapping and the use of molecular markers and other genomics in potato breeding have both been hindered by these genetic traits. Different molecular techniques are described for genotype-phenotype associations, candidate genes, describing loci, and alleles in potato.

Keywords

Potato, Eradication, Heterozygosity, Molecular markers, Genetic traits, Loci, Allels, Biotechnology

Introduction

The most significant crop that is not a grain is the potato. It was mostly discovered in the Titicaca Lake region near the Andes Mountains in Bolivia and Peru [1]. Early in the 17th century, Portuguese traders brought it to India for the first time. They brought this crop as a temperate crop, however, due to the long day condition of the crop made it unsuitable for growing in the sub-tropical part of the country [2]. Afterward, potatoes took over the maximum area in India where potatoes get the optimum temperature, and the areas like Calicut (Kerala), Kolkata (West Bengal), Dehradun (Uttarakhand), Nilgiri (Tamil Nadu), Ooty (Tamil Nadu). Its cultivation also increased throughout the country in past when it was declared as a vegetable of garden in the some part of India.

Potato crop can be helpful to overcome the poverty and hunger issues in the world [3]. Furthermore, it is majorly used in the industries like food processing, and bioenergy production (biofuels). In addition, starch is a stabilizing and thickening agent in foods and it is also used for making papers, cosmetics, textiles, and plastic industries. Because to its great value in the market, the main focus is given to the improvement of new cultivars of the potato; however, conventional breeding is time taking method. That's why; genetic engineering offers the pos-

sibility of discovering genes of interest without change in the allelic combinations which further demonstrates the success of commercial cultivars.

Numerous breeding and molecular techniques have been used for the improvement of traits of potatoes. Conventional breeding is an approach that helps to enhance the yield, storage-quality, and processing of potatoes [4]. In contrast, conventional breeding sometimes involves incompatibilities and inbreeding depression in intra-species that further obstruct trait assimilation in polyploidy crops. Both biotic (insects, pests, bacteria, and fungus) and abiotic (temperature, rainfall, drought, salt, and some post-harvest issues) factors have an impact on these crops. Biotechnological approaches are crucial to resolving all of these issues and improving potato output and quality [5].

The advancement of DNA-based technologies is referred to as "biotechnology". The genetic makeup of organisms can be altered through contemporary biotechnology in agriculture for either the cultivation or post-harvest process of agricultural commodities. By inducing nucleic acids into the some viruses, microbial plasmid, or several other platforms beyond their cells to enable the incorporation into the host in which they cannot normally arise but are continuing to proliferate, genetic engineering creates distinctive combinations of heritable material.

During meiosis, homologous chromosomes are paired randomly. This crop is extremely heterozygous also in many cases; their pollens are found to be sterile. The heterozygous nature and occurrence of inbreeding depression makes it complicated to get the homozygous lines. In addition, inbreeding or selfing can also lead to the loss of vigor of non-flowering and progeny [6]. With the help of the clonal propagation of the tubers, it is easy to preserve the heterozygosity among the commercial cultivars.

The potato crop is prone both biotic and abiotic stresses *viz.* bacterial wilt, potato cyst nematodes, late blight, early blight, and numerous soil and tuber-borne diseases. Out of them, late blight as well as cyst nematodes are most harmful in potato crop which can be controlled by identifying the genotypes, methods like phenotypic screening have been extensively used. Moreover, to identify the resistant genotypes for future breeding to produce new varieties, marker-based selection was used. Potato cyst nematode was mostly examined in the soil through the induction of the root ball technique for late blight, and this sort of phenotypic screening for the illness is primarily conducted in the areas that are most impacted by the disease [7].

Potato can be propagated through tubers (mini and micro), synthetic seeds, apical meristem, and seed. As the crop is basically propagated clonally and heterozygous in nature, the success in breeding to produce new varieties is very low. Less genetic diversity among the potato cultivars incorporates in crop improvement, mainly when to increase protect from new insects, diseases, and climate change. Moreover, due to their poor germination and large variation in segregant generations, potato seeds are not used commercially. Synthetic seeds can be a substitute for this and solve the problem. A single somatic

embryo in a capsule is a synthetic seed [8]. The standard description of synthetic seeds today is "artificially encapsulated somatic embryos, sprouts, and other tissues that can be used for planting *in vitro* or *ex vitro*" because they are clonal products and might be used as the primary seeds for transportation and storage purposes, *in vitro* as well as *ex vitro* planting purposes.

Biotechnological Applications

Tissue culture

This method used on a large scale for the multiplication of cells and plants. This is mainly done in aseptic and controlled conditions, and then allows adapting to the new environment, this is known as plasticity, and the development of the whole plant through the explants is called totipotency [9]. The growth media used in the tissue culture is Murashige and Skoog medium, which contains all the minerals, sucrose, and vitamins which help in proper formation of the *in vitro* formed plant. It is also known as micropropagation which is found to be helpful and have great value in terms of practical applications [10]. Studies showed that improving potato through micropropagation method and had been published in different papers. Under *in vitro* culture, potatoes grow with meristem culture, and plant material is later multiplied and acclimatized under the controlled conditions for further multiplication of mini-tubers and later will be distributed to the farmers [11]. But *in vitro* technique is still a challenge because of fungal, viral, and bacterial contaminations.

There are numerous techniques, depends on the main plant material (tissue or organ) *viz.* calluses, somatic embryogenesis, protoplast culture, microspores (anthers, pollens, or ovaries), roots, node cuttings, meristem tips of shoots, and seeds [12]. Out of these anther cultures, protoplast culture and mainly somatic embryogenesis might be find successful in breeding applications of potato [13].

Embryo culture

Embryo culture is useful for avoiding various kinds of interspecific compatibilities. *Solanum tuberosum* was effectively used to introduce potato leaf roll resistance into *S. tuberosum*. A superior example of how this culture is utilized to cure in the restoration of broad hybrid is found to be most successful crossing of disomic hexaploid *S. nigrum* as a female plant using tetraploid potato.

Protoplast culture

The production of interspecific hybrids between exotic and domesticated species, which is not achievable by conventional breeding, is the major goal of protoplast culture. In potato crop, protoplast culture is mainly done to get desirable traits and introduced them to the commercial genotypes by using the wild *Solanum* species. A technique of protoplast culture was found by Fish et al. [14] which are also helpful for the dihaploid potato genomes. Most protoplasts are separated from mesophilic cells of *S. tuberosum* and *S. brevidens* (wild diploid species), which are then fused together when pH will be high and also high calcium concentrations. After fusion,

potential hybrid will be chosen, and 11 somatic hybrids were identified using morphological characteristics and isoenzymatic patterns.

Somatic hybridization

Somatic hybridization by protoplast fusion is a crucial method for plant improvement, as it allows scientists to combine somatic cells from several cultivars, genera or species to create unique genetic combinations like symmetric hybrids, cybrids, or asymmetric hybrids. Somatic hybridization has a unique ability to combine cytoplasmic and nuclear genes at the same time which is different from sexual hybridization or genetic engineering. This strategy can ease breeding and gene transfer, bypassing issues related to conventional sexual breeding like sexual incompatibilities, polyembryony, and male or female sterility [15].

Due to autotetraploidy, agronomic qualities pyramided in one genotype during potato breeding, a substantial selection in a population is required. The effective introduction of a variety of beneficial features into cultivated potatoes from a wild or closely similar domesticated species has demonstrated the potential benefits of somatic hybridization. For instance, *S. tuberosum* resistance to bacterial wilt was only achieved by the hybridization of *S. phureja* and *S. stenotomum*. In *Solanum*, sexual crossover is more challenging because of the endosperm balance number. In order to overcome these incompatibilities brought on by the difference in endosperm number and ploidy, scientists were successful in creating cross between *S. tuberosum* and *S. commersonii*. Most of the fusion hybrids showed resistance against bacterial wilt but, few hybrids which were fertile and resistant were hybridized with *S. tuberosum* to get seeds which are viable.

The main motive of somatic breeding is to introduce Late Blight, Colorado Beetle, PVY, and Bacterial wilt resistance into the *S. tuberosum*. The most effective hybrids between PI 24939 (a clone of *S. tuberosum* which is resistant to a virus) and a *S. tuberosum* is dihaploid crossed with *S. berthaultii* to get hybrid clone. Cytological and molecular examination revealed that the trispecies cultivars had the anticipated ploidy ($2n=4x=48$). Cultivated potato is not resistant to bacterial wilt. Iovene et al. [16] effectively developed haploid species from the protoplast fusion of *S. bulbocastanum* and *S. tuberosum* hybrids in order to integrate the best traits from both parents. From *S. melongena* bacterial wilt resistance is successfully transferred to the dihaploid *S. tuberosum*. Additionally, Ahn and Park [17] used the somatic hybridization approach to successfully transfer the susceptibility of common scab by crossing *S. brevidens* and *S. tuberosum*.

Somatic hybridization has previously been used to introduce numerous resistant genes from native species into the potato, includes tolerant to frost, show tolerance towards herbicide, also to potato late blight diseases, and defense against pests.

For genetic improvement and research, the protoplast fusion strategy provides tremendous promise. According to Muralidhar and Panda [18] it is especially helpful for microorganisms that are used industrially. Plants of many different species protoplasts may now be used to regenerate new plants.

Therefore, *in vitro* protoplast fusion is a viable option for genetic modification. This field is intriguing because it allows for the creation of novel genome combinations that are not possible through traditional by fusing together protoplasts from various sources. So, new germplasm may be added to breeding operations through protoplast fusion. In different horticultural crops, somatic hybridization has been extensively used to produce new cultivars that gives great yield and resistant to several diseases. Somatic hybridization is also being utilized to improve rootstock, increase quality, transmit cytoplasmic male sterility, and increase salt tolerance.

Somaclonal variation

Somaclonal potato varieties comprise genetic, epigenetic, and physiological alterations connected to stages of plant transformation including cell culture and shoot regeneration. Because not all polymorphisms have a genetic foundation, lines that exhibit phenotypic modifications must be established across several field conditions to perform stability. Stable phenotypic changes of both inherited and epigenetic basis can be caused by point mutations, chromosomal abnormalities activation of transposable elements, gene amplification, and DNA methylation modifications.

These changes in potato callus can be optimized to identify desired variants with suitable characteristics like salt stress or drought. In potato breeding, assessing somaclonal variation might be useful. According to the reports somaclonal diversity in potatoes can provide disease resistance. Research was conducted by Rosenberg et al. [19] to find out the variations that could withstand both early and late blight. With the help of biotechnological techniques such as tissue culture and genetic variation. The desired qualities such as quality, quantity, genetic composition, and protective strategy against infectious agents can be improved in potatoes [20]. Early detection of somaclonal variations in potato transformation can help to eliminate undesirable variants that could alter morphological characters and agronomic functions. Somaclonal genetic diversity is often seen as a result of the propagation methods in different plants.

RAPD has been proven to be helpful and successful method in regard to genetic variation. Micro propagated plant cultures produce somaclonal genetic diversity [21]. The effective use of RAPD has revealed genetic similarity and differences in plants propagated through tissue culture. The number of subcultures in micropropagation protocols increases the rate of somaclonal variation in plant tissue culturing [22].

Intragenics and cisgenics

In spite of sudden increase in global use of genetically modified techniques in agriculture, including the potato, the use of genetically modified crop production has raised various questions [23]. One of the main root causes about the transfer of genes across extremely large taxonomic barriers is the insertion of bacterial genes into plant genomes. According to Holme et al. [24], the ethical implications of gene exchange between plants belonging to the same species are lower than those of gene exchange across species. Agrobacterium-mediated transformation vectors made of DNA from which plants have been

produced to alleviate concerns about GM technology. Plant transformation caused by agrobacteriums is well understood. Cisgenic and intragenic techniques have been established as means of transferring DNA inside a plant species [25]. Agrobacterium is used to transfer the t-DNA and intragenic or cisgenic traits into the host plant. The main difference between the two strategies is the use of plant-derived sequences that serve as t-DNA and contain plant-derived genes of interest (intragenic) as opposed to the use of t-DNA obtained from bacteria to host the gene being transferred (cisgenic). Every gene from a wild relative that confers an interesting characteristic has the potential to improve excellent potato germplasm through cisgenics and intragenics. Like, various wild varieties of potatoes have been shown to be resistant to late blight. *S. bulbocastanum* and *S. demissum* have also produced genes that give resistance to late blight. Park et al. [26] propose these resistance (R) genes as outstanding possibilities for cisgenic potato development.

Somatic cell selection

This selection has been used to improve the potato tuber, by focusing on selection of disease resistances. When pathogen culture filtrates or phytotoxins are introduced to a large population of cultivated potato cells, only the uncommon survivors are kept. Somatic cell selection is a technique used to improve potato cultivars that results in the creation of clones resistant to the disease like common scab [27].

Potato is a vegetatively propagated crop for large-scale production. But this type of method may attract bacteria, fungi, and other virus-related infections which simultaneously leads to the death of the plant or may reduce the crop yield [28]. Therefore, it is crucial to use material that is virus-free and of excellent genetic, physiological, and phytosanitary quality to that exhibits its high production.

Transgenic plants

Transgenic methods of genetic engineering include transferring a foreign gene from a different species. The majority of genetically modified products produced during the last two decades belong to the genetic engineering category [29].

Since 1996, when transgenic crops were first cultivated commercially, more than 70 nations have planted or imported transgenic crops. In 1996, 1.7 million hectares of these filed crops were grown by the six founder nations of biotech crops-Mexico Argentina, the United States, Canada, China, and Australia. In 2018, 70 nations accepted biotech crops, 26 countries growing them and 44 importing them. 26 nations - 21 developing and 5 industrial-produced biotech crops across 191.7 million hectares in 2018. Biotech crops have grown 113 times in the 23 years since they were first planted, spanning 2.5 billion hectares worldwide, which makes them the fastest-growing crop technology in the world.

In the past 20 years, sudden progress has been found mainly in the areas of pest resistance, herbicide tolerance, improved nutritional quality, and nutrient value, knowledge of gene activity and metabolic pathways, high photosynthetic rates, raised defectiveness of bio-control agents, sugar and starch synthesis.

In potato the most prevalent insect pests are potato aphids, the polyphagous *Myzus persicae* (Hemiptera: Aphididae). Through piercing and sucking the phloem, aphids can directly harm the plants. The main fact that *M. persicae* acts as a vector for more than one hundred plant viruses, roughly twelve of which affect directly the tubers, and numerous leaf-roll viruses are also shown to be toxic for the crop, makes it even more dangerous.

The outcomes showed that aphid efficiency differed substantially more among common potato varieties than desired. It is essential to contrast various GM activities with the non-transformed kind since insertion could have unforeseen consequences.

Genetics and genomics

Molecular technologies have capabilities for accelerating conventional breeding programs. The molecular identification of naturally occurring allelic variation can be a potent tool to speed up the breeding process for better cultivars. Genetically superior plants can be found early in the plant's development before the phenotype can be recognized to the correlation of a genotype with the interested phenotype. In the process of plant breeding, both time and money can be saved by being able to recognize superior plants and reject inferior ones.

Molecular genetics

The genetic complexity of the cultivated potato has impeded genetic mapping. Most of the cultivars and lines obtained from breeding are autotetraploid or have an elevated genetic weight [6]. In potatoes, it is not possible to use tools like mutant lines (homozygous), recombinant genetic lines, or almost isogenic lines. Recently, attempts have been undertaken to investigate the genetics of traits utilizing heterozygous lines derived from diploid (wild) potato species and tetraploid *S. tuberosum*. This has mostly been carried out in separating diploid F1 mapping samples of potatoes using bi-parental crossings of heterozygous lines. The earliest examples of diploid linkage maps by using RFLP markers of potato [6], using tomato RFLP markers and taking advantage of the high degree of synteny between the two plants, and were using morphological traits and molecular (RFLP) markers in one genetic map. RFLP-based genetic linkage maps for potatoes were quickly followed by maps utilizing AFLP markers [30].

Since, the first RAPD markers in potato and had been utilized to locate SSR markers on linkage group by separating from mapping population and also for fingerprinting and recognizing potato germplasm accessions and varieties.

They may perform on DNA-amplified fragments following PCR as a straightforward closed-tube test without the need for sample separation or processing. For the purpose of genotyping and alternative scanning in tetraploid potatoes as well as in diploid, De Koeber et al. [31] developed HRM analyses for five genomic markers. They demonstrated how HRM-based candidate gene results effectively gives all information on allele dosage and distinguishes between different haplotypes. The HRM methods were successful in assessing the influence of total carotenoid concentration on the allele

dosage of the recessive gene zeaxanthin epoxidase (Zep1) in tetraploid potatoes with yellow-fleshed germplasm.

Quantitative trait loci (QTL)

Quantitative characteristics are those whose regulation or change of the observable phenotype is often mediated by the action of several genes. QTL comprise regions within a genome that include one or more genes that affect a characteristic's outward appearance. In potatoes, markers of choice and offspring lines resulting from a biparental crossing that segregates for the desired feature are used to ascertain each individual's genotype. There are numerous elucidation of QTL mapping in potato species that are diploid or tetraploid. Recent QTL mapping research used RFLP and RAPD markers for locating QTLs for *Phytophthora infestans* resistance, as well as RFLP markers to localize QTLs in color of chip and tuber dormancy. Werij et al. [32] used QTL analysis and a candidate gene technique to successfully investigate the genetic basis for numerous tuber indicators of quality in the diploid mapping sample.

For molecular breeding, gene mapping is essential. The genetic mapping of potatoes is change by their high heterozygosity, severe inbreeding depression, and complex tetrasomic inheritance. Linkage mapping is the study of how traits and separating alleles of DNA markers are related genetically in a given mapping sample. The first linkage map was discovered by Bonierbale et al. [30] employing tomato RFLP markers in diploid species (*S. tuberosum* subspecies *tuberosum*). Next, unknown PCR-based molecular markers such as diversity array technology (DArT), AFLP, and SSR were utilized for mapping. Using more than 10,000 AFLP markers, an ultra-high-density genetic and physical map of the potato was created, and the genome of the potato was sequenced using this map. Sharma et al. [33] developed a detailed physical and genomic mapping for a diploid backcross generation using 2,469 markers (SSR/AFLP/DArT/SNP). Several QTLs had been identified in potatoes for numerous characteristics, including resistance to drought and late blight. For a variety of agronomic traits, such as resistance to Verticillium wilt and diploid/tetraploid clones have been employed in potato association mapping [34].

Marker-assisted selection (MAS)

In contrast to other crops, potatoes have an extremely limited usage of molecular markers in tetraploid potato breeding programs because of the increase amount of natural allelic diversity in potatoes affected by the autotetraploid habit of potatoes also due to their tetrasomic habit. In potato breeding, more than 40 features are regarded as crucial. Conventional breeding takes a long period since it requires the clonal selection and years of field testing. Therefore, it is thought that the best MAS results come from discovering closely connected markers with a target gene for a characteristic. By making early selections, MAS enables a large reduction in field exposures, which in turn shortens breeding cycles and field exposures. For hereditary characteristics including viruses, potato cyst nematode resistance, and late blight, many linked genomic markers had been generated or employed into the potato [35].

For different traits like heat, drought, yield, cold stress, and nutrient use efficiency, however, there are insufficient details available on MAS.

Potato genome sequencing/resequencing

An effective method for changing the genome to include desirable gene combinations is genome editing. Transcription Activator-Like Effector Nucleases, and Zinc Finger Nucleases, two earlier sequence-specific nucleases techniques, were utilized. The most popular genome editing technology now in use is called Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR associated protein 9 and uses an RNA-guided method to target DNA/RNA regions. According to Aversano et al. [36] these days around 100 species of *Solanum* which are sequenced or resequenced by considered Illumina as a platform such as like wild *S. commersonii* and *Solanum* species which are tuber bearing, and also *S. chacoense* which is generally called as "M6" [37]. *S. pinnatisectum* is developed from clonal hybrid. The identification of new genes, markers, and haplotypes has been stimulated by the quick development of sequencing and bioinformatics to help us better understand the biology of potatoes [38].

Conclusion

Reduced potato yields are commonly linked to factors such as a lack of superior seed, undeveloped cultivars, decrease in price of fertilizer application and water supply, as well as problems with insects and diseases. The utilization of biotechnologies, including cell culture and tissue culture and combination of all the biotechnological technologies, marker-assisted technologies, genetic engineering can increase potato production and quality. It is crucial that underdeveloped nations have access to these biotechnologies. The potato will remain at the forefront of advancing technology, even while changes in emerging countries bring interesting challenges for future crop enhancement, environmental sustainability, and advancement in science.

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Conflict of Interest

There is no conflict of interest.

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