

Biotechnological Approaches to Improve Potato: Review Article

Kanko Chuntale

Department of Plant Breeding, Southern Agricultural Research Institute, Arba Minch Agricultural Research Center, Arba Minch, Ethiopia

Abstract

This article was planned to summarize available information's on biotechnological approaches to improve potato. The potato (*Solanum tuberosum* L.) is a major world food crop. It gives an exceptionally high yield and also produces more edible energy and protein per unit area and time than many other crops. The developed countries make the most diversified use of potatoes as food, feed and raw material for processed products, starch and alcohol, while the developing countries are increasingly adopting potato cultivation primarily as a food crop. Potato is propagated by both sexually (using true potato seed) and asexually (vegetatively). Conventional propagation of potato is done vegetatively using seed tubers which ensure uniformity of the crop in terms of growth and yield, but results in degeneration of the crop due to virus infection. The availability of tissue culture technology for rapid multiplication of disease free planting material has facilitated potato seed production to a great extent. Potato is an ideal crop for the introduction of traits using biotechnology. Conventional potato breeding as it is practiced worldwide is an inefficient, slow process that has changed little in the past century. Potato breeding efforts have historically focused on yield, fresh market and processing quality, and storability as well as disease resistance. Genetic variation for these traits in commercial cultivars is low, but related wild species contain many traits not found in cultivars and represent an especially rich source of disease resistance and tuber quality genes. Combining tuber quality traits desired by consumers and processors with the agronomic performance and disease resistance preferred by farmers remains the most significant challenge in potato breeding. Fortunately, the tremendous amount of genetic diversity in wild and cultivated relatives of potato allows for relatively easy identification, isolation, and introduction of new genes for a specific trait using biotechnology.

Keywords: Biotech potato, Genetic modification, Biotechnology, True potato seed, Seed tubers

1. Introduction

The potato (*Solanum tuberosum* L.) is a major world food crop. In global food production during 2005, potato (324.5mt) was exceeded only by maize (711.7 mt), wheat (630.5mt) and rice (621.6 mt) (<http://faostat.fao.org>) cited by (Naik and Karihaloo, 2007). Potatoes are consumed by over one billion people world over, half of whom live in the developing countries. Potato gives an exceptionally high yield and also produces more edible energy and protein per unit area and time than many other crops. While the developed countries make the most diversified use of potatoes as food, feed and raw material for processed products, starch and alcohol; the developing countries are increasingly adopting potato cultivation primarily as a food crop. The share of developing countries in world potato area rose from 15.1% in 1961 to 51.0% in 2005. In 1961, potatoes produced in the developing countries accounted for 10.5% of the global output. Today, they produce about 47.2% of potatoes in the world (Naik and Karihaloo, 2007).

Potato is a semi perishable crop susceptible to many diseases and insect pests. Shortage of good quality seed has been recognized as the single most important factor limiting potato productivity in the developing countries. The availability of tissue culture technology for rapid multiplication of disease-free planting material has facilitated potato seed production to a great extent (Dodds 1988). Meristem culture is being successfully employed to obtain virus-free potato clones (Mori et al.1969). Rapid multiplication of these disease-free clones using micropropagation coupled with conventional multiplication methods has now become an integral part of seed production in many countries (Donnelly et al. 2003).

Potato is propagated by both sexually (using true potato seed) and asexually (vegetatively). Conventional propagation of potato is done vegetatively using seed tubers which ensure uniformity of the crop in terms of growth and yield, but results in degeneration of the crop due to virus infection. The rates of degeneration vary from place to place and from one growing season to other growing season. The viruses are transmitted through different ways as well as by planting infected tubers. If the seed stock is not maintained well or frequently replaced with fresh ones, the virus infiltration can reach up to 100% in 3- 4 successive crop seasons resulting in almost half or one third yields (Biniam,T. and M. Tadesse, 2008). This is the major problem faced by seed producers.

The other major production problems that account for low yield are unavailability and high cost of seed tubers, lack of well adapted cultivars, poor agronomic practices, diseases, insect pests, inadequate storage, transportation and marketing facilities (Tekalign T., 2005). Late blight of potato is the most important and most destructive disease of potato worldwide. The disease caused by the oomycete *Phytophthora infestans* (Mont.) de

Bary is still the greatest threat to the potato crop, accounting for significant annual losses in worldwide (Hijmans et al., 2000a, b).

Low soil fertility is one of the most important constraints limiting potato production in Eastern Africa (Muriithi MM, Irungu JW, 2004). and also (Tekalign et al., 2001) reported that nitrogen and phosphorus are deficient in most Ethiopian soils and thus application of these nutrients could significantly increase crop yields.

The purpose of this review is to provide readers with an overview of biotechnological approaches to improve potato and biotech potato including its history, past and potential impact on the industry, targeted traits, consumer perception, and biotech crop safety.

2. Development of Biotechnologies in potato

Potato is an ideal crop for the introduction of traits using biotechnology. In fact, after virus resistant tobacco (China in 1992) and the FlavrSavr tomato (U.S. in 1994), potato was one of the first crops to be genetically modified; it was grown commercially as NewLeaf™ by Monsanto in 1995 (Dennis et al, 2016). Conventional potato breeding as it is practiced worldwide is an inefficient, slow process that has changed little in the past century. Potato requires considerable inputs of nutrients, pesticides, and water to maintain yield, quality, and protection from diseases and insects. Potato breeding efforts have historically focused on yield, fresh market and processing quality, and storability as well as disease resistance. Genetic variation for these traits in commercial cultivars is low, but related wild species contain many traits not found in cultivars and represent an especially rich source of disease resistance and tuber quality genes (Hanneman 1989; Jansky 2000). Efforts have been made to introgress nutritional qualities and resistance to pests and abiotic stresses from wild species into cultivated potato, but popular cultivars have few traits derived from wild germplasm due to their genetic complexity, unpredictable expression in adapted backgrounds, and a desire by industry to limit variability in processing quality. In the U.S., the availability of effective pesticides, fungicides, fumigants, synthetic fertilizers, and irrigation systems has meant that market-driven traits, such as yield, are often given higher priority than biotic and abiotic stress resistances. Combining tuber quality traits desired by consumers and processors with the agronomic performance and disease resistance preferred by farmers remains the most significant challenge in potato breeding. Fortunately, the tremendous amount of genetic diversity in wild and cultivated relatives of potato allows for relatively easy identification, isolation, and introduction of new genes for a specific trait using biotechnology. For example, genes from wild potato relatives can contribute resistance to late blight, Verticillium wilt, potato virus Y, water stress, and cold-induced sweetening. The fact that genes of interest can be derived from wild relatives of potato allows for the production of biotech varieties by inserting potato DNA. This is contrasted with traditional transgenic plants that use DNA derived from bacteria, viruses, or other organisms.

In addition to the abundance of traits available for potato improvement, potato can be propagated easily in tissue culture, making it straightforward to integrate specific genes and recover plants from transformed tissue (Chakravarty et al. 2007). Some cultivars are more amenable to tissue culture than others, but with appropriate protocol modifications, most are capable of undergoing transformation using *Agrobacterium tumefaciens* and regeneration of plant tissue. The use of *Agrobacterium* to introduce genes of interest is the most common method of stable transformation in potato, although other methods such as particle bombardment, protoplast transformation, and microinjection have been successful. Regardless of the method, these approaches require regulatory clearance of the resulting potato variety before wide-scale release and production. Regulatory clearance in the U.S. can involve up to three federal agencies: the Environmental Protection Agency (EPA), the Food and Drug Administration (FDA), and the US Department of Agriculture (USDA). Relatively new methods to specifically edit regions of the plant genomes are also being developed and may provide a method for genetic improvement that fits outside the traditional regulatory process (Waltz 2012).

The biotech potatoes commercialized in the mid-1990s (Toevs et al. 2011b) were a technological success and provided benefits to producers, consumers, and the environment, but anti-GMO pressure regarding the safety of biotech food crops led to their removal from the market in 2002, and their status remained unchanged for more than a decade. With the widespread approval and adoption of other biotech crops, there is a renewed interest in the development of biotech potato which has led to the arrival of biotech potatoes back on the market in 2015 (Dennis et al, 2016).

2.1. History of Biotech Potato

In 1995 Monsanto released the first biotech potato used in agricultural production, the Russet Burbank variety containing the CryIIIA gene to provide resistance to Colorado potato beetle (USDA-APHIS, 2015). Named NewLeaf™, this marked the introduction of the company's first biotech crop of any type. Nature Mark, a wholly-owned subsidiary of Monsanto, eventually marketed three varieties of CPB-resistant potato – Atlantic, Russet Burbank, and Superior and branded them with the New Leaf™ trademark. In regions where CPB was a problem, NewLeaf™ potatoes quickly became popular among growers. The product was very effective at

preventing CPB damage and U.S. plantings of Nature Mark potatoes expanded rapidly, from 1,800 acres in 1995 to 55,000 acres in 1998. Three years after rolling out its biotech potatoes, Nature Mark varieties comprised four percent of the U.S. crop. By 1991, Monsanto had developed potatoes resistant to both CPB and potato leafroll virus (PLRV) (Perlak et al. 1993; Kaniewski and Thomas 2004). In 1998, Nature Mark introduced NewLeaf Plus™, a Russet Burbank variety with resistance to both CPB and PLRV.

Other Nature Mark products in development at that time included resistance to late blight and tubers with increased specific gravity (Kaniewski and Thomas 2004). Not surprisingly, Monsanto was not the only organization investing in biotech potatoes. Scientists with other firms and universities around the world were conducting research and field testing biotech potatoes. Much of the effort went into pest resistance, especially late blight resistance, but processing traits were also targets.

Anti-GMO activism fueling public debate regarding the safety of biotech crops eventually led to problems with marketing NewLeaf™ potatoes used for processing. The food industry, consumer groups, and anti-biotech activists, who remained quiet at first, began voicing opposition to products derived from biotech potato. Quick service restaurants reacted by moving away from frozen fries made with biotech potatoes. Fueling the debate surrounding biotech potato products, one frozen potato processor tried to differentiate its fries by guaranteeing that they were GM-free (Guenthner 2001). The North American fresh market continued to accept biotech potatoes, but with processed potato markets closing, growers became reluctant to take on the risk of planting biotech potatoes. Surrendering to dwindling marketability for their products, Monsanto closed its NatureMark potato business in the spring of 2001.

There were also problems with other processed potato markets. Raw product for dehydrated potato processing comes mostly from fresh packers who sort out potatoes that don't meet fresh quality standards. In the infancy of biotech crop commercialization, there was no perceived need for identity preservation (IP) or directed marketing program for NewLeaf™ potatoes. There was general mixing of biotech and conventional potatoes within the dehydration supply chain (Toevs et al. 2011a). Although Monsanto had received Japanese government approvals for most NewLeaf™ potato varieties, when NewLeaf™ potatoes were withdrawn from the market, they were also withdrawn from domestic and international regulatory processes. After withdrawal, an unapproved event was found in dehydrated potatoes and the potato import tolerance level for that event was 0 %. The consequences of this action included rejected shipments and expensive product testing for the North American potato industry.

At about the time that Monsanto withdrew from the biotech potato business, the J.R. Simplot Company began efforts on product development, testing, and regulatory submissions. Learning from the marketing difficulties encountered by Monsanto, Simplot focused on consumer traits rather than producer traits for its first biotech potato products. Simplot also used only potato genes for trait introduction in order to address the public's concerns regarding biotech food safety.

One of the first consumer traits focused on by Simplot was potatoes that had a lower propensity for the formation of acrylamide, a substance linked to birth defects and cancer in mice and rats (National Toxicology Program 2011), and common in foods cooked at high temperatures. Anticipating the need for low-acrylamide raw product for its potato processing business, Simplot scientists successfully developed potatoes with a lower potential for producing acrylamide. A second consumer trait of interest to Simplot was black spot bruise resistance, which could reduce food waste during processing and open new avenues for marketing fresh cut potatoes.

In 2013 Simplot submitted a petition to the U.S. Department of Agriculture, Animal and Plant Health Inspection Service (USDA-APHIS 2015) seeking non regulated status for its Innate™ 1.0 potato with low acrylamide potential and black spot bruise resistance traits. In 2014, Simplot received deregulation from the USDA. This was followed by completing the food and feed safety consultation with the Food and Drug Administration (FDA) in 2015, opening the door for Simplot to commercialize Innate™ 1.0 in Atlantic, Ranger Russet, and Russet Burbank potatoes.

In May 2015, the Innate™ 1.0 potatoes entered the fresh and chip market channels as a limited commercial launch. Simplot implemented a directed marketing stewardship program to keep the biotech potatoes out of the dehydration and frozen processing market channels. The company also submitted a petition to USDA APHIS for Innate™ 2.0 potatoes that have the same 1.0 trait but add late blight resistance and cold storage capability.

Efforts to develop and commercialize biotech potatoes continue around the globe, but outside North America only three varieties have received government approval as of May 2015 (ISAAA 2015). One is Amflora, a high-starch potato developed by BASF and approved in Europe, but no longer marketed. The other two, Elizaveta Plus and Lugovskoi Plus, are insect-resistant varieties developed by the Russian Academy of Sciences. Other biotech potato varieties have been developed, but have not completed regulatory clearances. In addition to Amflora, BASF also developed the varieties Modena, Amadea (both with increased amylopectin), and Fortuna (with late blight resistance). However, BASF halted its pursuit of regulatory approval of all biotech potato varieties in 2013 "because continued investment cannot be justified due to uncertainty in the regulatory

environment and threats of field destructions” (BASF 2013).

The perseverance of biotech potato development and marketing should serve as a clear indication of the desire for improved potato varieties by growers and producers. Consumer acceptance of biotechnology is increasing and the coupling of new technologies with an increasingly scientifically literate public and a focus on consumer-oriented traits should favor widespread approval of biotech potato on the market place.

2.2. Cell and tissue culture to potato improvement

Tissue culture is the regeneration of plants in the laboratory from disease free plant parts (cells, tissue). This technique allows for the reproduction of disease free planting material for crops. Over the past 50 years the application of cell and tissue culture techniques has been most conspicuous in potato than any other crop species. The first successful establishment of tissue culture from potato tubers was reported as early as in 1951 (Steward and Caplin, 1951), cited by Srivastava AK et al., 2012 and since then *in vitro* cultures of potato were developed from different plant parts such as petioles, ovaries, anthers, stems, roots, and shoot tips (Bajaj, 1987). Due to its high amenability to *in vitro* manipulations, a range of techniques had been perfected in this crop over the years. These techniques are of differing degree of complexity forming a complete spectrum of technologies. While some of these technologies have been applied to improved potato production by mean of micro-propagation and pathogen elimination, others are still being refined and improved. The use of *in vitro* technique for virus elimination (meristem culture) and clonal mass propagation (micropropagation) is the most prominent application in potato. *In vitro* produced disease free potato clones combined with conventional multiplication methods have become an integral part of seed production in many countries (Naik and Sarkar, 2000).

2.2.1. Pathogen elimination through meristem culture

As early as in 1952, Morel and Martin observed that many viruses were unable to infect the apical and/or auxiliary meristem of a growing plant, and that of a virus free plant could be produced if a small (0.1 -0.3 mm) explants of meristematic tissue was propagated *in vitro* (Srivastava AK et al., 2012). Since more than 30 viruses are known to infect potatoes, this technique found its immediate application for virus elimination. In the absence of chemical control of viral diseases, meristem culture technique is the only effective method available to date that eliminates virus infection from the systematically infected potato cultivars. In general, larger the size of the meristem, more the chances of its survival *in vitro*, whereas, smaller the size of the meristem, lesser its chances of being virus-infected (Mellor and Stace-Smith, 1987). Although theoretically it is possible to eliminate viruses from potato plants following meristem culture alone, this procedure is almost always combined with thermotherapy and/or chemotherapy to increase the likelihood of obtaining virus free plants *in vitro* (Khurana and Sane, 1998). Thermotherapy involves growing whole plants or *in vitro* cultures at high temperature close to the threshold of normal plant growth. The effectiveness of thermotherapy is due to disruption in the synthesis of viral ssRNA and/or dsRNA, production or activity of virus encoded movement proteins and coat proteins. For most plant cultivars, thermotherapy is usually done at 37°C, however, the exact temperature and length of treatment varies with the virus and heat tolerance of the plant. In contrary to thermotherapy, antiviral chemicals are used in chemotherapy to inhibit or interfere with virus replication or movement in plant tissues. These chemicals can either be sprayed on growing plants or incorporated into tissue culture media. The virus free clones of selected potato cultivars are maintained and multiplied *in vitro* for specialized utilization in potato seed production, international exchange and other activities. The protocol for potato meristem culture consists of i) selection and testing of apparently healthy plants from the field, ii) establishment of *in vitro* cultures and iii) meristem culture (Srivastava AK et al., 2012).

i) Selection and testing of plants

1. Select apparently healthy plants from the field or sample tubers.
2. Test these plant/tubers for freedom from viruses using ELISA.
3. If no plants/tuber is found free from all the viruses then one has to resort to meristem culture.
4. Select a plant/tuber that is infected with minimum (one or two) viruses for use in meristem culture.

ii) Establishment of *in vitro* cultures

1. **From infected plant:** When starting with plant, excise nodal segments from the third and fourth node from the stem apex with a scalpel. Each nodal cutting should be 1-2 cm long, and the leaves should be detached. Such single node cuttings (SNCs) are used to initiate *in vitro* cultures.
2. **From infected tubers:** Treat the freshly harvested tubers with 0.2% Bavistin for 15 min and dry them. If not required for immediate use, the tubers can be stored at 4°C for about 1 year. For immediate use, allow the tubers to sprout at 24°C. Since most cultivars are dormant after harvest, sprouting will initiate at least after 2 months. Harvest sprouts measuring about 2-3 cm long.
3. In the laminar flow clean air work station, surface sterilize the nodal segments/sprouts for 10-15 minutes in 20% of commercial grade of sodium hypochlorite solution (4% w/v available chlorine), rinse in sterile distilled water for three times. Trim both ends of the explants by a scalpel and place the explants into individual culture tubes (25 x 150 mm) each containing 13 ml of semi-solid nutrient

medium.

4. The culture is based on MS (Murasighe and Skoog, 1962) basal nutrients supplemented with D-calcium pantothenate (2mg/l), Gibberellic acid (0.1 mg/l), alpha-naphthalene acetic acid (0.01 mg/l) and 30g/l sucrose. The medium was solidified with 7.0 g/l agar.
5. Incubate the cultures under a 16h photoperiod from cool white fluorescent lights (approx. 50-60 mmol/sq m/s light intensity) at 24°C.
6. Allow the explants to grow up to 6-8 nodes/stem stage, and then subculture through SNCs on fresh medium under the same culture conditions. Shoot cultures can be maintained and multiplied *in vitro* by sub culturing on fresh medium every 3 weeks.

Virus elimination

Thermotherapy: It can be given to *in vitro* cultures or tubes prior to meristem culture. This is done as follows

1. Place 7 day old cultures in a BOD incubator at 37°C under a continuous photoperiod (approx. 20 mmol/sq m/s light intensity), and incubate for three weeks.
2. Treat infected tubers with GA₃ (2 mg/l) and allow them to sprout at 37°C under dark till 8-10cm long sprouts are formed.
3. After thermotherapy, meristems are dissected from *in vitro* plantlets and/or sprouts by the method described below.

iii). Meristem excision and culture

1. Excise meristem (terminal as well as axillary) from thermo-treated *in vitro* plantlets under laminar flow cabinet using a stereoscopic zoom microscope, scalpel and needle. Protective leaves on the buds are removed carefully using needle. Use a drop of sterile distilled water to avoid meristem desiccation during excision.
2. Trim the meristematic dome plus one set of leaf primordial with a scalpel to 0.2-0.3 mm.
3. In case of sprouts, surface sterilization of sprouts using 20% of commercial grade of sodium hypochlorite solution is essential before dissecting meristems.
4. Place the excised meristems on semi solid meristem culture medium in a culture tube (1 meristem/culture tube), and incubate the cultures under a 16 h photoperiod (approx. 50-60 mmol/sq m/s light intensity) at 24°C.
5. The meristem culture medium is based on MS basal nutrients supplemented with 2 mg/l D-calcium pantothenate, 0.1 mg/l GA₃, 0.01 mg/l NAA and 30g/l sucrose, and solidified with 6.0 g/l agar.
6. It takes about 5-6 months for meristems to grow into full plantlets (mericlones). At this stage sub-culture the plantlets, maintain their clonal identity.
7. Test meristem derived plantlets for presence or absence of viruses by ELISA and/or ISEM.
8. Multiply and maintain virus-negative counterparts of meristem-derived clones by shoot cultures *in vitro* as described above.

2.2.2. Micropropagation

Micropropagation allows large scale asexual multiplication of pathogen free tested potato cultivars. At an interval of every 21 days of sub culturing, minimum 3 nodal cuttings are obtained from a single microplant. Therefore, theoretically 3¹⁵ (43 million) microplants can be obtained from a single virus free mericlone in a year. Various techniques have been developed for producing large number of micro plants on nutrient medium under aseptic conditions. Nodal segment culture in which axillaries and terminal buds grow into new plants is predominantly used for initial shoot multiplication. The method involves culturing of nodal explants of disease free micro plants on semisolid (agarified) or liquid culture medium. Considerable research has been done on the nutritional, hormonal and physical aspect of the culture media and their effects on explants growth. Murasighe and Skoog medium is most widely used for potato micro propagation. Semi solid medium is used for initial nodal segment propagation; however liquid medium fosters higher growth rate of potato micro shoots (Rosell et al., 1987). *In vitro* derived micro plants are used as i) explants source for the production of micro tubers *in vitro*, ii) direct transplants in the greenhouse for the production of mini tubers, iii) mother plants for further *in vitro* multiplication through single node cuttings (SNCs) and iv) source material for production of synthetic seed.

i. Micro tuber production in vitro

Micro tubers are miniature tubers developed under tuber inducing conditions *in vitro*. These small tubers are particularly convenient for handling, storage and distribution. Unlike micro propagated plantlets, they do not require time consuming hardening periods in greenhouses, and may be adapted easily to large scale planting in the field. Many protocols have been developed to induce micro tubers in potato. Explants can be nodal cuttings, excised stolons, micro shoot cuttings or whole micro plants.

The harvested micro tubers are dormant, and therefore, required to be stored at 5-6°C for 3-4 months before planting on nursery beds or in the field. In order to avoid weight loss of micro tubers during storage, micro tubers are greened before harvesting by incubating 50-65 days-old induction cultures under 16h photoperiod at 24°C for 10-15 days (Naik and Sarkar, 1997). Greening improves the storage of micro tubers in terms of reduced

biomass loss due to shrinkage and better sprout emergence. Thickening or suberizing the periderm of micro tubers during greening is mainly responsible for making them more tolerant to evaporative water loss. Moreover, glycoalkaloids produced during greening protect the micro tubers from bacterial and/or fungal damage.

Micro tuber production technology involves i) initial multiplication of virus-free plantlets on semi-solid medium, ii) mass multiplication of *in vitro* plantlets in liquid medium, iii) production of micro tubers, iv) harvesting and storage and v) field planting (Srivastava AK et al., 2012). These are depicted in fig 1.

Initial multiplication

1. Multiply disease-free stock plants (obtained from meristem culture) through nodal cuttings on semi solid MS medium in culture tubes (25 x150 mm). To economize on space, tubes and other inputs use ordinary cane sugar in place of sucrose and inoculate 3 nodal cuttings in each tube.
2. To maintain varietal identity edible colours of 25mg/l can be mixed in the medium before autoclaving.
3. Incubate the cultures under a 16h photoperiod from cool white fluorescent lights (approx. 50-60 mmol/ sq m/s light intensity) at 24⁰C.

Mass micro propagation in liquid medium

1. When large number of plants is produced in initial multiplication, liquid cultures are initiated for mass micro propagation.
2. Initiate liquid propagation cultures in 250 ml Erlenmeyer flasks or magenta box, pour 20ml liquid propagation medium (composition same as in semisolid medium except agar) and autoclave.
3. Inoculate 10-12 stem segments (each having 3-4 nodes) obtained from six 21-day-old plantlets in each flask/box
4. Incubate the liquid cultures under the same cultural conditions as in semisolid propagation.
5. In about 3 weeks all axillary buds grow into full plants and fill the container.

Micro tuber production

1. After 21 days of incubation, decant the liquid propagation medium from the Erlenmeyer flask or magenta box under aseptic conditions of a laminar flow workstation, and pour in 40ml of micro tuber induction medium. The micro tuber induction medium is based on MS basal nutrients supplemented with 10mg/l N⁶-benzyladenine (BA), 500mg/l chlorocholine chloride (CCC) and 80g/l sucrose (commercial sugar).
2. Incubate these induction cultures under complete darkness at 20⁰C. Micro tubers start developing epigeally at the terminal or axillary ends of the shoots within 8-10 days and they are ready for harvesting after 60-90 days depending upon genotype. In general, 15-20 micro tubers with an average weight of 100-150mg are produced in each flask or magenta box.

Harvesting and storage

1. Before harvesting, green the micro tubers *in vitro* by incubating the induction cultures under a 16h photoperiod (approx. 30 mmol/ sq m/s light intensity) from cool white fluorescent lights at 24⁰C for 10-15 days.
2. Hand-harvest the green micro tubers in plastic trays. Avoid damaging the micro tubers during harvesting.
3. Wash the harvested micro tubers in running tap water to remove adhering constituents of the medium. Treat the harvested micro tubers with 0.2% Bavistin for 10 minutes and allow them to dry in the dark at 20⁰C for 2 days.
4. Pack the dried micro tubers in perforated polythene bags and store at 5⁰C in a refrigerator for 4-5 months under dark for breaking dormancy.
5. After 3-4 months of storage, the sprouted micro tubers are ready for field planting.

Field planting

1. Plant the sprouted micro tubers (1 micro tuber/plastic bag) in perforated plastic bags (8x4cm) filled with 1:1:1 mixture of farmyard manure, sand and soil, and grow for 3 weeks in the greenhouse.
2. Transplant the established plantlets into the field by removing the plastic bags without disturbing the root-soil mass.
3. Irrigate the plots by furrow irrigation throughout the growing period according to evapo- transpiration requirements to avoid drought stress during crop growth and grow the crop following recommended package of practices. At maturity harvest normal sized tubers.

Aeroponics in potato

Aeroponics culture refers to soil less culture for producing mini tubers. It involves spraying plant roots with a fine mist of a complete nutrient solution. Aeroponic systems feature the roots of the plant growing in lightproof, sealed box or container where they are continuously or intermittently misted with nutrient solution. This solution is continuously recirculated through the system. The top portion of the plant is exposed to the open air and a light source (either artificial light or natural sunlight). It does not need any excess area for aeroponic based healthy seed production. Only one percent of conventional water usage is required which is basically recycled water. It is the ideal technology for cost-effective production of quality seed in the present era. Advantages of this system are i) tropical states which do not have isolated and virus-free potato growing areas can also produce quality seed

ii) early supply of nucleus seed to commercial growers by reducing the field exposure time iii) improved tuber quality and reducing the load of degenerative diseases iv) utilize the resources and trained manpower round the year and v) vertical growth and reduction in pressure on land.

Soil less production techniques, such as Nutrient Film Technique (NFT) and aeroponics have been successfully employed in tuber production with good prospects for certified seed production (Boersig and Wagner, 1988), cited by (Srivastava AK et al., 2012). Aeroponic system has comparatively higher multiplication rate as compared to conventional micro-propagation system. Harvesting in aeroponics is convenient and allows a greater size control by sequential harvesting. It has added advantage such as solution recirculation, a limited use of water and is safe from soil borne diseases.



Figure 1. Potato seed production through micro tubers

2.3. Genetic engineering to potato improvement

Scientists have learned how to move genes from one organism to another. This has been called genetic modification (GM), genetic engineering (GE) or genetic improvement (GI). Regardless of the name, the process allows the transfer of useful characteristics (such as resistance to a disease) into a plant, animal or microorganism by inserting genes (DNA) from another organism (Li and Gray, 2005). Virtually all crops improved with transferred DNA (often called GM crops or GMOs) to date have been developed to aid farmers to increase productivity by reducing crop damage from weeds, diseases or insects.

Potato was one of the first crop plants in which plants were successfully regenerated (Shaning and Simpson 1986). Using recombinant gene technology, genes of interest are substitute to cogenes and some new traits were introduced in potato cultivars. For almost all transgenic potato plants developed today kanamycin resistance has been used as the selectable marker gene for the selection of transformed events. Different transformation protocols have been developed using other selectable markers or potato organs.

The following schema presents briefly the steps for *agrobacterium* mediated potato transformation on leaves and internodes (CIP protocol).

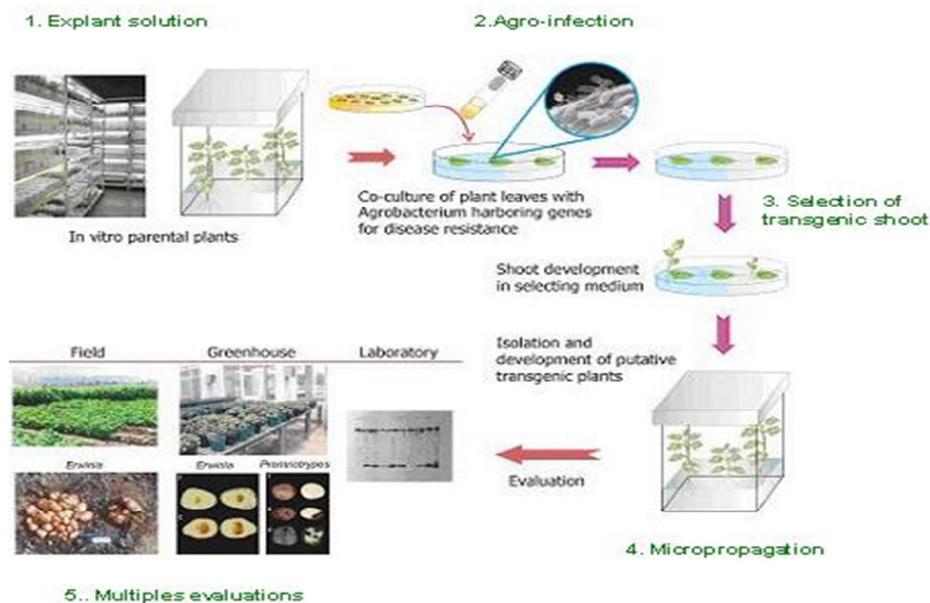


FIGURE 2. THE STEPS FOR AGRO BACTERIUM MEDIATED POTATO TRANSFORMATION ON LEAVES AND INTERNODES (CIP PROTOCOL)

2.4. Molecular marker system to potato improvement

Genetic markers are the biological features that are determined by allelic forms of genes or genetic loci and can be transmitted from one generation to another, and thus they can be used as experimental probes or tags to keep track of an individual, a tissue, a cell, a nucleus, a chromosome or a gene. Genetic markers used in genetics and plant breeding include morphological markers, biochemical markers (alloenzymes and other protein markers) and molecular markers (based on DNA-DNA hybridization). DNA markers have developed into many systems based on different polymorphism detecting techniques or methods such as RFLP, AFLP, RAPD, SSR, SNP, etc (Farokhzadeh et al, 2014).

The use of molecular markers in potato breeding offers new opportunities for the selection of genotypes. To date, many markers linked to useful traits have been found. Indeed, the potato molecular map is saturated with more than 350 markers uniformly distributed on 12 chromosomes. More than 25 single dominant genes have been localized on the potato map, most of them being pest-resistance genes, together with some quantitative trait loci (QTL) controlling yield and tuber quality traits. Molecular markers linked to these genes are now available for rapid, efficient assisted selection (positive assisted selection). Moreover, in order to transfer useful genes, interspecific crosses between wild and cultivated genotypes are often performed, and in this case the use of species-specific molecular markers would allow the wild genomic content to be reduced in few backcross generations (negative assisted selection)

3. Conclusion

The potato (*Solanum tuberosum* L.) is a major world food crop. It gives an exceptionally high yield and also produces more edible energy and protein per unit area and time than many other crops. The developed countries make the most diversified use of potatoes as food, feed and raw material for processed products, starch and alcohol, while the developing countries are increasingly adopting potato cultivation primarily as a food crop.

Potato is propagated by both sexually (using true potato seed) and asexually (vegetatively). Conventional propagation of potato is done vegetatively using seed tubers which ensure uniformity of the crop in terms of growth and yield, but results in degeneration of the crop due to virus infection. The availability of tissue culture technology for rapid multiplication of disease free planting material has facilitated potato seed production to a great extent.

The major production problems that account for low yield are unavailability and high cost of seed tubers, lack of well adapted cultivars, poor agronomic practices, diseases, insect pests, inadequate storage, transportation and marketing facilities (Tekalign T., 2005). Late blight of potato is the most important and most destructive disease of potato worldwide.

Potato is an ideal crop for the introduction of traits using biotechnology. Combining tuber quality traits desired by consumers and processors with the agronomic performance and disease resistance preferred by farmers remains the most significant challenge in potato breeding. Fortunately, the tremendous amount of genetic diversity in wild and cultivated relatives of potato allows for relatively easy identification, isolation, and

introduction of new genes for a specific trait using biotechnology. The biotechnological methods used to improve potato are cell & tissue culture, genetic engineering and molecular marker assisted selection.

References

- Naik, P.S. and Karihaloo, J.L. 2007. Micropropagation for Production of Quality Potato Seed in Asia-Pacific. Asia-Pacific Consortium on Agricultural Biotechnology, New Delhi, India.p. 47+viii.
- Bajaj, Y.P.S. (1987). Biotechnology and 21st century potato. In: Biotechnology in Agriculture and Forestry, Vol. 3: Potato (ed. Bajaj, Y. P. S.), Springer-Verlag, Berlin, pp. 3-22.
- BASF (2013). BASF to expand its plant biotechnology research into fungal resistance in corn.
- Biniam, T. and M. Tadesse (2008). A survey of viral status on potatoes grown in Eritrea and in vitro virus elimination of a local variety 'Tsaeda embaba'. *African J. Biotechnol.*, 7(4): 397-403.
- Chakravarty, B., G. Wang-Pruski, B. Flinn, V. Gustafson, and S. Regan (2007). Genetic transformation in potato: approaches and strategies. *American Journal of Potato Research* 84: 301-311.
- Dennis Halterman, Joe Guenther, Susan Collinge, Nathaniel Butler, David Douches (2016). Biotech Potatoes in the 21st Century: 20 Years since the First Biotech Potato. *American Journal of Potato Research*, Volume 93, Issue 1, pp 1-20.
- Farokhzadeh, S., AliFakheri, B., (2014). Marker-assisted Selection for Disease Resistance: Applications in Breeding. Faculty of Agriculture, Zabol University. *Intl J Agri Crop Sci.* Vol., 7 (14), 1392-1405.
- Guenther, J. 2001. The international potato industry. Woodhead Publishing, Cambridge UK.
- Hanneman Jr., R.E. 1989. The potato germplasm resource. *The American Potato Journal* 66: 655-667.
- Hijmans RJ, Forbes GA, Walker TS (2000b) Estimating the global severity of potato late blight with GIS-linked disease forecast models. *Plant Pathol* 49:697-705, doi:10.1046/j.1365-3059.2000.00511.x
- Hijmans RJ, Grunwald NJ, van Haren RJF, MacKerron DKL, Sherm H (2000a). Potato late blight simulation for global change research. *GILB Newsl* 12:1-3
- ISAAA (2015). Potato (*Solanum tuberosum* L.) GM events. <http://www.isaaa.org/gmapprovaldatabase/crop/default.asp?CropID=16&Crop=Potato>. Accessed 22 April 2016.
- Jansky, S (2000). Breeding for disease resistance in potato. *Plant Breeding Reviews* 19: 69-155.
- Kaniewski, W., and P. Thomas Khurana, S.M. Paul and Sane, A. (1998). Apical meristem culture- a tool for virus elimination. In: Comprehensive Potato Biotechnology (eds. Khurana, S. M. Paul., Chandra, R. and Upadhyaya, M. D.), Malhotra Publishing House, New Delhi, pp. 207-232.
- Mellor, F.C. and Stace-Smith, R. (1987). Virus-free potatoes through meristem culture. In: Biotechnology in Agriculture and Forestry, Vol. 3: Potato (ed. Bajaj, Y. P. S.), Springer-Verlag, Berlin, pp. 30-39.
- Muriithi MM, Irungu JW (2004). Effect of integrated use of inorganic fertilizer and organic manures on Bacterial Wilt Incidence (BWI) and tuber yield in potato production system on hill slopes of central Kenya. *J. Mountain Science*. 1: 81-88.
- Naik, P.S. and Sarkar, D. (1997). Influence of light-induced greening on storage of potato micro tubers. *Biol. Plant.*, 39:31-34.
- Naik, P.S. and Sarkar, D. (2000). In vitro propagation and conservation of genetic resources in potato. In: Biotechnology in Horticultural and Plantation Crops, (eds. Chadha, K.L., Ravindran, P.N. and Sahijram Leela), Malhotra Publishing House, New Delhi, pp. 369-406.
- National Toxicology Program (2011). Report on Carcinogens, Twelfth Edition. <http://ntp.niehs.nih.gov/ntp/roc/twelfth/profiles/Acrylamide.pdf>. Accessed 15 April 2016.
- Perlak, F.J., T.B. Stone, Y.M. Muskopf, L.J. Petersen, G.B. Parker, S.A. McPherson, J. Wyman, S. Love, G. Reed, D. Biever, et al. (1993). Genetically improved potatoes: protection from damage by Colorado potato beetles. *Plant Molecular Biology* 22: 313-321.
- Srivastava AK, Diengdoh LC, Rai R, and Bag TK (2012). Tissue Culture –Technology Harnessed for Potato Seed Production. Central Potato Research Station, 5th Mile, Upper Shillong, Shillong – 793 009.
- Tekalign M, Teklu E, Balesh T (2001). Soil fertility and plant nutrition research on Teff in Ethiopia. Proceeding of the international workshop on Teff Genetics and Improvement, October 16-19, 2001, Debre Zeit, Ethiopia, pp: 191-200.
- Tekalign Tsegaw (2005). Response of potato to paclobutrazol and manipulation of reproductive growth under tropical conditions. PhD thesis. pp. 2-3.
- Toevs, E., J. Guenther, A. Johnson, C. McIntosh, and M. Thornton (2011a). Identity preservation systems for genetically modified potatoes. *American Journal of Potato Research* 88: 303-308.
- Toevs, E., J. Guenther, A. Johnson, C. McIntosh, and M. Thornton (2011b). An industry perspective of all-native and transgenic potatoes. *AgBioforum* 14: 14-19.
- USDA-APHIS (2015). Petitions for determination of nonregulated status. http://www.aphis.usda.gov/biotechnology/petitions_table_pending.shtml. Accessed 21 April 2016.
- Waltz, E. (2012). Tiptoeing around transgenics. *Nature Biotechnology* 30: 215-217.