

ISSN 1980 - 6477

Journal homepage: www.abms.org.br/site/paginas

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How to cite

VIANA, P. A.; GUIMARÃES, P. E. O.; MENDES, S. M. Maize cultivars with native insect resistance – potential, advances and challenges. *Revista Brasileira de Milho e Sorgo*, v. 21, e1250, 2022.

MAIZE CULTIVARS WITH NATIVE INSECT RESISTANCE – POTENTIAL, ADVANCES AND CHALLENGES

Abstract – The development of resistant cultivars is one of the strategies applied in pest control. The method has the advantages of reduced cost and the lack of unwanted effects on the environment. Over the past decades, significant effort has been made toward developing the natural maize resistance to pests by evaluating germplasm and cultivar selection. This review highlights a maize breeding program, potential, advances, and challenges in addressing these characteristics. Also, it describes the main components and procedures applied in the mass rearing of insect pests of maize, artificial diets, techniques of artificial infestation employed in genotype selection, and methods to evaluate the mechanisms and causes of resistance. Studies on the inheritance of resistance, the breeding methods, and the potential for integrating classical and transgenic resistance are also emphasized.

CULTIVARES DE MILHO COM RESISTÊNCIA NATIVA A INSETOS – POTENCIALIDADES, AVANÇOS E DESAFIOS

Resumo – Entre os métodos de controle de pragas, o desenvolvimento de cultivares resistentes é o método que apresenta as vantagens de custo reduzido e não causar efeitos indesejáveis ao ambiente. Ao longo das últimas décadas, tem sido realizado um grande esforço para o desenvolvimento de resistência natural do milho às pragas através de avaliação de germoplasma e de seleção de cultivares. Este capítulo destaca a potencialidade, avanços e desafios de um programa de melhoramento de milho com esta característica. Descreve os seus principais componentes e os procedimentos usados na criação massal das principais espécies de insetos e as respectivas dietas artificiais utilizadas, as técnicas de infestação artificial empregadas na seleção de genótipos e os métodos para avaliação de resistência, mecanismos e causas. Também são enfatizados os estudos de herança de resistência, os métodos de melhoramento empregados e o potencial para a integração de resistência clássica e a transgênica.

In Brazil, the technology used in maize crop production systems has experienced expressive changes in recent years. This dynamic has modified the incidence of pests in the crop by using cultivars with different resistance levels and adopting new crop practices, such as the no-tillage system in the second crop in irrigated areas, with at least two crop seasons per year. Among the insect pests of maize crops, the fall armyworm *Spodoptera frugiperda* is of the most significant economic importance, causing losses of up to 34% (Carvalho, 1970). According to Cruz (2008), the armyworm can cause grain yield losses from 17.7% to 55.6%, depending on the hybrid, the developmental stage of the plant, and the growing season. The lesser cornstalk borer, *Elasmopalpus lignosellus*, is another relevant pest that can destroy the crop (Viana, 2004, Viana and Mendes, 2020).

Furthermore, the sugarcane borer, *Diatraea saccharalis*, and the cotton bollworm, *Helicoverpa armigera*, have assumed primary pest status in some maize production regions. The damage caused by the sugarcane borer feeding on corn stalk hinders the transport of photoassimilates and predisposes the plant to stalk breakage and lodging (Cruz, 2007; Mendes et al., 2014). Finally, *H. armigera* is a species recently identified in Brazil that has caused considerable losses in the production system. This polyphagous species feeds on the reproductive structures of plants and has caused damage to soybean, cotton, and maize crops (Ávila et al., 2013).

In addition, many other insect pests such as the corn rootworms, *Diabrotica* sp., the corn earworm, *Helicoverpa zea*, the corn leafhopper, *Dalbulus maidis*, the green-belly stink bug, *Dichelops melancanthus*, can also cause significant losses to the maize crop, depending on the region. Furthermore, although it is considered

a secondary pest, the corn aphid, *Rhopalosiphum maidis* can also damage the crop, and its effects depend on the population (Racliffe, 2001).

The development of resistant cultivars is a method for pest control that has the advantages of reduced cost and the absence of undesirable effects on the environment. Over the past decades, significant efforts have been made for developing natural maize resistance to pests by evaluating germplasm and selection of cultivars. Recently, genes codifying different proteins active against insects have been incorporated in diverse plant species, including maize, resulting in what is known as transgenic (genetically modified) plants.

The resistance of plants to insects is defined as the relative sum of hereditary qualities of the plant that affect the resulting degree of damaging that the insect causes (Painter, 1951). A plant resistance program aims to develop cultivars resistant to insects and maintain or enhance agronomic traits. The role of resistance in plants in a breeding program varies according to the crop and the pest species (Ortman and Peters, 1980).

Main components of a maize program for insects resistance

The success of a maize program for insects resistance requires a extensive knowledge of target plants and insects. Thus, pest biology and population, infestation, rearing, pest damage evaluation methods, plant germplasm, resistance, and inheritance mechanisms must be known. This level of knowledge requires a cooperative and interactive multidisciplinary team composed of entomologists, breeders, biochemists, statisticians, and other scientists considered essential for the program's success.

Information concerning the biology, habits, distribution, and control measures for maize pests is available in various publications (Cruz et al., 2008, Viana et al., 2008, Viana and Mendes, 2020). Although various insects attack the crop, only some are considered economically important or primary pests. The main species that attack the initial stage of the plant are the lesser cornstalk borer, the fall armyworm, the corn rootworm, and the green-belly stink bug. During the vegetative and reproductive phases, injuries caused by caterpillars and sucking insects attacking the leaves, stalks, and ears predominate. Depending on the region, the infestation of the sugarcane borer has increased in recent years, leading to risks of losses for maize growers (Cruz, 2007).

Advances for identifying both natural resistance and the resistance of genetically modified maize depend on the ability to distinguish the most resistant genotypes during selection. For that reason, it is necessary to have a laboratory infrastructure for rearing insects that allows them to be used in the infestation of plants and an effective method for evaluating plant resistance related to pest damage. In addition, it is necessary to establish uniform levels of infestation that must be used at the appropriate phenological stage of the plant, allowing selection of resistant genotypes, reducing or eliminating the chances of escape, and allowing the accumulation of genetic resistance (Ortega et al., 1980).

In the following, we discuss the main requirements for developing a program of plant resistance to insects.

Artificial diets and procedures used in mass rearing

One of the most critical components necessary

to identify or develop maize germplasm with resistance to insects is efficiently rear the pest species in the laboratory, aiming at their use in artificial infestation (Davis, 1989; Mihm, 1989a). For the primary caterpillars that attack maize, artificial diets have been created consisting of various ingredients that can be prepared in the laboratory and allow the production of many insects for research studies using artificial infestations. For the fall armyworm and the corn earworm, the diet used in the laboratory of plant resistance to insects of Embrapa Milho e Sorgo was proposed by Burton (1967), as it is easily prepared and provides high viability. The diet modified by Chalfant (1975) and the rearing method adapted by Viana (1999) is used for rearing the lesser cornstalk borer. The same methodology and diet as in CIMMYT (MIHM, 1989a) can be used for the sugarcane borer. For raising *Diabrotica* spp., maize seedlings feed the larvae and common bean plants are used to feed the adults (Ávila et al., 2000).

For any insect to be reared in the laboratory, it is essential to emphasize that each species has a specific requirement concerning the procedure used in raising it. For example, some species have cannibalism as larval development proceeds, and thus the feed requirements and the substrate for oviposition of the adults differ, affecting egg production. In addition, the requirements of temperature and photoperiod, sanitary control to avoid the appearance of fungi, bacteria, viruses, and other particular aspects vary for each insect. Therefore, each species' biology and needs must be well known to produce many insects by the rearing methods used. For example, protocols on the methods used for rearing the main pests of the maize crop are described by Burton and Perkins (1989) and Mihm (1989a).

Procedures used in artificial infestation

In addition to efficient mass rearing, the program for insects resistance also requires methodologies allowing infestation and rapid evaluation. Thus the plant selection is conducted in a greenhouse and under field conditions. It should be emphasized that damage “screening” carried out under controlled laboratory conditions and in a greenhouse needs to be confirmed in field trials due to possible interactions of biotic and abiotic factors existing under natural conditions.

For trials conducted in the field, the mean number of insects used in the infestation is always greater than under controlled conditions. The infestations can be carried out with eggs and newly-hatched larvae. However, the method used must allow an infestation of many plants without spending a great deal of time. For most caterpillars that attack maize, the equipment developed in The International Maize and Wheat Improvement Center – CIMMYT called “bazooka” can be used. In this case, newly-hatched larvae are mixed in ground maize cobs and then applied to the plant. This methodology can be used for the fall armyworm, corn earworm, and sugarcane borer. For trials conducted in the field, 30 to 45 larvae, on average, are used per plant (Mihm, 1989b). However, in trials in a greenhouse, where there is nearly no effect of the biotic and abiotic factors, a smaller number of larvae should be used, around ten larvae per plant. Excessive larvae may destroy the plant due to very high selection pressure, making it impossible to differentiate genotypes. The plant infestation by the fall armyworm and sugarcane borer should ideally be carried out in the V4 to V5 of maize leaf stage (open-leaf), and for the corn earworm, soon upon the emergence of the styles-stigmas.

For the lesser cornstalk borer, infestation in a

greenhouse should be with five eggs near hatching or two newly-hatched caterpillars per plant. The caterpillars are placed on the maize seedling at the beginning of emergence using a brush, whereas the eggs deposited on the oviposition substrate (paper) are glued on plastic stakes and stuck in the ground around the plant. Two caterpillars per plant are used to perform the infestation in the field. In that case, it is recommended that the caterpillars be kept in the laboratory in a coffee cup containing the artificial diet for five days. Then, the diet and the caterpillars are poured on the soil beside the plant stalk (Viana, 1999).

Resistance trials carried out with the corn rootworm generally use many eggs in the infestation. Generally, 600 to 1200 eggs per plant diluted in an agar solution is recommended, injected in the soil at 25 days after maize plant emergence (Branson and Sutter, 1989). The effect of the plant on larvae mortality and biology may also be used to evaluate resistance. In this situation, the number of larvae per plant can be reduced. Field trials can be conducted with a natural infestation, sowing crops considered attractive to the *Diabrotica* adults, such as cucurbits and sunflower, around the plots. This technique increases the insect population in the experimental area and makes it more uniform.

Infestations with specimens collected in the crop field are generally used to evaluate resistance to sucking insects, confining them in small cages on the leaves or screened cages over the plants. Screened-type structures are also used with plants inside, in which the insects are released.

Methods for evaluating resistance

Like the method used in artificial infestation, evaluation of resistance must be easy to use and allow

the evaluator to select a large number of plants rapidly. The methodology most used for evaluating maize plants with resistance to pests is through of visual damage scales. Different possible scales are in use. However, the best scale separates the resistant genotypes from intermediate and susceptible genotypes. Among the various possibilities, the scales described below have been adopted in studies on resistance developed at Embrapa Milho e Sorgo.

For the fall armyworm, a scale from 0 to 9 is used to evaluate the leaf injury caused by the caterpillar, where a score of 0 means no damage on the leaves and score 9, extensive lesions, consumed (dilacerated) parts on most of the leaves, and dead plants (Williams et al., 1983). Evaluation should be made around 14 days after artificial infestation. That time is sufficient for the larval phase to end, considering that temperatures are generally higher during the maize crop seasons.

For the sugarcane borer, the procedure initially used to evaluate resistance was through the opening of stalks and measuring the extension of the galleries. However, that method was considered laborious and consumed much time, mainly when many genotypes were evaluated. After that, research results showed there to be a high and significant correlation between the extension of the gallery caused by the borer with the number of galleries, number of internodes bored into and leaf damage caused by the caterpillar before penetrating the plant stalk (Hinderliter, 1983). Therefore, the procedure most used in selecting genotypes with resistance to the borer is a visual scale of leaf damage. One of the most used scales is proposed by Mihm (1989b), ranging from 1 to 9, with a score of 1 representing no damage or a few small perforations in the leaves and a score of 9 representing most of the leaves with elongated lesions. The evaluation should be performed around 14 days after artificial infestation.

To evaluate the damage from the corn earworm, a revised scale from Widstrom (1967), cited by Mihm (1989b), is used. The evaluation is performed three to four weeks after infestation by removing the husk from the ear and using a scale for determining the damages in both grains and styles-stigmas. The score 0 means no injury on the ear; score 1, damage only to the styles-stigmas; score 2, damage of up to 1 cm to the ear; and score three or more, increase the value by 1 for each centimeter of damage on the ear.

The initial studies for the selection of maize with resistance to the larvae of *Diabrotica* used different evaluation methods. These methods included root size, the ability to regenerate secondary roots after the attack, lodging, resistance to uprooting, and the ability of the plant to survive the attack of the larvae (Ortega et al., 1980; Branson and Sutter, 1989). Currently, evaluation of the damage caused by the larvae on the roots is the most used method: collecting roots at around 55 days after the maize sowing, then washing the roots, and evaluating the larvae severity-attack through a visual scale. The scale from 1 to 6 proposed by Hills and Peters (1971) and cited by Branson and Sutter (1989) is one of the most used. Score 1 represents no damage or only some signs of feeding on the roots, and score 6, three or more root nodules are destroyed. Another easy-to-use scale to measure the degree of root-damages ranges from 0 to 3 (Oleson et al., 2005) has also been used.

The method used to evaluate the resistance of maize genotypes to the attack of the lesser cornstalk borer is through the number of plants effectively attacked by the caterpillar up to the third week after artificial infestation. Therefore, the evaluations under field conditions should begin one week after

the artificial infestation and be performed three times a week to prevent the attack from being confused with other pests (Viana, 1999; Viana and Mendes, 2020).

The resistance of maize to the corn leafhopper, *D. maidis*, has been indirectly evaluated through maize bushy stunt and determined based on the percentage of plants with symptoms of the disease. The severity is determined, and scores are attributed from 1 to 6, referring to the mean level of the symptoms on the plants, where 1: absence of symptoms; 2: plants with at least 25% of the leaves with symptoms, that is, reddish or yellowish leaves, or exhibiting chlorotic streaks at their base; 3: plants with 25% to 50% of the leaves with symptoms; 4: plants with 50% to 75% of the leaves with symptoms; 5: plants with more than 75% of the leaves with symptoms; and 6: plants with early death caused by maize bushy stunt (Silva et al., 2003).

Resistance mechanisms

Although knowledge of mechanisms, inheritance, and resistance causes is not limited to developing a breeding program aiming at insect resistance, when these parameters are clarified, they are handy for progress in the insects resistance program, contributing to the choice of the breeding method adoption, duration projections, determination of the resistance effectiveness, and assistance in the planning of new lines of activity to be followed in solving future problems (Smith et al., 1989).

The resistance mechanisms described are antibiosis, non-preference or antixenosis, and tolerance. The term “non-preference” expresses a behavioral reaction concerning the plant, whereas the other two mechanisms define a reaction of the plant to the insect (Lara, 1991).

Antibiosis indicates that when the insect feeds

on the plant, it experiences an adverse effect on its biology. This effect can be manifested directly or indirectly, resulting in mortality in the young phases and prevent the transformation to the adult phase, reduction in size and weight, reduction in fertility, and change in the proportion of the sexes and the life cycle.

The non-preference mechanism is characterized when the insect uses the plant less than another under similar conditions, for feeding, oviposition, and shelter.

The tolerance is the resistance mechanism that depends exclusively on the plant and does not act on the insect. The plant is considered tolerant when it suffers minor damage compared to others under the same level of infestation of the pest, and the damage does not affect its biology and its behavior. However, there are cases in which the resistant genotype bears significant damage and, through regeneration of the destroyed tissues or any other means, it exhibits less reduction in yield.

Studies of resistance mechanisms conducted by Viana and Potenza (2000) with the genotypes CMS 23, CMS 14C, CMS 24, Zapalote Chico, and BR201 indicated the CMS 14C population as that which most harmed the biology of *S. frugiperda*. The results also showed that Z. Chico and BR 201 were non-preference for feeding the caterpillars, while CMS 14C and Z. Chico exhibited a non-preference for oviposition. Siloto et al. (2002) evaluated the effect of 12 materials (commercial hybrids and other varieties) in larval development of this pest and reported that the hybrids Master and Z 8486 were those that most limited the development of *S. frugiperda*, whereas XL 212 was the one that most favored it.

Antibiosis was also found in a hybrid coming from lines with this mechanism (Guimarães et al.,

2004). However, the hybrid developed had the highest larval mortality (32%) and the lowest mean values for weight of the larvae at 11 days of age, corresponding to 41% of that shown by Z. Chico and 21% of that observed for the hybrid BR 201, the susceptible check cultivar.

Causes of resistance

Even when resistance may be present in a genotype, its causes are not always known and are conditioned by physical, chemical, morphological, and physiological factors of the plant and the response of the pest itself. Among the physical factors, especially the different radiations and shades of colors of the plants are prominent, for these differences can be differentiated by the insects. The chemical factors are substances of the plant that act on the insect's response and metabolism and promote a nutritional imbalance in the insect. Finally, morphological structures of the plant, such as types of epidermis, dimension, and arrangement, are factors determining resistance. Additionally to the plant factors, those inherent to the insect's behavior, such as selectiveness towards hosts, are known as the Hopkins principle.

There are still many aspects to be investigated related to the causes of resistance in maize, especially in the level of identification of substances involved in the phytochemical mechanisms of resistance as an instrument for entomologists, breeders, and biotechnologists in the search for new cultivars with resistance to pests (Reesse, 1989; Bergvinson et al., 1997; Arnason et al., 1997; Snook et al., 1997; Warnock et al., 2001; Prates, 2002). Studies performed by Niemeyer (1988) showed that maize lines and varieties had exhibited phytochemical properties that limit the damage brought about by insects. The hydroxamic and phenolic acids of

natural origin have proven to reduce reproductive potential and, consequently, in the damage brought about by phytophagous insects (Philogène and Arnason, 1995). Hydroxamic acids are present in maize roots (Xie et al., 1991) and leaves, constituting up to 10% of the total dry weight of the plant. The concentration varies according to the line, varieties, altitude, and longitude (Philogène and Arnason, 1995). Two compounds, DIMBOA ((2,4-dihydroxy-7-methoxy-(2H)-1,4-benzoxazin-3(4H)-one) and MBOA (6-methoxy-2-benzoxazolinone) are active against other relevant maize pests in other countries, such as *Ostrinia nubilalis* (Guthrie et al., 1986; Barry et al., 1994), *Diatraea grandiosella* (Hedin et al., 1984), and *Diabrotica virgifera* (Niemeyer, 1988; Bjostad and Hibbard, 1992). The maysin flavonoid (luteolin 6-rhamnosyl-4-ketofucoside), isolated from the styles-stigmas, is reported as having activity against *Helicoverpa zea* (Snook et al., 1989). Lopez et al. (2007) reported that the herbivory of Lepidoptera in the resistant Mp708 maize genotype resulted in a rapid accumulation of the defense cysteine protease enzyme (Mir1-CP) in the vascular tissues. The chlorogenic acid described in the literature as a natural metabolite with feeding deterrence activity was identified in some leaf extracts of maize with resistance to *S. frugiperda* (Machado et al., 2014).

Inheritance of resistance and breeding methods

The gene action conditioning resistance for most of the insect pests of maize appears to be additive, indicating that procedures such as mass selection and various recurrent selections effectively accumulate the genes desirable for this trait (Ortega et al., 1980). According to Santiago et al. (2008), recurrent selection can be determinant in changing

the phenolic concentrations conferring resistance to the Mediterranean corn borer *Sesamia nonagrioides*. Similar results were obtained for lines resistant to maize pests in Africa, such as *Chilo partellus* and *Busseola fusca*, considering the predominant additive gene action (Karaya et al., 2009).

Guimarães et al. (2004) evaluated the combining ability of six maize lines of the CMS 23 population for larval development and the biological cycle of *S. frugiperda*. They reported significant variability for diallel analysis in diverse characteristics related to the development and cycle of this insect and that it was possible to select lines and hybrid combinations with more significant potential for acting against its biology. The two lines that exhibited the best general combining ability for various characteristics related to the antibiosis mechanism were also the progenitors of the hybrid with the most unfavorable set of CEC and heterosis values for larval development and the life cycle of this insect. This hybrid was also that which had the most significant mortality of the larvae (32%) and the lowest mean values for larval weight (67 mg) at 11 days of age, corresponding to 41% of that shown by Z. Chico (164 mg) and 21% of that observed for the hybrid BR 201 (315 mg), the susceptible check cultivar.

Integration of classic and transgenic resistance

Up to the advent of genetic engineering, prospecting sources of resistance to insect pests was carried out using only the plant species diversity. With transgenics, it can be affirmed that all the ecosystem's biodiversity is available for prospecting (Waquil et al., 2019).

The bacterium *Bacillus thuringiensis* (Berliner) (*Bt*) has been used as a bioinsecticide for decades

(Feitelson et al., 1992) and is registered, without limitation for use, for control of various pest species of Lepidoptera. Various *Bacillus* species were found, and within these species, many populations and hundreds of isolates from the most diverse regions are now registered in the literature. The active fractions produced by *Bt*, which are the accumulated proteins in crystal form within the cells, can constitute more than 30% of the total proteins of the cell (Hermstadt et al., 1986).

Currently, there is the option for developing maize cultivars resistant to *S. frugiperda* through the use of *Bacillus thuringiensis* (*Bt*) genes codifying insecticide proteins. The first events expressing the *Bt* toxins in maize were mainly aimed at controlling the European corn borer, *Ostrinia nubilalis*. After that came the incorporation of new toxins, opening the possibility for use in the control of various other species. Currently, various *Bt* events are expressed in maize plants (Waquil et al., 2002; 2004; Villela et al., 2002). Incorporating *Bt* genes in elite public lines are considered strategic for developing resistant cultivars, also bringing the possibility of developing more resistant hybrids by combining parental lines with classic and transgenic resistance (Williams and Davis, 1990). The strategy adopted by CIMMYT, through the IRMA project, is transferring the resistance of genetically modified maize, based on *Bt*, to existing populations with multigene resistance to pests, aiming at increasing the level of the resistance durability. (Mugo et al., 2001).

Advances, potentialities, and challenges in genetic resistance of maize to insect pests

Improving maize cultivars with durable multiple resistance to insects and diseases is considered of prime importance (Miedaner and Juroszek, 2021) and

constitutes a significant challenge (Kim et al., 2021). Broadly, breeding for resistance has been conducted to develop hundreds of resistant cultivars, increasing the yield and stability in production, associated with economic savings and good production standards, minimizing the damage caused by pests. In order to improve plant resistance to insects, it is essential to identify sources of genes conferring resistance. As sources of variability, the primary gene pool is the first choice of the breeder, as that may not only improve the crop agronomically but also confer resistance to insects. Transferring resistance from a secondary gene pool to the desired genotype is frequently time-consuming and laborious (Sandhu and Kang, 2017).

Various sources of resistance to the attack of pests have been identified for the maize crop. Genotypes with the trait called “bitter” have been reported as the most promising for resistance to *S. frugiperda* (Bertels, 1956). Materials of the “Antigua” group are also reported as sources of resistance to this pest (Wiseman, 1985; Wiseman and Davis, 1990). The genotypes Antigua 2D-118, MpSWCB-4, Pio. X304C, Mp 496, Zapalote Chico 2451, and MP 701-707 have been listed as sources of resistance to armyworms identified by various researchers (Wiseman, 1985). Boiça Jr. et al. (1993) identified the materials Zapalote Chico and TL 87-A-1855-7 as the least attacked by the pest among 24 genotypes evaluated. Osuna et al. (1995) evaluated 98 half-sib families of the Flint composite, aiming at resistance to *H. zea* and *S. frugiperda*, and they showed that the genotypes under selection had good variability. Viana and Gama (1988), Viana and Potenza (1992), Viana and Guimarães (1997), and Costa et al. (2007) found resistance to *S. frugiperda* in tropical maize. In the United States, various maize cultivars have been registered and released for public use that has resistance to *H. zea*, *S. frugiperda*, and *D. grandiosella* (Wiseman

and Davis, 1990). For *E. lignosellus*, few studies have been performed. Viana and Gama (1991) showed that this pest least attacked the variety Zapalote Chico and the CMS 15 population. New populations were selected as sources of resistance to the lesser cornstalk borer (Viana and Guimarães, 1997). Studies performed with 15 lines derived from backcrossing were evaluated for resistance to the main maize caterpillar pests (Abel et al., 2000). The lines selected with *S. frugiperda* resistance were 100-R-3, 116-B-10 for *S. frugiperda* and *D. grandiosella*, and 81-9-B and 107-8-7 for *H. zea*. The experimental maize hybrids IL1411, IL1477, IL1500, IL1409, IL1457, and IL1397 (Viana et al., 2014), and the lines 51206413 and 51205324 (Viana et al., 2016) suffered minor leaf damage caused by fall armyworm and had the most significant impact on the reduction of larval development. According to Ni et al. (2014), lines coming from tropical germplasm have been evaluated and found to be a source of natural resistance to *S. frugiperda* and *H. zea*. Of 15 genotypes evaluated for resistance to the corn earworm and the fall armyworm, one was selected for use in advanced breeding with high-yielding cultivars, including *Bt* hybrids grown in the southeast of the United States (Farias et al., 2014).

The existing programs use different breeding methods. At USDA-ARS, Mississippi, USA, is based on obtaining resistant homozygotic lines through selection in the successive generations of self-fertilization (Willians and Davis, 1989 and 2000). The lines Mp 713 and 714 were obtained with selection for resistance to *D. grandiosella* and *S. frugiperda* in eight generations of self-fertilization. The leaf damage caused by *S. frugiperda* in these lines and the susceptible check Ab24E were 4.6, 5.5, and 7.9, respectively (Willians and Davis, 2000). The

USDA-ARS program, Georgia, USA, uses a recurrent selection of S1 progenies in two populations, and in one of them, the mass selection was also applied, which proved to be ineffective. CIMMYT, Mexico, in a multiple resistance program, improved the MBR (multiple borer resistance) composites through two primary lines: recurrent selection of full-sib progenies evaluated in international trials; and obtaining and evaluating “per se” lines for the formation of synthetics as new sources of lines, and in crosses – test for determination of heterotic groups and hybrid formation (Smith et al., 1989). Dekalb-Pfizer selected lines from elite germplasm introgressed with the resistant lines were synthesized by the USDA-ARS, Mississippi (Overman, 1989). Kumar and Kumar (2002) compared the performance of Ag × Ag and Ag × R hybrids, synthesized with CIMMYT lines of the “Ag” (elites for agronomic characteristics) and “R” (resistant to *S. frugiperda*) types. Ag × R hybrids had minor leaf damage and lower yield than the Ag × Ag. The authors suggested the development of resistant lines and lines with desirable agronomic characteristics through the backcrossing process for obtaining high-yielding and resistant hybrids. The Embrapa Milho e Sorgo developed maize lines resistant to *S. frugiperda* extracted from the CMS 23 and MIRT populations (Guimarães and Viana, 1994; Viana and Guimarães, 1994). Since these sources did not have satisfactory agronomic performance, the program currently recycles resistant lines with elite lines for agronomic performance. From 2016, the fall armyworm, which had previously been under quarantine measures, became one of the main pests in the maize crop in Africa. CIMMYT evaluated, under artificial infestation, around 3500 hybrids for native resistance to this pest. Eight were selected and evaluated in trials conducted under-screened

enclosures and in the field. In the trial conducted under screening and artificial infestation, three hybrids selected for native resistance yielded 7.05 to 8.59 t/ha, whereas three commercial check cultivars yielded 0.94 to 1.03 t/ha. At field trials under the low incidence of natural infestation, significant differences in yield were not observed between the three hybrids selected and the commercial check cultivars (CIMMYT, 2020). In the United States, around 30 maize cultivars with resistance to *H. zea*, *S. frugiperda*, and *D. grandiosella* were registered and released for public use (Wiseman and Davis, 1990). The line Mp716 was registered as a source of resistance to *D. grandiosella* and *S. frugiperda* (Willians and Davis, 2002) and the population Zapalote Chico 2451F for resistance to *Euxesta stigmatias*, *S. frugiperda*, and *H. zea* (Widstrom et al., 2003). For *E. lignosellus*, it was shown that the variety Zapalote Chico and the CMS 15 population were less attacked by this pest (Viana and Gama, 1991). After that, new populations were selected as sources of resistance to the lesser cornstalk borer (Viana and Guimarães, 1994). Currently, there are options for developing maize cultivars resistant to *S. frugiperda* through *Bacillus thuringiensis* (*Bt*) genes codifying insecticide proteins (Waquil et al., 2002; 2004 and Vilella et al., 2002). In addition, there is the possibility of developing more resistant hybrids by combining parental lines with classic and transgenic resistance (Willians and Davis, 1999). This procedure may retard the breakdown of resistance of transgenic *Bt* maize to the pests, as later reported by Tabashnik et al. (2009) and Storer et al. (2009). For *H. armigera*, which has a history of a rapid selection of resistance to chemical insecticides and *Bt* proteins in GM plants (Alvi et al., 2012; Kriticos et al., 2015), natural resistance would be a sustainable strategy for its management in the context of IPM (Integrated

Pest Management).

According to Stout et al. (2009), plants undergo changes in gene expression and primary and secondary metabolism after losses caused by arthropods. One of the forms of resistance is direct, induced resistance. In that case, the plant reduces plants' suitability or palatability for the herbivores. Another form is indirect, induced resistance, where the plant can improve the effectiveness of natural enemies of the herbivores. Both responses are frequently systemic. For Farinelli and Fornasieri (2006), resistant plants affect the pest, producing less vigorous individuals more susceptible to the chemical treatments, making for more efficient use of those treatments.

A new source of natural resistance of maize to the attack of *D. virgifera virgifera* was identified by Tollefson (2007). According to the author, this source may be an alternative for regions with low or moderate populations of the pest and in the cases that genetically modified maize is not permitted or not preferred by the growers.

There is little information in the literature regarding resistance to the corn leafhopper and methods for evaluations of this resistance. Maize seedlings have been used to evaluate resistance to *D. maidis* (Silva et al., 2003). A collection of maize hybrids evaluated for resistance to the corn leafhopper showed a significant difference among the genotypes evaluated. A more extended period of development was found for the nymphs developed in the hybrid Pioneer 3027 (27.15 days) in contrast with those developed in the other five hybrids (mean of 24.82 days) (Zurita et al., 2000).

In recent years, outbreaks of infestation of maize by the aphid *R. maidis* have been recorded in Brazil (Pereira et al., 2006). Studies have shown that maize hybrids show differences in the degree of constitutive

resistance to *R. maidis*. Field evaluations have shown that the hybrids P30F53H, STATUS VIP, BM9288, DAS2B587HX, DKB175PRO, AS1633PRO, and DKB390PRO2 had the lowest percentages of plants with aphids, indicating that these hybrids were resistant to *R. maidis* (Bôer, 2017).

According to Carena and Glogoza (2004), plant breeding aiming at the resistance of maize to the aphid *R. maidis* continues to be a challenge due to dependence on the natural infestation. Nevertheless, new methods of analysis and maintaining colonies and artificial infestation that are being developed may change this scenery, making both selection of resistant types and support for breeding programs viable to avoid commercialization of more susceptible cultivars.

In addition to these advances in research, the genetic resistance of maize to insect pests has enormous potential to improve resistant lines or varieties with desirable agronomic traits and high yield. The big challenge is to make these products effective and available for additional studies and evaluations, developing and recommending these materials for research for insects resistance and breeding programs of the local, national, and multinational seed companies, intending to provide the farmer with high-yielding cultivars with genetic resistance to the main pests without causing impacts on the environment.

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