

Application of Biotechnology on Potato Crop Improvement

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Abstract

Potato (*Solanum tuberosum* L.) belongs to the Solanaceae family. Potato is a crop of major economic importance worldwide. It play major role in national food and nutrition security, alleviation of poverty, generating income, and providing job opportunity in line with production, processing and marketing sub-sectors. Aim of this review is to how potato crop improved through biotechnology. Potato is a self-pollinated crop but is conventional propagated. Self-pollination or in breeding in potato leads to loss of vigour of the progeny and non- flowering. Plant biotechnology has traditionally encompassed the application of cell and tissue culture for crop improvement. Through applications of biotechnologies such as tissue and cell culture, genetic engineering, marker-assisted technologies, genome-assisted technologies or a combination of all the technologies improve resistance to major pests and diseases, tuber quality traits, nutritional value, salinity tolerant potato of potato yield and yield components. We conclude that application of biotechnology on potato plant improves yield, yield components and quality.

Keywords: Biotechnology; Improvement; Potato

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Citation: Bekele D (2021) Application of Biotechnology on Potato Crop Improvement. J Plant Sci Agri Res Vol.5 No.5:63

Received: June 29, 2021; **Accepted:** July 13, 2021; **Published:** July 20, 2021

Introduction

Potato is a member of the *Solanaceae*, a large plant family with more than 3000 species. It has one of the richest genetic resources of any cultivated plant, with about 190 wild and primitive species in the section Petota of the genus *Solanum* [1]. Potato (*Solanum tuberosum* L.) is ranked fourth among the major staple crops after maize, rice, and wheat. It can provide more carbohydrates, proteins, minerals, and vitamins per unit area of land and time as compared to other potential food crops [2]. Potato is a self-pollinated crop but is vegetatively propagated. The cultivated tetraploid varieties are highly heterozygous. Most of them are also pollen sterile. Selfing or inbreeding in potato leads to loss of vigour of the progeny and non-flowering. This sexual reproduction creates an abundance of diversity by recombining the variants of genes that arose by mutation, and as a consequence, potatoes are highly heterozygous individuals that display inbreeding depression on selfing.

Applications of plant biotechnology over the past five decades have helped facilitate interspecies crosses and to augment and broaden the cultivated gene pool. Plant biotechnology has traditionally encompassed the application of cell and tissue culture for crop improvement. From the mid-1980s,

the development and application of transgenic plants was the dominant research activity associated with plant biotechnology. More recently research has expanded to include the application of genomics technologies. Members of the *solanaceae* family, especially tobacco and petunia, have been the subject of many of the developments in plant biotechnology, primarily due to their high propensity for growth and development in cell culture. Similarly, many elite potato genotypes are highly responsive in cell culture and provide opportunities for applications of biotechnology to potato improvement [3].

Several breeding and molecular approaches have been employed for trait improvement in potato. Conventional breeding techniques for potato improvement are directed to increase yield, processing, and storage-quality [4]. The disadvantages of conventional breeding may include intra-species incompatibilities and inbreeding depression that causes failure in trait incorporations in polyploid crops. The sustainable potato production faces a number of challenges due to biotic stresses (viruses, bacteria, fungal, insect pests) and abiotic stresses (drought, salinity, temperature, frost and post-harvest problems, i.e., accumulation of reducing sugars during cold storage). So, to improve yield, yield components and quality of potato application of biotechnology very pertinent.

Application of Biotechnology on Potato

Tissue culture for potato improvement

Embryo culture: The Endosperm Balance Number (EBN) plays an important role in the speciation of tetraploid from diploid *Solanum* species. Upon hybridization, the EBN should be in a 2:1 maternal to paternal ratio for normal endosperm development and successful seed production. The hybridization barriers between disomic (2 EBN) and tetrasomic (4 EBN) tuberous *Solanum* species can be overcome by double pollination and rescue of aborting embryos via tissue culture [5]. Embryo culture has also been valuable for circumvention of other forms of interspecific incompatibility. For example, resistance to potato leaf roll virus was successfully introgressed from *Solanum tuberosum* to *S. tuberosum* via embryo culture [6]. An extreme example of the use of embryo culture to aid the recovery of wide hybrids involves the successful hybridization of the disomic hexaploid *Solanum nigrum* (black nightshade) as a female parent with tetraploid potato [7].

Somaclonal variation: The historical dogma was that all plants regenerated from somatic tissue are identical to the parent plant. However, the contrary view was popularized by Larkin and Scowcroft, who described the high frequency of phenotypically variant plants observed among those regenerated from cell culture and coined the term 'somaclonal variation' for the phenomenon. For the 'optimists', somaclonal variation was seen as a new approach for generating novel variation in plants, especially in clonally propagated crops such as potato. For the 'pessimists', it was seen as an inherent curse for other applications of cell culture for crop improvement [8].

Explanations that account for the observed phenotypic changes among somaclonal potato lines involve physiological, epigenetic or genetic changes associated with the cell culture and shoot regeneration phase of plant transformation. As not all variants have a genetic basis, lines exhibiting phenotypic changes need to be grown over several field seasons to ensure stability of performance. Stable phenotypic changes of either heritable and/or epigenetic origin may arise through ploidy changes, chromosomal aberrations, gene amplification, activation of transposable elements, DNA methylation changes or point

mutations and can also occur during the long-term propagation of potato from internodal stem cuttings [9,10]. Considerable effort was devoted to recovering useful variants in a wide range of cultivars, and variants with improvements in specific useful traits were reported. Examples include improved resistance to *Alternaria solani*, *Verticillium dahliae*, late blight and more recently tuber morphology. However, it has been recognized over time that the recovery of a somaclonal line exhibiting beneficial traits without other simultaneously arising negative attributes is very rare. Nowadays, the phenomenon of somaclonal variation is widely seen as an inherent negative feature of regeneration from cell culture and considered as something to be avoided. Strategies to minimize the impact of somaclonal variation on plant performance are routinely implemented during other applications of cell culture for potato improvement [11,12].

Somatic hybridization: The regeneration of somatic hybrid plants from these cells is possibly provided the two parental species are closely related, even if they cannot be sexually hybridized. Such somatic hybrid plants offer new sources of germplasm for the introgression of traits into crop plants, although this is often very challenging due to the poor fertility of the initial somatic hybrids. Somatic hybridization has provided some new opportunities for introgression of novel sources of disease and pest resistance into cultivated potato from accessions of taxa possessing sexual reproductive barriers with potato. Resistances to diseases caused by leaf roll virus, potato virus Y, early and late blight, soft rot, Columbia root-knot nematode and Colorado potato beetle have been introduced through somatic fusion of potato protoplasts with protoplasts of wild relatives, including *Solanum palustre* (formerly *S. brevidens*) and *Solanum bulbocastanum* [13].

Somatic cell selection: The application of somatic cell selection to potato improvement has mainly focused on selection for disease resistance in potato. This has involved the exposure of large populations of cultured potato cells to pathotoxins or culture filtrates of pathogens, followed by the selection of rare surviving cells. An excellent example of the application of somatic cell selection for potato improvement involves the development of clones with resistance to common scab disease [14,15].

Table 1: Identification of toxic plants with their scientific names, Arabic and French names and corresponding families

Scientific name	Arabic name	French name	Toxic organs	Family
<i>Nerium oleander</i> L.	Defla	Oleander	All the organs are poisonous and especially the leaves.	<i>Apocynaceae</i>
<i>Conium maculatum</i> L.	Dayl gat	Great Hemlock	All the organs are poisonous and especially the root.	<i>Apiaceae</i>
<i>Peganum harmala</i> L.	Harmel	Harmel	All organs are toxic and especially the seed.	<i>Zygophyllaceae</i>
<i>Hyoscyamus albus</i> L.	Hebbala	White henbane	All organs are toxic and especially the seed.	<i>Solanaceae</i>
<i>Rhamnus alaternus</i> L.	Melelis	Alaterne	Ripe fruits and bark (of the plant)	<i>Rhamnaceae</i>
<i>Thapsia garganica</i> L.	Dreyes or bounaffa	False fennel or Thapsia	All the organs are poisonous and especially the root.	<i>Apiaceae</i>
<i>Ruta graveolens</i> L.	Figel	Foul street	All the organs are poisonous and especially the leaves.	<i>Rutaceae</i>
<i>Datura stramonium</i> L.	Msikra	Sorcerer's Grass or Mole Grass	All organs are toxic and especially the seed.	<i>Solanaceae</i>
<i>Alocasia macrorrhizos</i> L.	Elephant ear	African mask	All organs are toxic and especially the stem and leaves.	<i>Araceae</i>
<i>Carlina gummifera</i> L.	Addad	Glue thistle	All organs are toxic and especially the rhizome.	<i>Asteraceae</i>

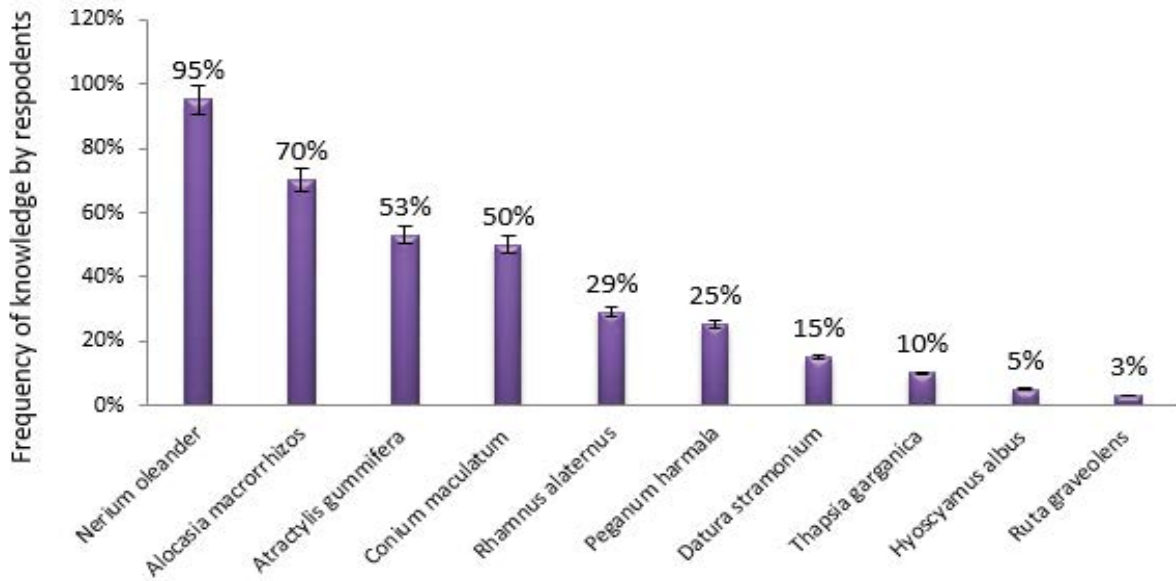


Figure 1: Frequency of knowledge of toxic species by respondents.

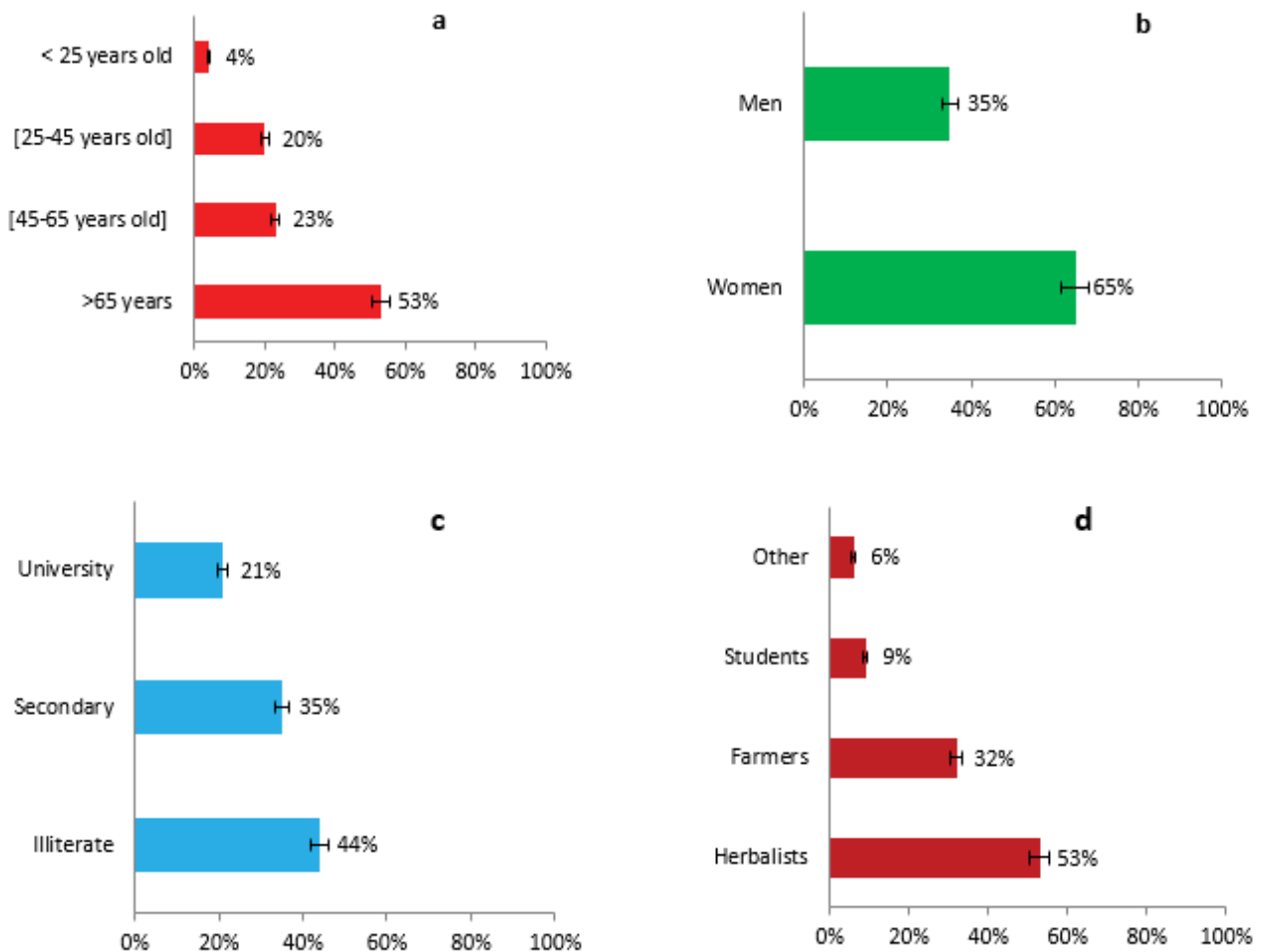


Figure 2: (a): Distribution of ethnoecological knowledge of poisonous plants according to age, (b): Sex, (c): Intellectual level and (d): Profession.

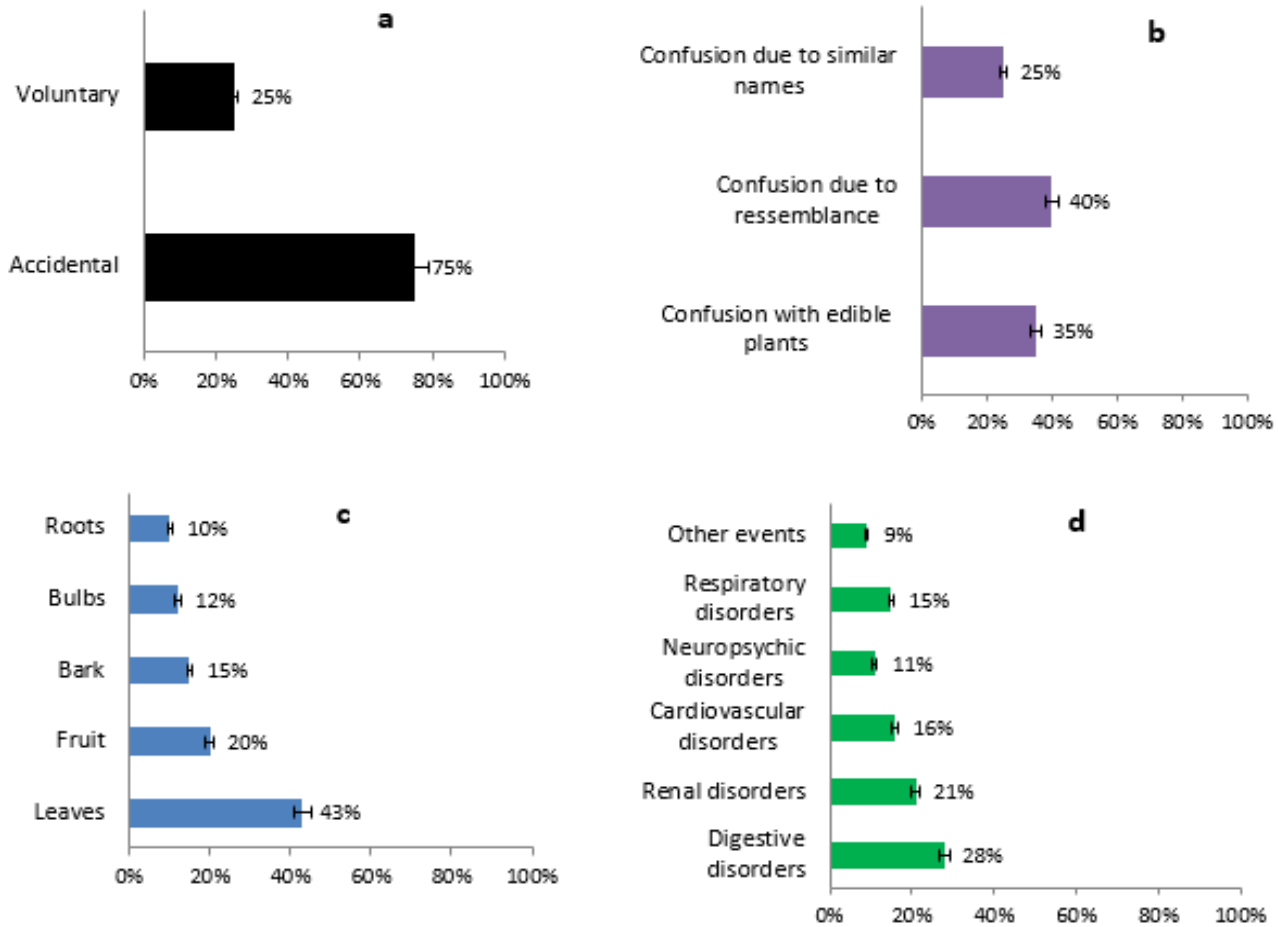


Figure 3: (a): Transmission of ethnobotanical knowledge according to the circumstances, (b): The nature of the confusion, (c): The organs used and (d): The clinical signs.

Application of Genetic Engineering in Potato

Gene transfer to potatoes by transformation

Development of potato transformation systems potato was one of the first crops for which transgenic plants were regenerated, and *Agrobacterium*-mediated gene transfer protocols were quickly adapted for important cultivars throughout the world [16]. This progress was motivated by the advantages that transformation offered for the genetic improvement in potatoes relative to the genetic limitations associated with traditional potato breeding. Potato transformation has also been achieved by direct DNA uptake [17], and success has recently been reported for the generation of transgenic potato using *vir* gene-mediated gene transfer from *Rhizobium* species and *Ensifer adhaerens* [18,19]. However, *Agrobacterium*-mediated gene transfer is the preferred approach and is routinely performed in laboratories worldwide. The source of explant tissue for potato transformation is usually derived from *in vitro* plants, maintained with pathogen-free (virus free) status. This eliminates the need for surface sterilization of the plant tissue, which saves time, reduces the possibility of

contamination and minimizes stress on the plant tissue from the chemical treatment.

Resistance to major pests and diseases

The major driver for research and development into this aspect is evident, due to the substantial crop losses attributable to the effect of pathogens, not only during the growing season but, in the case of potato tubers, also during storage. However, a secondary factor in the substantial research effort made in this area is that many of the resistance mechanisms could be introduced by a single gene, which was the only available strategy until the development of methods for introducing multiple genes was demonstrated on an efficient scale in the late 1990s. A further consideration is that much of the (expensive) research has been undertaken in laboratories associated directly, or indirectly, with the major agrochemical manufacturers.

Resistance to colorado potato beetle

Transgenic resistance was developed with the introduction of a gene that encodes the *Cry3A* protein derived from the bacterium *B. thuringiensis var. tenebrionis* and expressed in potato using the constitutive 35S cauliflower mosaic virus (*CaMV 35S*) promoter.

The strategy was so successful that such plants were the first GM potato varieties to be commercialized by Monsanto using the varieties Russet Burbank, Atlantic, Snowden and Superior in North America from 1995 to 2001.

Resistance to potato tuber moth

Potato tuber moth (*Phthorimaea operculella*) is a troublesome pest of potatoes and is found in warm tropical and subtropical climates. It is the most damaging pest of potatoes in fields and stores in warm, dry areas of the world, such as North Africa and the Middle East, Mexico, Central America and the inter-Andean valleys of South America. Transgenic resistance has been provided by the Bt protein encoded by the *cry5* gene and by the *cry1Ac9* gene, again using the constitutive *35S CaMV* promoter. Davidson demonstrated that their transgenic potato lines exhibited stable resistance to larvae across field seasons, between affected plant organs and between plant organs of different ages.

Resistance to viruses PLRV and PVY

Potato Leafroll (PLRV) and potato Y (PVY) are the two most serious virus diseases of potatoes worldwide. PLRV causes both qualitative and quantitative damage and is transmitted in a persistent manner by several aphid species. Sense and antisense RNA-mediated resistance to PLRV was engineered into Russet Burbank potato plants in 1991, and this work was expanded to generate Russet Burbank potatoes in which CPB resistance was combined with resistance to PLRV provided by a construct designed to prevent virus replication using the constitutive Figwort Mosaic Virus (FMV) promoter. Russet Burbank and Shepody potatoes have also been produced with combined CPB and PVY resistance, the latter provided by the PVY coat protein gene, again using the FMV promoter. In both these examples, the process started with about 3000 original transgenic potato clones in 1991 from which six were finally selected for commercialization. Trait stability has been demonstrated in field trials over a number of years, as has the greatly reduced use of pesticides.

Resistance to bacteria and fungi

Owing to the complexity of the host pathogen response, progress in engineering bacterial and fungal resistance into potato (and other crop plants) has been less rapid than with other traits.

Tuber Quality Traits

Anti-bruise potatoes

Bruise resistance is important in potatoes, as mechanical damage initiates enzymic browning which results in the production of black, brown and red pigments and either crop rejection by processors or waste during processing. A transgenic solution to the probe can be provided by the down-regulation of PPO gene expression so that the reaction leading to pigment production is no longer catalysed by the enzyme PPO [20].

Reduced glycoalkaloid content

Steroidal glycoalkaloids are a class of potentially toxic compounds

with a bitter taste, which are found throughout the family *Solanaceae*. Cultivars vary with regard to their inherent tuber glycoalkaloid content. Levels above 20 mg per 100 g fresh weight are considered unsuitable for human consumption as they can cause various symptoms typically associated with food poisoning. Although breeders check potential cultivars for unacceptably high levels, particularly where pedigrees involve wild species, a transgenic option for further reduction would be useful. Initial reports of down-regulating a gene encoding a Sterol Alkaloid Glycosyltransferase (*Sgt1*) and an almost complete inhibition of solanine accumulation which was compensated by elevated levels of chaconine have been made; however, further transformation will be required to inhibit chaconine accumulation [21]. Of more general interest, transgenic potato plants overexpressing a soybean (type 1 sterol methyltransferase (*GmSMT*)1) cDNA were generated and used to study sterol biosynthesis in relation to the production of toxic glycoalkaloids [22].

Nutritional Value

Protein and amino acid content

Chakraborty S reported improvements in the nutritive value of potato through transformation with a non-allergenic seed albumin gene (*AmA1*) from *Amaranthus hypochondriacus*. The seed protein has a well-balanced amino acid composition with no known allergenic properties. Five- to ten-fold increases in transcript levels in tubers were achieved using the tuber-specific GBSS promoter compared with the *35S CaMV* promoter. Significant two to four fold increases were achieved in the lysine, methionine, cysteine and tyrosine content of the protein amino acids, and a 35-45% increase was achieved in total protein content [23].

Insulin

Insulin is a mixture of linear fructose polymers with different chain length and a glucose molecule at each C2 end. Insulin belongs to the fructan group of polysaccharides and serves as carbohydrate storage in many plant species. Compounds such as insulin reduce the energy density of food and are used to enrich food with dietary fibre or to replace sugar and fat have developed transgenic potato tubers which synthesize the full range of insulin molecules naturally occurring in globe artichoke (*Cynara scolymus*) [24].

Carotenoids

Yellow and orange flesh colour comes from a class of pigments known as carotenoids (e.g. zeaxanthin) which are also antioxidants with health-promoting attributes. Hence, their enhancement by breeding provides an opportunity to improve the nutritive value of potatoes and processed foods made from potatoes. Transgenic approaches are also possible as Romer discovered that tuber-specific down-regulation of the zeaxanthin epoxidase gene in *S. tuberosum* increased not only the amount of zeaxanthin that accumulated but also the total carotenoid level by up to 5.7-fold of the controls. Recent work on the underlying mechanisms of

carotenogenesis during tuber development and storage in potato has been reported [25], and in further work by the same group, it was found that in developing tubers of transgenic Desiree (using an *Erwinia uredovora crtB* gene encoding phytoene synthase) lines, carotenoid levels reached 35 g carotenoid g DW, and the balance of carotenoids changed radically compared with controls. The *crtB* gene was also transformed into *S. phureja* (cultivar Mayan Gold), again resulting in an increase in total carotenoid content to 78 g carotenoid g DW in the most affected transgenic line [26].

Starch

Starch is the primary storage compound in tubers. It is also widely used for a range of industrial processes. The physical properties of starch vary with plant source, but there are considerable opportunities to generate novel starches for use in food and non-food market sectors [27]. Genetic engineering has already generated novel potato starches of which the two extremes are high amylopectin starch and high amylose starch. High amylopectin starch was produced by the down-regulation of the *GBSS* gene that controls amylose synthesis. In contrast, to produce high amylose starch, it was necessary to concurrently down-regulate two starch branching enzymes, A and B [28]. Field trialling confirmed the stability of the modification over years and demonstrated an increased tuber yield, reduced starch content, smaller granule size and an increase in reducing sugars [29]. Potatoes containing starch with a very low degree of branching, such as 0.3%, were not suitable for commercial cultivation due to severe starch yield reduction and other effects.

Reducing sugars

Ideally, the potato industry would like to store tubers at low temperatures (about 4°C) to minimize sprout growth and eliminate the need to use chemicals to suppress the sprouting process. However, low temperatures induce glucose and fructose accumulation, and these reducing sugars are primarily responsible for non-enzymic browning through a typical Maillard reaction that occurs at temperatures required to generate potato chips (crisps) and French fries. Whilst breeders have been able to select for lower levels of reducing sugars out of cold storage, transgenic approaches are also possible based on an understanding of primary carbohydrate metabolism. Increased tuber starch content and lowered the levels of reducing sugars by expressing an *E. coli glgC16* mutant gene that encodes for the enzyme ADP glucose pyrophosphorylase and increases the production of ADPglucose, which in turn becomes incorporated into the growing starch granule were able to minimize the conversion of sucrose to glucose and fructose by expressing a putative vacuolar invertase inhibitor protein from tobacco, called *Nt-inhh*, in potato plants under the control of the *CaMV35S* promoter [30].

Obtaining Salinity Tolerant Potato through Genetic Engineering

Some scientists believe that salinity tolerance of crop plants must be accompanied by plant transformation. The basis for

this argument is that lack of success through conventional breeding may have resulted from out-dated concepts. For potato, we can safely conclude that the basis for failure in achieving salinity tolerance has involved the complexity of this multigenic characteristic, the apparent lack of outstanding salinity tolerance in wild *Solanum* species, the lack of coordinated effort and the modest effort invested to date. Molecular engineering has resulted in major improvements in salinity tolerance in model plants such as *Arabidopsis* [31].

Molecular Marker Technologies and Applications

Genetics and genomics

Molecular technologies have huge potential for speeding up the process of conventional plant breeding. Identification of naturally existing allelic variation at the molecular level can provide a powerful tool to accelerate the process of breeding for improved varieties. The association of a genotype with a phenotype of interest allows genetically elite plants to be identified early in plant growth before the phenotype can be observed. The ability to identify elite plants and discard nonelite plants saves both time and money in the process of plant breeding.

Molecular genetics

Traditionally, linkage mapping has been the most commonly used way to correlate natural variation in phenotype with genotype [32]. Genetic mapping in cultivated potato has been hindered by its complex genetics. Most cultivars and breeding lines are autotetraploid and carry a high genetic load [33]. Tools such as (homozygous) mutant lines, recombinant inbred lines and near-isogenic lines are not available in potato. Diploid lines derived from tetraploid *S. tuberosum*, and diploid (wild) species of potato have been used over recent decades to unravel the genetics of traits. In potato, this has been performed predominantly in segregating diploid F1 mapping populations, established using bi-parental crosses of heterozygous lines. Early examples of diploid linkage maps include [34] who took advantage of the high level of synteny between potato and tomato and used tomato Restriction Fragment Length Polymorphism (RFLP) markers for map construction, Gebhardt who used potato RFLP markers and Jacobs who combined molecular RFLP markers with morphological traits in one genetic map. RFLP-based genetic linkage maps were soon followed by potato maps containing amplified fragment length polymorphism markers [35].

Randomly Amplified Polymorphic DNA (RAPD) markers were first reported in potato by Provan and have been used since for locating SSR markers on linkage groups using segregating mapping populations, as well as for identifying and fingerprinting potato germplasm accessions and cultivars [36].

It can be performed as a simple closed-tube assay, on DNA amplicons post-PCR without the need for separation or processing of the samples developed HRM assays for five molecular markers/candidate genes for genotyping and variant scanning in diploid,

as well as tetraploid potato and demonstrated that HRM-based candidate gene analysis efficiently provides information on allele dosage and discriminates different haplotypes. HRM technology was also successfully applied to examine the effect of allele dosage of the zeaxanthin epoxidase gene (*Zep1*, a recessive gene) on total carotenoid content in yellow-fleshed tetraploid potato germplasm [37].

Quantitative traits refer to traits that are often attributed to more than one gene controlling or influencing the observed phenotype. Quantitative Trait Loci (QTL) are regions of the genome that contain gene(s) influencing the phenotypic expression of the trait. In potato, progeny lines from a biparental cross segregating for the trait of interest are assessed, and markers of choice are used to genotype individuals in the population. Numerous examples exist for QTL mapping in potato in both diploid and tetraploid populations. Early QTL mapping studies include using RFLP and RAPD markers to determine QTL for chip colour and tuber dormancy, and RFLP markers to identify QTLs for resistance to *Phytophthora infestans*. More recently, QTL analysis in combination with a candidate gene approach was successfully used by Werij to analyse the genetic basis of various tuber quality traits in a diploid mapping population.

Marker-Assisted Selection (MAS) and potato breeding

The relative lack of implementation of molecular markers in tetraploid potato breeding programmes compared with some other crops is mainly due to the high level of natural allelic variation in potato, caused by the autotetraploid nature of cultivated potato and its tetrasomic inheritance [38].

Genomics

The genome sequence of potato (The Potato Genome Sequencing Consortium, 2011) was determined using a homozygous doubled monoploid (DM1-3 518 R44 or 'DM', as well as a heterozygous diploid line (RH89-039-16 or 'RH'). The elucidation of the reference potato genome, including the annotation of around 39,000 protein-coding genes, has opened up opportunities to rapidly identify candidate genes in regions associated with a trait of interest. For example, the identification of both the *StSP6A* gene for tuber initiation and the *StCDF1* gene responsible for plant maturity phenotype was greatly aided by the genome sequence [39].

The Single Nucleotide Polymorphism (SNP) frequency is very high in the potato genome (The Potato Genome Sequencing Consortium, 2011). A SNP chip based on the 'DM' genome sequence has been developed and contains 8,303 SNPs, including many targeted to candidate genes. The positions of these SNPs on the 'DM' genome are known, which allows for rapid identification of genomic regions of interest. The first genetic maps based on diploid biparental populations using the SNP chip include over 4,400 markers and refined the anchoring data of the potato genome sequence. The high frequency of SNPs in the potato genome implies that selection for favourable alleles based on

one single SNP is unreliable because it may not in all cases be indicative of the desired phenotype.

Conclusion

Current potato production in temperate regions, such as northern Europe and North America, under optimized agricultural practices including irrigation when necessary, can yield more than 40 tonnes of potato tubers per hectare within 4 months of planting whereas in developing countries like Ethiopia national average of potato production is 13.39 tones/ha. This implies that the lower yields of potato are typically attributed to lack of high-quality seed, unimproved cultivars, lower rates of fertilizer use and irrigation, as well pest and disease problems. Through applications of biotechnologies such as tissue and cell culture, genetic engineering, marker-assisted technologies, genome-assisted technologies or a combination of all the technologies for the improvement in potato yield and quality. Access to these biotechnologies is vital importance for developing countries. Potato will continue to be at the forefront of transformation technology, and developments in developing countries area offer exciting challenges for future crop improvement, sustainability and scientific advancement.

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