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REVIEW

Improvement for Bacterial Wilt Resistance in Potato By Conventional and Biotechnological Approaches

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Abstract Bacterial wilt (BW) of potato caused by the bacterium *Ralstonia solanacearum* (*Rs*) is considered a serious problem particularly in tropical, subtropical and warm temperate regions. Chemical-, cultural- and biological control of BW has limited success. Thus, the control of BW through resistance breeding and biotechnology is considered to be very important and necessary. *Rs* is considered a 'species complex' and has significant variation at physiological, serological and genetic levels. The bacterium has an unusually wide host range with over 400 hosts belonging to more than 50 botanical families. A large number of *Solanum* species have been screened for resistance to this bacterium, but so far no *Solanum* species has been found to have complete immunity. A high degree of resistance to *Rs* was found only in *S. phureja*, a diploid relative of cultivated tetraploid potatoes. The resistance has been transferred from *S. phureja* to cultivated potatoes through introgression breeding as well as somatic hybridization. Although moderate to highly resistant potato varieties have been released, high frequency of latent infection in tubers is still a major problem. Further, the resistant cultivars are not adapted to different agro-climatic zones and are not effective against all the strains of the pathogen. Biotechnological approaches involving the use of antimicrobial peptides, plant defence genes and plant resistance genes are being tried. This paper reviews the global situation with regard to screening of genetic resources and their utilization in resistance breeding for BW in potato and also the status and the opportunities that biotechnology offers to combat this disease.

Keywords Genetics · Brown rot · Breeding · Transgenics · Potato · *Solanum* spp. · *Ralstonia solanacearum*

Introduction

Potato (*Solanum tuberosum* L.) is the third most important food crop of the world after wheat and rice, which is roughly half the world's annual output of all root and tuber crops. Its production is 330 million tons fresh tubers from 19.7 million hectares with a productivity of 17.0 t/ha (FAOSTAT, www.faostat.fao.com). However, the potential production could exceed 400 million tons if the diseases that reduce the yield by approximately a quarter could be controlled [2]. It is the leading vegetable crop in acreage, but the productivity and quality of potatoes in the tropics are limited by a number of constraints including biotic stresses, such as debilitating diseases and insect pests, and abiotic stresses, such as high temperature, high humidity, excessive rainfall, drought, low light intensity and poor soil conditions. Of the major diseases of potato and other Solanaceous crops, bacterial wilt (BW) caused by the bacterium *Ralstonia solanacearum* (*Rs*) (formerly *Pseudomonas solanacearum* and *Burkholderia solanacearum*) [163], first reported in 1914 in South Africa, is considered a serious problem [74]. It has been estimated to affect 1.53 million hectares of potato crop in approximately 80 countries with global damage estimates exceeding $950 million per annum (APHIS, PPQ Action plan 2005, Fig. 1). According to the CIP (International Potato Centre, Lima, Peru) survey, BW, especially in developing countries,

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Fig. 1 Worldwide distribution of potato bacterial wilt disease (APHIS, PPQ, 2005)

is a top priority among the 5 most important challenges for high yields of potato. It causes severe crop losses in tropical, subtropical and warm temperate regions. The disease may also occur in cooler climates such as relatively high elevations in the tropics or higher altitudes. BW agent, R3bv2 (race 3, biovar 2 of *Rs*), is also considered a serious quarantine pest by the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA), by the North American Plant Protection Organization (NAPPO) and by the European Plant Protection Organization (EPPO) [26]. European Union in its decision 98/503/EC (Commission EC, 1998) has demanded that potatoes should be produced in the so-called pest free areas (PFA's) according to FAO standards, where the disease should be known not to occur (via testing) (EU Communities, 2005) [37]. French [45] considered four factors as important in the control of BW of potato: temperature, bacterial survival in the field, host range and resistance of the host, and also considered some other factors that may influence disease incidence, i.e. moisture content and flow, soil type and salinity, strain aggressivity, inoculum potential, mechanical damage to hosts, transmission in seed tubers and nematode interaction. New chemical-based control by soil fumigants, antibiotics and copper compounds was tried for control of BW, but without much success [104]. In addition, most chemical pesticides have hazardous effects on the environment, non-target beneficial organisms and human health. Cultural and biological control of bacterial diseases has also been tried by many investigators as an alternative solution, but again with limited success [100, 121, 161]. Thus, control of BW through resistance breeding and biotechnology is considered to be very important and necessary.

Pathogen

Ralstonia solanacearum [139, 163] is a Gram-negative, rod-shaped, chemoorganotroph and strictly aerobic bacterium that is 0.5–0.7 × 1.5–2.0 μ in size. The bacterium is soil dwelling that enters the plants to roots and colonizes in xylem tissues. The pathogen can be found in six of the seven continents [39]. The origin of *Rs* is not clear, but Hayward [59] suggests it predates the geological separation of the continents as the bacterium has been found in virgin jungle in South America and Indonesia. *Rs* is considered a 'species complex' due to significant variation at different levels (physiological, serological, genetic characteristics and host range) within the group [39]. In order to describe this intra-specific variability, several systems of classification have been proposed. Traditionally, the pathogen has been subdivided into five races on the basis of differences in host range [15] and seven biovars on the basis of carbohydrate utilizations [59]. But, this old classification system is unsatisfactory because it is not predictive and some groups (e.g. race 1) contain very large variation and because of this overlapping there have been no tests to define 'Race' of an isolate. Moreover, races and biovars are thought to be informal groupings at the intra-subspecific level that are not governed by the code of nomenclature of bacteria [91]. The modern techniques of molecular biology enable the construction of tree or dendrogram depicting evolutionary relationships at different levels or depths. On the basis of sequence analysis of 16S–23S ITS and endoglucanase gene, Fegan and Prior [39] proposed a hierarchical classification for *Rs* into four phylotypes belonging to β-subdivision of the class proteobacteria at the highest level. The four phylotypes broadly reflect the ancestral relationships and geographical origins of the strains, namely phylotype I strain originated in Asia (Asiaticum), phylotype II strain originated in Americas (Americanum), phylotype III strain originated in Africa (Africanum) and phylotype IV strain originated in Indonesia (Indonesian) [39, 90]. Phylotypes are further subdivided into sequevars based on the sequence of the endoglucanase (*egl*) gene [39].

There is no general correlation between races and biovars; however, biovar 2 strains are almost always race 3 (vice versa), but with the evidences found so for, it can be concluded that biovar 1 and 2 are less nutritionally versatile than biovars 3 and 4 [59]. Multilocus sequence typing and other analyses have confirmed that this system of classification reflects the phylogeny of the group. The electrophoretic pattern of the membrane proteins differs somewhat between biovars [34] and biovar 1 and 2 are distinct from biovars 3, 4 and 5 on the basis of DNA probes and RFLP analysis [29]. Race 1 is a poorly defined group with a very wide host range and is endemic to lower elevations, i.e. the southern United States as well as Asia, Africa and South America. These strains are limited to tropical, subtropical and warm-temperate locations and usually cannot survive under cool temperate conditions.

Race 2 principally attacks bananas and is found mainly in Southeast Asia and Central America. Race 3 (biovar 2) strains of *Rs*, which affect mainly potato, but occasionally tomato and other Solanaceous crops and weeds, is distributed worldwide and are most common in higher elevations of the tropics (up to 3,400 m asl). This group of strains is very homogeneous, possesses a narrow host range and is highly virulent mainly towards potatoes and tomatoes. The race 3 (biovar 2) has been typed on the basis of genetic sequencing as Phylotype II, sequevar 1 [39, 142].

Hosts

The bacterium has an unusually wide host range with over 400 hosts belonging to more than 50 botanical families. Species belonging to the *Solanaceae* are particularly threatened, including cultivated species such as potato, tomato, eggplant and tobacco [59]. Weed hosts of *Rs* are *S. dulcamara*, *S. nigrum*, *Portulaca oleracea* and *Rumex dentatus* [37]. Volunteer plants or (in colder climates: perennial) weeds can be a reservoir and responsible for the transmission of the pathogen through successive seasons [92]. It has been reported on several commercially important woody perennials like cashew, custard apple, Alexandra palm and strawberry [136]. In India, nearly 24 weed and non-host species are reported including *Datura stramonium*, *Solanum xanthocarpum* spp. *antirrhinum*, *Capsicum baccatum*, *Ageratum conyzoides* and *Ranunculus sceleratus* [145]. Race 4 affects ginger in much of Asia and Hawaii and race 5 effects mainly correspond to geographical origin and are thought to be not catastrophic [39]. Race 5 is reported to affect mulberries in China [32]. Crops highly susceptible to race 1 (biovars 1, 3 or 4) of *Rs* are potato, tobacco, tomato, eggplant, chilli, bell pepper and groundnut. Till date, 33 strains of biovar 3 and biovar 2 have been isolated from ginger, paprika, chilli, tomato, *Chromolaena* sp. and potato from various parts of India [79]. These have been well characterised phenotypically and genotypically. The strains of bacterium present in India appear to be the most virulent [135] and mainly caused by biovar 2 [79]. A wide range of hosts for race 3 (biovar 2) have been reported by various researchers around the world (Table 1). R3bv2 probably originated in the Andes, and sequevar 1 was apparently disseminated worldwide on potato tubers; this group now occurs in tropical highlands and in subtropical and warm-temperate areas throughout the world, except in North America [15, 29]. Considering the devastations of race 3 (biovar 2) on economically important crops like potato and tomato, an 8× draft of UW551 (*Rs* R3B2 strain) genome [48] has been sequenced and released to the public. This information may facilitate the identification of race-specific genes and to the development of race 3-specific molecular diagnostic assay.

Resistance Sources

At the beginning of the twentieth century, a few tuber-bearing wild *Solanum* species were reported to possess the BW resistance [128]. So far, no complete immunity has been identified, but some degree of tolerance has been reported in several tuber-bearing *Solanum* species [61]. Various standardized methods/procedures are available for field, glass house and in vitro screening of potatoes for BW resistance [28]. Some of the wild *Solanum* species extensively tested for BW reaction are discussed below.

Solanum tuberosum

It is the main cultivated species of potato. So, if resistance is found in this species, it is easy to utilize as it will not involve the problem of cross compatibility and ploidy level differences. Jaworski et al. [67] screened 51 potato cultivars for reaction to BW under high disease pressure in field plot near Tifton, Georgia for 2 years. All were susceptible except cultivar Ontario in which only 1 and 8 % of infected plants wilted in the consecutive years. Green Mountain, Snow chip and Sebago also showed some resistance, but not enough to be used in breeding programme. Gunawan and Smith [55] screened many clones for resistance to BW and identified 52–152 and 52–221 as resistant. Michel and Mew [100] tested 52 clones to BW in East Africa and CIP720118, CIP800212, CIP800223 and CIP800224 were identified as resistant. These clones were products of crosses between *S. phureja* and Atzimba (CIP720054). Tung et al. [151] screened 12 resistant clones at three locations of Philippines. Stability analysis indicated that the genes for heat tolerance were crucial for resistance to *Rs* race 1. This study showed that resistance to BW, both derived from *S. phureja* and other sources, tends to break down at high temperatures, and the genes for adaptation are involved in expressing resistance. Clones with genes for both resistance and heat tolerance resisted wilt better under hot conditions than those with resistance alone. This was observed in clone CIP378597.1, which has resistance genes from *S. phureja* and is also heat tolerant. Though in this study clones used carried resistance genes from *S. chacoense*, *S. raphanifolium* and *S. phureja*, the study did not clearly show whether genotypes with a range of resistance genes from several species have a more stable resistance than genotypes with resistance from one species. Theoretically, however, it would be expected that a wide genetic background would make the resistance more stable. Spooner and Hijmans [141] identified 12 clones out of 500

Table 1 Reported hosts of *Ralstonia solanacearum* (race 3 biovar 2)

Natural host	Family	Occurrence
Solanaceous crops		
Capsicum annuum	Solanaceae	Rare, S. America
Lycopersicon esculentum	Solanaceae	Widespread
Solanum tuberosum	Solanaceae	Widespread
Solanum melongena	Solanaceae	Rare, S. America, France
Solanum phureja	Solanaceae	Rare, Colombia
Solanum dulcamara	Solanaceae	USA
Pelargonium hortorum	Solanaceae	USA
Solanaceous weeds		
Cyphomandra betaceae	Solanaceae	Rare, Colombia
Urtica dioica	Solanaceae	Rare
Tropaeolum majus	Solanaceae	Rare
Stellaria media	Solanaceae	Rare
Portulaca oleracea	Solanaceae	Rare
Polygonum capitatum	Solanaceae	Rare
Melampodium perfoliatum	Solanaceae	Rare
Drymaria cordata	Solanaceae	Rare
Chenopodium album	Solanaceae	Rare
Cerastium glomeratum	Solanaceae	Rare
Datura stramonium	Solanaceae	Rare, Georgia (USA)
Physalis sp.	Solanaceae	Rare, Georgia, USA
Physalis angulata	Solanaceae	Rare, S. Africa
Solanum carolinense	Solanaceae	Rare, Georgia, USA
Solanum cinereum	Solanaceae	Australia only
Solanum dulcamara	Solanaceae	NW Europe
Solanum nigrum	Solanaceae	Widespread
Non-solanaceous natural host plants		
Bidens pinnata	Compositae	Rare, Georgia, USA
Brassica rapa	Cruciferae	India
Chenopodium spp.	Chenopodiaceae	Nepal
Melampodium perfoliatum		Costa Rica
Momordica charantia	Cucurbitaceae	Philippines
Pelargonium zonale (= *P. x hortorum*)	Geraniaceae	USA
Phaseolus vulgaris	Leguminosae	Philippines
Portulaca oleracea	Portulacaceae	Kenya
		Egypt
		Nepal
Salvia reflexa	Labiatae	Australia
Natural latent hosts		
Cleome monophylla	Capparadidaceae	Kenya
Galinsoga ciliata	Compositae	Nepal
Nicotiana glutinosa	Solanaceae	S. America
Nicotiana rustica	Solanaceae	S. America
Polygonum capitata	Polygonaceae	Nepal
Solanum sisymbriifolium	Solanaceae	Brazil
Urtica dioica	Urticaceae	Rare Netherlands
Beta vulgaris	Chenopodiaceae	Sweden
Brassica juncea., *B. Napus*	Cruciferae	Nepal, Sweden,

Table 1 continued

Natural host	Family	Occurrence
Brassica rapa	Cruciferae	AI
Browallia speciosa	Solanaceae	AI
Cerastium glomeratum	Caryophyllaceae	Nepal
Chenopodium ambrosioides, *C. amaranticolor*, *C. paniculatum*	Chenopodiaceae	AI
Cucurbita pepo	Cucurbitaceae	Japan
Drymaria cordata	Caryophyllaceae	Nepal
Erodium moschatum	Geraniaceae	AI
Eupatorium cannabinum	Compositae	AI
Galinsoga parviflora	Compositae	Nepal
Glycine max	Leguminosae	AI
Gnaphalium elegans	Compositae	AI
Helianthus annuus	Compositae	AI
Hordeum vulgare	Graminae	AI
Ipomea sp.	Convolvulaceae	Peru
Lycopersicon chilense	Solanaceae	AI
Nicandra physaloides	Solanaceae	AI
Nicotiana alata	Solanaceae	AI
Phaseolus vulgaris	Leguminosae	AI
Phaseolus multiflorus	Leguminosae	AI
Physalis floridana	Solanaceae	Chile
Pisum sativum	Leguminosae	AI
Rumex spp.	Polygonaceae	AI
Salpiglossis sinuata	Scrophulariaceae	AI
Spergula arvensis	Caryophyllacae	AI
Solanum capsicastrum	Solanaceae	Chile
Solanum caripense	Solanaceae	Colombia
Solanum luteum	Solanaceae	AI
Solanum sarrachoides	Solanaceae	Chile
Solanum xanthophyllum	Solanaceae	Nepal
Soliva anthemifolia	Compositae	AI
Tagetes sp.	Asteraceae	Peru
Tropaeolum majus	Tropaeolaceae	AI
Verbena brasiliensis	Verbenaceae	AI
Vicia faba	Leguminosae	AI
Vigna sinensis	Leguminosae	AI

AI artificial inoculation

screened in Brazil using race 1 biovar 1. The most promising clones were CIP377835.1 (BR63.65 × Atlantic), CIP382292.99 (BR69.84 × India 853), CIP382293.20 (India 853 × BR69.84), CIP282299.103 (PSP30.10 × BR68.84), CIP382303.94 (377835.9 × AVRDC1287.19), CIP382305.110 (377835.7 × AVRDC1287.19), CIP 3822309.75 (Serrana x AVRDC1287.19) and CIP 382314.5 (377836.1 × AVRDC1287.19). Lin et al. [89] tested 5 populations in green house and reported the highest level of resistance in clones I1, I2 from population I (CIP385312-2) and in clones II1 from population II (CIP388285-14). Quezado et al. [119] reported high resistance in six clones namely, BP88166-2, CIP800966, BP88068-3, BP88166-5, BP88074-1 and CIP382303-1. Of 15 cultivars screened in Kenya, Kenya Dhamana, Mauritious and Cruza, CIP-720118 had low BW severity and were rated tolerant compared to other moderate and susceptible cultivars [9]. The resistance, however, was shown to be very unstable due to its strong host–pathogen environment interactions [151].

In India, a total of about 6,500 accessions including several *tuberosum* and *andigena* clones, polyhaploids, hybrids and irradiated materials were screened under natural field conditions and by artificial inoculation, but no useful source of resistance was found [80, 108, 153]. However, five accessions (CP 1636, CP 3088, CP 3144, CP 3159 and CP 3171) were reported to be moderately resistant. Chakrabarti et al. [23] screened 61 clones by root injury method under 28–30 °C. None of the clones were resistant, but 3 clones of *S. tuberosum* (CP 2342, CP 1227 and CP 1241) along with 4 clones of *S. sucrense* and one clone of *S. juzepczukii* were found to be highly tolerant. Nagesh et al. [108] in 8 years of evaluation identified 5 clones, namely CIP382381.13, CIP381381.20, CIP382193.9, CIP378699.2 and CIP387792.5, as resistant to late blight and BW. Gadewar et al. [49] screened 230 accessions against *Rs* by root injury method in glass house. Three accessions CP1818, CP2024 and CP2345 had the longest incubation, but wilted when inoculated by stem stab method.

Solanum phureja

It is a diploid relative of cultivated potato and belongs to the South American centre of origin. Samsen et al. [129] showed that the First Division Resistance (FDR) 2*n* pollen can transfer bacterial wilt resistance from diploid to tetraploid progenies by 4× × 2× matings. In the testing of a wide diversity of germplasm of cultivated and *Solanum* species and inter-specific hybrids against race 3 of *Rs*, a high degree of resistance was found only in *S. phureja*. It has been widely used in breeding programmes for the last 4 decades because of its potential BW resistance character [124, 148]. Nearly 850 (238 clones of *S. tuberosum*, 364 clones of *S. andigena*, 190 clones of *S. phureja*, 43 clones of other wild species and 226 clones of inter-specific hybrids) clones from Colombian Potato Collection were tested for BW resistance in green house experiments at Bogota, Colombia. Of the 190 clones of *S. phureja* tested, however, many showed resistance with varying degrees. Six clones (C.C.C. 1339, C.C.C. 1350, C.C.C. 1386, C.C.C. 1388, C.C.C. 1395 and C.C.C. 1449) showed the resistance consistently [148]. The resistance derived from *S. phureja* has been found to be adequate for the Andean highlands [46]. Unfortunately, this resistance is temperature sensitive and is effective only at higher elevations or in cooler climates [46]. Three RAPD markers ($OPG05_{940}$, $OPR11_{800}$ and $OPO13_{770}$) and an SSR (STM0032) marker linked to resistance gene or the reciprocal loci have been identified in *S. phureja* [51]. AFLP markers have also been identified by bulked segregant analysis [52]. These markers were found to be on chromosome 1 and 12.

Solanum commersonii Dun.

It is a wild tuber-bearing species native to Uruguay [58]. It possesses many desirable traits, including tolerance to low temperatures reported as early as 1930 [123] and resistance to nematode *Ditylenchus destructor*, fungus *Alternaria solani*, bacterial pathogen such as *Rs* and potato viruses X and Y [81]. Leaf extracts of several Uruguayan *S. commersonii* accessions collected from different geographical locations were shown to produce an inhibitory effect on the growth of *Rs* suggesting the presence of constitutive compounds associated with resistance [137]. Esposito et al. [38] have shown the presence of metabolites specially expressed in the *S. commersonii*-resistant genotypes, which could be involved in plant-pathogen incompatible reaction. cDNA-AFLP approach was used to study transcriptome variation in resistant and susceptible interactions. A specific EST collection of the *Ralstonia*–potato interaction has been built up. Two different steps leading to resistance could be distinguished: (1) the disease is stopped at the recognition of the infection and (2) the development of symptoms is delayed and reduced. Recently, Siri et al. [138] screened 30 accessions of *S. commersonii*, and different levels of resistance were found ranging from delayed wilting to asymptomatic reaction. The genetic variation and the relationships among individuals in this germplasm collection were studied by different molecular markers, viz. RAPD, AFLP and SSR [138]. All markers grouped *S. commersonii* accessions into two clusters regardless to the marker type. The distribution into two main clusters showed high correlation with geographical origin of the accessions. These were further used for screening in polyhouse and 9 most promising lines were identified [138].

Solanum stenotomum

It is one of the seven cultivated species of potatoes [144] and believed to be the first tuber-bearing species, which had been domesticated around lake Titicaca in Andean high plateau, astride the border between Peru and Bolivia [58]. This diploid species, also called *S. tuberosum* group Stenotomum, is thought to be the progenitor of the cultivated potato, *S. tuberosum* [58]. It is reported to possess the resistance against BW, which was transferred to *S. tuberosum* through breeding [95] and somatic hybridization [43]. Its somatic hybrids with *S. tuberosum* showed higher resistance to *Rs* race 1 and 3 [44].

Solanum sucrense

It is a tetraploid wild species from southern Bolivia and is thought to be a member of *S. brevicaule* complex. It is thought to be a hybrid between *S. oplocense* and *S.*

tuberosum subsp. *andigena* [58]. Jaworski et al. [67] screened *S. sucrense* for BW resistance. Clones G1451, G 2084 and G 2368 showed moderate resistance at a pathogen load of 1×10^9–3×10^9 cfu/g. Chakrabarty et al. [23] screened 61 accessions against *Rs* (race 1) and a few clones that could delay wilt appearance even in the presence of *M. incognita* were identified. Four clones of *S. sucrense* (G 446, G 953, G 1042 and G 1050) along with 3 clones of *S. tuberosum* and one clone (CP 2342/Zugucha) of *S. juzepczukii* were found to be highly tolerant. However, all of these harboured high load of *Rs* (4×10^5–4×10^7 cfu/g) in their stem.

Solanum microdontum

This diploid wild species of potato is native to Bolivia and Argentina [58]. From 1973 to 1984, Shekhawat et al. [135] screened 7,183 clones of the species against bacterial wilt of which only one accession (SS-529-1) showed good resistance. This was used by Tyagi et al. [152] for transferring resistance to cultivated tetraploids. A diploid clone of *S. tuberosum* (PH 54-30) was used for the crossing. Nearly 500 seeds were obtained from the crossing PH 54-30 and *S. microdontum*. The resulting lines, however, showed inconsistent resistance when tested under different agro-ecological zones.

Other Species

At the University Wisconsin-Madison, 1,573 accessions of 102 species from IR-1 (Inter-Regional) were tested against BW. Seedlings were grown for 21 days in a green house at 22 °C. The survivability varied from 1 to 100 % and 41 were identified as most resistant accessions including 19 of *S. demissum*, 6 of *S. phureja*, 3 of *S. commersonii*, 2 of *S. polytricon*, 2 of *S. raphanifolium* and one each of *S. berthaultii*, *S. blanco-galdosii*, *S. boliviense*, *S. brachycarpum* and *S. chacoense*. At International Potato Centre, Lima, Peru, 85 intra-specific crosses between 31 tuber-bearing *Solanum* species were screened. Seven families that had high levels of resistance involved 11 *Solanum* species namely *acaule*, *boliviense*, *bukasovii*, *candolleanum*, *coelestipetalum*, *leptophytes*, *peloquinianum*, *phureja*, *raphanifolium*, *sparsipilum*, *sogarandinum* and *tapojense* [47]. Three of the families were inter-specific crosses involving different accessions of *boliviense*, *peloquinianum* and *sogarandinum*. Other species reported to be resistant by CIP are *S. bulbocastanum*, *S. capsicibaccatum*, *S. curtilobum*, *S. jamessi*, *S. microdontum* and *S. stenotomum* [45]. Spooner and Hijmans [141] screened around 80,000 potato clones for BW resistance at CIP since 1985 and identified 30 clones as most resistant.

Genetics

BW resistance in potato is very complex in nature. Initially, it was thought to be controlled by three independent dominant genes [15], but later it was reported to be controlled by four major genes [53]. Recently, Guidot et al. [54] showed around 70 genes and 15 inter-genes specific to the potato brown rot pathogen by microarray technique. Of these, 29 genes were part of mobile genetic elements. The evidence to be considered by all researchers is that genes for pathogenicity in *Rs* are not resident on simple plasmids. But *Rs*, like *Rhizobium*, harbours a megaplasmid with molecular weight larger than 4.5×10^8 Da [126]. There is some evidence that most of the genes involved in the control of pathogenicity are clustered together on the megaplasmid in a 80-kb area the deletion of which also gives resistance to acridine orange [13]. BW resistance is apparently of a polygenic and quantitative type involving genes with major effects as well as genes with minor effects [151]. The major genes have been evolving independently from the pathogen interaction, whereas minor genes are thought to operate in a gene to gene way with the pathogen. There is evidence that in the inheritance of resistance to wilt, non-additive gene action is important [151]. Chakrabarthi et al. [24], however, reported significant general and specific combining abilities for BW resistance indicating that both additive and non-additive gene actions are important in conditioning resistance expression. There was evidence that epistasis is an important component of the non-additive gene action in the inheritance of resistance [151]. Resistance to BW is temperature dependent, which was shown in a study using race 1 and race 3 isolate of *Rs* to test the resistance under warm temperatures. Results obtained also indicated partial dominance of resistance [151]. The pathogen also interacts with other pathogens and pests such as bacterial soft rot caused by *Erwinia* spp. and root knot nematodes (*Meloidogyne* spp.). These interactions often result in sever losses to potato crop [130]. There is also interaction between biovar and planting season and between biovar and potato cultivar.

Introgression Breeding

A complexity of host–pathogen–environment interaction has made breeding potato for BW resistance extremely difficult. The clones found resistant to BW in 1 year/environment or location succumb to the disease in the other year/environment or location and are not resistant against all strains of pathogen [92]. Nevertheless, keeping in view the importance of breeding for resistance to this important pathogen, interest in the use of known resistant species for

breeding began over 150 years ago when Sabine (1824) from Germany pointed out that new introductions were needed to rejuvenate potato stocks and help to combat the known diseases. Wild *Solanum* species have proven to be valuable in breeding potatoes for disease resistance, environmental tolerance and other agronomic traits of interest [141]. In the last four decades, breeding for wilt resistance has been mainly based on resistance derived from *S. phureja* with few breeding programmes involving other wild species. Sexual hybrids of potato with *S. chacoense*, *S. sparsipillum*, *S. raphanifolium*, *S. microdontum* and *S. multidissectum* achieved only a moderate level of resistance, together with some undesirable wild traits, such as high glycoalkaloid content [47, 130]. Tung et al. [151] suggested that widening the genetic base for both resistance and adaptation is important in breeding for resistance to BW. Later, Tung et al. [151] screened 30 F1 progenies from different parents with different levels of BW and heat resistance. These were tested against race 1 and race 3 of *Rs*. The existence of strong interaction between resistance genes and genes for heat resistance was found. The reciprocal differences observed were not significant, suggesting the absence of cytoplasmic effect on the expression of resistance. Watanabe et al. [157] transferred the resistance to BW found in tuber-bearing *Solanum* species (both 2× and 4× bearing clones with diverse genetic background generated at CIP) into diploid potato breeding population. This transmission of resistance for BW to tetrapliod progeny was done via FDR 2*n* pollen [157]. The resistant genotypes were identified using virulent race 3 (CIP-204). These resistant diploids were then crossed with cultivated tetraploids. Resistance in the offsprings obtained varied from highly susceptible to highly resistant. Based on the frequency of the crosses and the genetic mode of FDR 2*n* pollen, at least five to six loci were associated with the resistance [157]. Considering the components of the quantitative resistance to BW, the genotypes showing resistance to multiple pests (other than to BW) and characterized by short day adaptation and crossability to tetraploids were selected under green house tests for BW resistance, followed by field trials. Watanabe et al. [158] screened a total of 517 clones representing diploid F1 families, derived from crosses between *S. tuberosum* haploid line and seven wild *Solanum* spp. and fifteen clones of these were highly resistant to BW. Watanabe et al. [158] crossed diploid potato lines resistant to BW, PTM (potato tuber moth) and root knot nematodes to 4× cultivars via FDR. Among 557 clones, 114 clones showed combination of two resistance traits: BW + RKN—85 clones, LB + BW—14 clones, BW + Glandular trichomes—1 clone and RKN + Glandular trichomes—14 clones. Clones (381077.1 × XY.16).28, (382302.2 × XY.9).55 and (382291.1 × XY.16).68 did not have any BW latent infection. Watanabe et al. [158] reported that some diploid genotypes selected for BW resistance showed a comparable yield to that of local standard cultivars under subtropical conditions. A diploid genotype 90.12.52, which gave higher yields than standard cultivar in Lima, gave significantly lower yields at San Ramon. So, they concluded that there was no consistent relationship between ploidy levels and yield [158]. The study also revealed that the resistance to RNK is associated with resistance to BW which is a desirable trait for potato growers in tropical and subtropical regions. Heat tolerance was also reported to be linked with the BW resistance as heat tolerant LT-7 and AVRDC 1287.19 showed the highest resistance to BW and a negative correlation ($P = 0.5$ $r = 0.34$) between BW incidence and altitude. Resistance to latent infection was also studied. Four of the nine resistant lines showed latent infection continuously for three consecutive years. Of 18 potentially bacterial wilt-resistant CIP clones tested in field tests at four locations in Indonesia, where BW is a constraint to potato production, eight clones, CIP 390774, CIP 390775, CIP 390791, CIP 390811, CIP 390812, CIP 390814, CIP 390817 and CIP 390818, were the most resistant with a wilt incidence of less than 30 %. Of these, six clones (CIP 390785, CIP 390811, CIP 390812, CIP 390814, CIP 390815 and CIP 3908116) yielded on average more than 200 g/plant. Five clones were selected according to their rank in BW infection, tuber yield, percentage of marketable tubers and tuber uniformity [127]. However, performance of these five clones in different agro-ecological zones remains unknown. Prior and Fegan [118] evaluated advanced clones at CIP for 3 years (crosses of *S. tuberosum* with wild species) for BW resistance (R3B2A). All clones showed moderate to high resistance, but had high frequency of latent infection. Kim Lee et al. [77] selected tetrapliod hybrids between *S. tuberosum* and BW-resistant *S. commersonii*. Three highly resistant BC1 clones were back crossed to cultivars. Seven clones were resistant or highly resistant for both R1 and R3. Of these seven, three were selected for further testing. Most recently, Felix et al. [40] screened five Irish potato cultivars grown in most of the African countries: Tigoni (CIP-381381.33), Asante (CIP-381381.20), Kenya Karibu, Kenya Sifa and Dutch Robjyn, and found that none of the potato cultivars was resistant to BW and reactions to wilt varied from cultivar to cultivar and environment to environment. Kinya Sifa and Kenya Karibu were found to be most tolerant to BW, while Dutch Robjyn and Tigoni were most susceptible. Tigoni was bred to tolerate late blight [94], but it seems to lack tolerance to bacterial wilt. In India, seedlings of 44 *S. tuberosum* × *S. andigena* crosses were screened against brown rot and other important diseases of potato, but none of these gave a promising resistance to BW [107].

The above account shows that, till now, BW resistance that is available in wild species has not been utilized to its fullest extent [43, 92]. Although moderate to highly resistant potato varieties have been released in different countries, high frequency of latent infection in tubers is still a major problem [118]. Some of the resistant varieties of potato released are MEX-Cruza 148, CIP720118 and Ndinamagara in Africa, Caxamarca, Molinera, Ampola and Huanuquena in Peru, Kenya Dhamana in Kenya, Domoni in Fiji, Chicua Irazu in Costa Rica and Prisca and Kinga in Madagascar [59]. Achat, a multiviral resistant variety, is also resistant to BW [91]. Achat is more resistant than Elvira or Baronesa, the other two popular varieties in Brazil and other South American countries [91]. Cruza (CIP 720118) and clone MB 03 are the most resistant in Brazil. Sequoia is a BW-resistant variety in Papua New Guinea; Red Pearl is resistant to common scab, corky ring spot and BW [13]. Kangqing, a medium maturing variety with strong resistance, from the cross BR63.5 × 104.12L3 [60], have been released in China. Some of the cultivars which showed moderate to high resistance to BW have become susceptible in the course of time, like Katahdin in USA, Red Pontiac and Kennebec in Uruguay and Renacimiento in Peru [46]. Shekhawat et al. [135] tested the cultivars Molinera, Caxamarca and Serrana, resistant in South America against Indian strains and found that they wilted within 5–10 days. Rao [122], while testing tomato and brinjal varieties for resistance to the disease, observed that cultivars reported to be resistant in other countries were highly susceptible to Indian isolates of bacterium. Jenkins and Nesmith [69] compared isolates of the bacterium from USA and India for their virulence on resistant cultivars of tomato and brinjal. All the resistant cultivars included were susceptible to Indian isolates. They concluded that Indian isolates of bacterium were more virulent than US isolates.

Somatic Hybridization

The introgression of resistance gene from wild *Solanum* species into *S. tuberosum* by classical breeding methods is time consuming, laborious and may encounter difficulties, particularly differences in the ploidy level or in EBN (endosperm balance numbers) [158]. Crosses between 4 × *S. tuberosum* and hybrids from 2 × *S. commersonii* and 2 × *S. tuberosum* di-haploids (2EBN) have repeatedly failed [20]. Therefore, somatic fusion is expected to provide a possibility for increasing the nuclear and cytoplasm genetic variability, and also a means of transferring the desirable agronomic traits into potato [43]. Usefulness of somatic hybridization is examined based on the following criteria: resistance must be stable through somatic hybridization, and somatic hybrids must be fertile with *S. tuberosum* to allow eventual introgression of desirable traits through conventional breeding. Potato is one of the few agriculturally important crops where somatic hybridization is extremely used. The potential use of somatic hybridisation has been demonstrated by the successful introduction of traits such as resistance to viruses [154] and frost [117] from *S. brevidens*, resistance to *Phytophtora infestans* and *Globodera pallida* from *S. circaeifolium* [96], and insect resistance from *S. berthaultii* [132]. Kim et al. [75] produced somatic hybrids between *S. commersonii* and *S. tuberosum* by electrofusion. Laferriere et al. [81] reported that somatic hybrid plants were vigorous, their BW resistance level was similar to *S. commersonii* and were also male and female fertile. However, stability of resistance under different field conditions and temperature regimes remain unknown. Nyman and Waara [112] produced *S. tuberosum* + *S. commersonii* somatic hybrids and have demonstrated that frost tolerance along with BW resistance could be recovered in these hybrids. This also showed that wilt resistance was stable through somatic hybridization. Results from all these studies suggest that *S. commersonii* and *S. tuberosum* somatic hybrids may be useful as sources of BW resistance in potatoes.

Fock et al. [43] produced somatic hybrids between *S. tuberosum* and *S. phureja* and obtained five tetraploids and an amphidiploid. These were screened with R1B3 and R3B2 race/biovar of *Rs*. Disease incidence was evaluated at 30 days after inoculation as percentage of wilted plants and bacterial population in the roots were estimated. They concluded that though *S. phureja* was tolerant to R1 and moderately susceptible to R3, amphidiploids showed resistance to both races, whereas all tetraploids appeared to be susceptible to both. Somatic hybrids between a dihaploid clone of potato (*S. tuberosum*) cv. BF15 and *S. stenotomum* were produced by electrofusion of mesophyll protoplasts [43]. The hybrids produced exhibited high vigour and showed morphological intermediate characters. DNA analysis by flow cytometry revealed that 25 were tetraploids (4×; 48 chromosomes), three hexaploids (6×; 72) and two aneuploids (<4×; 48). Their hybrid nature was also confirmed by examining isoenzyme patterns for esterases and analysis of DNA simple sequence repeat (SSR) markers. When these were screened with race 1 and 3, interestingly all somatic hybrids tested showed a resistance level as high as that of the wild species. These also produced bigger tubers compared to the small tubers obtained from wild species [43]. *S. stenotomum* was used by Fock et al. [44] for somatic hybridization with *S. tuberosum* and recorded that the hybrids expressed the same level of resistance as the resistant parent. Further, these somatic hybrids maintained in vitro for 5 years still carried the same level of resistance along with all other

agronomically superior characters like high tuber number and size.

Biotechnological Approaches

In recent years, genetic engineering for disease resistance, particularly, the use of many potent antimicrobial peptides from plant resources, such as enzyme inhibitors, lectins, pathogenesis-related proteins and thionins, has been demonstrated [12]. Control of bacterial diseases has been made possible through genetic engineering using genes found in fungi, insects, animals and other plants. Antimicrobial proteins, peptides and lysozymes that naturally occur in insects [68], plants [14], animals [156] and humans [109] are now a potential source of plant resistance. Use of these in potato and some other crop plants for *Rs* and other bacterial pathogens is discussed below.

Expression of Antimicrobial Proteins

Antimicrobial peptides (AMPs) with α-helical structures are ubiquitous and found in many organisms. AMPs have been isolated from frogs, insects and mammalian phagocytic vacuoles [149]. AMPs are selective for prokaryotic membranes over eukaryotic membranes due to the predominantly negatively charged phospholipids on the outer surface of the prokaryotic membrane [149]. Such preference is considered a regulatory function in target selectivity. AP1 (Antimicrobial Peptide 1) is a plant endogenous antimicrobial protein isolated from BW-resistant potato clone MS42.3. Transgenic potato expressing AP1 gene showed increased resistance to BW [88]. INF1 elicitin, a well-characterised, class IA, 10 kDa extracellular protein produced by *Phytophthora infestans* induces hypersensitive response (HR) and systemic acquired resistance (SAR) in *Nicotiana* species and few other genera [72]. INF1-treated tomato and -potato exhibited resistance to BW disease. INF1 activates the jasmonic acid and ET-mediated signalling pathways without the development of hypersensitive reaction or cell death [73]. Jia et al. [70] expressed Cecropin B and Shiva genes in six transgenic Chinese potato cultivars by *Agrobacterium* mediated transformation. Transgenic potatoes were resistant to BW against race 3, and also tested new peptides ABP3, Shiva 2A and WHD with strong antibacterial activity. These were designed and synthesized chemically. Potato plants were transformed with these genes using *A. tumafeciens*. Transgenics were evaluated both in green house and field nurseries using race 3. Three clones with enhanced resistance were selected after 3 years of testing. Allefs et al. [6] fused the sequence encoding a synthetic tachyplesin I gene with that of the barley hordothionin signal peptide. A low expression of this chimaeric gene in three potato cultivars revealed slight inhibitory effects to *E. carotovora* subsp. *atroseptica*. Tachyplesin was also found to be effective in controlling the growth of bacteria that are typically found in vase water like *Bacillus*, *Enterobacter* and *Pseudomonas* spp. [42]. Tachyplesins are a family of antimicrobial peptides first isolated from acid extracts of hemocytes of the Japanese horseshoe crab (*Tachypleus tridentatus*). These strongly basic 2.3-kDa peptides (17–18 residues) with two disulphide bridges primarily inhibit the growth of both Gram-negative and Gram-positive bacteria by forming a complex with bacterial lipopolysaccharides or with phospholipid membranes [113].

Magainins

Magainin is a defence peptide secreted from the skin of the African clawed frog (*Xenopus laevis*), first discovered by Zasloff [165]. The mechanisms of action of magainin peptides are well studied. Magainins and their analogues have been studied as a broad-spectrum topical agent, a systemic antibiotic, a wound-healing stimulant and an anticancer agent [66]. One of the important natures of these magainin peptides is the selective toxicity to bacteria, and the non-toxicity to plants in in vitro plant tissue studies [159] and mammalian tissues [165]. Magainin peptides and derivatives have been reported to enhance broad spectrum resistance to a range of phytopathogens (both bacterial and fungal) in transgenic tobacco [30], potato [85], tomato [4], grape [71] and banana [25, 30]. Megainin II transferred into potatoes is reported to give a high resistance to a range of pathogens [10]. Interaction studies between the microbial communities and the transgenic potatoes containing megainin II gene have been studied by Callaghan et al. [16], which showed that targeted inhibitory effect on pathogens. Philippa et al. [116] transferred an analogue of magainin II, magainin D into potato. Three consecutive years of pathogen assays of field-grown transgenic potato tubers identified lines with improved resistance to *E. carotovora* and *Rs*. Western analyses showed high levels of expression of the magainin D peptide in these resistant lines. Li et al. [85] have reported disease resistance, to both a fungal and a bacterial pathogen, conferred by the expression of a magainin analogue, Myp30, in transgenic potato. Another analogue MSI-99, when expressed in tobacco via chloroplast transformation conferred both in vitro and in planta resistance to plant pathogenic bacteria and fungi. However, some amount of growth retardation was also reported [30]. Recently, MSI-99 was also expressed in two important Indian potato cultivars, Kufri Bahar and Kufri Jyoti resulting in increased resistance to

fungal and bacterial diseases [50]. However, more studies need to be done in transgenic potato expressing analogues of meganins, on their expression strategy, mode of action and site of accumulation, etc., so as to use them efficiently.

Cecropins

These are probably the best-known antibacterial peptides of insect origin. These are synthesised in lipid bodies and accumulate in the haemolymph of the giant silkmoth (*Hyalophora cecropia*), the silkworm (*Bombyx mori*) and fruitfly (*Drosophila melanogaster*) in response to infection. These short, linear peptides (31–39 amino acids) interact with the outer phospholipid membranes of both Gram-negative and Gram-positive bacteria and modify them by forming a large number of transient ion channels [35]. Native CB (cecropin B), mutant (SB37 = 38 aa, MB39 = 39 aa) and synthetic (Shiva-1 peptide = 38 aa, D4E1 = 17 aa) cecropins are active in vitro against a wide range of plant pathogenic Gram-negative bacteria [115] including *E. amylovora*, *E. carotovora* subsp. *carotovora* and *atroseptica*, *E. chrysanthemi*, *P. syringae* (several pathovars), *R. solanacearum* and *X. campestris* (several pathovars) [120]. They exert no toxicity at bactericidal concentration to cultured cells or protoplasts of several plant species [115, 120]. Therefore, these are considered as potential candidates to protect plants against bacterial pathogens. Transgenic tobacco plants expressing cecropin B (MB 39) had increased resistance to *P. syringae* pv. *tabaci*, the cause of tobacco wildfire [64]. Synthetic lytic peptide analogues, Shiva-1 and SB-37, produced from transgenes in potato plants reduced bacterial infection caused by *E. carotovora* subsp. *atroseptica* in transgenic potato plants [8]. The Shiva gene in tobacco has been reported to confer resistance to *Rs* pv. *tabaci* [162], whereas the SB37 gene has shown activity in potato to *E. carotovora* subsp. *atroseptica* [8]. Transgenic apple expressing the SB-37 lytic peptide analogue showed increased resistance to *E. amylovora*, pathogen for fire blight, in field tests [110]. A high degree of resistance to *E. carotovora* was also observed in potato tubers expressing the 34-aa chimaeric peptide MsrA1, which contains a C-terminal eight-aa segment from cecropin A and an N-terminal 16-aa segment of melittin and a 26-aa antibacterial peptide from bee venom [114]. The expression of the D4E1 in poplar has resulted resistance to *A. tumefaciens* and *X. populi* [98]. Sessitsch et al. [134] found that the expression of cecropin B in transgenic potato plants gave a resistance to *Rs* and had no negative affect on the other beneficial organisms in the rhizosphere. They also found an unintentional beneficial effect against non-target organisms in tomato [115].

Attacins

In response to bacterial infection, insects that synthesize sarcotoxins also produce attacins, which belong to another family of six 20-kDa antibacterial proteins. Attacins alter the structure and permeability of prokaryotic membranes by binding to lipopolysaccharide in the bacterial envelope and inhibiting the synthesis of the outermembrane proteins [17]. Attacin expressed in transgenic potato enhanced its resistance to bacterial infection by *E. carotovora* subsp. *atrospetica* [8]. Transgenic pear and apple-expressing attacin genes had significantly enhanced resistance to *E. amylovora* in in vitro and greenhouse [78]. In field tests, reduction of fire blight disease has been observed in transgenic apples expressing attacin genes [110]. Transgenic apple expressing attacin targeted to the inter-cellular space, where *E. amylovora* multiplies before infection, had significantly reduced fire blight, even in apple plants with low attacin production levels [78]. A number of distinct, antibacterial peptides or proteins have been described in other insects including apidaecins from honeybees [22] and moricin from silkworm [56], but there are no reports of these being used in plants.

Lysozymes

Lysozymes are a ubiquitous family of enzymes that occur in many tissues and secretions of humans, animals, as well as in plants, bacteria and phage. Lysozyme attacks the murein layer of bacterial peptidoglycan resulting in cell wall weakening and eventually leading to lysis of both Gram-negative and Gram-positive bacteria [62]. Hen egg-white lysozyme (HEWL), T4 lysozyme (T4L), T7 lysozyme [64], human and bovine lysozyme genes have been cloned and transferred to enhance bacterial or fungal resistance in plants. The lysozyme genes have been used to confer resistance against plant pathogenic bacteria in transgenic tobacco plants [150]. T4L, from the T4-bacteriophage, has been reported to enhance resistance of transgenic potato against *E. carotovora*, which causes bacterial soft rot [36]. Transgenic apple plants with the T4L gene showed significant resistance to fire blight infection [78]. High extracellular secretion of HEWL in transgenic tobacco resulted in growth inhibition of *Clavibacter michiganense* and *Micrococcus luteus* [150] in laboratory assays. Potato plants transformed with the HEWL gene showed increased resistance to *E. carotovora* subsp. *atroseptica*, and the level of resistance correlated with the level of transgene expression in the nine lines tested [133]. Human lysozyme transgenes have conferred disease resistance in tobacco through the inhibition of fungal and bacterial growth, suggesting the possible use of the human lysozyme gene for controlling plant disease

[109]. There is evidence of efficacy of bovine lysozyme isozyme c2 (BVLZ) transgene against a variety of *X. campestris* strains in both monocotyledon and dicotyledon crops including tomato, tobacco, rice and potato [101]. Since this bactericidal transgene has been shown to function in monocots and has clear efficacy against at least several strains of *X. campestris*, its usefulness as a transgene for resistance to *X. campestris* in *Musa* has a high probability of success. Interestingly, the effect of T4 lysozyme expressing transgenic potato plants on non-target bacteria in the rhizosphere has been intensively studied. According to one standpoint, the T4 lysozyme was secreted from plant roots and was toxic in vitro to both Gram-negative and Gram-positive bacteria [31]. In contrast, in a field trial of transgenic potato, no negative influence was found on the genetic make-up of antagonistic and plant-associated soil bacteria [93]. It can be concluded that in plants a lysozyme might confer resistance to leaf pathogens without a major influence on soil bacteria.

Thionins

Thionins are the best characterized plant antimicrobial proteins, which are able to inhibit a broad range of pathogenic bacteria in vitro [42] and other phytopathogens [12, 19]. Barley μ-thionin gene in transgenic tobacco enhanced resistance to two pathovars of *P. syringae* in laboratory assays [19]. Yuan et al. [164] expressed thionin *Thi2.1* gene from *A. thaliana* in tomato. The transgenics showed high resistance against bacterial wilt and Fusarial wilt. Unfortunately, most thionins can be toxic to animal and plant cells and thus may not be ideal for developing transgenic plants [21]. Another class of antimicrobial peptides called fabatins, a defensin-type pseudothionin from potato that are active against both Gram-negative and Gram-positive bacteria [103], may be more suitable for this purpose. Based on the first positive results obtained in transgenic tobacco against *P. syringae* pv. *tabaci* [102], lipid transfer proteins and snakins may be good candidates for use against some plant pathogenic bacteria.

Expression of Plant Defence Genes

Plants have their own networks of defence against plant pathogens that include a vast array of proteins and other organic molecules produced before infection or during pathogen attack. Recombinant DNA technology allows the enhancement of inherent plant responses against a pathogen by either using single dominant resistance genes not normally present in the susceptible plant or by choosing plant genes that intensify or trigger the expressions of existing defence mechanisms [125]. In the innate immune response, only one peptide, i.e. defensin has been shown so far to be highly conserved among plants, invertebrates and vertebrates. It was hypothesized that defensins from these different eukaryotic kingdoms arose from a common ancestral gene [147]. Defensins were first identified as a family of peptides in rabbits [131] and subsequently in other higher vertebrate species, including humans. Earlier defensin genes were identified and isolated from larger organisms, but Mygind [105] was the first to isolate the defensin from a fungus, i.e. plectasin from the saprophyte *Pseudoplectania nigrella* (commonly known as ebony cup). Till now, three types of defensins have been characterised namely α-, β- and δ-defensins. These have antimicrobial activity against some Gram-positive and Gram-negative bacteria. Harder et al. [57] reported the antimicrobial activity of defensins against yeast. However, mode of action of these antimicrobial defensins is still not clear. In the model for the antimicrobial mechanism proposed by Hoover et al. [63], defensin molecules form positively charged octomers that neutralise the anionic lipid head groups of bacterial membrane. This neutralization disrupts the integrity of the lipid bilayer, causing membrane permiabilization. As such, plant defensins are not only structurally homologous to β-class of human defensins but also to the insect defensin-like peptide heliomicin [82]. Moreover, several research groups have reported an increased resistance to fungal diseases in plants by overexpressing different types of plant defensins [51, 86]. A similar protective effect has been reported for plants expressing plant defensin-like proteins from insects [83]. An et al. [7] expressed the human β-defensin gene in *A. thaliana* to obtain resistance to bacterial and fungal diseases. Several enteric β-defensin genes have been identified and characterised like from bovine neutrophils and macrophages [131]. All characterised β-defensins have broad-spectrum antimicrobial activity [131]. Enteric β-Defencin (EBD) gene isolated and characterised from murine, which was reported to have the antibacterial properties [3], was used against the potato bacterial wilt (S. K. Chakrabarti, personal communication). The transgenic potato plants expressing EBD gene have shown higher levels of resistance against BW both in vitro and in glass house trials. A two-component system (Barnase and Barstar), based on the expression of a bacterial ribonuclease gene (*barnase*) driven by a pathogen-inducible promoter (*prp1-1*) and an inhibitor of barnase (*barstar*) driven by a constitutive promoter, to avoid deleterious effects of background activity of *barnase* in uninfected tissue, has been used successfully to protect transgenic potato plants from the fungus *P. infestans* (the causal agent of potato late blight) [143]. A similar strategy could be developed against bacterial diseases if specific promoters are made available.

Plant Resistance (*R*) Genes

Plant disease resistance genes are an agriculturally important class of genes that are increasingly well characterised at molecular level. These include *R* genes that mediate resistance to bacterial, fungal, viral and nematode pathogens. To date, several *R* genes and QTLs conferring resistance to bacterial diseases have been cloned [33, 140, 146]. Many *R* genes against *Rs* have also been identified from various plant species, including *RRS1* gene from *A. thaliana* [33], *AP1* gene from potato [41] and QTL *BW_1*, *BW_3*, *BW_4*, *BW_5*, *Bwr_3*, *Bwr_4*, *Bwr_6* and *Bwr_8* from tomato [18]. Basically, all these genes seem to encode components of receptor systems and form part of a signal transduction pathway, which triggers general defence reactions such as reinforcement of the cell wall, synthesis of phytoalexins and oxidation of phenolic compounds, activation of defence-related genes and the HR. Proteins such as iron ABC transporter and ferredoxin-I protein were speculated for bacterial wilt resistance [1]. Xa21 gene is member of rice family that provides broad spectrum *Xanthomonas* resistance in rice. Resistance genes from monocots and dicots are highly conserved, suggesting their common functional domains [140]. Kinase activity of the Xa21 is very important for full resistance and it had been reported in orange for the production of resistance against bacterial canker [97]. Very recently, Xa21 gene was over expressed in transgenic tomato, which resulted in very high resistance against the *Rs* [1]. Berrocal et al. [11] expressed *snakin1* and *snakin2* in potato and reported that expression of these genes increased the resistance to BW. Very recently, Li et al. [87] have identified several genes by Suppression Subtractive Hybridization (SSH) and microarray techniques in potato which give resistance to *Rs*. *STA51*, *STC84* and *STD62* are reported to be major genes involved in resistance along with *StSN2* (STM21). These genes are reported to be involved in pathogen recognition, signal transduction, transcription factor functioning, HR (hyper sensitive reaction), SAR (systemic acquired resistance), etc. As discussed above, bacterial wilt resistance in potato is a complex trait (quantitative) and involve as many as 60–70 resistance (*R*) genes and most of these genes are highly conserved among the plant species [87]. This hypothesis was given credence when the first cloned disease resistance gene *Pto* (a tomato resistance gene against *P. syringae* pv. *tomato*) was found to function both in *Nicotiana tabacum* and *N. benthamiana* [125] suggesting that disease resistance functions are conserved in a wide range of plant species. Many of these *R* gene products share structural motifs, which indicate that disease resistance to diverse pathogens may operate through similar pathways. Pathogen *Avr* genes undergo some times strong diversifying selection pressure to avoid recognition by the host. To overcome this problem, *R* gene pyramiding approach is being used, e.g. 4 different *R* genes conferring resistance to bacterial blight in rice have been incorporated together [85]. The *Pto* gene encodes a serine/threonine protein kinase that confers resistance in tomato to *P. syringae* pv. *tomato* strains that express the type III effecter protein AvrPto [76]. Overexpression of *Pto* in tomato under control of the cauliflower mosaic virus (CaMV) 35S promoter has been shown to activate defence responses in the absence of pathogen inoculation. *Pto*-overexpressing plants show resistance not only to *P. syringae* pv. *tomato* but also to *X. campestris* pv. *vesicatoria* and to the fungal pathogen *Cladosporium fulvum* [106]. Therefore, *Pto* genes are considered as potential candidates to protect plants against pathogens. Caffeoyl CoA 3-O-methyltransferase (*CCoAOMT*) gene from tomato confers resistance to *Rs* by increasing the lignin deposition [99]. The *Bs2* resistance gene of pepper specifically recognizes and confers resistance to strains of *X. campestris* pv. *vesicatoria* that contains the corresponding bacterial avirulence gene, *avrBs2* [146]. Transgenic tomato plants expressing the pepper *Bs2* gene suppress the growth of *Xcv*. The *Bs2* gene is a member of the nucleotide binding site-leucine-rich repeat (NBS-LRR) class of *R* genes. Some of the ROS (reactive oxygen species) genes also have been known to induce the bacterial resistance in plants [155]. The production of large amounts of hydrogen peroxide has been induced in transgenic potato plants by the expression of a glucose oxidase (*GO*) gene from *Aspergillus niger* [160] that resulted in an increased level of resistance to *E. carotovora*. Indeed, even though GO was produced constitutively and extracellularly, a significant increase in the hydrogen peroxide level was only detected following bacterial infection. Over expression of PPO (Polyphenol oxidase enzymes) in tomato leads to a significant increase in resistance to *P. syringae* pv. *tomato* in compatible interactions [84]. EF-Tu receptor (EFR) gene which is thought to be highly conserved among the prokaryotes, if expressed in plants would increase resistance by activating the PAMP triggered immunity in the plants against all prokaryotes. Very recently, a synthetic EFR (*elf18*) was expressed in transgenic tomato and tobacco and very high broad spectrum resistance was obtained against all prokaryotic pathogens [5]. The problem associated with R genes is potential fitness cost and pleiotropic effect associated with their introduction. This fitness reduction could be bigger problem for farmers than the disease for which the plant is resistant [5]. Researchers are looking for the solution to overcome this problem.

Conclusions

Control of potato brown rot has proven to be very difficult and puzzling task. Although a number of sources of

resistance to BW have been reported, resistance breeding has only been reasonably successful in tropical crops like eggplant, tomato, peanut, pepper and to a very small extent potato in South America and Asia. Further, the resistant cultivars developed are not adapted to different agroclimatical zones and are not effective against all strains of the pathogen. Hence, development of transgenics, containing genes imparting stable resistance, seems to be the only effective alternative at present.

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