Necesidades de investigación futuras

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Gracias al trabajo de todos los profesionales y científicos dedicados al estudio y control de la mosca blanca *Bemisia tabaci* y de los geminivirus que esta especie transmite, hemos logrado acumular un conocimiento extenso sobre estos problemas. La red Mesoamericana y del Caribe, coordinada por el Dr. Luko Hilje de CATIE, ha logrado aunar esfuerzos y diseminar información en toda la región. El Proyecto Suizo (COSUDE) de PROFRIJOL, hizo igualmente posible el desarrollo de variedades resistentes al mosaico dorado-amarillo en la América Latina. Finalmente, El Proyecto Global de Mosca Blanca, financiado por la Agencia de Desarrollo Danesa (DANIDA), y coordinado por la Dra. Pamela K. Anderson desde el CIAT, ha realizado un trabajo exhaustivo de investigación y compilación sobre *Bemisia tabaci* y los geminivirus que atacan cultivos básicos e industriales en el mundo, considerando el volumen de información generado. Ahora necesitamos proyectos que nos permitan hacer un análisis intensivo de toda la información recogida y, más importante, necesitamos aplicar los conocimientos adquiridos a nivel de campo.

Una de las áreas más afectadas por las crisis económicas que han venido afectando a la América Latina, ha sido el mejoramiento genético. Son pocos los cultivos afectados por geminivirus en el mundo, donde se pueda mostrar tanto progreso en la generación de variedades resistentes, como en el caso del frijol. Existen aún algunos programas de mejoramiento de frijol que buscan mejorar esta leguminosa por su resistencia a virus transmitidos por mosca blanca, como son los de la Escuela Agrícola Panamericana (Zamorano) en cabeza del Dr. Juan Carlos Rosas; el de la Universidad de Puerto Rico, a cargo del Dr. James Beaver; el del Norte de México, llevado por el Ing. M.Sc. Rafael Salinas; y el del Proyecto Frijol, en el CIAT, liderado por los Drs. Shree Singh y Steve Beebe. Es necesario continuar el apoyo y reforzar estos trabajos de mejoramiento convencional, que tantos éxitos han tenido.

El uso de técnicas moleculares para el desarrollo de variedades de frijol resistentes a geminivirus, es promisorio, y felizmente cuenta con recursos económicos adecuados. Es necesario poner esta metodología al servicio de los programas nacionales y mejoradores de frijol en general.

Los trabajos pioneros de la Universidad de Wisconsin, coordenados por el Dr. Douglas Maxwell, han permitido vislumbrar la posibilidad de transformar plantas de frijol buscando su resistencia a geminivirus. Este ha sido un trabajo difícil, que ya encuentra eco en otros países de la América Latina, donde científicos como el Dr. Josias Faria, ha transformado frijol para el control del mosaico dorado del frijol. Igualmente se reconoce la labor que viene desarrollando el Dr. Robert Gilbertson, Universidad de California, Davis, para la comprensión de los mecanismos moleculares que gobiernan la interacción entre geminivirus y sus hospederos.

La epidemiología de los virus transmitidos por mosca blanca en frijol, ha sido el tema de los trabajos realizados por la Dra. Pamela K. Anderson, en Nicaragua y luego en el CIAT, Colombia. Actualmente se encuentra trabajando en el mejoramiento de un modelo matemático que nos permitirá tomar decisiones de control según los parámetros existentes en cada región afectada por estas plagas. Estos estudios se han beneficiado de la información que constantemente generan investigadores que trabajan a nivel de campo y/o laboratorio, como la Dra. Jane Polston y el Dr. Ernest Hiebert de la Universidad de Florida, o la Dra. Judith K. Brown, en la Universidad de Arizona. Estos científicos han estado constantemente involucrados con los problemas de la producción de alimentos causados por geminivirus en la región mesoamericana.

Se inició también en el CIAT, un estudio sobre la distribución espacial de las enfermedades causadas por geminivirus transmitidos por mosca blanca, utilizando sistemas de información geográfica (SIG). Estos estudios nos permitirán analizar mejor los factores que intervienen en el desarrollo de epidemias de geminivirus en diversos cultivos y regiones geográficas.

Por último, necesitamos desarrollar un sistema de monitoreo y de alerta sobre la posibilidad de ataques severos de geminivirus y mosca blanca, basados en los estudios y análisis epidemiológicos y espacio-temporales.

Nuestra mayor esperanza está basada en un gran número de investigadores latinoamericanos, los cuales se han formado en áreas relacionadas al conocimiento de los geminivirus y de los biotipos de mosca blanca que afectan los cultivos en la América Latina. Solo esperamos que se encuentre un balance entre los investigadores de campo y de laboratorio, para llevar a feliz término la misión que les ha sido encomendada: mejorar la productividad de los cultivos afectados por geminivirus, en especial los cultivos alimentícios básicos, como el frijol.

English summary

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Current situation of common bean production and whitefly transmitted begomoviruses affecting *Phaseolus vulgaris* in Latin America

Common bean research and production in Latin America

The common bean (*Phaseolus vulgaris* L.) remains one of the most important food crops in Latin America, where this legume provides rural and urban populations with most of the essential protein and carbohydrates. However, common bean production in Latin America has drastically declined in the past decade, as a response to the globalization of the economy. The new economic policies have resulted in decreasing financial support from national and international governments to agricultural research in Latin America. The limited resources available are being used to conduct research on non-traditional export crops or natural resource management, often, with a minimum research capacity that needs to be supplemented with special project funds. As a result, most of the main common bean producing countries in Latin America are currently importing this basic food to meet the internal demand. In Latin America, Argentina has become the main supplier of common beans, producing approximately 300,000 tons a year on average. Other common bean producing countries are Chile, Ecuador and Bolivia, but the total area devoted to the production of common beans for export only amount to approximately half a million hectares, whereas the total land abandoned to common bean production in Latin America in the last decade, exceeds a million and a half hectares.

Another negative aspect of common bean production in Latin America, is a notable decrease in the capacity to generate new varieties. Without strong and functional common bean breeding programs, Latin America will not be able to meet current or future research challenges posed by the continuous emergence of new biotic and abiotic production problems. Although the use of molecular techniques, such as molecular markers, is expected to compensate for the downsizing of common bean breeding programs, Latin

America still has a long way to go before these techniques are effectively used for common bean improvement purposes. Likewise, the successful model of common bean programs conformed by teams of scientists from different disciplines, has also disappeared from most national programs and even international institutions devoted to agricultural research in Latin America.

Begomoviruses infecting common bean and their control

In the meantime, the emergence of new geminiviruses and whitefly biotypes, continues to threaten the viability of common bean production in the lowlands and mid-altitude valleys of Latin America. Whereas *Bean golden mosaic virus* (BGMV) and *Bean golden yellow mosaic virus* (BGYMV) remain the most important geminiviruses of common bean, other geminiviruses transmitted by *Bemisia tabaci* have shown their capacity of adaptation and destructive potential in common bean. A clear example is the adaptation of *Squash leaf curl virus* (SLCV) to common beans in northwestern Mexico, and the resulting yield losses caused by the common bean variant of SLCV, known as *Bean calico mosaic virus* (BCaMV). In recent surveys of northwestern Mexico, conducted by the author, strains of SLCV can still be recovered from mosaic-affected common bean plants. This virus (SLCV) probably disseminated from northwestern Mexico and/or southwestern United States, and is now found affecting different cucurbitaceous crops in Central America, posing a threat to bean production in this important common bean-producing region.

Fortunately, private industry is currently financing some of the research activities abandoned by national programs, and we still have a few active and experienced bean breeders, who continue to generate excelent common bean varieties possessing resistance to different geminiviruses. This is the case of Ing. Agr. M.Sc. Rafael Salinas, common bean breeder located in Los Mochis, Sinaloa (N.W. Mexico), who has developed common bean cultivars highly resistant to BCaMV. Curiously, he has been able to use some of the sources of resistance identified for other begomoviruses of common bean, particularly red kidney genotypes of Andean origin.

Bean golden yellow mosaic virus (BGYMV) has its domains from southern Mexico, affecting all the Central American and Caribbean countries down to Colombia. Dr. Julio Bird first used this name to refer to the striking yellowing symptoms induced by a geminivirus (later selected as the type strain of BGMV) in *Phaseolus lunatus* and *P. vulgaris*, in Puerto Rico. Although this species has probably evolved since its original molecular characterization in the last decade, the most noticeable change in some isolates of BGYMV, has been their loss of reactivity with a monoclonal antibody selected seven years ago to distinguish BGYMV from other begomoviruses infecting common bean in Latin America (F.J. Morales, *unpublished results*).

The control of BGYMV in southern Mexico, Central America and the Caribbean region has been possible thanks to the international and national breeding for resistance project on BGYMV initiated in the mid-1970s in Guatemala. The BGYMV-resistant lines generated by this project have made an exceptional impact throughout this region, and even in South American countries, such as Argentina, where these lines have been equally resistant to the distinct *Bean golden mosaic virus* species. Although the output of this project has been significantly reduced due to the current financial situation, other projects, such as the Bean/Cowpea (CRSP) project has been successfully complementing these efforts, particularly in Puerto Rico (Dr. James Beaver, University of Puerto Rico) and in Honduras (Dr. Juan Carlos Rosas, Escuela Agrícola Panamericana).

Another potential threat to common bean production in the Caribbean region, is the presence of an Old World geminivirus introduced into the Dominican Republic from Israel: *Tomato yellow leaf curl virus* (TYLCV). This virus has already been isolated from diseased common beans in Spain, and the common bean production regions in Mesoamerica are usually close to the tomato growing areas affected by TYLCV. Fortunately, there seems to be a larger number of potential sources of TYLCV resistance in *Phaseolus vulgaris* than in tomato.

In South America, with the exception of Colombia, where BGYMV has been detected, the predominant species is *Bean golden mosaic virus* (BGMV). This virus was first reported in Brazil by Dr. Alvaro Santos Costa in the early 1960s, and later shown to be different to the "bean golden mosaic" viruses described in Mesoamerica (BGYMV and BCaMV). BGMV arrived in Northwestern Argentina in the early 1980s, and, during the last decade, to the department of Santa Cruz, Bolivia, following a horizontal dissemination pattern in the savanna regions of South America located between 10° and 30° latitude south. Another geminivirus that has caused significant yield losses in this region, particularly in northwestern

Argentina, has been *Bean dwarf mosaic virus* (BDMV). This virus, related to *Abutilon mosaic virus* (AbMV), was first described in Brazil, together with BGMV, but its incidence in Brazil was usually low. In the late 1970s, BDMV (or a closely related virus) caused severe yield losses in common bean fields located in northwestern Argentina. BDMV has been found in Colombia and in Nicaragua, infecting beans at a relatively low incidence. Other geminiviruses have been isolated from common bean in northwestern Argentina, including *Tomato yellow vein streak*, described later on from the State of São Paulo, Brazil.

The incidence of BGMV in the common bean production region of Santa Cruz, Bolivia, is relatively low at this time, mainly because some of their bean fields are at altitudes above 1,000 meters. At this altitude, the predominant whitefly species associated with common bean is *Trialeurodes* sp. and not the vector *Bemisia tabaci.* Nevertheless, the increasingly frequent phenomenon of "El Niño", which creates unusually dry conditions in some Andean highland regions, has made possible the increasing multiplication of *B. tabaci* populations above 1,000 m.

The development of BGMV-resistant common bean cultivars has been rather slower in South America than in Mesoamerica, due to different reasons. In Brazil, institutions such as CNPAF (National Center for Research on Common Bean and Rice) and IAPAR (Agronomic Institute of Parana), have developed a number of BGMV-resistant lines. Additionally, many BGYMV-breeding lines developed in Central America, have been taken to Brazil, Argentina and Bolivia, where they have performed well under BGMV pressure, suggesting the existence of similar mechanisms of resistance to BGYMV and BGMV. These breeding lines and BGYMV-resistant cultivars have also performed very well under BDMV pressure.

Geminivirus taxonomy

The taxonomy of geminiviruses has changed rather dynamically in the last decade, mainly, driven by advances in molecular techniques that facilitate the amplification of geminiviral DNA and its rapid sequencing. These techniques are now being used to characterize geminiviruses transmitted by *Bemisia tabaci* in Latin American countries, such as Mexico, Guatemala, Honduras, Costa Rica, Panama, Colombia, Venezuela, Brazil and Argentina.

Currently, the majority of geminiviruses affecting cultivated and wild plants in Latin America, are transmitted by *Bemisia tabaci.* All of these viruses are considered New World viruses, and are native to the American tropics and subtropics, where *B. tabaci* can thrive. The only exception is *Tomato yellow leaf curl virus*, introduced into the Americas from Israel. Considering that *B. tabaci* was introduced into the Americas, probably from Asia, it is understandable that whitefly-transmitted geminiviruses are also found outside the Americas, namely in Asia and Africa. However, the type strain of the newly recognized genus *Begomovirus*, which groups all of the whitefly-transmitted geminiviruses known world-wide, is the South American geminivirus *Bean golden mosaic virus* (BGMV), from which the genus derives its name. BGMV is considered a unique species, distinct from the geminivirus that induces similar symptoms on common bean throughout Mesoamerica, recently renamed *Bean golden yellow mosaic virus* (BGYMV), as it was first named by Dr. Julio Bird in Puerto Rico. The third distinct geminivirus species found in the Americas, causing "golden mosaic"-like symptoms on beans, is *Bean calico mosaic virus* (BCaMV), a more distant virus closely related to *Squash leaf curl virus* (SLCV).

Dr. Maria del Rosario Rojas has contributed a chapter on the molecular characteristics of begomoviruses, where she describes four filogenetic groups of bean geminiviruses, including the Mesoamerican BGYMV isolates, the South American BGMV, *Bean dwarf mosaic virus* (BDMV) from South and Central America, and BCaMV from N.W. Mexico.

Drs. Douglas Maxwell, Stephen Hanson, Josias Faria and Robert Gilbertson discuss the molecular techniques currently used by most researchers to detect and identify begomoviruses. Monoclonal antibodies (developed at the University of Florida under the direction of Drs. Ernest Hiebert and Dan Purcifull), specific and broad spectrum probes, and PCR, are the main diagnostic techniques used today by most laboratories. These techniques have been used to identify the main wild reservoirs of common bean begomoviruses, but, so far, only few wild hosts have been confirmed as such. The University of Wisconsin has also been a leading institution in the implementation of antiviral strategies and common bean transformation methods, based on the study of the genomic function of begomoviruses. Among the antiviral strategies mentioned are: capsid protein- mediated plant resistance, creation of non-functional Rep proteins, *trans*-dominant lethal *rep* gene constructs, and antisense strategies.

The whitefly vector

The discussion on the whitefly vector is led by Dr. Pamela K. Anderson, who points out the need to consider *Bemisia tabaci* not only as an insect pest but as a virus vector as well. Whitefly population management is necessary to reduce the intensive use of pesticides, and to prolong the useful life of the begomovirus-resistant common bean cultivars developed by national and international institutions in Latin America.

The identification of *B. tabaci* has also been facilitated by the development of taxonomic keys for both immature and adult specimens, thanks to the work of Dr. Rafael Caballero in Honduras (EAP). Dr. Anderson also discusses the current controversy among taxonomists, regarding the identification of the biotype B of *B. tabaci* as a distinct species named *Bemisia argentifolii* (Perring & Bellows). Whereas this possibility is not discarded, most whitefly taxonomists feel that further research is needed to settle this controversy. In the mean time, the name *B. tabaci* biotype B is retained in this publication. Nevertheless, the higher reproductive rate and broader host range attributed to biotype B of *B. tabaci*, requires further investigation from the epidemiological point of view.

The biology and ecology of *B. tabaci* is also described with special emphasis on the role of common bean as a reproductive host. Data collected in Mexico, Central America and Colombia, show that common bean is not a preferred reproductive host of *B. tabaci*, although this species can reproduce abundantly on common bean in the absence of more suitable reproductive hosts, as observed in Brazil.

Regarding the epidemiology of begomoviruses transmitted by *B. tabaci*, Dr. Anderson refers the reader to the chapter contributed in the first edition of this publication, produced in 1994, for complementary information. Here, she points out the need to identify the reproductive hosts of *B. tabaci* in each common bean production region affected by begomoviruses, because these hosts are not necessarily the same in all countries. She lists over 30 different plant species identified in Latin America as hosts to *B. tabaci*. Finally, Dr. Anderson lists the main categories of pesticides used in Latin America to control *B. tabaci*, and emphasizes the need to implement IPM practices to reduce the use of synthetic pesticides in common bean production regions. One of the most promising methods of control discussed, is the use of biological control agents, namely entomopathogens and predators of *B. tabaci*.

The identification of *B. tabaci* biotypes A and B, is the subject of the chapter contributed by Dr. Lee Calvert. He has implemented the RAPD-PCR method described by Dr. Paul De Barro in Australia, to differentiate the introduced biotype B from the local *B. tabaci* biotype. Dr. Calvert shows that the primers recommended by De Barro, can be used to differentiate biotypes A and B of *B. tabaci* in the Americas, but points out critical technical procedures and other considerations to be taken into account for the correct analysis of results. One of the main sources of error found, was the presence of other whitefly species on common bean in the Americas, particularly *Trialeurodes vaporariorum*, which can produce similar RAPD patterns with at least one of the primers used.

Integrated begomovirus and whitefly management

By far, the most successful approach to the control of geminiviruses affecting common bean, has been **breeding for disease resistance**. The implementation of this approach was possible thanks to the efforts of a number of donors, particularly US and Swiss donors, who funded CIAT and national programs in Mexico, Central America and the Caribbean region, to start a very successful breeding project in Guatemala. The first generation of BGYMV-resistant materials originated from a few black-seeded parental materials of Mesoamerican origin. One of these early lines, DOR-41, became a widely-adopted cultivar in Central América, the Caribbean, and even northwestern Argentina. Similar materials, were also adopted in southern Mexico. A second generation derived from the same sources of resistance to BGYMV, crossed to local landraces, yielded some excellent lines, such as ICTA-Ostúa, still under cultivation in Guatemala. The project, however, had to tackle a more difficult task: to breed for resistance to BGYMV in grain types other than black, mainly the highly prized red-seeded materials consumed in El Salvador, Nicaragua, Costa Rica and some Caribbean countries.

A breakthrough occurred when a non-black-seeded genotype, A 429, which had never been bred for resistance to BGYMV, showed unexpected high levels of resistance to this virus under field conditions. This line, originally selected for its superior plant architecture, was thoroughly evaluated at CIAT, together with its parental materials, to identify the source(s) of resistance. Following their BGYMV screening under controlled conditions, two parental genotypes were identified as sources of BGYMV resistance in A 429: Porrillo Sintético (a black-seeded resistance source used for the first generation of DOR lines), and "Garrapato", a rustic pinto variety of Mexican origin. The latter genotype was infected and had no pods due to the infection by BGYMV, but it did not show any yellowing symptoms. This rather questionable bean genotype, has become one of the main sources of BGYMV/BGMV resistance known to date.

Later on, a line selected for its resistance to BGYMV (derived from Porrillo Sintetico), DOR 303, showed some interesting reactions to the virus under field conditions. Basically, it did not have noticeable yellowing symptoms, but a few plants were frequently affected by severe stunting. A similar examination of this line and its parental materials at CIAT, revealed the presence of red kidney types of Andean origin, which condition the type of resistance to BGYMV displayed by this line, in combination with P. Sintetico.

A thorough search of bean genotypes available at the CIAT's bean germplasm bank, led to the evaluation of selected accessions in different countries of South America, Central America, the Caribbean, and in Mexico. Some additional 15 bean genotypes were added to the sources of BGMV/BGYMV resistance previously identified. Genetic studies conducted under the direction of Dr. Shree P. Singh at CIAT, made possible the combination of various mechanisms of virus resistance identified in bean genotypes belonging to different races of *Phaseolus vulgaris*. Additionally, research conducted at the University of Puerto Rico, led to the identification of specific genes responsible for the responses observed in selected sources of resistance to the inoculation of bean begomoviruses. This research resulted in the development of molecular markers/techniques (RAPD, QTL, SCAR, microsatelites) to expedite the breeding for BGMV/BGYMV resistance work (marker assisted selection).

Chemical control is still widely practiced in Latin America to control the whitefly-transmitted geminiviruses that affect common bean. However, considering the relatively low economic resources of most bean farmers in this region, the types of pesticides applied to control *B. tabaci*, are usually the compounds more likely to be deactivated by this whitefly species, generating an increasing problem of development of pesticide-resistant whitefly populations. The new chemicals, such as imidacloprid, are very effective but equally costly for Latin American bean farmers. Consequently, the use of non-synthetic pesticides and biocontrol agents is gaining terrain in Latin America, where botanicals, such as neem and tobacco extracts; predators, such as *Eretmocerus* and *Encarsia* spp.; and entomopathogens, such as *Verticillium lecanii* and *Beauveria bassiana*, are now being tested and applied more frequently. The use of some soaps to control *B. tabaci*, is another interesting method of control practiced by some small-scale farmers.

Cultural practices have not been widely adopted by bean farmers to control begomoviruses. The use of physical barriers, mainly anti-whitefly screens or screenhouses; live barriers or trap crops; sticky traps, planting density and planting dates, are seldom used in Latin America. Perhaps the most successful control practice implemented in certain countries of Latin America to control BGYMV, has been the enforcement of legal decrees banning the continuous cropping of whitefly-rearing hosts throughout the year. Such legal measures have been successfully implemented in the Dominican Republic, to break the *B. tabaci*-BGYMV cycle.

Future research needs

We hope that the breeding-for-resistance work that has been so successful in the past, will not be abandoned but, rather, expedited by marker assisted selection. We must make a concerted effort to maintain the existing whitefly/geminivirus research networks. There is the need to continue the basic research that is the foundation of the antiviral strategies, and to improve the available technology for common bean transformation. Finally, we need to advance our understanding of the ecology and epidemiology of whitefly-transmitted geminiviruses in different cropping systems. We sincerely hope that the information presented in this publication will help researchers in developing and industrialized countries to better define their research priorities.

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