Chapter 6
Potato Diversity and Its Genetic Enhancement

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Abstract Potato is the world's third largest food crop after rice and wheat widely grown across all continents. It belongs to the genus Solanum and section Petota that contain approximately 2000 species that are distributed from the South-western United States (38°N) to Chile (41°S) between 2000 to 4000 m altitudes. Potato has 6 cultivated species, 225 wild relatives and 110 wild tuber-bearing species. The main cultivated potato species *Solanum tuberosum* L., a tetraploid ($2n = 4x = 48$) originated from Andes of Peru and Bolivia in South America over 10,000 years ago. The ploidy of potatoes varies from diploid ($2n = 24$) to hexaploid ($2n = 72$) with majority being diploids. Potatoes were introduced to Europe in 1570s and by beginning of seventeenth century they spread to the other parts of the world. Systematic potato breeding started in 1807 in England followed by other parts of Europe, North America, India, International Potato Centre, Peru and China. There are two basic approaches to conserve potato genetic resources, viz. in situ and ex situ. Currently, cryo-conservation is being tapped for long-term conservation. Seven major potato gene banks are present worldwide to conserve existing diversity. Although more germplasm are being evaluated, the use of genetic resources has been much poorer to their evaluations mainly due to undesirable tuber traits of the wild species and crossability barriers. This has led to narrow genetic base of the cultivated potatoes. The 'Irish famine' of 1840s depicts the devastating effect of

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growing large areas under a single variety. Cultivated potato exhibits complex tetrasomic inheritance and high heterozygosity. Dihaploids of tuberosum cross readily with many diploid species thus providing opportunity for introgression of useful traits from alien sources to cultivated background. The other well-exploited techniques in potato breeding, viz. somaclonal variations, somatic hybridization, molecular markers, genetic transformation and RNAi approaches. Potato is one of the rare crops where maximum tissue culture and genetic engineering interventions have been connoted. Today, potato genome is sequenced and it opens up new vistas for developing tailor-made varieties in future.

Keywords Potato diversity · Germplasm conservation · Genetic stability · Abiotic stresses · Quality traits

6.1 Introduction

Potato rightly called, 'the vegetable that changed history' provided both the spark and the fuel for centuries to the social change. While conquering the world, it was banned and lauded, cursed and praised, feared and loved until humanity welcomed it into its home and hearth. Today, as one of the world's major non-cereal food crop, potato is grown in more than 148 countries in a wide variety of soils and climates surpassed only by wheat, rice and maize in total production. Yet till sixteenth century it was unknown to the people of Europe, Asia, Africa and North America. The crop has a very fascinating history of its origin, evolution and spread in the world, stretching to nearly 7000–9000 years back. Some of it is well documented while other has been chronicled from the archaeological remains and historical evidence (Hawkes 1990).

6.2 Origin

The potato is believed to be originated in the South American continent where it grows as wild in nature and represents the widest diversity of forms in tuber shape, size, colour, taste, etc. The mainly cultivated potato species *S. tuberosum* L., a tetraploid ($2n = 4x = 48$) is believed to have originated from the basin of lake Titicaca on Peru–Bolivian borders from its wild diploid ancestors many of which may be extinct now. Two main centres of diversity of tuber-bearing *Solanum* species are Central America and Andean region of North-western Argentina, Peru and southern Bolivia. The species grow in a wide variety of habitats from semi-desert conditions of northern Argentina, southern Bolivia and Mexico to the high rainfall receiving subtropical forests of Central and South America exhibiting a wide adaptation to altitude right from the sea level to nearly 5,000 m.

Archaeological evidence suggests that the potters from the Moche cultures in northern Peru (c. AD 1–600) and the Chimu people (c. AD 900–1450), as well as Huari or the Nazca valley in southern Peru (c. AD 650–700) obtained potatoes by barter or other means from farmers in the highlands where potatoes were actually 'cultivated'. Actual remains of the potatoes were also recovered infrequently from tombs, dwellings and rubbish heaps including chuño or tunta, from some archaeological sites. Archaeological remains of potatoes from the Chilca valley near Lima have been radiocarbon-dated to 7000 years before present (Hawkes 1990). There is much later evidence for potatoes from rubbish heaps, graves and food stores in 4500–3500 BC (Ugent et al. 1982). The first historical record of potato can be traced back to 1537, when a band of Spaniards led by Jiménez de Quesada penetrated into the highlands of Colombia. Later potatoes were accounted by López de Gomara (1552) in southern Peru and by Pedro Cieza de León (1553) in the southern Colombia and northern Ecuador. Potatoes in Chile received the first mention by Sir Francis Drake in 1578 (Drake 1628).

6.3 Early History

In South America, where it originated, potato was the most productive source of main food for centuries for the people in the high Andes and southern Chile. Potatoes were dried by Andean Indians to make chuño, a freeze-dried potato powder of the bitter, frost-resistant potatoes grown at 3,600–4,400 masl during food shortage and between successive crops during periods of scarcity caused by frost or other unfavourable growing conditions. Following the conquest of Peru, the Spaniards introduced potatoes in Spain and further spread it to many European countries including Italy, Belgium, Germany, France, Switzerland and Holland by the end of the sixteenth century. Initially, potato was grown only as a curiosity in the Europe's botanical gardens and remained a shunned plant—at best a food for swine and country bumpkins for next two centuries. The crop remained a botanical curiosity till about the mid-eighteenth century, and was not grown in any Western European country barring Ireland, where potatoes became the most profitable new crop, mainly for human consumption, and for pigs thriving well on potatoes. Throughout the eighteenth century, none seems to have been aware of the danger to the economy of a nation dependent on a single crop. The warnings of Curwen (1818) went unheeded till August 1845, when suddenly one warm, rainy day in August, an unknown malady (late blight) struck the Irish potato fields. Potatoes quickly rotted in the fields, sending an unbearable stench across the countryside and repeating the same scene across whole of Europe. This was also true in 1846, 1847 and 1848 resulting in famous famine and death of nearly 2.5 million and migration of one million Irish including the famous Kennedys and Reagans to North America.

6.3.1 Spread in Europe

Potato was introduced into Europe, first in Spain in *c*. 1570 and second in England in *c*. 1590 (Hawkes 1990). The Spanish introduction rests on market records from the Hospital de la Sangre in Seville, whilst the English records are extremely complex; nevertheless, we know from Gerard that he grew potato in his garden and described and figured it in *Herbal* of 1597.

Hawkes (1994) reports that European herbalists obtained their potatoes from the Flemish herbalist, Carol Clusius (1601), his specimens being derived from Spain via Italy. The early European potato came from the Andes, perhaps from the northern Colombian part. It is interesting to note that these first European potatoes were adapted to short (12 h) day of the Andes and not to long (16–18 h) day as present day potatoes of Europe, a fact which is also confirmed from the evidence from the Spanish archives. These potatoes were probably the Andean form of the tetraploid potato (*S. tuberosum* subsp. *andigena*), which further evolved through several centuries of 'unconscious' selection in Europe to adapt it to the long summer days of northern Europe showing morphological correlates to this evolutionary change, including reduced top growth, shorter internodes, larger leaves and reduced flowering and fruiting. Hence it was not until the late eighteenth and early nineteenth centuries that this new-day-length adaptation was complete, allowing potato cultivation on a large scale to spread into Central and Eastern Europe.

From Spain the potato was taken to Italy by the Carmelite Friars, as Clusius (1601) mentions that it was grown in Italy before 1587. Clusius received potato from Italy and sent to botanists in many parts of Germany and Austria. The Swiss herbalists C. Bauhin and J. Bauhin obtained tubers from Clusius in the late sixteenth century and sent them to France by about 1600. The Slavic nations seem to have obtained their potatoes from Germany since the names of potato are derived from German ones, e.g. *Kartoffel, Grundbirne,* etc. The potato was brought to Russia by Peter the Great from Holland at the end of seventeenth century.

After the introduction into England in 1590, it was not until the mid-eighteenth century that it was grown on a large scale. The same time scale is recorded for Scotland and Wales. In Ireland, it was grown on field scale by the early seventeenth century. From Scotland the potato was taken to Norway in the mid-eighteenth century and then to Sweden and Denmark.

6.3.2 Spread in Asia, Africa

The potato's global voyage began in the seventeenth century. While stay-at-home Europeans may have had misgivings about the new crop, the sailors, soldiers, missionaries, colonial officials and explorers quickly carried it to their foreign outposts. Thus, Belgian, British, Dutch, French, Portuguese and Spanish sailors

carried the potato first to ports in Asia and the South Pacific while trading, whaling and fishing and later inland to their homes.

Dutch settlers believed to have taken potatoes to the Penghu Islands in the Taiwan Strait as early as 1603 (Anonymous 2007). Belgian and French missionaries introduced them into Taiwan. Soon the crop had spread throughout China passing across Eastern Europe, over the Urals and into the steppes of Asia.

The potato arrived in Africa relatively late. A few grew in South Africa as early as 1830, but British and German colonists and missionaries did not introduce potatoes into East Africa until about 1880. In North and West Africa, the two world wars were the main stimulus for the crop's introduction. With supply lines from Europe cut, armies and colonial personnel were forced to grow their own *bombiderres*. While Africa is not a major producer in terms of volume, more African countries grow potatoes today than any other continent.

In the latter half of the twentieth century, the crop found a home in the arid Middle East, where it established itself as an important commodity in Jordan, Israel and other countries. It is even grown in climate-controlled facilities in the Gulf States.

In North America, potato was completely unknown until the early seventeenth century. It first received potatoes from England via Bermuda in 1621 where it was introduced in 1613. The first potatoes were grown in Virginia. Later in the century, there were more introductions from England and Ireland, but no records of an introduction were made from South America before Goodrich, in 1863, (Hawkes 1992) obtained some varieties in a Panama market.

6.3.3 Spread in India

In India, potato was introduced in the early years of the seventeenth century, most probably by either Portuguese sailors or by the Britishers to the hills of the north India and to Sri Lanka where it flourished in the colonial home gardens. The earliest reference of the potato occurs in account of the voyage of Edward Terry in 1655 (Upadhya 1974) who was chaplain to Sir Thomas Roe, British Ambassador to the court of the Mughal Emperor Jahangir from 1615 to 1619. Similarly, Fryer's travel records (1672–1681) mention the potato as a well-established garden crop in Surat and Karnataka in 1675.

By late eighteenth or early nineteenth century the potato was an important, established, vegetable crop in the hills and plains of India. Early introductions resembled the andigena potatoes and were adapted to short winter days having long dormancy and capable of withstanding higher temperatures under country stores. They were grown by various names in local dialects depicting some character, viz. *Phulwa*—flowering in the plains; *Gola*—round potatoes, *Satha*—maturing in 60 days, etc. and came to be known as *desi* varieties (Pushkarnath 1969).

Between 1924 and end of World War II, the state agricultural departments and other agencies introduced a large number of European potato varieties with a view

to selecting those suitable for local conditions. These efforts, however, proved of little value mainly due to poor adaptation of temperate long day adapted European varieties in the subtropical short days available in plains of India. Only few foreign varieties approached the yield levels of local varieties under commercial culture, however, none proved to be a very good yielder. Fast degeneration of seed stocks was another important factor in non-establishment of European varieties in the subtropics. Lack of adequate cold storages for storing seed potatoes over long periods of hot summer also posed problems. The local old varieties could be kept well in country stores, whereas the imported varieties could not survive these conditions. The introductions from Europe thus made no impact on potato culture in subtropical plains of India. However, a few introductions such as Magnum Bonum, Up-to-Date, Royal Kidney, Great Scot, Craig's Defiance, etc. survived in the hills. In India till 1950, 16 each of desi and European varieties were identified to be mostly under cultivation.

6.4 Genetic Diversity Among Cultivated and Wild Potatoes

There are seven cultivated tuber-bearing *Solanum* species, viz. *S. stenotomum*, *S. ajanhuiri*, *S. phureja*, *S. chaucha*, *S. juzepczukii*, *S. tuberosum* ssp. *andigena*, *S. tuberosum* ssp. *tuberosum* and *S. curtilobum*, occurring in a polyploid series ranging from diploid to pentaploid. Several of them are fairly similar to each other probably because they were initially confined to cool temperate climatic region of the Andes of South America and the lowlands of southern Chile and for that reason

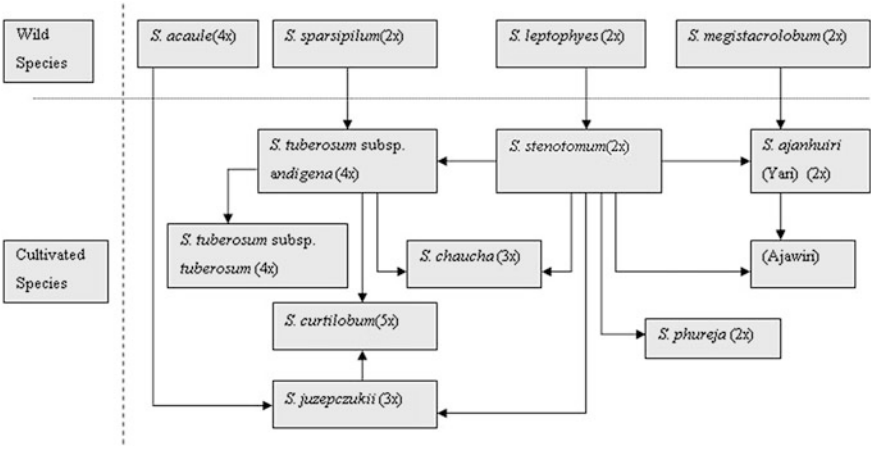

Fig. 6.1 Evolutionary relationships of cultivated potatoes and their ploidy levels (after Hawkes 1990)

were classified by Dodds (1962) as 'groups' of *S. tuberosum* rather than distinct species. Their probable evolutionary relationships are shown in Fig. 6.1.

The wild potatoes seem to have evolved by means of geographical and ecological isolation rather than by genetic incompatibility. The related wild species are much more widespread. The diploid species, *S. stenotomum* is grown from central Peru to central Bolivia and is believed to be the most primitive, probably having been derived from the diploid wild species, *S. leptophyes*, or possibly *S. canasense*, both of which still occur in the central part of its distribution area.

At least four wild potato species are widely believed to be involved in the process of evolution of the cultivated species of potato. Evidence indicates that hybridization of *S. stenotomum* with the weedy species *S. sparsipilum* and subsequent chromosome doubling produced the tetraploid *S. tuberosum* subsp. *andigena* in the central Andes (Cribb and Hawkes 1986). Some workers, however, consider that the tetraploid Andean potatoes are derived from *S. stenotomum* by simple chromosome doubling. This tetraploid subspecies was carried by ancient people into southern Chile, where it became adapted to the long-day length to evolve into subsp. *tuberosum*. A similar process in Europe caused the same development to take place under the long-day conditions. However, certain authors (Grun 1979) believe that subspecies of *tuberosum* from Chile and Europe differ from subspecies of *andigena* by certain cytoplasmic factors acquired from some wild diploid species, such as *S. chacoense*.

In pre-conquest days, the cultivated diploid species *S. phureja* evolved from *S. stenotomum* through a process of artificial selection by Andean farmers in lower, warmer eastern valleys and acquired shorter dormancy so that three crops could be grown in a year. In contrast, natural hybridization of *S. stenotomum* with the wild frost-resistant species *S. megistacrolobum* gave rise to the diploid *S. ajanhuiri*. The F_1 hybrid produced the 'Yari' group of varieties and a probable backcross to the cultivated parent gave rise to the 'Ajawiri' group of varieties. Similarly, the F_1 cross from a series of hybridizations between *S. stenotomum* and the wild tetraploid species *S. acaule* gave rise to a highly sterile triploid *S. juzepczuki*, which incorporated the strong frost resistance of *S. acaule*. A further natural cross between *S. juzepczukii* and *S. tuberosum* subsp. *andigen*a produced the only slightly less frost-resistant pentaploid species *S. curtilobum*. This evidently involved a 2*n* gamete from *S. juzepczukii* and a normal gamete from *S. tuberosum* subsp. *andigen*a (Hawkes 1990). A series of crosses between *S. stenotomum* and subsp. *andigena* have given rise to the triploid hybrids named *S. chaucha*.

We thus have a network of cultivated species or species groups, which evolved chiefly in the central Andes of Peru and Bolivia, involving four original wild species, viz. *S. acaule*, *S. sparsipilum*, *S. leptophyes* and *S. megistacrolobum*. All but two of these cultivated potatoes have always been confined to that central area. However, the diploid *S. phureja* has extended northwards into Ecuador, Colombia and Venezuela, whilst the tetraploid *S. tuberosum* spread into southern Chile.

A lot of studies have been done to assess genetic diversity among different cultivars grown across the world utilizing both conventional and molecular tools. Molecular markers have become important tools in studies of genetic diversity

(Bered et al. 2005), due to the high resolution and reliability in the identification of cultivars. They are also applied in the genetic characterization of potato (Ford and Taylor 1997, Schneider and Douches 1997). Random amplified polymorphic DNA (RAPD) markers have the advantage of detecting polymorphism simply and quickly (Demeke et al. 1996; Kujal et al. 2005), while simple sequence repeat (SSR) markers or microsatellites provide high reproducibility and genetic informativeness. Both the markers have been used in the molecular characterization of potato cultivars (Coombs et al. 2004) as well as of other species, e.g. soybean (Garcia et al. 2007). These studies have unanimously concluded that the cultivable varieties possess narrow genetic base. The European cultivated potato has been known to have arisen from a limited number of introductions (Glendinning 1983), resulting in a low level of genetic diversity, compared to the potato gene pool of the American countries. Moreover, the selection of genotypes which produced tubers under long-day conditions, combined with selection for superior agronomic traits, further narrowed the European gene pool (Provan et al. 1999; Spooner and Alberto 2006).

6.5 Maintenance of Diversity

6.5.1 Gene Banks

Up to early twentieth century, *S. tuberosum* L. was the only potato species known outside South America. It was only after the first expedition carried out in centre of origin by Prof. S.M. Bukasov and his co-workers in 1925–1926 that knowledge about the wealth of genetic diversity among the tuber-bearing *Solanum* started accumulating. Thereafter a number of N.I. Vavilov expeditions largely led by S.M. Bukasov and S.W. Juzepczuk were held in the Americas. This laid the foundation of the first gene bank in Leningrad starting in 1927. This gene bank still survives as N.I. Vavilov Institute of Plant Industry, Russia. Taking advantage of the pioneering Russian work, British Empire expedition held in 1939, collected materials in Mexico and the Andean countries of South America. As a result, Commonwealth Potato Collection was established in Cambridge and is now situated at Scottish Crop Research Institute, Pentlandfield, Scotland. Lately, the major bulk of collection has been mediated by the International Potato Centre (CIP), Lima, Peru, a CGIAR organization with the primary mandate of collection and conservation of vast potato genetic resources. Presently, CIP is the holder of the largest collection of the potato germplasm. In addition, large potato collections also exist at Inter-regional Potato Introduction Station (IR-1), Sturgeon-Bay, Wisconsin, USA; Instituto Nacional de Tecnologia Agropecuaria (INTA), Balcarce, Argentina; Chilean Potato Gene bank in Valdivia, Chile; Dutch-German Potato Collection, Braunschweig, Germany, and Institut fur Kartoffelforschung, Gross-Lusewitz, Germany. Some national potato programmes also maintain potato genetic resources, e.g. in India, Central Potato Research Institute, Shimla holds a modest collection of more than 4,100 accessions of elite potato varieties, parental lines as well as wild species imported from

Fig. 6.2 Variability in potato germplasm for tuber & flesh colour

40 countries with good variability in tuber skin and flesh colour (Fig. 6.2). This is the largest potato collection in Asia (Gopal and Gaur 1997). In spite of concerted efforts to collect the genetic variability, only about 130 out of the total known number of 235 potato species exist in gene banks. More efforts are needed to collect the others, which are known only as descriptors or dried specimens.

6.5.2 Conservation

There are two basic approaches for conservation of genetic resources: in situ conservation and ex situ conservation. In situ conservation refers to situations where the material is maintained in the natural habitat, within the community of which it forms a part. In situ conservation is based on the concept that it allows natural evolution to continue. In situ conservation is generally suggested only for wild relatives because they alone live in natural communities. It is carried out under the auspices of national governments through biospheres reserves, national parks, world heritage sites and other protected areas. It can also be carried out through farmers who manage land races and wild relatives in eco-geographic pockets of genetic diversity. In potato, there is no specific programme to follow this method of conservation because of the practical problems of maintenance, economic viability, etc. Further, in this population size should be large enough to avoid risk of inbreeding and of genetic drift as these lead to the decay of genetic diversity. It is not realistic to expect that all species and their component populations can be covered by natural reserves, national parks and other protected areas. Further this method cannot safeguard the species in face of unforeseen natural calamities.

Due to the practical limitations of in situ conservation, it is preferred to maintain variability under managed conditions (ex situ) in gene banks. This method, however, freezes the evolutionary process of species though it ensures conservation of its existing genetic variability. Potato genetic resources can be conserved as vegetative propagules or as true (botanical seeds). Potato is a highly heterozygous crop

due to which sexual reproduction results in segregating populations. So when the objective is to maintain the exact genotype of an accession, vegetative propagation (in vitro or in vivo) is the only option. However, if the objective is not to maintain the exact genotype, but total gene pool, germplasm can be conserved as true seeds.

In vivo propagation is done through tubers in glass house, as well as in fields. Genetic identity is maintained by roguing mixtures and clones are protected from diseases and pests by using various protective measures. Still, there is risk of exposure of germplasm to viral and mycoplasmal diseases from year to year, resulting in degeneration of the germplasm stocks. Loss of material due to natural calamities and loosing identity due to mechanical mixtures or wrong labelling are the other risks associated with this method. Labour and maintenance costs are also high. This is the traditional method of conservation. This method, however, provides a continuous opportunity to evaluate and compare the characteristics of different genotypes. Further, seed/propagules can be readily made available to the users.

The ability to grow plants under aseptic conditions has allowed the development of in vitro preservation techniques for germplasm conservation and exchange. In vitro maintenance of germplasm has several advantages. A large number of accessions can be conserved in a small space under disease free conditions irrespective of the crop season. The risk of loss due to biotic and abiotic factors is minimized and the possibility of cross infection between accessions is eliminated. In vitro materials can be made free of systemic bacteria, fungi, viruses and mycoplasmas and maintained as pathogen-free stocks (Khurana and Garg 1998; Jeffries et al. 2006). This eases the quarantine regulations for international distribution of genetic materials. A major limitation of in vitro germplasm conservation is the likelihood of genetic instability in the process of culture. In vitro potato germplasm can be conserved through slow-growth conservation for medium-term storage or cryo-preservation for long-term storage.

6.5.2.1 Slow-Growth Conservation

This is based on the micropropagation of apical or axillary buds (nodal cuttings) on modified Murashige and Skoog medium. In order to avoid frequent subculturing of micropropagated plants, subculture period is enhanced by following the slow-growth conservation strategy. For this, a number of approaches like low temperature storage, reduced light intensity, high sucrose concentration, use of osmoticums or growth retardants in the medium, increase in volume of medium, sealing of the culture vessels, mineral oil layer on the medium, etc. are used. The most commonly used protocol for in vitro conservation of potato is based on combination of low temperature, reduced light intensity and use of osmoticums. By this method, in vitro plantlets can be conserved for 2–3 years depending on the genotype (Fig. 6.3). In subtropics, where maintenance of low temperature is problematic and expensive due to high demand on energy, plantlets could be conserved at normal propagation temperatures using MS medium with osmoticum (4 % sorbitol, 2 % sucrose) for 12 months without subculture (Khurana et al. 1998).

Fig. 6.3 In vitro conservation of potato germplasm

6.5.2.2 Cryo-Preservation

By this method plant material is frozen at ultra low temperature around −196 °C of liquid nitrogen. At ultra low temperatures, the cells are in state of metabolic inactivity. Due to inhibition of cell division this method allows storage of material with minimal risk of genetic instability. In this method, meristematic tissue of in vitro material is usually used. The technique can also be used for preserving embryos, callus, pollen, cell suspension, etc. These are, however, rarely used as potato germplasm due to their intrinsic genetic variability. This is followed by pretreatment with cryo-protectants with low molecular weight such as glycerol and dimethyl sulphoxide, which penetrates the cell with ease and high molecular weight compounds such as polyvinyl pyrrolidone and dextran, which penetrates slowly and can reduce cryo-damage significantly. They protect surface membranes by reducing growing rate and size of ice crystals, and by lowering the effective concentration of solutes in equilibrium with ice inside and outside the cell. They also help to increase membrane permeability which aids removal of water from the cell and facilitates protective dehydration in the early stages of freezing. By pretreatment with cryo-protectants some degree of dehydration is induced in cells and tissues thereby avoiding the damage caused by the formation of ice crystals during the freezing and defrostation process. Freezing is the most crucial step in the whole process of cryo-preservation. This can be achieved through slow freezing where cultures are frozen by slow cooling at freezing rate between 0.5 and 4 °C per min, starting from 0 °C until the temperature reaches −100 °C, and finally transferred to liquid nitrogen; by rapid freezing wherein the materials contained in vials are lowered directly into a tank filled with liquid nitrogen. The temperature decreases rapidly at the rate of 300–1,000 °C per min. The stepwise freezing method combines both the procedures of slow and rapid freezing. Initially plant material is cooled slowly and stepwise (ca 1–5 °C per min) to an intermediate temperature, maintained at that temperature for 30 min, and then rapidly cooled by plunging it into liquid nitrogen. In the initial slow freezing, ice is formed outside the cells and the unfrozen

protoplasm losses water due to the vapour pressure deficit between the super-cooled protoplasm and the external ice. Vitrification is a process by which water undergoes a phase transition from a liquid to an amorphous 'glassy state'; in this form, water does not possess a crystalline structure. Vitrification occurs when the solute concentration becomes so high that ice formation is prevented and the water molecules form glass. For this, tissue is sufficiently dehydrated with a highly concentrated vitrification solution at 25 or 0 °C without causing injury prior to immersion in liquid nitrogen. Plant vitrification solutions like PVS2 and PVS3 have been developed which are glycerol based and less toxic. During encapsulation/dehydration, the cells/tissues are trapped into calcium alginate beads followed by incubation in 0.85 M sucrose (as sole source of cryo-protection) for 14–16 h, air-drying for 3–4 h in a laminar flow chamber and rapid freezing in liquid nitrogen. The above alternatives are also combined, e.g. encapsulation–vitrification, pregrowth–desiccation, droplet freezing, etc. to achieve the desired results. Thawing of the frozen material is achieved by transferring it to warm water at 37–40 °C. The optimal thawing rate is one that prevents ice formation by recrystallization in the process of warming. The recovery of thawed cultures can be improved by nursing them through an initial recovery period involving gradual dilution of cryo-protectants through several steps of washing and by keeping osmotic disruption to the minimum. Plants are regenerated from the recovered tissue by culturing on suitable tissue culture media (Khurana et al. 1998). To avoid somaclonal variations, shoot tips are directly regenerated into plantlets, without any adventitious growth or callus formation. This is ensured by developing suitable protocols of cryo-conservation and regrowth media for different cultivars/accessions.

6.5.2.3 Sexual Propagation

Potato true seed (botanical seed or TPS) is orthodox type, meaning it is able to tolerate a high degree of desiccation and storage at low temperature, i.e. between 5 and –20 °C without loss of viability. Due to this, preservation of potato germplasm through sexual propagation as true seed is less laborious and much cheaper than the preservation of vegetatively propagated material. In addition, it is easy to maintain the material free of pathogens this way, as only a few virus diseases are known to be seed transmitted. Seeds occupy relatively small space and their transport is also economical. However, majority of the wild species of potatoes are diploid and self-incompatible. In such species true seeds are required to be produced by sib-mating. The sample size should be large enough to maintain the heterozygosity and to preserve all alleles at the loci of interest. Sample size depends on the nature of genetic control of the character(s) concerned.

True seeds with low moisture content can be kept at low temperatures for many years. Successful seed storage depends on effective control of several factors including physiological maturity of the seed, temperature, seed moisture content, storage atmosphere, etc. It is desirable to harvest seeds at physiological maturity as germination, and vigour are maximum in fully mature seeds and longevity of

mature seeds is more than in those harvested at other stages. The seeds extracted from the mature berries should be dried in shade or in seed dryers at temperature <30 °C. Drying in direct sunlight or at temperatures above 30 °C can adversely affect the viability of the seed. The moisture content of the seeds can be further reduced by keeping them in silica gel for a week or so. Within certain limits, viability of the seeds increases by drying and storing them at low temperature. For long-term storage, potato seeds should have moisture content 5 ± 1 % and stored at −10 to −20 °C. For short to medium-term storage samples should be stored at 0–10 °C after drying up to 8 ± 1 % moisture content. Before storage, the samples should be hermetically sealed in airtight containers.

6.5.3 Genetic Stability of Conserved Germplasm

Preservation of the genetic integrity of plants generally multiplied by vegetative means is fundamental to long-term conservation. Field gene banks where potato accessions are maintained by propagation through tubers are generally considered safe from this angle as natural mutations are rare, and if there is any off-types can be rogued out. However, apprehensions are expressed about the genetic integrity of the material conserved in vitro. In slow-growth in vitro conservation, plantlets are grown under suboptimal conditions. Due to this they show signs of stress and have bush like appearance with thin stems and reduced or nil leaves. Continuous maintenance under these conditions may lead to physiological as well as genetic changes in the material. In the cryo-preservation protocols, genetic variations could occur during tissue culture before or after cryo-preservation or during eventual plant regeneration. During the storage phase, some genetic variations may be induced by the accumulation of mutations caused by background ionizing radiations, even in explants which were previously genetically stable. Bi-nucleation and abnormal chromosome distribution in DMSO (cryo-protectant) treated cells has also been reported.

Accurate characterization of the clone is required to answer questions related to genetic stability. Morphological analysis provides one approach for the description of the genetic stability when phenotypic variation is demonstrably linked to genetic variation. The expression of morphological characters, however, can be subject to environmental or physical effects. This and other limitations have led to research on methods of analysis based on more stable features of the genome. Non-specific proteins and isozyme analyses are also used, but these too suffer from environmental and developmental effects. Recently, molecular markers such as RFLP, RAPD, AFLP and SSR have shown their usefulness in the genetic characterization of plant material after long-term storage. RAPD and AFLP are dominant markers that help to determine genetic distance in terms of providing estimates of genetic differences rather than of genetic similarities. The advantage of co-dominant markers, such as microsatellites, is the possibility to display all four alleles at a single locus. Hence by their nature, microsatellites are more informative in detecting genetic changes, if any.

6.5.3.1 Evaluation of Germplasm

Evaluation for different traits

The species evaluated for various characters of resistance or adaptation are even fewer than those collected. Important sources of resistance to the major potato diseases and pests, and adaptation to environmental extremes are listed in Table 6.1.

Table 6.1 Sources of resistance to the major biotic and abiotic stresses

Biotic/Abiotic stress	Causal organism	Source of resistance
Fungus resistance	*Phytophthora infestans* (late blight)	
	i. Race specific	*S. cardiophyllum, S.demissum, S. ediense, S. stoloniferum* and *S. verrucosum*
	ii. Non-race specific	*S. berthaultii, S. bulbocastanum, S. chacoense, S. circaeifolium, S. demissum, S. microdontum, S. phureja, S. pinnatisectum, S. polyadenium, S. stoloniferum, S. tarijense, S. tuberosum* ssp *andigena, S. vernei* and *S. verrucosum*
	Alternaria solani (early blight)	*S. bulbocastanum, S. chacoense* and *S. tarijense*
	Synchytrium endobioticum (wart)	*S. acaule, S. berthaultii, S. boliviense, S. demissum, S. gourlayi, S. sparsipilum, S. spegazzinii, S. sucrense, S. tuberosum* (both subspecies) and *S. vernei*
	Fusarium spp. (Fusarium wilt)	*S. acaule, S. kurtzianum* and *S. spegazzinii*
Bacterial resistance	*Pseudomonas (Ralstonia) solanacearum* (bacterial wilt)	*S. chacoense, S. microdontum, S. phureja, S. sparsipilum* and *S. stenotomum*
	Erwinia carotovora (soft rot; blackleg)	*S. acaule, S. brevidens*, *S. bulbocastanum, S. chacoense, S. demissum, S. hjertingii, S. leptophyes, S. megistacrolobum, S. microdontum, S. phureja, S. pinnatisectum, S. tuberosum* ssp. *andigena* and *S. vernei*.
	Streptomyces scabies (common scab)	*S. chacoense, S. commersonii, S. jamessi, S. tuberosum* ssp. *andigena, S. yungasense* and various cultivated varieties
Virus resistance	Potato virus X	*S. acaule, S. berthaultii, S. brevicaule, S. chacoense, S. commersonii, S. curtilobum, S. phureja, S. sparsipilum, S. sucrense, S. tarijense* and *S. tuberosum ssp. andigena*
	Potato virus Y	*S. acaule, S. chacoense, S. demissum, S. gourlayi, S. phureja, S. rybinii, S. stoloniferum, S. tuberosum* ssp. *andigena*
	Potato virus S	*S. tuberosum* ssp. *tuberosum* varieties like Great Scot, Kerries Pink, Red Skin, Eclipse, British Queen, Up-to-date, Duke of York and Foxlyna etc.
	Potato leaf roll virus	*S. acaule, S. brevidens, S. chacoense, S. demissum, S. etuberosum, S. raphanifolium, S. stolonifrum* and *S. tuberosum* ssp. *andigena*
	Spindle tuber viroid	*S. acaule, S. berthaultii, S. gurreroense, S. hjertingii* and *S. multidissectum*

(continued)

Table 6.1 (continued)

Biotic/Abiotic stress	Causal organism	Source of resistance
Insect resistance	*Leptinotarsa decemlineata* (Colorado beetle)	*S. berthaultii, S. chacoense, S. commersonii, S. demissum, S. jamesii, S. pinnatisectum, S. polyadenium* and *S. tarijense*
	Myzus persicae, Macrosiphum euphorbiae (aphids)	*S. berthaultii, S. bukasovii. S. bulbocastanum, S. chomatophilum, S. infundibuliforme, S. lignicaule, S. marinasense, S. medians, S. multidissectum, S. neocardenasii, S. stoloniferum*
	Phthorimaea operculella (Tuber moth)	*S. chacoense, S. stenotomum* and *S. tuberosum* ssp. *andigena*
Nematode resistance	*Globodera rostochiensis, G. pallida* (potato cyst nematode)	*S. acaule, S. berthaulti, S. boliviense, S. bulbocastanum, S. capsicibaccatum, S. cardiophyllum, S. demissum, S. gourlayi, S. kurtzianum, S. leptophyes, S. multidissectum, S. oplocense, S. sparsipilum, S. spegazzinii, S. sucrense, S. tuberosum* ssp. *andigena* and *S. vernei*
	Meloidogyne incognita (root-knot nematode)	*S. bulbocastanum, S. cardiophyllum, S. chacoense, S. curtilobum, S. hjertingii, S. kurtzianum, S. microdontum, S. phureja, S. sparsipilum and S. tuberosum* ssp. *andigena*
Physiological characters	Frost	*S. acaule, S. ajanhuiri, S. boliviense, S. brachistotrichum, S. brevicaule S. brevidens, S. canasense, S. chomatophilum, S. commersonii, S. curtilobum, S. demissum, S. etuberosum, S. juzepczukii, S. megistacrolobum, S. multidissectum, S. pumilum, S. raphanifolium, S. sanctae-rosae, S. toralapanum and S. vernei.*
	Heat and drought	*S. acaule, S. bulbocastanum, S. chacoense, S. commersonii, S. gourlayi, S. megistacrolobum, S. microdontum, S. ochoae, S. papita, S. pinnatisectum, S. spegazzinii* and *S. tarijense*
	Lack of tuber blackening	*S. hjertingii*
	High protein content	*S. phureja* and *S. vernei*
	High starch content	*S. vernei*
	Low reducing sugars at low temperature storage	*S. phureja, S spegazzinni* and *S. vernei*
Quality traits	Chip making directly from cold storage	*S. medians, S. okadae, S. pinnatisectum, S. raphanifolium, S. sogarandinum*
	Use in medicine	*S. siparunoides, S. sisymbrifolium, S. stramonifolium, S. tuberosum*
	High carotenoid content	*S. phureja, S. stenotomum*
	High starch content	*S. phureja, S. vernei*
	High ascorbic acid content	*S. phureja, S. estoloniferum*

Information on usefulness of various accessions and species is getting enriched and published as more and more germplasm is evaluated. Where such information is not available, passport data on characteristics of the natural habitats of species are of great importance, for example late blight resistant species are found in Mexican gene pool where late blight fungus has been found to reproduce sexually. Similarly

frost resistance is found in species capable of growing at altitudes above 3,500 m. Species from dry and warm climate areas are tolerant to these stresses.

6.6 Utilization of Genetic Resources

Use of genetic resources has been even much poorer to their evaluation. Only 10 primitive cultivars or species had been used in the pedigrees of 62 % of the 627 cultivars listed in the Index of European Potato Varieties. Remaining 38 % cultivars involved only ssp. *tuberosum.* Only 13 species have been used so far in the variety improvement programmes of the world. Parentage of as many as 171 American varieties could be traced to a single variety Rough Purple Chilli introduced from Chiloe region of southern Chile. The wild species which occur in the pedigree of several cultivars are briefly described below.

S. demissum: A hexaploid, self-fertile spp. from Mexico. It is resistant to PVY and PLRV, hypersensitive and resistant to late blight, resistant to wart and both *Globodera* species; alkaloids in this confer resistance on Colorado beetle and other insects. It is moderately resistant to frost also. Genes of *S. demissum* have been incorporated into more than 50 % of the world's cultivars mainly for resistance to late blight and PLRV.

S. acaule: A tetraploid and also hexaploid, self-fertile spp. from Andes of Argentina to Peru. It is extremely resistant to PVX and resistant to PLRV, PSTVd, wart, both spp. of *Globodera* and frost.

S. chacoense: A diploid spp. from Argentina, Southern Brazil, Bolivia, Paraguay and Uruguay. It is extremely resistant to PVA and PVY, resistant to late blight, Colorado beetle, tuber moth and other insects. *S. chacoense* has been used mainly with *S. phureja* to produce a hybrid which served as a bridge between *S. demissum* and *S. tuberosum* to create tetraploid hybrids by the smooth euploid transfer of gene into ssp. *tuberosum.*

S. spegazzinii Bitt: A diploid spp, from Northwest Argentina. It is resistant to *Fusarium,* wart and *Globodera* spp and has low content of reducing sugars. This has been mainly used as a source of resistance to nematodes.

S. stoloniferum Schlechtd. et Beche: A tetraploid, self-fertile spp. from Mexico. It is extremely resistant to PVA and PVY, hypersensitive and field resistant to late blight. About 20 cultivars carry the gene *Ry* for extreme resistance to PVA and PVY derived from *S. stoloniferum.*

S. vernei Bitt. et Wittm.: A diploid spp. from Northwest of Argentina. It is outstanding for protein and starch content and resistant to late blight and both spp. of *Globodera*. It has low content of reducing sugars. It has been used in breeding mainly for resistance to nematodes.

In the utilization of gene pool, it is just as important to be aware of possible undesirable characters as it is to know of desirable characters from a specific source. Undesirable tuber traits of the wild species and crossability problem of certain species have acted as deterrents for their use by the breeders. Pre-breeding of the

wild species for combining resistance to various diseases and insect pests with agronomic characters requires special attention. This will undoubtedly lead to a closer association between gene banks and gene bank users and help in turning 'gene bank collections' into 'working collections'.

6.7 Potato Genetic Enhancement

Even though wild species of potato have been known to harbour myriad of genes for various biotic and abiotic resistance traits, the associated genetic load of poor yield and wildness traits renders them unfit for any breeding programme. Limited effort has been done to improve the tuber traits of these wild species through pre-breeding. These traits can be those missing from the advanced populations with resistance to pests, viruses or bacterial wilt, complementary genes for more durable resistance against late blight, resistance genes for emerging man-made constraints like heat, salinity, etc. or genes for improved nutritional value such as zinc and iron. Despite lack of pre-breeding in most of the cases, wild species have been used enough for transfer of the resistance genes to cultivated species.

6.7.1 Utilization of Wild Species for Improvement of Cultivated Potato

Conventionally, potato breeding has been restricted to the members of the cultivated tuberosum accessions. Choice of a parent is most often based on its de facto phenotypic expression of the characters of interest. Normally breeders try to pick pairs of clones/varieties for inter-mating based on complementary sets of characters with desirable level of expression. The ideal strategy would be to identify the superior parents based on their general combining ability and inter-mating them in all feasible combinations to select a few top combinations based on progeny test. This is followed by selection over successive generation for yield and other desirable traits (Bradshaw and Mackay 1994).

S. tuberosum is known to have a narrow genetic base and little improvement is observed in tuber yield among the recombinants produced by crossing among *tuberosum* parental clones. To overcome these problems attempts were made in Europe and North America (where the crop is grown under temperate long days) to use *andigena* in potato breeding programmes. But the short-day requirement for tuberization of *andigena* resulted in poor performance of *tuberosum* × *andigena* progenies. However, the use of *andigena* pre-selected for long days, in crosses with *tuberosum* led to progenies yielding higher than *tuberosum* x *tuberosum* progenies. In 1970s, the name *neo-tuberosum* was proposed for advanced *andigena* material adapted to long days. However, in the plains of subtropics, potato is grown during

winter under short days. Under these conditions, *andigena* adapted to short days can be used advantageously for exploiting heterosis. The progenies from such crosses, however, are generally late maturing. To overcome this drawback and avoid the undesirable tuber characters in t*uberosum x andigena* populations, there is a need to select early bulking/maturing *andigena* clones with desired tuber characters, like uniformly white or red skin and shallow eyes.

Utilization of wild species in genetic improvement of potato suffers from many impediments owing to its clonal propagation, reproductive barriers, EBN imbalances and complex inheritance pattern. To be able to make the desired cross for any genetic and breeding work, it is essential that the parental genotypes flower over a sufficient length of time and that the flowers do not drop, but develop into fruits. Genotype, day length and temperature are the major factors determining behaviour of flowering and fruiting in potato, though a number of other factors like inflorescence position, plant/stem density, competition between flower and tuber, precipitation, nutrients and date of planting are also known to influence production of flowers and fruits in potato.

In the tropics and subtropics, conditions conducive to flowering and fruiting are available only at high altitudes (>1500 m above sea level) where potato crop is grown during summer season. Even under such conditions, not all genotypes produce flowers and fruits. During the short days of autumn season when the potato crop is grown in the plains in subtropics, flowering genotypes are few. Attempts to induce flowering in non-flowering genotypes by planting on bricks, removal of tubers, grafting shoots on tomato stock, extending of photoperiod by artificial illumination and spray of hormones like gibberellic acid and 2,4-dichlorophenoxy acetic acid, etc. have been successful to certain extent.

Male sterility is a very serious constraint in potato breeding. Although both pollen and ovule sterility can occur, pollen sterility ranging from partial to complete absence of pollen grains is very common in potato. Almost one-third of the potato cultivars derived from *S. tuberosum* ssp. *tuberosum* do not form berries. Further potato suffers from crossability barriers. Both self- and cross-incompatibility conditioned by a series of allelomorphs are widely prevalent in potatoes. Most diploid species are obligate out-breeders due to self-incompatibility (Ross 1986).

Incompatibility barriers can be pre-zygotic or post-zygotic. Pre-zygotic barriers are expressed between pollination and fertilization as non-germination of pollen on stigma; germination, but no penetration into the stigma; penetration, but inhibition of pollen tube growth to different degrees and at different sites in the style, ovary or ovulum. Post-zygotic barriers are expressed during and after fertilization in ovules, or more specifically in embryo sacs and also during growth and flowering of plants (F1) produced upon hybridization. These barriers, like the pre-zygotic ones, may cause partial or complete failure of hybridization and introgression (Caligari 1992).

Diploid potato species are a rich source of many desirable traits. The dihaploid of the tetraploid cultivated potato can be utilized for mating with the diploid species. Dihaploids of potato are generally produced by their parthenogenetic development in $4x \times 2x$ crosses between S. *tuberosum* × *S. phureja* (pollinator). Anther culture has also been used to produce dihaploids in potatoes, but the application of

this approach is limited due to the lack of response of many genotypes to another culture. Dihaploids of *tuberosum* cross readily with many diploid wild spp. and thus provide the opportunity to introgression of useful traits from alien sources to cultivated background. Dihaploids with disomic rather than tetrasomic inheritance, facilitate the genetics and breeding of potatoes. Smaller populations are required to detect recessive genes in 2*x* crosses than in 4*x* intercrosses.

The final goal of breeding at the diploid level is to develop 2x clones with specific attributes, good breeding value for agronomic traits and 2*n* gametes. Production of 2*n* (unreduced) gametes by diploid–dihaploid hybrids is important to revert to tetraploid level through 4*x* × 2*x* or 2*x* × 4*x* (unilateral) or 2*x* × 2*x* (bilateral) polyploidization (Tai 1994). The natural production of 2*n* gametes in many tuber-bearing *Solanum* species is well established, although their frequency may vary greatly within and between species owing to genetic, environmental and physiological factors. Such 2*n* gametes may originate in different ways, but genetically the basic distinction is between 2*n* FDR (First Division Restitution) gametes (reduction of 2*n* to *n* chromosomes followed by restitution to 2*n* through incomplete first division) and 2*n* SDR (Second Division Restitution) gametes (reduction of 2*n* to *n* chromosomes followed by restitution to 2*n* through incomplete second division). It can be calculated that FDR gametes brought 80 % introgression of the intact parental genotype to the hybrid progeny and SDR gametes 40 %. This high percentage of intact gene transfer through FDR gametes is especially important when the parent plant carries many desirable genes for qualitative as well as quantitative characters. The analytical breeding scheme based on dihaploids recommends the use of 2*n* gametes for production of tetraploids with maximal heterozygosity.

Due to the plasticity of various causal organisms responsible for a number of maladies in potato, new pathotypes mainly through mutations keep on emerging in nature. The situation is further aggravated by the cultivation of resistant monocultures on a large scale, rendering the available resistant varieties infructuous. This phenomenon makes the breeding of resistant varieties against the biotic stresses a continuous process (Shekhawat et al. 2000). The breeding of new varieties, therefore, always requires search of new/appropriate genes obtaining resistance from wild/semi-cultivated species and their introgression in suitable agronomically superior background. In a number of instances, however, resistant genes are not available and in this context, biotechnological interventions in conjunction with conventional breeding techniques can be useful in transferring resistance across the species, genera, families, etc. Quantitative trait loci (QTLs) (Schafer- Pregl et al. 1998), a biotechnological tool, in potato, holds promise as most of the biotic/abiotic stresses are polygenic and inherited in quantitative manner. The QTL markers, if could be exploited in a manner as in case of Mendelian factors, can substantially reduce the time lag in breeding.

Despite the problems, conventional breeding has been successful to various extent in improving the yield and introgression of various desirable traits from diverse sources. A brief account of it is as follows.

6.7.1.1 Late Blight

Late blight is caused by *Phytophthora infestans* (Fig. 6.4) which is highly variable and therefore, breeding varieties against it has been a see-saw story. Pathological variants (races) could be detected universally after the resistance genes from *S. demissum* were transferred to the commercial cultivars. Towards the end of twentieth century, the racial complexity had reached its zenith in most of the countries. The fungus had developed virulences against almost all the known R-genes in potato. This may be due to the fact that A_2 mating type now occurs almost throughout Europe and even in some Asian countries and therefore, racial variability through sexual reproduction in these regions cannot be ruled out (Singh 2000).

In 1909, R.N. Salaman demonstrated the heritable nature of resistance to late blight in wild species *S. edinense* (Salaman 1929). Resistance to blight can occur both in foliage and tubers. However, breeders have largely neglected the latter. Broadly resistance can be grouped into two types: (i) race-specific resistance (also called vertical resistance or major gene resistance, qualitative or discontinuous resistance) and (ii) race non-specific (also called horizontal resistance or minor gene resistance, field resistance, polygenic resistance, quantitative resistance or partial resistance).

The race-specific resistance based on gene-for-gene relationship was initially identified in hexaploid ($2n = 6x = 72$), wild species *S. demissum*. It is expressed in the form of hypersensitive response of the tissue to all races of *P. infestans* that did not possess the corresponding virulence to the resistance genes (R-genes). Specific resistance is conditioned by a series of major dominant genes each of which is brought into action by distinct pathotypes; currently 11 such genes (ex-demissum) are recognized. *S. stoloniferum* has also been found to possess similar, if not identical, resistance genes.

However, the R-genes wherever deployed were defeated in due course of time. In India, the process of transfer of R-genes from *S. demissum* background started in mid-1950s and the first set of late blight resistant varieties was released for commercial cultivation in 1968. Of these, cv. Kufri Jyoti (possessing R-genes 3.4.7) became the most popular which is still grown in several parts of the country. Since

Fig. 6.4 Late blight infested potato leaf

then a lot of varieties carrying R-genes have been bred and deployed across the country. In cv. Kufri Jyoti, matching virulences (3.4.7) were detected immediately after 5–6 years of its cultivation both in North-eastern and North-western hills. Both frequency of matching virulences, their combinations and disease increased rapidly making it completely susceptible by 1988. In India, this problem has been avoided by making adjustments in screening methodology (Singh and Shekhawat 1999). The seedlings in F_1C_1 are challenge-inoculated with the most complex race (8–9 gene complexes) (Fig. 6.5). The seedlings showing either complete susceptibility or immunity are discarded. The selected seedlings possess both R-genes coupled with a high degree of field resistance.

Race non-specific resistance is a quantitative and multifaceted trait, probably governed by many genes, it is, therefore, difficult to analyse in Mendelian ratios. Field resistance to late blight operates mainly through four factors, viz. infection efficiency, incubation period, colonization rate and sporulation efficiency. Many host factors, environmental aspects, edaphic, nutritional and climatic have an effect on these four components of resistance. Besides, components of field resistance to tuber blight include the depth in the soil at which the tubers are produced, the ease with which the spores are washed down from the canopy into the soil, the rapidity of periderm formation and the resistance to wounding. Although, at the phenotypic level, both types of resistances can be easily identified, at the genotypic level these are almost similar. Genetic analysis of resistance to late blight using DNA markers showed that major genes for resistance (R-gene) are closely linked to the factors controlling quantitative resistance suggesting that there is no real difference between qualitative and quantitative resistance to late blight as far as the nature of the genes involved. The differences observed at the phenotypic level may be the result of various allelic and non-allelic interactions. Thus a complex picture emerges, which renders both selection for and evaluation of blight resistance a slow process.

Several wild *Solanum* species possess high degree of resistance to late blight. Species like *S. bulbocastanum*, *S. demissum*, and *S. stoloniferum* had clones, which

Fig. 6.5 Late blight screening chamber showing F1C1 seedlings inoculated with *P. infestans*

possessed low infection frequency. Besides clones of *S. bulbocastanum* and *S. demissum* developed only small lesions, whereas clones of *S. stoloniferum* possessed high degree of resistance to tissue colonization. Umaerus demonstrated that *S. demissum* is a treasure of resistance. Besides R-genes, it also possesses field resistance, which operates primarily through low infection frequency at seedling stage. In German-Dutch potato collection nothing encouraging was found in *S. tuberosum* ssp. *andigena* and the primitive cultivars except an accession of *S. phureja*. However, resistance was detected in wild diploid Mexican species like *S. pinnatisectum*, *S. bulbocastanum*, *S. polyadenium* and *S. verrucosum*. It was also detected in Bolivian and Argentinean species, including *S. chacoense*, *S. berthaulti*, *S. microdontum* and *S. vernei*. Although initially *S. tuberosum* ssp. *andigena* was thought to be not having any resistance to late blight, there has been renewed interest in it as andigena potatoes respond well to selection for the resistance and produced a number of highly resistant clones. Resistance has also been reported in the Russian diploid sources, including *S. polytrichon*, *S. simplicifolium* and *S. microdontum*, which showed resistance to tuber blight as well.

6.7.1.2 Viruses

Viral diseases are an important constraint for potato crop because of their systemic distribution in the host and are mainly responsible for the degeneration of seed stocks (Khurana 1999, 2008; Jeffries et al. 2006). At least 12 viral diseases are known to infect potato crop in India and elsewhere. Among them, PVX, PVY, PVS, PVA, PVM, leaf roll (PLRV) and apical leaf curl viruses (PALCV) are important (Figs. 6.6, 6.7 and 6.8). Introducing resistant cultivars is one of the most efficient ways of reducing the losses caused by viruses. Resistance genes to different potato viruses have been identified in many wild potato species. Some of these genes have been incorporated in many of the recently released potato cultivars.

Fig. 6.6 PVY infected potato plant

Fig. 6.7 PALCV infected potato plant

The nature of resistance against viruses is of several types: (i) tolerance, (ii) resistance to infection, (iii) hypersensitivity usually giving field immunity and (iv) extreme resistance or immunity. Tolerance to viruses in potatoes is usually considered a dangerous type of resistance. Resistance to infection can be defined as the type in which only a small percentage of infection appears in the field and is governed by polygenes. Hypersensitivity and immunity are on the other hand due to mostly single dominant genes. Out of the four types of resistance, immunity gives almost complete elimination of virus and is preferable over other types. However, in recent years, more emphasis is being given to vector resistance where the resistance sought is against the vectors (aphids and other vectors), the carrier of viruses and not against viruses themselves.

There are three main groups of strains of PVY, viz. PVY^O (common strains), PVY^N (tobacco veinal necrosis strains) and PVY^C (stipple streak strains). The strain-specific resistance is controlled by the resistance gene N_Y while the extreme resistance is controlled by the gene R_Y. *Ny* genes are found in large number of cultivars including Pentland Crown, Pentland Ivory, King Edward and Cana and in hybrids derived from wild species like *S. chacoense*, *S. demissum* and *S. microdontum*. The sources of *Ry* gene are *S. stoloniferum*, *S. hougasii* and

S. tuberosum ssp. *andigena*. Accordingly the genes are named as Ry_{sto}, Ry_{hou} and Ry_{adg}. *Ry* is inherited as a single dominant gene and hence easy to breed.

There are mild, moderate and severe strains of PVA. They differ in severity of symptoms produced in potato cultivars. The gene *Na*, present in many cultivars, protects the plant from infection under natural pressure from PVA by means of a hypersensitive response. The gene *Na* is linked to gene Nx_{tbr}, which controls the resistance to PVX.

PVX strains can be separated according to their serological reaction into two main pathotypes: 1 and HB. Cockerham (1970) identified several genes conferring hypersensitivity, viz. $Nx_{tbr,}\, Nb_{tbr,}\, Nx_{chc}$ *and* Rx^{n}_{acl}. Similarly, genes conferring extreme resistance are known to occur in several species (Rx_{adg}, Rx_{acl} and Rx_{scr}).

Strains of PLRV differ in the severity of the symptoms they produce on potato and on test plants. Resistance to PLRV has been found to be oligogenic and has been detected in *S. brevidens* and *S. tuberosum*. Transfer of resistance genes from *S. brevidens* has been achieved through protoplast fusion but from *S. tuberosum* it has been possible only through bridge crosses. Among the hexaploid somatic hybrids derived from *S. tuberosum* and *S. brevidens* by protoplast fusion, some hybrids with high PLRV resistance were obtained.

Potato apical leaf curl disease, first reported in northern India by Garg et al. (2001) has been associated with a geminivirus, which was confirmed to be a strain of tomato leaf curl New Delhi virus (ToLCNDV) belonging to the genus Begomovirus (Usharani et al. 2003). The virus is transmitted by whiteflies and the affected plants show curling/crinkling mosaic of apical leaves.

Solomon-Blackburn (2001) and Tiwari et al. (2012) have excellently reviewed on prevalence of resistance against different virus among potato species and the nature of genes controlling them. Both major genes and quantitative trait loci (QTLs) have been found to govern virus resistance. The two major QTLs, *Plrv.1* and *Plrv.4* confer resistance to PLRV. Major genes Rl_{adg} and Rlr_{etb} confer high resistance to PLRV infection. At present there are four different known *R* genes: Ry_{adg}, Ry_{sto}, Ry_{hou} and Ry_{chc}, which confer extreme resistance to PVY in potato. In addition, Ny_{chc}, Ny_{dms}, Nc_{tbr}, Ny_{adg} Ny_{tbr} and *Ny-1* confer hypersensitive resistance on PVY. The gene Ny_{adg} controlling hypersensitive resistance to PVY^{O} is epistatic to Ry_{adg}. As a result, the genotypes carrying both Ry_{adg} and Ny_{adg} exhibited extreme resistance to PVY. The *R* genes Rx_{adg} (*Rx1*), Rx_{tbr}, Rx_{acl} (*Rx2*) and $Rx^{scr}_{HB}/Rx^{scr}_{CP}$ confer extreme resistance to PVX. The *N* genes Nx_{acl}, Nx_{chc}, Nb_{tbr}/Nx_{tbr}, Nx^{spl}_{tbr}, Nx_{phu} confer hypersensitive resistance to PVX. A single dominant gene *Ns* conferring hypersensitive resistance to PVS. The gene *Rm* confers hypersensitive resistance while gene *Gm* confers resistance to PVM infection. The *R* genes Ry_{sto}, Ra_{sto}, Ra_{adg} and Ry_{hou} confer extreme resistance while *N* genes Na_{adg}, Na_{sto}, Ny_{chc}, Na_{dms}, Ny_{dms}, Na_{tbr} and Na^{tbr}_{KE} confer hypersensitive resistance to PVA.

The development of cultivars with multiple virus resistance, however, remains a challenge for the breeders. This may be because the breeder select for many

Fig. 6.8 PLRV infected potato plant

important characters, therefore, introducing even a few genes for resistance to viruses becomes a difficult task. At CPRI, parental lines having virus resistance in duplex/triplex/tetraplex form have been developed. The progeny of triplex/tetraplex parents are being crossed with nulliplex parents to produce almost immune clones. After the evaluation for viral resistance in early generations, evaluation for horticultural traits is being done in later clonal generations.

6.7.1.3 Bacterial Wilt

Bacterial wilt or brown rot caused by *Ralstonia solanacearum*, first reported from India in 1892, is the most destructive of all bacterial diseases. Incidence of bacterial wilt is wide spread in all mid hill regions of the country and pockets of Assam, Meghalaya and Maharashtra. The disease damages the crop in two different ways—premature wilting of standing crop and rotting of tubers in fields, transit and stores (Fig. 6.9). It is primarily tuber borne, but survives equally well in soil. Host resistance is hard to find because of lack of co-evolution of the host and the bacterium, high variability in the bacterium and instability of the host resistance.

Resistance to bacterial wilt is a partially dominant character and is more of a polygenic type. Inheritance of resistance and its expression is complex and both additive and non-additive gene actions are involved, but the latter component is more important. A gene-for-gene relationship is not applicable to bacterial wilt. Certain genes other than those 'for resistance alone' have turned out to have the novel (pleiotropic) effects in conferring the resistance once the potato plant has come into contact with pathogen under a certain set of environmental conditions.

Fig. 6.9 Bacterial wilt infected field and tubers

These genes were eventually called 'genes for resistance' once a certain level of resistance was detected. The major or minor status of these genes depends on the particular genotype of the pathogen, and the particular environmental conditions that influence their expression. Attempts to transfer resistance from wild *Solanum* spp. into common potato resulted in excessive recombination and in breakdown upon intercrossing. Non-strain specificity and race cultivar specificity are the common features required for resistance to bacterial wilt. Thus, the host genotype x pathogen genotype interaction in potato, *R. solanacearum* system, seems to be artifactual. Both the host and pathogen are sensitive to environmental changes. Therefore, host genotype *x* pathogen genotype interaction may also be a result of host genotype x environment and/or pathogen *x* environment interaction.

The resistance in the clones of species such as *S. phureja* mainly and a few other *Solanum* species have been exploited extensively in the South American countries. But the Indian isolates of the bacterium have proved to be highly virulent making these sources ineffective. Resistance in *S. phureja*, the only species where it has been studied in detail, is strain- and temperature-specific, and it breaks down under the warm climates. Nematode injury also leads to its break down. A collection of nearly 500 clones of *Solanum* species which carry low to moderate degree of resistance, i.e. *S. phureja*, *S. microdontum*, *S. canasense*, *S. stenotomum*, *S. pinnetisectum*, *S. sparsipilum*, *S. kurtzianum*, *S. jamesii*, *S. polytrichon*, *S. vernei*, *S. acaule* and *S. stoloniferum* and interspecific hybrids between a number of above species and also resistant varieties developed so far in other parts of the world, viz. Prisca, Cruza, Caxamarca, Molenera and Ampola were screened against different isolates of the pathogen. All these cultivars/cultures proved susceptible to Indian isolates except for the one clone of diploid *S. microdontum* showing a moderate level of resistance. The efforts made to transfer the useful resistance from this source into the *tuberosum* background via dihaploids resulted in the development of two promising meiotic tetraploids. However, in field tests these were also proved to be susceptible.

6.7.1.4 Wart

Wart disease, of potato caused by a phycomycetous fungus, *Synchytrium endobioticum* (Schilb), was first reported from India in 1953 in North Bengal Hills. To avoid its further spread to other parts of the country, this area was brought under domestic quarantine in 1959. This has helped in containing this dreaded disease in Darjeeling district only. The disease causes cauliflower like growths on tubers, stolons and stem bases (Fig. 6.10). The heavy infection of disease causes rotting of entire products and results in total loss of crop. Cultivation of wart-immune varieties on a long-term basis is the only viable alternative. The resistance genes are available in a number of varieties of *S. tuberosum*. Besides, a number of wild species such as *S. boliviense*, *S acaule*, *S microdontum, S. demissum, S. sparsipilum*, *S. polytrichon*, *S. simplicifolium, S. chacoence* f.sp. *boergerii, S. vernei* and *S. spegazzinii* are known to have resistance to the disease. Monogenic dominant mode of inheritance for at least the control of necrotic response has been proved. However, modifying genes are also present which condition the nature and extent of response. Systematic breeding programme for wart immunity started in 1964. Since the pathogen survives in soil over long period, the only plausible defence mechanism is to develop varieties immune to it and saturate the area with the same. The crosses between wart-immune *tuberosum* parents result in recovery of quite high percentage of resistant clones than between resistant x susceptible parents. Using Adina × Ultimus, several late blight resistant and wart-immune hybrids were developed. One of them was released for commercial cultivation under the name Kufri Sherpa in 1983, which did not become popular because of its poor keeping quality, unattractive dull white skin, round tubers with medium deep eyes. Indian cultivars, viz. Kufri Jyoti, Kufri Chamatkar, Kufri Muthu, Kufri Sheetman, Kufri Bahar, Kufri Khasigaro and Kufri Kumar are immune to the race of *S. endobioticum* prevalent in the Darjeeling hills. These cultivars except Kufri Jyoti did not establish in the area because of local preference for varieties with red skin tubers. To develop a red tuber variety, pimpernel was used as one of the parents. One hybrid from cross

Fig. 6.10 Wart infected potato plant

SLB/Z 405a x Pimpernel was selected and has since been released as cultivar Kufri Kanchan. This variety is immune to wart and possesses high degree of field resistance to late blight.

6.7.1.5 Nematodes

About 90 species of nematodes belonging to 38 genera have been reported to be associated with potatoes. Among these, the root-knot nematodes and potato cyst nematodes have been recognized as the major pests.

At least nine species of root-knot nematode (*Meloidogyne spp.)* are known to infect potatoes. Among these, *M. incognita* is the most important throughout the world followed by *M. javanica*. The dominant root-knot nematode species affecting potato both in hills and plains is *Meloidogyne incognita* while *M. javanica* infestation is restricted to mid hills and plains.

Heavily infested plants are stunted with yellowish leaves while no visible tuber symptoms of nematode injury are seen under low infestation levels. The galls on potato roots are small and often go unnoticed. Wart-like structures are formed due to tuber infestation, which reduces the commercial value and keeping quality of tubers. The second-stage juveniles (hatched out from the egg masses laid by females) infest the young roots resulting in the formation of giant cells and the formation of syncytium and development of the nematode in the roots causes formation of galls or root knots. The nematode infection on tubers is characterized by the formation of typical wart-like pimples on the outer skin.

Work on breeding potato varieties resistant to *M. incognita* was initiated at the CPRI way back in 1961. Out of 101 hybrid cultures tested in a nematode infested field, only one line HC-294, a cross between Kufri Red × (Gladstone × Taborky) proved resistant to root-knot nematode. Further tests confirmed that only a few larvae invaded the roots of this selection and giant cells were not well developed. The resistance to root-knot nematode in this variety was inferred to be polygenic in nature. Later, another selection HC 115 from a cross of HB-289 × Kufri Red was also reported to be resistant to *M. incognita.* Both these lines were tolerant to heat, drought and frost. However, crosses involving HC-294 did not possess any resistance to root-knot nematodes indicating that the resistance was controlled by recessive genes. Further screening of accessions of tuber-bearing wild *Solanum* species indicated that a high degree of resistance was available in *S. spegazinii* and *S. vernei*, which were used as parental material for producing several crosses.

Potato cyst nematodes, *Globodera* spp. also known as golden nematode or potato root eelworms, is considered as one of the major pests throughout the world. Quarantine or regulatory actions are imposed against them in most countries. In India, the potato cyst nematode was first detected in 1961 by Jones at Ootacamund in Tamil Nadu. The nematode is reported to be present in about 3,050 hectare areas of Nilgiri and about 200 hectares of Kodaikanal hills of Tamil Nadu. Both these species *G. rostochiensis* and *G. pallida* are prevalent in these hills singly and also as mixed populations. The hatching of larvae from cysts (Fig. 6.11) is initiated by the

Fig. 6.11 PCN cysts in potato roots

root diffusates of potato or the members of the family Solanaceae. The nematode takes about 35 and 40 days for completion of life cycle during the crop season (Krishna Prasad 1993).

Heavy infestations in the absence of control measures often result in total crop loss. When the population in soil is sufficiently high, small patches of poorly growing plants may appear in the field. Temporary wilting of plants occurs during hotter parts of the day. Typical symptoms of heavy infestations are stunted plants with unhealthy foliage, premature yellowing, poor development of root symptom, reduction in size and number of tubers and poor yields. Since control of potato cyst nematode through chemicals is inadequate, expensive and environmentally hazardous, breeding cultivars resistant to the pest is the most effective way to control it.

The efforts to locate the source of resistance to cyst nematodes began in 1968, but a systematic breeding programme at CPRI was started in 1971. Over 2,000 genotypes comprising group tuberosum, group andigena and tuber-bearing *Solanum* species were screened and resistance was located in 20 accessions of 14 wild tuber-bearing species, viz. *S. ehrenberjii*, *S. vernei*, *S. chacoense*, *S. phureja*, *S. demissum*, *S. gourlayi*, *S. microdontum*, *S. sucrense*, *S. tarijense*, *S. acaule*, *S. fendleri*, *S. multidissectum*, *S. oplocense*, *S. sparsipilum* and some accessions of *S. tuberosum* ssp. *adigena*. Simultaneously, efforts were also made to procure resistant breeding lines to both the species from the Netherlands and USDA. A parental line VTn^2 62.33.3 (*S. tuberosum* × *S. vernei* hybrid) received from the Netherlands, having resistance to both the species, was extensively used in crosses with late blight resistant cultivar Kufri Jyoti resulting in release of hybrid Kufri Swarna for cultivation in cyst nematode infested areas of Nilgiri hills. Later one more variety, Kufri Neelima was released in 2010 for commercial cultivation in these hills using the same parental line.

Besides the important resistant lines from the Netherlands, nine resistant cultures with ssp. andigena in their pedigree were also received from Dr. Howard of Plant breeding Institute, Cambridge in 1976. Of these, two cultures, viz. D 40/8 and D 42/9, proved resistant to both the species of cyst nematodes. These are still used in the breeding programme.

6.7.1.6 Heat Stress

The potato has long been considered a crop for cool and temperate climates. Higher temperatures inhibit yield by overall reduction of plant development due to heat stress or by reduced partitioning of assimilates to tubers. Tuberization is reduced at night temperatures above 20 °C with complete inhibition of tuberization above 25 °C. Exposure of potato plants to heat stress alters the hormonal balance in the plants. As a result most of assimilated carbon is partitioned to above ground vegetative parts at the cost of the tubers. Aspects of heat tolerance that are considered important and should be taken into account in breeding programme includes ability of the plants to tuberize at night temperature of 22 °C and above (Fig. 6.12), low shoot/root ratio at high temperature, and early maturity of the crop.To breed heat-tolerant genotypes for Indian conditions, crosses were made amongst known heat-tolerant and local high-yielding genotypes. The known heat-tolerant genotypes used in the breeding programme were LT-1, LT-2, LT-5, LT-7, LT-8, LT-9, DTO-28, DTO-33 (received from CIP) Katahdin, Desiree and Kufri Lauvkar. The progenies were screened for heat tolerance by their ability to form tubers within 1 month after shifting to high temperatures. The selected genotypes were multiplied and further selected at early planting in Indo-Gangetic plains at Modipuram and Jalandhar under heat stress. Kufri Surya, a heat-tolerant variety (Minhas et al. 2006) has been released for cultivation as early planting in north-western plains as well as in rabi and kharif crops in peninsular India. This variety yields excellent defect-free

Fig. 6.12 Impact of heat stress (24 °C) on leaf bud tuberization in potato cultivar, *K. Surya* (heat tolerant) and *K. Chandramukhi* (heat sensitive)

tubers with high proportion of large (>85 mm) tubers suitable for processing into high quality French fries and chips. The reducing sugar content of tubers of this variety is less than 100 mg/100 g fresh weight and the tuber dry matter content is 20–21 % at harvest.

6.7.1.7 Drought Stress

Drought is a major limiting factor for potato production in the world influencing yield as well as tuber quality. Drought may occur due to erratic rainfall, inadequate irrigation techniques and lack of water supply. Even with good irrigation practices, water stress may occur because of high transpiration rates especially during mid-day, when root system cannot completely meet the transpiration requirements of the plant. Drought may affect potato growth and production by reducing the amount of productive foliage, decreasing the rate of photosynthesis per unit of leaf area and shortening the vegetative period.

Drought resistance can be the result of drought avoidance (e.g. closure of stomata, large root system) or drought tolerance (capacity for osmotic adjustment, rapid resumption of photosynthesis activity, etc.). Aspects of drought resistance that are considered important and should be taken into account in breeding programme includes the effect of short periods of stress on productivity and tuber quality, survival and recovery of the plants after water stress and water use efficiency. Experiments on reduction of leaf extension rate upon exposure to water stress and its recovery showed that potato cultivars could be placed in three groups. Group 'A' was characterized by minimum growth reduction under stress and rapid recovery on re-watering with final increase in the leaf length exceeding that of the unstressed controls. In group 'B' plants stress created moderate reduction in growth and on recovery the increase in leaf length became comparable to that of controls. Group 'C' was characterized by large reduction in growth and re-watering did not result in final leaf length increases comparable to that of controls. Both of these characters can be used as selection criteria in the breeding programme for drought tolerance.

6.7.1.8 Frost Tolerance

The problem of frost is known in almost every country where potatoes are grown. Temperatures below –2 °C in the field can produce partial or complete loss of the crop. In temperate zones, frosts can occur during spring when the crop is establishing itself, or during autumn when the crop is maturing. Higher crop losses occur in tropical highlands and subtropical plains where frosts can occur any time during the crop growth period. In India, more than 80 % of the potatoes are grown during winter in plains and the crop is prone to frosts during the months of December and January. Based on the field observations, two types of frosts are often distinguished. 'White frost' occurs when there is a decrease in temperature and relative humidity is high. 'Black frost' occurs under low temperatures and much drier conditions, hence

more damaging and severe, because plant tissue is darkened immediately. Acclimation or hardening may increase the resistance to frosts in many plants. Exposure of the plants to prolonged low temperature is effective in increasing resistance to frost injury in *S. tuberosum, S. multidisectum, S. chomatophilum, S. acaule and S. commersonii*. Genetic variability exists in the genus *Solanum* with respect to frost injury. *S. acaule* has the ability to withstand extracellular ice formation up to –5 °C, which gives this species frost tolerance. This species can be used in the breeding programmes to transfer frost tolerance to *S. tuberosum*. In the north of India, frost occurs during December and January in the plains of Punjab and Eastern UP. Cultivars Kufri Sheetman and Kufri Dewa released by the Central Potato Research Institute possess resistance to frost. High degree of frost resistance was observed in other 28 hybrids from crosses involving *S. acaule*.

6.7.1.9 Fertilizer Use Efficiency

The potato is considered to be heavy feeder on nutrients and requires high inputs of NPK and water for optimum production. This not only increases the cost of production but also causes environmental pollution. The application of high rates of N and K fertilizers and irrigation water on coarse-textured soils on which the shallow-rooted crop is often grown can result in loss of N and K, which represents an economic loss to the grower and may cause environmental degradation of groundwater. While numerous studies have explored N and K management practices as a strategy for minimizing N and K loss, there is potential for exploiting the genetic variability among cultivars of asexually propagated crop species for improved N and K uptake (Trehan 2009). A nutrient-efficient potato can produce higher yields per unit of nutrient, applied or absorbed even at a limited nutrient supply (Graham 1984). Such genotypes could reduce N fertilization and nitrate leaching (Duynisveld et al. 1988; Sharifi et al. 2007). The recovery of applied phosphorus by potato crop is not more than 15–20 % (Trehan et al. 2008). Numerous studies have demonstrated the existence of considerable variation for nutrient efficiency among crop species and cultivars within species, which suggests genetic control of inorganic plant nutrition (Errebhi et al. 1999; Trehan et al. 2005). Although plant breeders seldom select for nutrient use efficiency (NUE), breeding programmes that develop lines that produce high yields may result in unconscious selection of genotypes that use nutrients more efficiently (Batten 1993).

At CPRI, studies on nutrient use efficiency have shown that potato cultivars showed wide variation in agronomic use efficiency (AUE), nutrient uptake efficiency (NUE) and physiological use efficiency (PUE) with respect to nitrogen, phosphors and potassium. Kufri Pukhraj was the most N, P and K efficient cultivar among ten cultivars tested in the absence as well as the presence of green manure. The efficient cultivars gave higher tuber yield under nutrient stress (i.e. with less dose of N, P and K fertilizer) than less efficient cultivars. The main cause of higher nitrogen efficiency in the presence of green manure was the capacity of a genotype to use/absorb more N per unit green manured soil, i.e. the ability of the root system

of a genotype to acquire more N from green manured soil (NUE). The variation in potassium and phosphorus efficiency of different potato cultivars was due to both their capability to use absorbed K and P to produce potato tubers (PUE) and to their capacity to take up more K and P per unit soil (NUE). Breeders should combine parameters/characters (NUE and PUE) responsible for high nitrogen, phosphorus and potassium efficiency to breed multi-nutrient efficient potato cultivar. Kufri Sindhuri, a red-skinned potato variety released in 1967, is termed poor man's potato owing to its capacity to produce higher yield even under poorly managed growing conditions (Pushkarnath 1976). Recently CPRI has released potato variety Kufri Gaurav (Fig. 6.13) having higher nutrient use efficiency. The variety requires lower

Fig. 6.13 Leaf, flower, sprout and tubers of nutrient-efficient potato cultivar, *Kufri Gaurav*

doses of N, P and K than other cultivars to produce a particular fixed yield in the same field.

6.7.1.10 Quality Traits

Potatoes represent a non-fattening, nutritious and wholesome food, which supply important nutrients to the human diet. Tubers contain significant concentrations of vitamin C and essential amino acids. They are also a valuable source of at least 12 essential vitamins and minerals. Besides being important in human diet, potatoes are also used as animal feed and as raw material for starch and alcohol production. Potato quality parameters change according to the specific market utilization types, and are often referred by two major categories. The first category groups 'external quality', aspects comprising skin colour, tuber size and shape, eye depth. These traits are deemed very important for fresh consumption where external traits are most likely to influence consumer's choice. The second category comprises 'internal quality' aspects including nutritional properties, culinary value, after-cooking properties or processing quality. Internal quality is given by traits such as dry matter content, flavour, sugar and protein content, starch quality, type and amount of glycoalkaloids. Although quality is one of the most important characteristics of potato, it is probably the most poorly defined and least researched at the genetic level (Dale and Mackay 1994). There are several factors affecting tuber quality. They include the genetic makeup of the cultivar, crop maturity, agronomic practices, environmental conditions, storage temperatures, the presence of pests and diseases Traits that are genetically controlled can be grouped as biological traits (proteins, carbohydrates, vitamins, minerals, reduced amounts of toxic glycoalkaloids), sensorial traits (flavour, texture, colour) and industrial traits (tuber shape and size, dry matter content, cold sweetening, oil absorption, starch quality).

Breeding potato for quality traits requires a continuous flow of new genes and allelic diversity into the *S. tuberosum* gene pool. The genetic improvement of this crop is hampered by its tetrasomic inheritance, high level of heterozygosity, and incompatibility barriers. However, recent advances in plant biotechnology have significantly improved the possibilities of producing novel genetic variability and efficiently perform selection, especially when biotechnologists pool resources with breeders. Equally important is the fact that basic studies have contributed to elucidate our knowledge on the genetics, biochemistry and physiology of several quality traits, making breeding efforts less empirical and more predictable.

Use of *Solanum* species is particularly important in potato in that this crop has more related and cultivated and wild relatives than any other crop plant (Pavek and Corsini 2001), so that almost any trait that is important for breeding can be found in this germplasm. Since most *Solanum* species are diploid ($2n = 2x = 24$), a simple and efficient approach for their use is based on analytic breeding scheme involving first the production of *S. tuberosum* haploids ($2n = 2x = 24$) prior to crossing with compatible $2x$ species. Once the resulting diploid hybrids that produce $2n$ gametes at acceptable frequencies are selected for traits of interest (e.g. good chipping

ability, high dry matter content and resistance to diseases), the return to the tetraploid level of the cultivated potato may be achieved through sexual polyploidization schemes.

Lu et al. (2001) represents an example on the use of this approach. The authors produced *S. phureja–S. stenotomum* diploid hybrids and screened them for individual and total carotenoid content of tubers. They found a linear correlation between carotenoids and yellow-flesh intensity and selected hybrids containing 13 times more carotenoids than control cultivar 'Yukon Gold' (yellow flesh), and 22 times more carotenoids than 'Superior' (white flesh). The best hybrids also produced 2*n* pollen, and thus represent unique material for unilateral sexual polyploidization schemes aimed at producing 4*x* offspring for further selection.

Varieties obtained through sexual polyploidization approach are available worldwide. 'Yukon Gold', for example, is a yellow-flesh Canadian variety obtained through 4*x* × 2*x* crosses between 4*x* cultivar 'Norgleam' and a 2*x S. tuberosum–S. phureja* hybrid (Johnston and Rowberry 1981). Potato cultivars obtained through unilateral sexual polyploidization are also being released in China, now ranking first in the world potato production, (Jin et al. 2004). Up to now the 4*x* × 2*x* approach has been mainly used to transfer resistance traits. It also has a great potential for improving quality traits due to the high number of *Solanum* species with noteworthy quality characteristics (Table 6.1).

In potato genetic engineering techniques have been applied to produce routinely and several transformation protocols are currently available. Data published by Dunwell (2000) indicated that the potato ranks second, after corn, in the list of plant species for which field trials were carried out in the United States. In the past, most potato genotypes were transformed with genes for herbicide or insect resistance. However, much emphasis is now on quality traits. The production of starches with modified amylose-to-amylopectin ratio represents a good example of the possibilities offered by genetic engineering in improving potato quality traits. Lloyd et al. (1999) provided evidence that in transgenic potato lines where the activity of ADP-glucose pyrophosphorylase (AGPase) was reduced through antisense technology had a significant reduction of amylose. They also observed that in AGPase antisense plants, amylopectin accumulated shorter chains and that the size of starch granules was reduced. On contrast, the simultaneous antisense inhibition of two isoforms of starch-branching enzymes (SBE A and B) to below 1 % of the wild type activity increased amount of amylose in transgenic lines (Schwall et al. 2000). The amylose content of their transgenic lines was comparable to that of the high-amylose corn starch reported by Shi et al. (1998).

Great attention has been given to improve the essential amino acid composition of tubers and especially their lysine, tyrosine, methionine and cysteine content. Chakroborty et al. (2000) transformed a potato genotype with the gene *AmA1* from *Amaranthus hypocondriacus,* encoding a protein with a nutritionally balanced amino acid composition. The amino acid profile in tubers of both types of transgenic plants showed a 1.5- to 8-fold increase for all essential amino acids in the wild type.

Genetic engineering has been recently used to improve carotenoid content of tubers. In particular, to overcome the zeaxanthin deficiency of human diet, Römer

et al. (2002) downregulated the synthesis of zeaxanthin epoxidase specifically in tubers through antisense technology and co-suppression approaches. Both strategies achieved a decreased conversion of zeaxanthin to violaxanthin in transgenic tubers with a corresponding increase of zeaxanthin content of 4- to 130-fold. Due to the use of a tuber-specific promoter, leaf carotenoid content of all transformants was very similar to the control plants, and thus photosynthesis was not negatively affected by lack of violaxanthin.

One main constraint in the use of wild species is that, together with useful traits, they can transfer characteristics that are undesired from the commercial standpoint. In the case of *Solanum* species, traits such as long stolons, deep eyes, which are negative quality traits, can be transmitted. As reported recently by Pavek and Corsini (2001), transmission of undesired traits has very much limited the use of potato genetic resources. Therefore, after interspecific crosses, time-consuming evaluation and selection are necessary to eliminate unwanted wild type genes and restore the cultivated improved phenotypes.

Marker-assisted selection is perhaps the most powerful approach that uses DNA markers efficiently for selection of interspecific hybridization by reducing the linkage drug in terms of time and space. The use of DNA markers can be ascribed not only to the use of markers tightly linked to target genes (positive-assisted selection), but also to the use of markers specific for the wild donor parent to perform selection against the wild genome (negative-assisted selection; Barone 2004). For example, *S. commersonii*, a diploid wild species possesses several useful traits like frost resistance, acclimation capacity, high dry matter content of tubers, resistance to *Ralstonia solanacearum* along with high content of demissine, tomatine and commersonine, glycoalkaloids which are extremely toxic to humans and animals. To efficiently identify desirable *S. tuberosum–S. commersonii* hybrids, a negative-assisted selection approach was followed to estimate estimating the wild genome content of each hybrid by using *S. commersonii* specific AFLP markers with assumption that hybrids with low wild genome content may show only in minimal part the negative traits associated to the genome of the wild parent. This approach helped to identify hybrids combining low wild genome content with resistance and quality traits from *S. commersonii* (Barone et al. 2001; Carputo et al. 2002).

A very exciting development in the context of efficient selection has been the generation of a molecular-linkage map based on functional gene markers involved in carbohydrate metabolism and transport (Chen et al. 2001). Using diploid mapping populations for which molecular maps were already available, the authors performed CAPS, SCAR and RFLP marker assays for 69 functional genes previously studied and identified (among the others *AGPase*, *SssI*, *GbssII*, *Dbe*, *UGPase*, *Ppc*, and *Cis*). This work allowed the identification of 85 genetic loci covering a considerable amount of the potato genome. The availability of this molecular function map allowed a candidate gene approach to be used for studying starch- and other sugar-related agronomic traits in potato. Chen et al. (2001) compared the QTL map for starch content previously published (Schäfer-Pregl et al. 1998) with the

molecular function map, and various correlations between the map positions of 14 QTLs for tuber starch content and function-related loci were found.

A candidate gene approach has been also used by Menendez et al. (2002) to study cold sweetening in potato. Using RFLP and AFLP markers, they generated a QTL and linkage map of two segregating diploid populations previously evaluated for sugar content after cold storage. The authors mapped ten potato genes with known map position and unknown function in carbon metabolism or transport, and tested them for their effects on sugar content. Results displayed linkage between glucose, fructose and sucrose QTLs and all of eight candidate gene loci (*AGPaseS*, *AGPaseB*, *SbeI*, *GapC*, *Invap*, *Ppa1*, *Sut1*, *Sut2*). The authors pointed out that their results provide a basis for performing marker-assisted selection using allelic variants of candidate genes in the *Solanum* gene pool.

6.8 Biotechnology in Potato Genetic Enhancement

Potatoes are highly amenable to tissue culture and have been used widely for the development of biotechnology techniques. These techniques are now becoming handy in the potato variety improvement programmes. Various techniques have been used in potato breeding programmes includes somaclonal variations for creating useful variability, somatic hybridization for overcoming barriers to normal crossing, molecular markers for marker-assisted selection and genetic transformation for transfer of single, specific genes from any source to the existing varieties.

Use of biotechnology in potato improvement is already a reality, but these techniques are likely to remain as adjuncts to the more classical methods of breeding, which themselves will continue to evolve in efficiency as the underlying mechanisms and properties of genetic systems become more clearly known.

We are not dealing herewith about potato improvement through biotechnology for want of space, and also volume of data generated beyond the scope of this chapter and the readers are therefore suggested to refer to recent papers and more specific review chapters/books on the subject.

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