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Bibliographic review

Genetic improvement methods in the cultivation of common beans (*Phaseolus vulgaris* L.) against the Bean Yellow Golden Mosaic Virus (BGYMV)

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ABSTRACT

The common bean (*Phaseolus vulgaris* L.) is one of the most important crops in Latin America, within the legumes due to the high content of nutrients it has, its production has been limited by the presence of multiple diseases, highlighting among them Bean Yellow Gold Mosaic Virus (BGYMV). Genetic improvement has allowed the efficient use of available genetic material, incorporating new resistance genes to this disease in our country, in order to generate cultivars with high genetic resistance. Molecular marker technology has revolutionized these programs, serving as a fundamental tool in the identification and selection of these genes for crop improvement.

Key words: beans, molecular markers, virus resistance

INTRODUCTION

The common bean constitutes the most important species within the legumes due to the high content of nutrients it has. This grain provides an essential source of protein, vitamins and minerals to the diet of populations in America. It is suggested that the protein content in the dry seeds of beans ranges around 20 %, a proportion that is significantly favorable since it places it in an advantageous position compared to other foods of plant origin in low-resource countries, especially in developing countries (1,2).

P. vulgaris is one of the oldest crops; archaeological findings indicate that it was known at least 5000 years before the Christian era. The genus *Phaseolus* is native to the

American continent and a large number of species were in Central America found. The genus comprises around 70 species, of which five were domesticated and cultivated (*Phaseolus vulgaris* L., *Phaseolus dumosus* Macfad., *Phaseolus coccineus* L., *Phaseolus acutifolius* A. Gray and *Phaseolus lunatus* L.). From the five species, *P. vulgaris* is the one that is mainly cultivated around the world (about 90 % of the cultivated area). This cultivation is currently distributed in the five continents and is an essential component of the diet, especially in Central and South America (3).

Studies in this century have been carried out experiments and the results ratify the Mesoamerican and South American origin of *P. vulgaris*, which gave rise to the gene pools currently known (4).

World production of this crop exceeded 26000000 t in 2016 with a yield of 0.91 (t ha^{-1}). In Cuba during that year the common bean planted area reached 122545 ha, achieving a production of 136570 t, with an agricultural yield of 1.11 t ha^{-1} where the private sector planted 117753 ha, which represents more than 95 % of the total. Production in this sector increased to 130225 t and the yield was 1.11 t ha^{-1}. The state sector planted 4792 ha, reaching an agricultural yield of 1.32 t ha^{-1} (5,6).

The common bean in Cuba is consumed in the form of dry grains and is exclusively dedicated to human consumption. It is considered a strategic food and constitutes, together with rice, the basic diet of Cubans, in which beans contribute around a fifth of the total protein consumed (5).

In our country, production is guaranteed mainly by the non-state sector, gaining importance in recent years. The production of this grain faces problems of low yields related in addition to the low fertility of the soils, the drought and the affectations by pests (3).

Meeting the growing food demand of the human species is an issue of extraordinary importance and validity at all times. For this reason, throughout the world, numerous researchers work incessantly in the search for agroecological solutions, since over the year's farmers at the field level have used chemicals with various doses to counteract diseases and thus obtain higher yields to meet and satisfy food demands (5).

Diverse insects are pests, diseases and viruses that damage bean cultivation. Among them those are the commonest: chrysomelid (*Diabrotica balteata* Leconte), common chrysomelid of beans (*Cerotoma ruficornis* Olivier), whitefly (*Bemisia tabaci* Gennadius), leafhopper of the beans (*Empoasca kraemeri* Ross and Moore), Melon thrips (*Thrips palmi* Karny), Common miner (*Liriomyza trifolii* Blanchard), Fusariosis (*Fusarium solani* f. sp. phaseoli SCHLTDL.). Also, Powdery mildew (Erysiphe betae (Vanha) Weltzien) Bean rust (*Uromyces appendiculatus* (Link) Unger), Ashy blight

(*Macrophomina phaseolina* (Tassi) Goid), Southern blight (*Corticium rolfsii* (Curzi) CCTu & Kimbr), Common bacteriosis (*Xanthomonas axo-nopodis* pv. *Phaseoli* Pammel & Dowson), Common mosaic (*Bean Common Mosaic Necrosis Virus*- BCMNV), Bean Golden Yellow Mosaic Virus (*Bean Golden Yellow Mosaic Virus* BGYMV), Yellow Mosaic (*Bean Yellow Mosaic Virus* BYMV) can appear. The latter is reported as determining in the low production of the crop in Cuba, causing severe damage and large losses (7).

The most important viral disease caused by begomoviruses that affects beans in Latin America is the golden yellow bean mosaic (BGYMV), first reported in Brazil, which spread to producing regions of Argentina and Bolivia, where it reached values of incidence of 80 % and losses between 40-100 %, and became the most devastating crop disease of all time in the 1980s (8).

Since the beginning of the seventies of the last century, the symptoms of yellowing in beans were reported in Cuba as caused by the golden mosaic of beans associated with populations of whiteflies (9). This virus caused, in the campaigns of the years 1989-1990 and 1990-1991, maximum percentages of infection, which led to the destruction of more than 1000 ha of the crop. Subsequently, with the presence of the B biotype of *Bemisia tabaci* Gennadius, in the late nineties, the disease affected 90 % and became the main common bean viral pathogen in the country (8).

The studies to date in Cuba related to this virus were on epidemiological aspects based, without references on characterization and diagnostic methods for its detection. On the other hand, the measures that have been in the region implemented to protect the crop from viral diseases have been on the use of genetically improved varieties with resistance to golden mosaic based, the application of pesticides to reduce the levels of the vector insect and the implantation of the integral handles (8).

The introduction of cultivars with better behavior against this disease and the implementation of preventive and control measures in an integrated management program allowed to reduce productive losses and improve the sustainability of the crop (9).

Bean Genetics

The bean is a diploid species (2n=2x=22), annual and predominantly autogamous. The bean genome is small (635 Mpb/haploid genome) and similar in nature as a true diploid to that of rice (*Oryza sativa* L.) (340 to 560 Mpb/haploid genome). The size of the mitotic and meiotic chromosomes is approximately 1-3 µm in length, the majority of chromosomes are metacentric or submetacentric, and have an average of 1.9 chiasms for bivalent chromosomes in meiosis due to crosslinking by arm of the chromosome. Regarding genomic studies in common beans, small families of genes have been recognized, such as the actin genes where six members were described. Large families have also been described, such as analog resistance genes and genes that code for kinases. In addition, many genes involved in resistance, isoflavone production and testa color have been identified. Regarding testa color, for example: white seed color has been found to be controlled by a single dominant gene. The testa color is controlled by two pairs of genes, RR and CC that produce white (RC), cream (rC), Red (Rc), and pink (rc) (10).

Beans have a broad genetic base, which is safeguarded in different germplasm banks, where CIAT stands out. It has more than 37.000 accessions, of 70 different bean species with origins from 112 different countries, which they include both wild species and accessions of the five domesticated species: *Phaseolus vulgaris*: common bean; *P. coccineus*: ayocote; *P. dumosus P. acutifolius*: tepari beans and *P. lunatus*: Lima beans. In order to provide genetic diversity for research and development, they are available to the international scientific community (11,12).

Insect affectations

Since the last decades, the cultivation of beans (*Phaseolus vulgaris* L.), chili (*Capsicum annuum* L.), tomato (*Solanum lycopersicum* L.), and other vegetables, have been affected by different harmful insects that have caused great damage to the crops. Leafhopper of the bean (*Empoasca kraemeri* Ross and Moore), Thrips of the melons (*Thrips palmi* Karny) and Whitefly (*Bemisia tabaci* Gennadius) stand out, the latter being reported as a determinant in the low production of the crop in Cuba, because it is vector of various viruses (13).

Whitefly (Bemisia tabaci Gennadius)

Distribution and importance

The *B. tabaci* species is a complex of many and different biotypes, some specialized on certain hosts and other polyphages. In the mid-1990s, one of them (the “B” biotype) was chosen as a new species by some researchers with the name of B. argentifolii. This biotype is characterized by being extremely polyphagous and fertile, in addition to inducing phototoxicity symptoms in some plant species (for example, *Cucurbita* spp.), which has been used as a method to differentiate it from other biotypes. It has also been verified that the individuals of this biotype do not differ, and can be crossed with individuals from at least one other different biotype, originating fertile offspring. For these reasons, most authors continue to consider *B. argentifolii* as a biotype of the *B. tabaci* species and not a new species. *B. tabaci* biotype B is widespread throughout the country and is potentially a transmitter of large numbers of viruses. More than 60 viruses can be transmitted to various crops. It can mention: Cotton leaf curl disease virus (CLCuV) and Cotton leaf crumple virus (CLCV) in cotton, African cassava mosaic virus (ACMV) in cassava, Tomato yellow leaf curl virus and Tomato mottle virus in tomato cultivation, Tobacco leaf curl virus in tobacco cultivation, Squash leaf curl virus (SLCV) in zucchini and Bean Golden mosaic virus (BGMV) in beans (7).

Damage to the bean crop due to *Bemisia tobacco*

B. tabaci is a vector insect of geminiviruses like BGYMV and that causes considerable economic damage due to its high resistance to systemic insecticides. Furthermore, it is an important pest due to its high fertility, fertility, and virus transmission capacity. The whitefly is estimated to have around 500 plants as hosts having an invasive capacity of 200 km radius (14).

The damage they cause is due both to the insect's effect on the attacked plants and their role as vectors of viral diseases. In its diet, the whitefly causes a weakening in the plant by the extraction of nutrients, affects the growth and production of the crop, physiological disorders and the transmission of viruses (15).

Over the past three decades, *B. tabaci* has caused millions of dollars in losses from growing *P. vulgaris*. However, the real estimate of the economic impact of their populations on world agriculture has been difficult to obtain due to the large number of affected areas, the number of crops and ornamental plants involved, and the different monetary systems.

The damage it causes in bean cultivation is due to *biotype B* feeding directly on the phloem, causing physiological disorders, and indirectly, it excretes molasses that favors the growth of fungi (eg *Capnodium* spp.), in addition to the transmission of viruses belonging to seven groups including *Begomovirus* (15).

Bean Yellow Gold Mosaic Virus (BGYMV)

Distribution of the BGYMV virus

Many of the plant pathogenic viruses are transmitted by insects, among which is the whitefly, *Bemisia tabaci* (Genn.), which is considered a cosmopolitan, polyphagous and one of the most devastating pests, due to its ability to acquire and spread a variety of viruses to healthy plants. This insect is capable of transmitting many species of viruses belonging to the genus *Begomoviruses*, *Ipomoviruses*, *Crinivirus*, *Carlavirus* and *Torradoviruses*. *Begomoviruses* are viruses made up of DNA that cause diseases that cause significant economic losses for farmers. Due to the characteristics of the phytopathogenic viruses, the diversity of species and biotypes of the *B. tabaci* complex, molecular techniques are used to identify them due to their high sensitivity and reproducibility (16).

In Central America and the Caribbean, the bean golden yellow mosaic virus (BGYMV) is one of the most important diseases in bean cultivation. This virus is transmitted by the whitefly (*Bemisia tabaci* Genn). Infection can be perceived by inducing symptoms of leaf yellowing and chlorosis and stunted growth. Post-infection leaf development is deformed and its tissue eventually dies. Virus infection affects pod development, reduces weight, causes deformation, and reduces grain yield. BGYMV is the most important viral disease in Central America. It can cause losses of 30 to 100 % depending on the variety, age of the plant and the population of the whitefly vector (17).

Appearance in Cuba

The genus Begomovirus is the largest and most important in the family Geminiviridae. Numerous begomovirus species have been detected in Cuba, affecting crops of great economic importance (18).

This virus is present throughout the national territory, constituting since 1989 the disease that attacks different crops of agricultural importance in the country such as beans, soybeans (*Gycine max* L.) and tobacco (*Nicotiana tabacum* L.) (7).

Symptoms and Harm of BGYMV

The main symptomatology is caused by the BGYMV, a Mesoamerican species, due to the fact that two species of the virus that cause the golden mosaic symptom are distinguished in bean cultivation: the South American, called Bean golden mosaic virus (BGMV), and the Mesoamerican, named Bean golden yellow mosaic virus. The first is transmitted only by whiteflies and is distributed in the countries of Brazil, Argentina and

Bolivia, while the Mesoamerican by whiteflies and by mechanical means, and is found in the Dominican Republic, Nicaragua, Costa Rica, Puerto Rico, Haiti and Mexico.

Like BGMV, symptoms are manifested by a marked deformation of the leaves, many of which may occasionally turn completely yellow or almost discolored. Some cultivars have less intense mosaic symptoms, and at a later stage of development may have some recovery. The pods of infected plants are very misshapen. The seeds can discolor, deform and decrease in size and weight. Some plants infected at an early stage can be severely stunted and often do not produce pods (15,19).

The BGYMV infected plant shows symptoms of chlorosis with dark green mottles on the leaves that later spread and turn yellow, producing dwarfism due to the inhibition of photosynthesis, since the virus replicates in most of the lamina foliar. The leaves may curl to the bottom, causing the veins to turn whiter than normal. Pods tend to produce small, misshapen seeds and lose color. It can even see root necrosis and necrotic streaks that can spread to the stem and meristem in hypersensitive varieties. Finally, the plant withers and dies causing significant losses in production (14).

Young plants show symptoms on the first trifoliate leaves, with veins turning light yellow. Generally, this process begins in the middle of the leaf near the tip. After 3 or 5 days, this chlorosis in the veins extends to cover a large part of the leaf, contrasting with the internervial areas that are dark green. This chlorosis then spreads out in the form of a golden mosaic that gives the plants a striking color. After the first leaves develop symptoms, young leaflets stop growing and curl downward, becoming stiff and leathery and sometimes necrotic. If infection is early, pimple formation is very poor or absent, and the pods become deformed. In general, the plants do not grow, the leaves show abnormal colors, the flowers can abort and the fruits deform (7).

Control methods

Given the incidence of these new diseases, the management given to the problem of *Begomovirus* in beans and other crops has mainly emphasized the development of resistant varieties, which are obtained through the combined incorporation of the dominant genes for hypersensitivity I and resistance genes recessive (bc-u, bc-1, bc-11, bc-2, bc-22 and bc-3) to bean varieties susceptible to viruses (20).

One of the main sources of resistance to BGYMV is found in the Creole bean Garrapato (G2402), which contains the recessive gene bgm^{-1}, demonstrating that it is a stable and valuable gene for generating tolerant varieties of the golden yellow mosaic of the bean.

The selection process for resistant bean varieties can be accelerated by molecular marker assisted selection (SAMM). Molecular markers linked to some BGYMV resistance genes are currently available (21).

Integrated pest management

Among the objectives of Integrated Pest Management (IPM) is to reduce the population density level of pest organisms so that their damage decreases, as well as the costs of protection and the undesirable effects of control actions, mainly of pesticides. IPM is a system that integrates different control tactics that producers select according to their decision-making according to their local conditions and the type of pest, seeking to use control tactics based on a cost-benefit analysis that takes into account the interests of producers and society and the impact on the environment. In addition, it is based on natural control because it is sustainable, in combination with cultural control, genetic control (use of resistant plants), ethological control (the sterile insect technique [TIE]. As well as, the use of semi or chemical), biological control (use of predators, parasitoids, and pathogens) and the use of legal control that includes the use of quarantine measures that mitigate the risk of pests entering free zones; and as a last option, chemical control (22).

Despite the pest control measures used to decrease BGYMV infection rates, the incidence of various typical yellowish symptoms has been increasing. The presence of *begomovirus* has been observed in surveys carried out in areas of main bean production in the last three years. Recently, three new species have been reported in different geographic regions in Cuba: the severe common bean mosaic virus (CBSMV), the common bean mottle virus (CBMV) in the western region (18).

Genetic improvement

Man more than 1000 years ago has been concerned with improving the plant species he used to satisfy his various needs with the least amount of resources possible. Food production since 1900 has made great progress, which has benefited both consumers and producers, since plant genetic improvement has led to increasingly productive varieties, lines and hybrids with resistance to various pathogens, insects, cold, heat, soil salinity, low fertility, etc. Thus allowing agriculture to be possible in marginal places. However, it is necessary to continue researching to continue meeting the food needs of the world population, which is in continuous increase (2).

The progress of the improvement depends on the genetic systems and the selection methods available, when the characteristics are inherited in a single-dose dominant gene and when the presence of such genes results in complete protection against a defined disease or clear reaction. On the contrary if, this protection or clear reaction of the plant does not exist or in the absence of the gene, the progress in the improvement can be done quickly. The improvement process is much slower when the genetic system is polygenic and the reaction of the plants is intermediate (23).

Genetic improvement is the main tool to launch new cultivars on the market. Bean breeding programs emphasize the exploration of genetic varieties through the hybridization of superior and contrasting varieties (24).

Having genetic variability is the main step in an improvement program, which allows the selection of cultivars for various purposes, such as higher yield, protein or oil content, tolerance to biotic or abiotic factors, among others (24).

The importance of genetic improvement lies in being able to correct those undesired characteristics by means of hybridizations and specific selection methods in order to decrease grain losses at harvest, increase resistance to pests and diseases, and create tolerant varieties that avoid contamination and spread of pathogens resistant to chemical applications. The first step in an improvement program is to use phenotypic evaluations to identify resistant genotypes (14).

Phenotypic evaluation in the field for resistance to BGYMV consists of identifying plants with genetic potential, estimating the incidence and severity of the virus using standardized scales. Likewise, the evaluation of the effect of BGYMV on yield is an important variable since the final objective of every producer is to obtain crops with the highest possible yield (14).

Genetic improvement tools

Among the genetic improvement tools available to increase genetic diversity, hybridization, recombination and natural or induced mutation are mentioned as most important (10).

Because this resistance depends on the incidence of viruliferous whiteflies and the time of infection, when whitefly populations are very high and the virus is present, complementary chemical control measures should be used. Black grain materials generally have an appreciable level of strength. Materials of Mexican origin (Pinto type, Red Mexican, Great Northem) and some Andean ones (Red Kidney types), also provide genes for resistance to BGYMV in beans. It is recommended to sow beans in rainy seasons or immediately after a rainy season, when whitefly populations are usually low (7).

Authors suggest that sowing of beans should be done by treating the seed with a systemic insecticide. About 15 days after seedling emergence, another application of a new generation (low environmental impact) systemic insecticide should be made. Depending on the whitefly population, a second foliar application can be made in the following two weeks, or biological insecticides can be used in the final stages of cultivation. In the presence of an appreciable population of *B. tabaci*, during the initial growing cycle, it is necessary to protect the bean plants until the pod formation stage (7).

Molecular markers in Genetic Improvement of bean cultivation

A molecular marker is simply a segment of DNA with a specific location on a chromosome that allows differentiating between one genotype and another at the DNA level, these can be genes or DNA segments without any known function or coding. Furthermore, they are anchor points or labels when they are attached or close to the sequences of the genes. Molecular markers have become powerful tools to make it possible to determine the genetic characteristics of plants and to select by genotype rather than phenotype. DNA markers are primarily based on the analysis of differences in small DNA sequences between individuals (25).

Genetic Marker Assisted Breeding (SAM, according its acronyms in Spanish) is a promising technology that enables the development of new varieties with specific traits to be accelerated without the need to intervene in the genome of currently existing varieties. This technique is based on identifying a singular or unique DNA (molecular marker) sequence close to a character of agronomic interest (a resistance gene). Once this is achieved, plants bearing this agronomic character are marked, and quickly selected in segregating populations according to whether or not they present the marker, developing new varieties with the desired genes (10).

They are based on the amplification of the DNA or part of it, which can be coding, non-coding regions, or known sequences that allow comparing genomes within and between species. The amplification of the DNA segments is performed with the PCR technique (Polymerase Chain Reaction), as in the case of Random Amplified Polymorphism DNA (RAPDs) or in the generation of cut sites in the DNA sequence. In the case of restriction fragment length polymorphisms or RFLPs (Restriction Fragment Length Polymorphisms) and within them several types can be differentiated as we will see later. Molecular markers are characterized by their quick and easy detection, many are codominant, there is an absence of pleiotropism and epistasis, expression is early, their distribution is homogeneous in many cases and they have a high polymorphism (pleiotropy= a single pair of genes acts in the manifestation of various characters) (10).

The integration of SAMM with classical genetic improvement in *P. vulgaris* allows determining genotypes of durable resistance to diseases. Bean breeders also have the opportunity to use resistance genes from two different gene pools (Mesoamerican and Andean) to obtain resistance to the various bean pathogens. However, the additive effects of genes linked in the repulsion phase can cancel the effect of another, a result of locus over-dominance. Furthermore, it is common to detect QTLs (Quantitative Trait Loci) associated with disease resistance; although their strong association with the environment makes them inconsistent and this is a limitation for use them in SAMM (26).

In the case of the bean yellow gold mosaic virus, epistatic interactions between different sources of resistance occur, making it difficult to determine which sources can be combined in breeding. A source of resistance with the *bgm*–1 gene is "Garrapato" that does not develop mosaic symptoms. This gene can be identified by inoculation with different viral strains and ranges of molecular marker labels are available for each gene (26).

The complex interaction of multiple genes and their recessive nature allowed SAMM for the rapid development of resistant varieties. Gene pyramiding is applicable in bean breeding for virus resistance with several independent resistance genes as they provide different patterns of virus resistance (26).

For the sustainable development of genetic improvement programs in bean cultivation, it is required: the development of more reliable and complex procedures for direct (phenotypic) selection and for SAMM of resistance characteristics. Besides, a better understanding of inheritance and resistance mechanisms especially for stress complexes; relevant molecular and genomic genetic studies to gain a better understanding of resistance genetics and physiology and integrate the added markers to the improvement to complement the classical improvement (26).

Other methods of genetic improvement

Currently, there is still no efficient protocol for the genetic transformation of beans. The first bean transformation report was made in the 1990s using particle acceleration by bombardment. In several countries, investigations using the particle bombardment technique to produce transgenic beans have generated lines with resistance to yellow gold mosaic virus (BGYMV); however, the reported transformation efficiency is still lower than 0.7 %. To carry out genetic transformation, there are two ways, through direct techniques such as electroporation, microinjection and biolistics, or by agro infection, that uses the bacterium *Agrobacterium tumefaciens*. The latter is the most versatile and widely used due to its repeatability, as it is known that this bacterium is capable of introducing a fragment of DNA into plant cells and inserting it into the genome (10).

The biolistics or particle acceleration technique involves bombarding cells or tissues with gold or tungsten particles, coated with DNA. The particles are by the use of an inert gas accelerated, which causes the impact on the plant material and allows the particles to penetrate the cell wall, the cell membrane and the nuclear membrane, so once in the nucleus, the DNA is released and Recombination occurs for incorporation into the cell genome (10).

Mutation induction is an alternative to generate genetic variability not present in nature or to obtain genotypes that can be used as parents in breeding programs, by producing new genetic combinations or by increasing variability in a population (27).

Mutagens can be physical and chemical. Among the physical mutagenic agents are X-rays, gamma, ultraviolet, as well as carbon ions. The most common chemical mutagens are ethyl methanesulfonate, dimethyl sulfate, and diethyl sulfate. The application of mutagenesis in genetic improvement and agricultural production began in the early twentieth century in crops such as corn (*Zea mays* L.), wheat (*Triticum* sp.), Barley (*Hordeum vulgare* L.), cotton (*Gos-sypium* sp. L.). In ornamental plants the gamma rays are the most, while in the cultivation of beans, studies have been carried out on their effect in treatments that promote the development of the embryo and reduce germination limitations, to achieve uniformity and increase germination (27).

CONCLUSION

The development of a genetic improvement program for the cultivation of common beans allows the identification of sources of resistance to the bean golden yellow mosaic virus using molecular markers.

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