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REVIEW: PLANT RESISTANCE TO AGRICULTURE INSECT PESTS

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ABSTRACT

Competition and interaction among organisms in nature results to selection for traits which confer added advantage. As a result of such interactions between plants and their natural enemies, plants have developed defensive mechanisms, both chemical and physical, to minimize pest damage. Intensive researches were carried out to identify and isolation the gene responsible for resistance plant to insects' infestation which is used in breeding programs for crops improvement. It can be summarized as follows:

1- Corn ear worm, *Helicoverpa zea* (Boddie) can't cause damage to certain corn strain due to maysin, a C-glycosyl flavone, and related compounds in the silks that inhibit corn earworm larval growth. Scientist can identify corn chromosome regions associated with silk maysin concentration, and then used it in producing new hybrid.

2- Other insect such as the fall army worm, *Spodoptera frugiperda* can't cause damage to resistance corn due to some factors as high hemicelluloses content, low protein content and leaf toughness. They can also identify a gene code which is controlled by the resistance traits of corn to the fall army worm.

3- However, the European corn borer, *Ostrinia nubilalis* (Hubner) can't cause damage to certain corn varieties due to high concentrations of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) in the leaves of mid-whorl stage plants. Scientist indicated that resistance to the European corn borer was identified in Peruvian corn germplasm and the GEM (Germplasm Enhancement of Maize) corn collection, then make backcross to produce new resistance strain.

4- Other crops as tomato infested by *Tuta absoluta* (Meyrick) but some tomato varieties as *Lycopersicon esculentum* can resist it due to anti-xenosis factor which interrupted tomato leaf miner from feed, oviposition and shelter.

5- Russian wheat aphid, *Diuraphis noxia* (Mordvilko), can't cause damage to some wheat and barley and others crop due to antibiosis and anti-xenosis factors. Six sources of resistant wheat, each governed by a different, single gene, have been identified.

6- Two-spotted spider mites, *Tetranychus urticae* (Acari: Tetranychidae) and Whiteflies, *Bemisia tabaci* (Hemiptera: Aleyrodidae) are herbivorous pests. Additional whitefly infestation to spider-mite

infested plants resulted in a reduced attraction of predatory mites (*Phytoseiulus persimilis*). This interference was shown to result from the reduction in (E)- β -ocimene emission from plants infested by both spider mites and whiteflies.

7- Transgenic crops expressing a protein from the bacterium *Bacillus thuringiensis* Berliner (Bt) were carried out into potato, maize, cotton and soybean to be reduced their insect infestation.

Keywords: Resistance factors, Morphological trait, Plant volatiles, Historical cases, Maize pests, Tuta absoluta, Wheat pests, Transgenic, Risks, Precaution.

Contribution/ Originality

This study contributes in the existing literature which interested to study factors of plant resistance to agriculture insect pests and its' role to improve new plant strains.

1. INTRODUCTION

Plants and insects have coexisted for as long as 350 million years, if the earliest forms of land plants and insects are included, and have developed a series of relationships which affect the organisms at all levels, from basic biochemistry to population genetics. With the domestication of plants for agriculture, farmers always selected plants which withstood adverse environmental factors, including insects and diseases. Those plants which were susceptible to pests generally died, and only resistant plants survived until crop harvest.

Insect-resistant crop varieties suppress insect pest abundance or elevate the damage tolerance levels of these plants. In other words, insect-resistant plants alter the relationship an insect pest has with its plant host. How the relationship between the insect and plant is affected depends on the kind of resistance, e.g. antibiosis, antixenosis (non-preference), or tolerance.

Plant resistance is defined as the heritable characteristics possessed by a plant which "influence the ultimate degree of damage done by the insect" ¹[1] or "reduce the probability of successful utilization of the plant by the insect"²[2]. These definitions differ in that Painter included tolerance, where the plant "shows an ability to grow and reproduce itself, or to repair injury to a marked degree, in spite of supporting a population approximately equal to that damaging a susceptible host"². Beck recognizes that tolerance "implies a biological relationship between insects and plants that is quite different from resistance in the strict sense", and excludes it from his definition of resistance.

Tolerance does not involve the degree of insect utilization of the plant, or the amount of biting damage or stylet feeding done, but lies in the response of the plant to a given level of biting or stylet feeding. As rapid foliage growth in Lucerne (*Medicago sativa*) attacked by alfalfa weevil³[3] or rapid tiller production after attack by the sorghum shoot fly on sorghum (*Sorghum bicolor*)⁴[4].

Plant breeding, is the science of altering the genetic pattern of plants in order to increase their value. Increased crop yield is the primary aim of most plant-breeding programs; advantages

of the hybrids and new varieties developed include adaptation to new agricultural areas, greater resistance to disease and insects, greater yield of useful parts, better nutritional content of edible parts, and greater physiological efficiency. Other goals are adaptation of crops to modern production techniques such as mechanical harvesting and improvement in the market quality of the product⁵⁻⁷[5-7]. Consequently, the term “plant breeding” is often used synonymously with “plant improvement” in modern society.

The plant breeding techniques have been increasingly sophisticated since 1900 and have routinely employed technique such as cell fusion since 1909, mutation via X rays since 1927 and embryo rescue since the 1960's. The latest technique to be introduced to facilitate plant breeding is genetic engineering; by which genetic materials from other organisms are inserted into plant to allow it express novel traits⁸[8].

The Objectives of the Present Review: To analyze those factors which influence pest incidence infestation to plants, how these plants can resist them and how breeding could be realized.

II.1. Resistance Factors:

Resistance factors are the inherited plant characters which affect resistance at a phase or phases of the insect/plant relationship. Resistance factors of plant including:

a- Plant Morphological Traits: The morphological attributes of plants may have some direct impact on herbivores. For example, leaf pubescence⁹[9] and lack of cuticular wax blooms¹⁰[10] can impair the development of herbivores. Of particular interest is the impact that these morphological traits can have on the natural enemies of herbivores. If morphological barriers interfere with the action of natural enemies, the benefits of these ‘defenses’ may be reduced. Understanding the compatibility of plant resistance factors with biological control and the interactions of biological control enhancing features, such as leaf domatia, with plant resistance remains an important challenge for plant biologists^{11,9,10}[9-11].

b- Plant Volatiles: Plants respond to initial attack by herbivores and pathogens by increasing their levels of defense¹²[12]. For example, volatile compounds emitted by herbivore-infested plants that are attractive to natural enemies of herbivores are hypothesized to be an evolved response to herbivory. Although the net costs or benefits in plant performance of such induced volatile responses have not been identified, intricate and highly specific interactions between constituents of herbivore saliva and plant responses provide circumstantial evidence of their importance.

Insect herbivorous active induced defense both locally and systematically by signaling pathways involving system in, jasmonate, oligo-galacturonic acid and hydrogen peroxide. Plants also respond to insect attack by producing volatiles, which can be used to deter herbivorous to communicate between parts of the plant or between plants, to induce defense responses¹³[13].

Recent physiological studies have linked the plant signal transduction pathways that result in induction of direct defenses in leaves to indirect defenses that act through the production of volatiles which attract natural enemies of herbivores. Jasmonic acid (JA) is a key component of the highly conserved octadecanoid (fatty acid) pathway in plants that attraction of parasitoids was also shown to be associated with foliar defenses that negatively affected several herbivores by increasing their development time^{14&15}[11, 14].

In general, the bouquets of volatiles which are emitted from damaged plants come from at least three biosynthetic pathways: first, the fatty acid (or octadecanoid) pathway produces leafy green volatiles and jasmone; second, the shikimic acid (or tryptophan) pathway produces indole and methyl salicylate; and thirdly, the isoprenoid-derived pathways produce terpenes¹⁶[15].

III. Summarization of Some Historical Cases of Insect/ Resistance in Selected Agricultural Crops.

1- **Corn Earworm:** The corn earworm, *Helicoverpa zea* (Boddie), is native to the Americas and occurs wherever corn, *Zea mays* (L), is grown. Adult females prefer to lay eggs on fresh silks and emerging larvae move from the exposed silks to a more protected position in the silk channel formed by the husk extension. If silk quantity is sufficient, larval development may be completed in the silk channel; but, if the amount of silk is limited or if the husks are sufficiently loose, larvae will move to and feed on developing kernels resulting in significant economic impact. In addition to direct kernel damage, corn earworm damage enhances introduction of secondary pests and micro-organisms such as *Aspergillus flavus* (Link) and *Fusarium moniliforme* Sheldon which produce mycotoxins.

2- In the southeastern USA, susceptible corn hybrids serve as a nursery for the development of large corn earworm populations, which not only cause considerable damage to corn, but produce large populations of adults which infest other crops and subsequently cause substantial economic losses in cotton (*Gossypium hirsutum* L), peanut (*Arachis hypogaea* L), sorghum (*Sorghum bicolor* L), soybean [*Glycine max* (L)], and many vegetables and ornamental crops.

3- Host plant resistance to corn earworm is due to maysin, a C-glycosyl flavone, and related compounds in the silks that inhibit corn earworm larval growth¹⁷[16]. Upon wounding of silk tissue, such as with insect chewing, maysin and related compounds are believed to be oxidized by polyphenol oxidases to quinones, which are responsible for the silk-browning reaction^{18&19}[17, 18]. In the larval gut, quinones apparently bind to -SH and -NH₂ groups of free amino acids and proteins, reducing their availability to the insect and thus inhibiting larval growth and development²⁰[19]. Quantitative trait locus methodology has been used to identify corn chromosome regions associated with silk maysin concentration. In the population (GT114 × GT119) F₂,²¹[20] studied maysin inheritance by associating phenotypic values of individual plants with genotypic variation at flavonoid pathway loci. Using RFLP markers, they found that the *p1* region of chromosome1 accounted for 58% of the phenotypic variance for the trait and

detected a second QTL on the short arm of chromosome 9 that showed significant epistasis with *p1*.^{18,22,23}[17, 21, 22], demonstrated that the primary locus controlling the synthesis of apimaysin is located on maize chromosome 5.^{24&25}[23, 24] documented that the interaction between *p1* and *a1* express quantitative genetic control over maysin, apimaysin, methoxymaysin and chlorogenic acid, and confirmed that *p1* and *a1* are major QTLs controlling maysin concentration in populations (GE37 × 565) F₂ and (SCI 02 × B31857) F₂. Other loci with significant associations with resistance in corn to the corn earworm and maysin production include *umc105a* on chromosome 9S,¹⁸[17] *asg20* on 2L,²¹[20] *wx1* located on 9S,^{18&23}[17, 22] *bnl5.71* on 5C-5L,²³[22] *umc85* on 6S,²³[22] *npi286* on 1S,²⁵[24] and *csu1066* on 2C-2L^{26&27}[25, 26].

2. FALL ARMY WORM

Corn and grain sorghum are grown on more than 95 million acres in USA. The fall armyworm, *Spodoptera frugiperda* (JE Smith) is one of the most economically damaging insect species of the tropical and subtropical regions of the Western Hemisphere and is an especially important pest of corn and sorghum). An average annual crop loss to the fall armyworm in USA exceeds \$300 million, but during particularly severe outbreaks, losses attributed to this pest may exceed \$500 million annually.

ARS scientists Scott, Davis and Williams released the first corn germ plasm with resistance to fall armyworm^{26,28,29}[25, 27, 28]. Since then, numerous inbred have been developed from Antigua germplasm. Factors associated with the resistance, such as high hemicelluloses content, low protein content and leaf toughness, are correlated with reduced larval growth^{30&31}[29, 30].

Recently, a gene coding the 33-kD cysteine proteinase has been cloned from corn genotypes resistant to the fall armyworm. When larvae were reared on callus expressing the proteinase, their growth was inhibited 60 to 80%³²[31].

3. EUROPEAN CORN BORER

The European corn borer, *Ostrinia nubilalis* (Hubner), was introduced into the USA in the early 1900s and has spread across the corn-producing areas of the country from the east coast to the Rocky Mountains.

Estimated annual losses in the US Corn Belt alone ranged from \$200 to \$500 million annually³³[32]. Losses resulting from physiological damage due to feeding injury to the plant, reduced quality due to direct kernel damage and to dropped ears.

Extensive host plant resistance research on the European corn borer identified antibiosis resistance³⁴[33] due to high concentrations of 2,4-dihydroxy-7- methoxy-1,4-benzoxazin-3-one (DIMBOA) in the leaves of mid-whorl stage plants³⁵[34]. Commercial seed companies have incorporated this resistance into commercial corn varieties³³[32]. In addition, European corn borer resistant composite populations Mo-2ECB and Mo-2 ECB-2, and inbred Mo45, Mo46 and

Mo47 have been released with excellent resistance to both leaf feeding and sheath and collar feeding³³[32].

More recently, resistance to the European corn borer was identified in Peruvian corn germplasm³⁶[35] and the GEM (Germplasm Enhancement of Maize) corn collection³⁷[36]. Antibiosis and feeding non-preference were identified as the mechanism of resistance in the Peruvian germplasm³⁸ [37]. All of the Peruvian lines had low concentrations of DIMBOA in the leaves, indicating that another mechanism was responsible for the resistance. Several of these corn lines also had resistance to other pests³⁹[38]. A backcross breeding program with this germplasm using B94 or B97 as the recurrent parent was extremely successful, with 15 lines identified with leaf feeding resistance and eleven lines with both leaf feeding and sheath/collar feeding resistance to the European corn borer^{36,39}[35, 38].

Two of these lines [(PI 503720 × B97) // B97 and (PI 503806 × B94)//B94] were also resistant to leaf feeding by the fall armyworm and another [(PI 503731 × B94) // B94] was resistant to silk feeding by the corn earworm⁴⁰[39]. Thus, several of these lines offer the potential to develop commercial lines of corn with multiple pest resistance.

4. TOMATO LEAF MINER

The tomato leaf miner *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is one of the major pests attacking the commercial tomato (*Lycopersicon esculentum* Mill.), and it occurs (causing damage to tropical and subtropical regions). The larvae damage the leaves creating perforations in the form of galleries, since they feed on mesophyll tissues⁴¹[40].

New shoots, flowers and fruits are also attacked⁴²[41]. Plant resistance may occur by antixenosis and antibiosis mechanisms. Antixenosis is characterized by lower utilization of the host by insects, for food, oviposition and shelter⁴³[42].⁴⁴Antônio, et al. [43] determine the inheritance of resistance by antixenosis in tomato plants (*Lycopersicon esculentum*) to tomato leafminer [*Tuta absoluta* (Lepidoptera: Gelechiidae)]. Evaluations were performed for tomato plants of the generations P1, P2, F1, F2, RC1 and RC2. The measured characteristic in the parents, BGH-1497 (P2 male) and 'Santa Clara' (P1 female), and in the F1, F2, RC1 and RC2 generations was the number of eggs per plant. This number was converted to the oviposition non-preference index. The inheritance of antixenosis resistance of genotype BGH-1497 is ruled by a gene of greater effect and polygenes in epistatic interactions, with a phenotypic proportion of 13:3 between susceptible and resistant genotypes, respectively.

5. GREEN BUG AND RUSSIAN WHEAT APHID

Several species of aphids are important, primary pests of small grain grown in the USA. The green bug, *Schizaphis graminum* (Rondani) feeds on sorghum, wheat, barley and other small grains and was the most important small grain aphid pest until the discovery of the Russian wheat aphid, *Diuraphis noxia* (Mordvilko), in Texas in 1986⁴⁵[44]. Since its introduction, the Russian wheat

aphid has spread throughout the Western USA and has become a serious, perennial pest of both wheat and barley^{45,46} [44, 45]. The small grains germplasm collection maintained by USDA-ARS has proved to be of vital importance in the identification of resistance to both important aphid pests. At the time of its introduction, all commercial cultivars of wheat and barley were highly susceptible to the Russian wheat aphid⁴⁵ [44]. Subsequently, the entire working collection of wheat and barley from the USDA-ARS National Small Grains Collection was screened for resistance, and several different sources of resistance were identified. Initial screening efforts identified resistance in five barley accessions from Afghanistan (PIs 366444, 366447, 366449, 366450 and 366453) and one accession from Iran (CI1412). PI 366450 had a high level of resistance and was selected for use in an accelerated breeding program to provide resistance to this insect in barley. Selection for uniformity of resistance led to the release of STARS-9301B⁴⁷ [46]. Resistance is due to tolerance, antibiosis and antixenosis, resulting from the action of two genes, *Dnb1* and *Dnb2*,⁴⁸ [47] controlled by recessive epistasis of the dominant gene *Dnb2* on the incompletely dominant *Dnb1*. Another source of resistance, STARS-9577B, was released in 1999⁴⁸ [47]. Resistance in this line is primarily due to tolerance, but a low level of antibiosis is also present. Several important sources of resistance to the Russian wheat aphid have been identified in wheat. Resistance in PI 140207 and PI 137739 is due to antibiosis and is controlled by a single dominant gene, *Dn1*⁴⁵ [44].⁴⁹ Porter and Webster [45] reported that a 24-kD protein complex was inhibited in a susceptible wheat genotype after the Russian wheat aphid fed, but persisted after feeding in PI 140207. They concluded that feeding by the Russian wheat aphid selectively inhibits normal metabolic functions in susceptible plants, but not in the resistant genotype.⁵⁰ Webster and Porter [48] found that resistance to the Russian wheat aphid in STARS-9302W does not confer resistance to other important species of aphid, the greenbug, bird cherry-oat aphid, *Rhopalosiphum padi* (L) or the yellow sugarcane aphid, *Sipha flava* (Forbes).

The green bug is still a serious pest, especially in the Southern Great Plains where periodic outbreaks result in loss of millions of dollars due to damage and costs of control^{51,52} [49, 50]. It has been able to adapt to changes in the environment and resistance in hosts, resulting in the development of several biotypes. Currently 11 biotypes of the green bug have been identified⁵³ [51]. Six sources of resistant wheat, each governed by a different, single gene, have been identified: DS 28A,⁵³ [51] PI 578213 (Amigo),⁵⁴ [52] PI 268210 (Largo, CI 17895),⁵⁰ [48] CI 7959,⁵⁵ [53] CI 1788240 and PI 561948 (GRS 1201)⁵² [48, 50].⁵³ Porter, et al. [51] chronicle the development of the different green bug biotypes and the reaction of each biotype to the six sources of resistance in wheat. Currently, there are three predominant greenbug biotypes, E, I and K.⁵² Webster and Porter [50] reported that 'GRS 1201' and 'Largo' were resistant to these three biotypes, but 'GRS 1201' had a much higher level of combined resistance than did 'Largo.' Pyramiding genes in wheat for green bug resistance did not enhance resistance to the various greenbug biotypes over that provided by a single gene for resistance⁴⁹ [54].⁵⁶ Dubcovsky, et al. [55] reported the translocation of a greenbug resistant gene *Gb5* from *Triticum speltoides* (Taush)

Gren to wheat. The translocated *Gb5* gene was located on the long arm of chromosome 7A, and RFLP markers were identified to assist in efficient marker-assisted breeding to transfer the resistance gene to new cultivars with resistance to the green bug.

6. WHITE FLY AND SPIDER MITES

Two-spotted spider mites, *Tetranychus urticae* (Acari: Tetranychidae) and Whiteflies, *Bemisia tabaci* (Hemiptera: Aleyrodidae) are herbivorous pests. Plants under herbivore attack are able to initiate indirect defense by synthesizing and releasing complex blends of volatiles that attract natural enemies of the herbivore. However, little is known about how plants respond to infestation by multiple herbivores, particularly if these belong to different feeding guilds. Zhang, et al. [56] report the interference by a phloem-feeding insect, the whitefly *Bemisia tabaci*, with indirect plant defenses induced by spider mites (*Tetranychus urticae*) in Lima bean (*Phaseolus lunatus*) plants. Additional whitefly infestation to spider-mite infested plants resulted in a reduced attraction of predatory mites (*Phytoseiulus persimilis*) compared to attraction to plants infested by spider mites only. This interference is shown to result from the reduction in (*E*)- β -ocimene emission from plants infested by both spider mites and whiteflies. When using exogenous salicylic acid (SA) application to mimic *B. tabaci* infestation, we observed similar results in behavioral and chemical analyses. Phytohormone and gene-expression analyses revealed that *B. tabaci* infestation, as well as SA application, inhibited spider mite-induced jasmonic acid (JA) production and reduced the expression of two JA-regulated genes, one of which encodes for the *P. lunatus* enzyme β -ocimene synthase that catalyzes the synthesis of (*E*)- β -ocimene. Remarkably, *B. tabaci* infestation concurrently inhibited SA production induced by spider mites. They conclude that in dual-infested Lima bean plants the suppression of the JA signaling pathway by whitefly feeding is not due to enhanced SA levels. A few studies documented effects of infestation by phloem-feeding insects on plant responses induced by other herbivore species⁵⁸⁻⁶⁰[57-59]. For instance, *B. tabaci* suppressed the emission of three terpenoids that were induced by simultaneously feeding caterpillars in cotton plants⁶⁰[58]. In contrast, the aphid *Myzus persicae* caused an increased emission of volatiles triggered by spider mites in pepper plants, and consequently increased the attraction of predators to plants infested with aphids and spider mites [57]. However, the signal-transduction mechanisms underlying these effects of phloem-feeding insects remain unknown, although interactions between different defense signal-transduction pathways have been suggested to play a role⁶⁰[58].

7. INSECT RESISTANCE IN TRANSGENIC CROPS

Transgenic crops expressing a protein from the bacterium *Bacillus thuringiensis* Berliner (*Bt*) have been commercially available since the mid-1990s and have been readily accepted by both the American producer and consumer. The *Bt* bacterium is ubiquitous and is unique in that it produces a crystalline (Cry) protein during sporulation that is toxic to certain insects. In 2001,

genetically engineered crops were grown on 52.6 million hectares (130 million acres) worldwide, up 19%, or almost 20 million acres from 2000 ⁶¹[60]. Of this total, 88.2 million acres were planted to transgenic crops in the USA in 2001 and included soybean, cotton, corn and potato (*Solanum tuberosum* L). Of the total acreage planted to transgenic crops, herbicide resistance accounted for 77%, *Bt* crops for 15%, and stacked genes for herbicide and insect resistance accounted for 8%. Growers who planted *Bt* cotton reduced insecticide applications by an estimated 2.7million pounds and made 15 million fewer insecticide applications each year than those that planted conventional cotton ⁶²[61]. The US Environmental Protection Agency (EPA) recently renewed registration of *Bt* crop varieties for another 7 years [62]. ARS scientists have played an important role in the evaluation and development of genetically engineered crops, development of insect resistant management (IRM) programs, monitoring for resistance to the cry proteins and monitoring for adverse effects on non-target organisms and the environment. Boll- Gard cotton containing a *cry1Ac* gene was the first transgenic crop commercialized. It is very effective in controlling the tobacco budworm, *Heliothis virescens* (Fab), but is less effective in controlling the cotton bollworm, *H. zea*⁶³[63]. Recent evaluation of Boll- Gard II transgenic cotton containing both Cry1Ac and Cry2Ab proteins showed control of cotton bollworm, fall armyworm and beet armyworm, *S. exigua* (Hübner), better than control of these insects with Boll Gard which only expressed Cry1Ac. ^{64&65}[64, 65]. Scientists in the NC205 regional research committee (Ecology and Management of European Corn Borer and Other Stalk-Boring Lepidoptera), which included ARS scientists, led the effort to establish a practical IRM program for corn growers. A unified approach to *Bt* corn IRM has gained wide stakeholder acceptance and increased grower compliance. Close collaboration with EPA has allowed the NC205 committee to identify important research areas which the EPA has addressed in its amended registration document for *Bt* corn⁶⁶[62]. In both laboratory and field tests, transgenic field corn was almost immune to damage by the southwestern corn borer, *Diatraea grandiosella* Dyar, and highly resistant to the corn earworm and fall armyworm ⁶⁷⁻⁶⁹[66-68]. A combination of traditional resistance plus *Bt* transgenes was more effective in control of the fall armyworm than either component alone ^{70&71}[69, 70]. Transgenic sweet corn containing a gene for Cry1Ab production was extremely resistant to the corn earworm and highly resistant to the fall armyworm ⁷⁰⁻⁷²[69-71]. Resistance to the *D. grandiosella* in *Bt* hybrid field corn did not reduce alfa-toxin contamination when plants were inoculated with *A. flavus* spores or *A. flavus* spores and *D. grandiosella*⁷²[71]. However, reduced *Fusarium* ear infection and fumonisin in the kernels was noted in *Bt* corn lines expressing Cry1Ab protein as compared with near-isogenic, non-transformed corn lines ⁷³⁻⁷⁵[72-74].

Recent research showed that current *Bt* proteins produced in EPA-approved commercial corn hybrids pose a minimal threat to Monarch butterfly, *Danaus plexippus* (L), larvae. Results from collaborative ARS and university studies suggest that *Bt* pollen densities in excess of 1000 grains cm⁻² would be required to have an adverse effect on Monarch larvae ⁷⁶[75]. Under field conditions, pollen contamination of milkweed average 10 to 425 grainscm⁻²59 ⁷⁷[76]. Thus, the

2-year collaborative research project suggested that *Bt* corn pollen produced by current EPA-approved commercial hybrids would have a negligible effect on Monarch populations ⁷⁸[77]. In Northeastern Louisiana, evaluation of efficiency of transgenic in reducing insect damage recorded by ⁷⁹Huang, et al. [78] who collected field strain of the European corn borer, *Ostrinia nubilalis* (Hübner) from corn fields. Susceptibilities of the field strain and a corresponding laboratory strain of the borer to Cry1Ab protein in DK69-70 *Bacillus thuringiensis* (Bt) corn hybrid were determined by exposing neonates to intact leaf tissues from whorl stage plants. Mortality and growth of larvae were evaluated after 2 and 4 day post treatment in the bioassays by using intact leaf tissues. All neonates of *O. nubilalis* were killed on the diet treated with Cry1Ab protein at 0.5 and 1 mg/kg. Larval growth on Cry1Ab-treated diet was inhibited. In Egypt, ⁸⁰Saker, et al. [79] carried out transgenic tomato (CV.Money maker) over expressing Bt (Cry2Ab) gene which was produced using *Agrobacterium* -mediated transformation method. They confirmed through molecular and biochemical analysis that the expression and integration of the transgene into tomato genome. They also evaluated the Obvious effects of Cry 2Ab on the mortality of the American bollworm *Helicoverpa armigera* (Hübner) and the potato tuber moth *Phthorimaea operculella* (Zeller), when fed on Bt tomato. These results indicate that a significant amount of Bt protein was present in all of the transgenic lines and that plants expressing Cry 2Ab gene could be used for management of the target lepidopteran insect pests. In Egypt, ⁸¹El-Shazly, et al. [80] evaluated insect resistance transgenic expression Bt protein to maize hybrid. They found that the duration of larvae and pupae of *Sesamia cretica* affected by transgenic hybrid maize (IYG) and local AYG (transgenic maize), beside increased the larval mortality reached to 100 % when larvae were fed on transgenic hybrid maize (IYG and AYG) under laboratory conditions. They also recorded the reduction in population fluctuation of pink corn borer in (IYG) and local AYG (transgenic maize) under field conditions.

8. RISKS AND PRECAUTIONS

Researchers and governments tried to find solutions to the problems of food gap. Host plant resistance to pests has long been one of the main tools used in pest control. Plant breeding is one of agriculture tools which are targeted to the improvement of quality and quantity of crops.

Notably, the plant breeding programs are progressed and developed of the molecular biology to facilitate manipulation of genes.

Recent advance in genetic engineering as powerful modality for combating some of the important insect pests, but we must recognize that it may have some unpredicted risks.

The risks of modern plant breeding (as recorded by USDA, EPA and FDA) can be summarized as follows:

- 1-The GE trait could be passed to a wild or weedy relative and increase its weediness or invasiveness, or the GE plant itself could become weedy or invasive.
- 2-The GE trait could negatively impact non-target organisms in the environment.

3- Organisms that the GE trait is intended to harm could develop resistance to the trait.

So, Precautions which should be followed to avoid probability of GM risks can be summarized as follows:

- 1- It must follow regulatory tools which minimized if the product is declared.
- 2- It should be done with rigorous scientific analysis to establish that the GM product posses no more health or environmental risk than its conventional counterpart.
- 3- GM crop should be cultivated in isolated area to avoid GM trait passes to wild plant relatives (pure line gene).
- 4- World regulation of GM crop must be followed.
- 5- Reject null hypothesis that GM crop possess no significantly risk.

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