Conventional breeding for resistance to whitefly-transmitted geminiviruses

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Abstract

The whitefly *Bemisia tabaci* Genn. is a pervasive pest and vector of plant viruses. The existence of *B. tabaci* biotypes and numerous whitefly-transmitted geminiviruses (begomoviruses) affecting food and industrial crops, has become a major constraint to agricultural development in tropical and subtropical regions of the world. The predominant whitefly and begomovirus method of control has been the application of insecticides. The excessive use of agrochemicals over the past decades, has resulted in an exponential increase in *B. tabaci* populations and incidence of begomoviruses transmitted by this whitefly vector. Under these conditions, biological and integrated whitefly/geminivirus control practices have not met expectations. Incorporating begomovirus resistance in a relatively small number of crops improved by conventional plant breeding methods has been a sustainable and efficient disease control strategy. This review discusses some of the conventional intra- and inter-specific hybridization strategies followed to incorporate genetic resistance to begomoviruses in three major crops: cassava, common bean and tomato.

1. Introduction

The whitefly *Bemisia tabaci* Genn. (Homoptera:Aleyrodidae) is one of the main pests of the 20th century. Since the 1950s, *B. tabaci* has caused significant crop losses in tropical and subtropical agricultural regions in the five continents of the world (Brown, 1994). *B. tabaci*, the sweet potato, tobacco or cotton whitefly, was originally described in Greece, in 1889 (Gennadius, 1889). In tropical/subtropical environments, *B. tabaci* can produce an average of 15 generations in one year, with females depositing an

average of 200 eggs in a 3-6 weeks lifespan. Following a brief "crawler" stage and four subsequent sessile instars, winged adults emerge, which may cause direct damage (plant nutrient loss; physiological disorders; honeydew excretions, etc) or act as virus vectors (Brown, 1994). *B. tabaci* is a polyphagous whitefly that colonizes mostly annual, herbaceous plant species numbering over 500 in 74 families (Mound and Halsey, 1978; Brown and Bird, 1992). *B. tabaci* is an efficient vector of numerous geminiviruses. These viruses consist of two quasi-isometric 'twined' or 'geminate' particles, encapsidating one or two single-stranded DNA genomes. Geminiviruses transmitted by *B. tabaci* belong to the genus *Begomovirus* (sigla for the type species, ***Be****an* ***go****lden* ***mo****saic* ***virus***), according to the latest taxonomic classification (Murphy et al., 1995; Regenmortel et al., 2000). These viruses are highly 'plastic', being able to adapt to a large number of different cultivated plant species, following their transmission by *B. tabaci* from wild or other cultivated hosts (Padidam et al., 1999). Currently, over 100 begomoviruses are known to be transmitted by at least two biotypes of the whitefly *B. tabaci*, to more than 20 different cultivated species of socioeconomic importance. Some of the main crops affected by whitefly-transmitted geminiviruses are: common bean, mung bean, blackgram, lima bean, soybean, cowpea, tomato, potato, eggplant, pepper, chili peppers, melon, watermelon, squash, okra, cassava, cotton, and papaya (Muniyapa, 1980; Brown, 1994).

2. Controlling *B. tabaci*-transmitted geminiviruses

The extreme pathogenicity, virulence and severe yield losses caused by begomoviruses in susceptible plant species, and absence of immune cultivars in most of the commercial plant species attacked, has forced farmers and agronomists to resort to other pest and disease control methods. Considering that begomoviruses are not controlled by agrochemicals, it is understandable that their vector, *B. tabaci*, has become the target of most pest management strategies deployed so far around the world. Unfortunately, the most widely used practice for controlling whiteflies has been the application of insecticides, often at doses exceeding the recommended formulations (F. J. Morales, *personal observation*). As a result, *B. tabaci* has developed resistance to most of the insecticides developed to date. Furthermore, biological control practices are ineffective under these high pesticide-input conditions, and pesticide contamination of agricultural produce and the environment have greatly increased (Traboulsi, 1994).

3. Conventional breeding for begomovirus resistance

Despite the unavailability of immune cultivars observed for the majority of the commercial crops affected by *B. tabaci*-transmitted geminiviruses, breeding for disease resistance has proven the most complementary and sustainable of the integrated whitefly/begomovirus control methods implemented to date.

3.1. Conventional breeding for resistance to African cassava mosaic virus

Cassava (*Manihot esculenta* Crantz) is one of the earliest crops to be bred for resistance to an important group of distinct but related begomoviruses, collectively known as *African cassava mosaic virus* (ACMV). ACMV is transmitted by the whitefly *B. tabaci*, but the main method of dissemination is through the vegetative propagation of ACMV-infected cuttings (Swanson and Harrison, 1994).

The search for resistance to African cassava mosaic viruses began in East Africa in the 1920s. Initially, several ACMV-tolerant cultivars were identified in large cassava germplasm collections evaluated in Madagascar and Tanzania (Jennings, 1994). Cultivars such as Bouquet de la Reunion, Java 12/28, and Criolina, in Madagascar; and Mpezaze, Msitu, Aipin Valencia, F 100, and F 279, in Uganda, helped stop the ravages of ACMV (Cours-Darne, 1968). Higher resistance levels were achieved following intra-specific crosses, such as the case of the highly tolerant selection 37244E, obtained from a cross between F 100 and Mepezaze. Cours (1951) associated mosaic incidence with some phenotypic traits, such as color of the stigma and the root bark, and the fertility of the male flowers. He suggested that selection for red stigmas, grey root bark, and fertile male flowers would lead to higher levels of resistance to ACMV.

Despite the initial successes in achieving higher levels of ACMV tolerance or resistance (*sensu lato*), cassava breeders were still hoping to find a source of ACMV immunity. To this end, they made inter-specific crosses using *Manihot glaziovii* (Ceara rubber), *M. dichotoma* (Jaquie Manicoba rubber), *M. catingae*, and a genotype called “tree cassava” (probably a natural *M. esculenta* X *M. glaziovii* hybrid), in search for higher levels of cassava mosaic resistance (Jennings, 1957). The hybrids obtained in the first generation of *M. glaziovii* X *M. esculenta* crosses produced in 1937, had non-tuberous roots and became

infected by ACMV under field conditions. Backcrossing was necessary to recover the main agronomic traits found in *M. esculenta*, particularly root quality. Resistance to ACMV improved slightly during the three generations of backcrosses; probably due to the use of *M. esculenta* parents with intermediate levels of ACMV resistance.

Crosses with *M. dichotoma* were made at Amani, Tanzania, in the same year. 32 F_1 hybrids remained symptomless for 22 months, but lacked vigor and restoring their root quality proved more difficult than in the case of *M. glaziovii*. Following three generations of backcrossing, the resulting materials had acceptable agronomic characteristics, but no higher levels of ACMV-resistance than the *M. glaziovii* X *M. esculenta* hybrids. The *M. dichotoma* X *M. esculenta* hybrids were not selected for further breeding work. Hybrids with the "tree cassava" were similar to *M. glaziovii* X *M. esculenta* hybrids, and only a few selections were made.

Unfortunately, the high expectations for the *M. glaziovii* X *M. esculenta* hybrids, were realized in some but not all regions of East Africa. For instance, a high proportion of the hybrid cassava lines selected, succumbed to ACMV in the coastal areas of Kenya. Understandably, after three backcrosses, the average proportion of *M. glaziovii* genes in the progenies, would have been reduced to 1/16. Moreover, resistance to ACMV appeared to be multigenic and recessive. Thus, the expression of *M. glaziovii* genes was probably low, and the resistance achieved was the result of the accumulation of genes from moderately resistant *M. esculenta* parents used for backcrossing. Later, some of the most resistant backcross hybrids were intercrossed to concentrate genes for resistance, which may have become dispersed among the various breeding lines, as well as to increase the levels of homozygosity of recessive resistant genes. However, there are different ACMV strains and, more important, distinct viral species in what was originally considered as *African cassava mosaic virus* (Hong et al., 1993). The existence of different virus species, thus, might have also contributed to the differential reaction of the tolerant lines selected in Tanzania to the ACMV variant found in the coastal areas of Kenya.

In Ivory Coast, the four cassava cultivars showing the highest degree of resistance under severe ACMV pressure, were: Aipin Valenca-19 from Brazil, Garimoshi from India, Mwakasanga-13 from Kenya, and the inter-specific hybrid 5318/34-12 from crosses made in East Africa. Most South American cultivars evaluated in Ivory Coast proved susceptible, which has been explained by their lack of co-evolution with

ACMV. However, some South American cultivars have shown adequate levels of resistance to ACMV in West Africa (Fargette et al., 1996), which suggests that additional introductions from South America should be evaluated for ACMV resistance.

3.2. Conventional breeding for resistance to begomoviruses infecting common bean

Since the middle of the 20th century, different geminiviruses transmitted by *B. tabaci* have been reported to attack common bean (*Phaseolus vulgaris* L.) in the Americas (Morales, 2000). *Bean golden mosaic virus* (BGMV) was first noticed in 1961, in Brazil (Costa, 1965). This virus became the most limiting problem of common bean production in Brazil, Argentina and Bolivia, within the next two decades, due to the exponential expansion of soybean, a preferred breeding host for *B. tabaci* in the region. A similar begomovirus, originally thought to be BGMV, but now recognized as *Bean golden yellow mosaic virus* (BGYMV), infects common bean in southern Mexico, Central America and the Caribbean region (Morales, 2000). *Bean dwarf mosaic virus* (BDMV), first described in Brazil (Costa, 1965), became economically important around 1980, when it caused the loss of over 40,000 hectares of common bean in northwestern Argentina (Morales, 2000). *Bean calico mosaic virus* (BCaMV) is a geminivirus transmitted by *B. tabaci* to common bean in northwestern Mexico (Brown and Bird, 1992). This begomovirus was initially thought to be BGMV, but it was later shown to be a distinct virus species related to *Squash leaf curl virus* (Loniello et al., 1992). BCaMV caused widespread epidemics in common bean plantings in the states of Sinaloa and Sonora, Mexico (Morales, 2000).

Initial attempts to breed common bean for BGMV resistance in Brazil were disappointing. Pompeu and Krantz (1977) initially selected symptomless individual plants within field populations of three common bean cultivars: Rosinha G2, Aetê 1, and Carioca 99, under natural BGMV pressure. However, the selected lines were shown to be susceptible to the virus in subsequent evaluations (Costa, 1987). Some of these selections, namely Rosinha G2/69 and an individual selection of Carioca 99, were used in subsequent breeding programs. This observation demonstrates the limited value of selecting individual plants within susceptible plant populations, where some susceptible plants usually escape virus infection despite high *B. tabaci*/begomovirus pressure. Another plant improvement strategy pursued in Brazil was the use of

radiation to create genotypic mutants resistant to BGMV (Tulman-Neto, 1979). One of these common bean mutants, TMD-1, showed partial resistance to the virus but its yielding ability was poor, and its use in conventional breeding programs did not produce any outstanding progenies.

A parallel breeding project was initiated in Guatemala, in 1974, to solve the bean golden yellow mosaic problem in Central America, Mexico and the Caribbean. This project was financed by the Rockefeller Foundation, USAID, UNDP and, later on, by the Swiss Development Agency (COSUDE). Approximately, 7,000 germplasm bank accessions of common bean were evaluated under natural disease pressure in southeastern Guatemala, but no immune genotypes were observed. Among these accessions, a group of black-seeded genotypes, namely Turrialba 1, Porrillo 70, Porrillo Sintetico, ICA-Pijao and ICA-Tui, was selected for their better performance under natural BGYMV pressure. Due to their tolerance (acceptable yielding ability despite expressing noticeable foliar yellowing) Porrillo Sintetico and ICA-Pijao were ultimately selected, together with Turrialba 1, as potential parental materials (Yoshii et al., 1979). The best lines derived from different crossed between the selected parental genotypes: DOR 41 (Porrillo Sintético X ICA-Pijao), DOR 42 (ICA-Pijao X Turrialba 1) and DOR 44 (sister line from the cross ICA-Pijao X Turrialba 1), were soon released in Guatemala as cultivars ICTA-Quetzal, ICTA-Jutiapan and ICTA-Tamazulapa, respectively (Yoshii *et al*., 1980). In the absence of pesticide applications, ICTA-Jutiapan, ICA-Pijao and the local black-seeded cultivar Rabia de Gato, sustained yield losses of 38%, 53% and 86%, respectively.

Despite initial successes in developing BGYMV-resistant common bean genotypes, two constraints remained. First, yield losses for the DOR lines fluctuated proportionally with viruliferous *B. tabaci* populations. Second, there was no progress in breeding for BGYMV resistance in red-seeded common bean cultivars. Red-seeded beans are in great demand in Costa Rica, Nicaragua, Honduras, and El Salvador (Morales, 2000). This situation persisted for some years, with only some agronomic improvements to the first generation of black-seeded DOR lines, such as the recovery of 'earliness' in ICTA-Ostua (still grown in Guatemala) and Negro Huasteco-81, released in the Gulf region of southern Mexico (Yoshii, 1982).

A serendipitous event in the mid 1980s, changed this situation. A common bean line improved at CIAT, Colombia, for upright architecture (A 429), showed an unexpected high level of BGYMV resistance under field conditions in Guatemala. Evaluation of the parental materials originally selected to produce A 429,

did not reveal genotypes possessing a high level of resistance to BGYMV. However, one parent, a common bean genotype belonging to the Mexican Durango race (Singh et al., 1991), did not react with the characteristic yellowing when inoculated with BGYMV, despite being systemically affected by plant malformation and flower abortion caused by the virus. This Mexican common bean genotype, called Garrapato, and another parent of A 429, the Mesoamerican black-seeded cultivar Porrillo Sintetico, were associated with the high level of BGYMV resistance found in A 429 (Morales and Niessen, 1988). A 429 soon became one of the most widely used sources of begomovirus resistance in common bean breeding programs in Latin America (Singh et al., 2000). The gene *bgm-1* was shown to condition mosaic resistance in Garrapato (Morales and Niessen, 1988; Blair and Beaver, 1993a).

Later, a red kidney line, DOR 303, was also selected for its high level of BGYMV resistance under field conditions. An evaluation of the parental materials selected to produce this line, revealed the presence of Red Kloud, a red kidney genotype of Andean (race Nueva Granada) origin (Singh et al.,1991), besides the traditional black-seeded source of resistance, Porrillo Sintetico. In subsequent tests, Red Kloud was shown to be tolerant to BGYMV, producing flowers and pods despite striking mosaic/yellowing foliar symptoms (Morales and Niessen, 1988). Porrillo Sintetico has considerable vigor, which often allows plants to escape infection, particularly when infection occurs after the first 2-3 weeks following emergence of the plants (Morales and Niessen, 1988). Thus, a second favorable interracial recombination of Mesoamerican and Andean genes produced a red-seeded common bean genotype possessing high levels of BGYMV resistance. The BGYMV-resistance gene in DOR 303 was later identified as *bgm-2* (Velez et al., 1998). Some Andean common bean genotypes also possess genes for resistance to the severe pod malformation induced by BGYMV in susceptible cultivars (Morales and Niessen, 1988). Molina and Beaver (1998) reported the presence of a dominant gene, *Bgp*, responsible for this trait, but, which seemed to require the presence of *bgm-1* for expression.

Based on the above findings, an extensive search for new sources of resistance was launched using the common bean collection maintained at CIAT. A selection of diverse grain types was evaluated in different countries of Latin America, from Argentina to northern Mexico, to identify different mechanisms of virus resistance and sources of resistance to begomoviruses infecting common bean in this region. At least 10 new sources of resistance were identified in the *P. vulgaris* accessions possessing grain colors different

than black. The most interesting bean begomovirus-resistance mechanisms were disease escape, low mosaic expression, hypersensitivity, low flower abortion, and low pod malformation (Morales and Niessen, 1988). The general combining ability of these traits was highly significant ($P<0.01$) and greater than values for specific combining abilities, suggesting that selection for the various traits was possible in true breeding lines, due to significant additive genetic variance (Morales and Singh, 1991). In subsequent studies, 83 recombinant inbred lines (RIL) selected from a population generated from the cross between a Mexican (Pinto UI 114) and a Mesoamerican (ICA-Pijao) common bean genotypes, were evaluated for their reaction to BGYMV. Of these lines, 11 did not show symptoms, 24 lines had mean disease incidence of 8%, 28 lines had a disease incidence of 26.6% and developed intermediate mosaic symptoms, and 20 lines were more susceptible than either of the parents. Thus, values for the 83 RILs transgressed the reactions observed for the two parents, showing both higher and lower levels of disease incidence and mosaic expression. These results suggested that the BGYMV-resistance genes in the two parental genotypes were different and complementary to each other and, consequently, that gene pyramiding might be a viable breeding strategy. Subsequent interracial crosses produced highly resistant lines, including DOR 482 (Don Silvio), Tio Canela 75, and Turbo III., which have become cultivars in different countries of Central America. Begomovirus replication in these improved genotypes was highly restricted according to nucleic acid hybridization tests performed on these line (Morales, 2000). This type of resistance has also been associated to quantitative traits (QTLs), which reduce symptom expression (Miklas et al., 1996). On the contrary, common bean genotypes derived from intraracial populations (*e.g.* DOR 41, DOR 390, DOR 500) usually behave as moderately resistant under severe whitefly/virus pressure (Singh, et al., 2000).

Some of the sources of resistance to BGMV and BGYMV identified in *P. vulgaris* are also effective against distantly related begomoviruses of common bean. For instance, Azufrado Higuera is a new cultivar developed from Nueva Granada (Andean) sources of resistance originally identified in South America, released in northwestern Mexico to control *Bean calico mosaic virus* (Morales, 2000).

3.3. Conventional breeding for resistance to tomato begomoviruses

The boom of non-traditional export crops has taken place in most of the agricultural regions affected by whitefly-transmitted geminiviruses around the world. One of the crops predominant these regions is tomato (*Lycopersicon esculentum* Mill.). This is a highly profitable but costly crop due to the great amount of chemical inputs usually required to protect tomato from the various pests and diseases that attack this crop. The well documented abuse of pesticides associated with tomato production, has greatly contributed to development of pesticide-resistant *B. tabaci* populations capable of vectoring over 20 different begomoviruses that attack tomato in tropical and subtropical regions of the world (Polston and Anderson, 1997; Zeidan et al., 1999).

Although most of the begomoviruses that attack tomato are found in the New World, relatively little breeding work has been done to minimize the severe damage that these whitefly-transmitted geminiviruses cause to tomato plantings in this region. Moreover, despite its tropical American origin, most of the tomato breeding work has been conducted in temperate countries. Hence, tomato growers in tropical America have relied mainly on pesticides and imported tomato varieties and hybrids resistant to Old World begomoviruses, to control *B. tabaci* and the begomoviruses that infect tomato in the Americas.

The situation in the Old World is different due to the severe damage caused by a group of begomoviruses collectively referred to as *Tomato yellow leaf curl virus* (TYLCV) in the Mediterranean region, the Middle East, north Africa, central Africa and southeast Asia (Czosnek and Laterrot, 1997). One of these TYLCV variants accidentally introduced in the last decade into the Americas (Nakhla et al., 1994; Polston et al., 1994), where it has already caused millions of dollars worth of industrial and fresh tomato production losses.

Early efforts to identify sources of resistance to TYLCV within *L. esculentum,* only revealed the existence of some moderately resistant or tolerant genotypes (Cohen and Harpaz, 1964; Nitzany, 1975; Abu-Gharbieh et al., 1978). However, Cohen and Nitzany (1966) observed that some wild relatives of tomato, namely *L. pimpinellifolium* and *L. peruvianum,* possessed a higher level of resistance to TYLCV, although they were not immune. Crosses between *L. esculentum* and *L. pimpinellifolium* (currant tomato/accession LA 121) and genetic analyses of F_{1-3} and backcross generations, indicated the existence

of incomplete dominance of resistance over susceptibility, suggesting a monogenic control of resistance (Pilowski and Cohen, 1974). A dominant gene, *Tylc*, was later proposed for the resistance gene in *L. pimpinellifolium* (Kasrawi, 1989). The progenies derived from this cross showed only moderate symptoms, but their yield was markedly reduced. Nevertheless, among the *Lycopersicon* species, *L. pimpinellifolium* is one of the most compatible for crossing with *L. esculentum* (Picó et al., 1996).

In contrast, the inheritance of tolerance to TYLCV in *L. peruvianum* (PI 126935) is controlled by five recessive factors, according to Pilowski and Cohen (1990). This breeding program initiated in 1977, released the commercial hybrid TY-20 in 1988. This hybrid delays symptom expression and viral DNA accumulation in infected plants, resulting in acceptable yields (Pilowski and Cohen, 1990). Other tolerant/resistant TY-lines generated by this breeding program are: TY172, TY197, TY198, and TY536 (Lapidot et al., 1997; Friedmann et al., 1998).

In 1991, other wild tomato species: *L. chilense* and *L. hirsutum*, besides *L. peruvianum* and *L. pimpinellifolium* were examined for viral DNA and symptom expression following inoculation with TYLCV. Approximately 85 days after inoculation, all inoculated species were infected and had detectable levels of viral DNA, but *L. chilense* and *L. hirsutum* remained symptomless and with low levels of viral DNA (Zakay et al., 1991). The TYLCV resistance gene in *L. chilense* was identified as *Ty-1* (Michelson et al., 1994). Resistance to this virus in *L. hirsutum* was dominant and controlled by more than one gene (Mazyad et al., 1982). *L. hirsutum* was crossed with *L. esculentum*, yielding tolerant and immune lines. One immune line was crossed with *L. esculentum* to produce the hybrid FAVI-9 or line F1-901. The immune reaction was associated with 2-3 additive genes (Vidavski and Czosnek, 1998). Another promising species evaluated for TYLCV resistance, *L. cheesmanii*, possesses recessive resistance to TYLCV. Breeding projects in the Mediterranean region have also selected *L. cheesmani, L. peruvianum* and *L. pimpinellifolium* to control TYLCV in this region (Laterrot, 1990, 1992, Laterrot and Moretti, 1996). Some of the TYLCV-resistant lines obtained from this project are Pimpertylc-J-13 and Chepertylc-92.

Interespecific hybrids obtained from crosses between *L. pimpinellifolium, L. peruvianum,* and *L. hirsutum*, show transgressive segregation for their reaction to TYLCV, suggesting that different but complementary genes condition resistance (Kasrawi and Mansour, 1994).

Muniyapa and coworkers (1991) reported that lines of *L. hirsutum* and *L. peruvianum* were resistant to another tomato geminivirus: *Tomato leaf curl virus* (ToLCV). The resistance mechanism in these wild species was associated with production of exudates from trichome glands on the leaf surface, in which whiteflies became entrapped (Channarayappa and Shivashankar, 1992). This is one of the few examples for which genetic resistance to a viral disease has been achieved indirectly by incorporating genetic traits against *B. tabaci*. Nevertheless, there is circumstantial evidence showing that different cultivars of common bean and tomato, interact differentially with *B. tabaci*. For instance, In Sinaloa, northwestern Mexico, the common bean cultivar Azufrado Peruano-87, had 16% more nymphs per leaf than the BCaMV-resistant common bean cultivar Azufrado Higuera (Lopez, 1996). Similar data have been obtained for tomato, although *B. tabaci* preference for some tomato cultivars, was not related to virus resistance/susceptibility traits in the tomato cultivars evaluated (Avilés, 1996).

4. Discussion

The crop improvement strategies described above, present interesting similarities and differences worthy of discussion. First, begomoviruses are highly infectious pathogens that cause disease and significant yield loss in most of the susceptible plant species. For example, *Bean golden mosaic virus* can infect more than 20,000 common bean accessions screened thus far (F.J. Morales, *unpublished data*). Despite the existence of tolerant or moderately resistant genotypes of cultivated plant species, plant protection specialists and breeders have been reluctant to use symptomatic genotypes as sources of begomovirus resistance. In the search for begomovirus immunity, significantly higher levels of resistance have been found among the wild relatives of the cultivated plant species. Understandably, plant breeders have used these symptomless, but often susceptible, wild species as sources of begomovirus resistance, in hopes of recovering the commercial characteristics of the susceptible cultivars, through conventional genetic improvement methods, such as backcrossing.

4.1. Cassava

Cassava breeders have apparently achieved higher levels of African cassava mosaic resistance. However, it is not clear whether moderate levels of ACMV-resistance are the result of the backcrossing of hybrid genotypes with moderately resistant *M. esculenta* genotypes, or of the expression of hybrid vigor from the original interspecific crosses with *M. glaziovii*. Moreover, the undesirable agronomic characteristics of these wild relatives, expressed in the resulting ACMV-tolerant genotypes, forced most African farmers to maintain the preferred ACMV-susceptible cassava cultivars, albeit at a low level. Whereas, this practice maybe desirable from the point of view of *in situ* conservation of genetic resources, these highly susceptible land races constitute an important source of ACMV inoculum, as suspected during the recent epidemics of African cassava mosaic in Uganda (Harrison et al., 1997). Finally, it is apparent that there are still some cassava cultivars in Africa (F.J. Morales, *personal observation*) and South America (Fargette et al., 1996), which may be potential sources of ACMV resistance. Until new breeding strategies are implemented, African cassava mosaic may remain "an under-estimated and unsolved problem" (Thresh et al., 1994).

4.2. Common bean

As in the case of cassava, bean breeders and pathologists soon realized, that there was no immunity to whitefly-transmitted geminiviruses in *P. vulgaris*. They were also aware by the late 1970s, that related species, such as *P. coccineus* and *P. acutifolius*, could contribute higher levels of resistance (Yoshii et al., 1979; Yoshii, 1984). However, these *Phaseolus* species belong to secondary or tertiary gene pools and, thus, crossing with *P. vulgaris* often results in embryo abortion (Debouck, 1991; Barcala and Ron, 1996; Dinca and Raducanu, 1997). Moreover, embryo rescue techniques were not used in the early breeding programs, and recovery of commercial grain types in *P. vulgaris*, is still difficult through genetic recombination, particularly from interspecific crosses. Thus, the incorporation of resistance to begomoviruses in *P. vulgaris*, has been primarily intraspecific. Following the discovery of tolerance in

closely related black-seeded genotypes of Mesoamerican origin, in the late 1970s, no further progress seemed possible for almost a decade. Fortunately, the fortuitous discovery of non-black-seeded common bean genotypes possessing high levels of begomovirus resistance, made possible a closer examination of genetic diversity within *P. vulgaris*. Detailed analyses of the effect of begomoviruses on individual yield components in *P. vulgaris*, led to the identification of corresponding resistance traits in different gene pools and races of this legume species (Morales and Niessen, 1988). Subsequently, the genetics of these resistant traits was studied to exploit the general combining ability and additive genetic variance in races of the common bean (Morales and Singh, 1991; 1993). Gene pyramiding has resulted in the development and release of common bean cultivars possessing resistance to BGMV, BGYMV, BCaMV and BDMV during the past 15 years. Currently, with improved plant tissue culture techniques, breeders should be able to expedite the introgression of useful genes from other *Phaseolus* spp., into common bean, including genes for resistance for begomoviruses (Miklas and Santiago, 1996; Bianchini, 1999).

4.3. Tomato

Tomato is a high value crop and, as such, it has had the financial support of the industrial and commercial sectors, and several agricultural research institutions. The urgency to solve the tomato yellow leaf curl problem, led to a relatively rapid and satisfactory introgression of TYLCV-resistance genes from wild relatives. However, this process has not been easy due to the complicated genetics of resistance involved in interspecific hybridization, and agronomic traits that must recovered from susceptible tomato cultivars to satisfy consumer preferences and industrial demands. Perhaps, Vidavski and Czosnek (1998) best summarized the situation of tomatoes and TYLCV as follows. “After more than 25 years of effort, the best cultivars and breeding lines available show tolerance to the virus rather than resistance. Moreover, these tolerant cultivars need to be protected from viruliferous insects with insecticides or nets during the first months after planting”. Although these introductory remarks were used to announce the development of TYLCV-resistant and immune lines from interspecific (*L. esculentum* X *L. peruvianum*) crosses, 25 years of interspecific crossing in tomato has not significantly improved this crop more than the intraspecific breeding approach followed in the case of common bean.

5. Conclusion

Many scientists have implied that begomovirus resistance is largely unavailable for most susceptible crops, probably in reference to the difficulty in identifying 'immune' plant genotypes. It is important to note that 'resistance' is a relative term, which can span a range of disease reactions from a low to a high level of symptom expression. 'Tolerance' is another term which is often misused in reference to symptom expression. This term should be used to refer to the ability of a diseased plant genotype to yield an acceptable or expected quantity or quality of product (*e.g.* seed, fruits, flowers, etc.). Both genetic resistance and tolerance can be found in most cultivated species affected by whitefly-transmitted geminiviruses.

Selection of plant genotypes for begomovirus resistance started in Kenya as early as 1929, where some local cassava cultivars were found to be moderately resistant to ACMV. Further screening of over 100 cassava genotypes did not result in higher levels of ACMV resistance in these introduced materials, relative to the resistance available in local cassava cultivars. Hence, interspecific hybridization was attempted based on the previous successful introgression of mosaic resistance in sugarcane (*Saccharum officinarum*) from *S. spontaneum* (Nichols, 1947). This breeding strategy is still practiced without much effort to exploit all the mechanisms of 'resistance' and/or 'tolerance' that potentially exist in all cultivated plant species. The successful strategy followed to incorporate high levels of begomovirus resistance in common bean, strictly through the intraspecific recombination and pyramiding of different resistance traits found in diverse gene pools of *Phaseolus vulgaris*, confirms the feasibility of this approach. Moreover, the underlying mechanism of resistance (*i.e.* restricted virus multiplication) is similar in the three crops discussed here (Zakay et al., 1991; Fargette et al., 1996; Lapidot et al., 1997; Morales, 2000).

Undoubtedly, there is both direct and circumstantial evidence indicating the existence of adequate genetic variability in the primary and secondary gene pools of most cultivated species. This genetic variability can be exploited within and between cultivated species and their relatives. Interspecific hybridization in cassava, common bean and tomato, can be utilized to breed for resistance to begomoviruses, and other pathogens and pests (Nichols, 1947; Debouck, 1991). For tomato, it is evident that the cultivars improved for TYLCV resistance, also exhibit acceptable levels of resistance to distinct New World begomoviruses

infecting tomato in the Americas and Asia (Muniyapa et al., 1991; Piven et al., 1995). Another neglected but potentially valuable begomovirus control method, is the incorporation of genetic resistance to the whitefly vector, *Bemisia tabaci*, as it has been suggested by limited investigations in cassava (Fargette et al., 1996), common bean (Blair and Beaver, 1993b), and tomato (Channarayappa et al., 1992). Undoubtedly, utilization of the genetic diversity present in the primary and secondary gene pools of these plant species, will require both conventional and advanced crop improvement techniques, such as molecular marker assisted selection (Chavarriaga et al., 1999; Singh et al., 2000; Zamir et al., 1994).

Finally, genetic engineering is the current method of choice to incorporate resistance to plant viruses into commercial crops, including cassava, common bean and tomato (Hong et al., 1996;Aragao et al., 1998; Noris et al., 1996; Duan et al., 1997). However, molecular biologists have been working for almost two decades on the transformation of plants for resistance to plant viruses, and there are only a few successful examples of commercial plant cultivars expressing a high level of resistance to begomoviruses. On the other hand, the efforts of several plant breeders, pathologists, entomologists and agronomists, using conventional breeding and virus screening techniques, has resulted in the release of a large number of begomovirus-resistant cassava, common bean and tomato cultivars. These cultivars are critical components of integrated pest and disease management programs and, more important, have greatly contributed to the alleviation of poverty in developing countries throughout the world.

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