

Review Paper

Morphological, Physiological and molecular markers for the development of resistance in cotton against insect pests.

Abstract

Cotton is a most-very important fiber and oilseed crop of many countries. Commerce-The economy of most countries dependsis dependent upon it. Both abiotic and biotic factors affect the crop yield and quality. In the biotic factors, sucking and chewing insect pests (white fly, jassids, pink bollworm, spotted bollworm and army bollworm) affect cotton crops drastically at early and reproductive stages. The farmer is always using chemical control method to control all these pests which increases the cost of production to manyfolds. In addition to this it also causes many environmental as well as health hazards. Built in In-built resistance in plants is offered by various mechanisms they-that can be of morphological, physiological and molecular origin-; these mechanisms can be exploited for the purpose of ameliorating the current scenario. They use of morphological, physiological and molecular markers for the ideotype plant development is something-regarded to be useful and practical. Morphological markers can be used by visual observation, e.g. leaf shape, color, structure, trichomes, and number of gossypol glands and amount of waxes etc. These can be widely and with great ease. Physiological markers used at the protein level and-evaluate its expression in cotton against insects. The plant produces thea special group of proteins as lecithins and other phenolic compounds that reduce the insect population to a great extent. Molecular markers used at the genetic level and-may evaluate the presence of genes that are involved in producing structures and chemicals to prevent the invasion of foreign pests. This-These has have the ability to express and play important role in cotton against the insect pests. Genes of interest can be transferred by genetic engineering in recommended varieties. This review paper covers the morphological, physiological and molecular markers associated with resistance development against insect pests in cotton.

Key words: Morphological, Physiological, molecular, Markers, Resistance, Insect Pests, Cotton

1. Introduction:

Cotton is the one of most important non-food and fiber crops around the globe. Due to its importance in the economic sector, it is also called "white gold". Pakistan had a great era of cotton production in the past that has been declined to a greater extent over the years. Even at At present 60% of foreign exchange is through-obtained from cotton. It is the backbone of the country's economy as it is involved in 8.2% of value addition and 2% in GDP [1]. Pakistan is at-number-fourranked fourth in the world for

cotton production and its role in GDP. The insect pest can destroy the agricultural products at different stages in different plants and cause a total loss of 30-40% in the production of agricultural products[2].

Insect pests are the main factor for the ~~deteriorating~~ deterioration in the productivity of ~~Cotton~~ cotton by decreasing quality as well as yield [3]. Cotton accommodates about 1326 insect species in the world that ~~cause~~ damage ~~to~~ it from sowing ~~stage~~ to maturity [4]. Nearly 145 species of insects damage the cotton in the Pakistan and cause various diseases [3]. The damage is caused by both sucking and chewing types of insects. The sucking type insects suck the cell sap containing food and other useful nutrients from the leaves and other soft parts of the plant and cause loss of vigor in plants ~~and~~ ~~resulting~~ in the plant ~~wilts~~ wilting and ~~drops~~ dropping its leaves [5]. Cotton is ~~the favorite crop of most susceptible~~ to the insect pests ~~such as~~ *Helicoverpa* species ~~which~~ can reduce the production by 60%, ~~even~~ ~~with~~ ~~irrespective of~~ all the efforts to control them [6]. Among sucking insects ~~following~~ ~~causing~~ critically damage ~~the~~ ~~to~~ cotton and ~~cause~~ 40-50% reduction in yield ~~include~~ *Aphis gossypi*, *Bemisia tabaci*, *Thrips tabaci* and *Amrasca bigutella* [7, 8,9]. The most common way of controlling the pests by the farmers ~~the is by~~ use of insecticidal sprays that are quick in eliminating these pests [10, 11]. The immense use of such chemicals poses serious harms to the human health and ~~resistance in these~~ insects ~~become resistant~~ to these chemicals. In addition to this, ~~the~~ environment is also polluted by them [12, 13]. These chemicals are non-degradable and very much poisonous ~~for the~~ ~~to~~ ~~some~~ beneficial insects, fishes and humans ~~also~~ [14]. ~~The death~~ rate in humans is increasing day by day due to these chemicals, the people involved in spraying, packing, loading and farm laborers confront these harmful chemicals [15]. ~~It is needed~~ ~~There is the need~~ to develop a strategy that controls these pests without the use of such detrimental chemicals [3].

~~There are Many many~~ other ways ~~can be there for~~ ~~to~~ controlling these pests that ~~could cause~~ decline ~~the~~ in cotton production. Plants ~~can~~ contain various mechanisms that can ~~control the pest~~ population, ~~-, this~~ ~~This~~ type of defense is called basal defense and is present in almost all the plants [16]. Some plants show special type of resistance in which ~~the~~ first step is to identify the pathogen and then regulate some action ~~due to some~~ ~~involving~~ ~~gene~~ ~~for~~ ~~gene~~ interaction [17]. These mechanisms could be ~~either~~ of morphological ~~or~~ physiological ~~and or~~ molecular basis. Host plant itself can have resistance against many pests [18, 19]. Some particular morphological traits such as density of hairs, length of hair, plant height, thickness of leaf lamina and number of gossypol glands control the insect pest population [20]. The plant derived chemicals which act as repellent can ~~be served~~ as bio pesticide ~~without any having no~~ ~~adverse~~ effects ~~on to the~~ environment [21]. Plants produce a class of special chemicals (proteins) called secondary metabolites that can avert the pests action. ~~One~~ ~~Some~~ of these proteins ~~are~~ ~~include~~ lectins or

agglutinins [22, 23,24]. This group has ~~an eminent a major~~ characteristic ~~that it can of~~ **identifying the special type** of pest and ~~can then~~ bind their internal carbohydrate structures [24]. Advancements in molecular techniques ~~lead led~~ to the development of Bt cotton that showed significant resistance levels without damaging environment. It can kill the pests that sucks its sap or eat its leaves [25]. This transgenic cotton is grown throughout the world [26]. The decline in the toxin production by the expression of toxin gene (cry1Ac) is observed. Recently it ~~is was~~ reported that the level of toxin produced ~~is was~~ not ~~adequate- sufficient~~ to kill the insect pests [27]. In this review the ~~markers related to~~ morphological, physiological and molecular ~~basis markers are discussed that are~~ involved in developing resistance against cotton insect pests are discussed.

2. Morphological Markers associated with resistance against insect pests

Many ~~characters-characteristics~~ in different plant ~~can could~~ be the source of resistance against pests. Hair density, length of hair, plant height, number of gossypol contents and thickness of leaf lamina are some important morphological features that are involved in offering resistance against pests naturally [20]. Varietal selection can play an important role in improving the resistance. High gossypol content ~~is involved in causes decreasing-decrease in~~ **the insect** population ~~especially,-;~~ whitesfly. Hence, the plants with higher gossypol content are required that may ~~support lesser or no reduce~~ insect population [28, 29]. In the case of bollworms, the high gossypol content is not preferred. It is observed that high quantity of gossypol along with hemigossypolone and heliocides is present in the leafy areas in minute quantities [30]. It is also seen that plants having no gossypol glands were more prone to insect attack. Gossypol **is** a crucial chemical compound that is phenolic in nature and is involved in developing resistance in plants against insect population (CICR,2011). The plants having higher gossypol glands disturb the growth and reproduction of the insect population and drastic reduction in survival of larvae and pupae. This is also involved in the reduction of weight of larvae and pupae and ~~they take more prolonged~~ time in completing the larval and pupal phases of life as compared to plants that have no gossypol glands [4]. In the same way longer plant height is related **to low** insect population [28, 29]. Hair density is related with insect population also; ~~the~~ higher the hair density ~~the~~ higher ~~will be~~ the insect population [31, 32]. Hair length is also positively associated with insect population. **It** is involved in increasing the jassids population [33]. The plants having **less** hair ~~showed-carried~~ lower number of eggs and larval infestation because higher hair density is required by insects to have a strong grip while laying eggs and retaining them on leaves in **a better** way than ~~on~~ smooth leaves [34, 4]. Leaf lamina thickness is also an important factor; ~~the~~ higher the thickness of leaf lamina ~~the~~ lower ~~will be~~ the insect population

of the plant [35, 3]. In addition to these the lower bract sizes is also desirable as it ~~is involved in~~ habours less-lower insect population [4].

From seedling stage to the maturity of the cotton plant jassids feed on it as it is the major pest of cotton. it inserts its style in the tissues of plant and sucks sap from it, ~~in addition it thus inserts injecting~~ some poisonous substances inside it [36]. As a result, the plant starts wilting and drying and gradually becomes ~~a week weak~~; and ~~turns curls~~ its leaves ~~as it can have been seen clearly from the heavily affected cotton by jassids and thereby decreasing yield is decreased by~~ many folds [37]. Resistance of the plant is determined by the amount of glandular trichomes present beneath the leaves as it is less preferred by the jassids to mate on such leaves [38, 39]. The trichomes are least preferred by the insect and they are present where trichomes are negligible [10]. Trichomes density is an important factor for controlling jassids. Plants having ~~the lowest~~ low number of trichomes on the lower sides of the leaves were ~~favorite-preferable~~ to jassids ~~and while~~ the plants with high number of trichomes ~~show usually haboured less-low~~ insect population [40].

Leaf size and shape also determine the insect pests attack. The size of the leaf is variable in different plants it can alter the mobility of insects [41]. In the same way narrow okra ~~leaved~~ and super-okra ~~leaved~~ plants usually exhibit higher resistance. Broad leaf laminae are more prone to attack by whitefly than narrow leaves because it offers greater surface area for landing and oviposition [42]. Insects cut the leaf disks of the same size and offers its young ones for food and uses it for oviposition [43]. But, when the plant is damaged its mechanical wounding starts a mechanism that forms a green volatile substance [44]. These behave either as repellent or attractant [45, 46, 47]. Some characters like leaf color, leaf shape, leaf size, trichome length and hair density are involved in the attraction of insects. Insect preference is for special traits of its choice for example, whitefly ~~use to lays~~ eggs near the trichomes because it is the area of high pressure of selection by the enemies and less prone to natural predators [48].

Leaf thickness is also a major factor involved in the preference by the insect. It has been observed that plants with thin leaf lamina possessed ~~the lesser-smaller~~ insect population than thicker leaves. The thin leaves were least preferred as they were less succulent and less tasty. The same thing has been reported in many other crops also such as mung bean, cucumber and black gram [49, 50, 51, 52]. In all these plants leaf thickness is directly proportional to insect population. The lamina of leaves reflecting longer wavelengths is considered to be more resistant than compared to that which reflects shorter wavelength and hence, the red colored leaf is resistant to insects in cotton [53]. The same thing was

observed in *Brassica oleracea* this red color proved as defensive trick as it ~~is~~ was considered a low quality plant [54]. Light green leaves ~~are~~ were attracted more by the whitefly as compared to dark green leaves [55, 56].

Waxes also play important role in the determining the resistance along with **the main** function of conserving the water. The plants having more waxes are susceptible to insect pest species than those having no or less waxes. In castor single bloom, double bloom and triple bloom varieties showed variable resistance against leaf hoppers [57].

3. Physiological markers Associated with insect pest resistance

As cotton plant is infested by numerous types of insect pests. Nearly each growth phase of cotton harbors a different insect pest. Plant behaves differently to these insect pests. Like plants have developed a wide range defense mechanism to counter insect **attacks**. These mechanisms could be categorized on the basis of before and after attacks of insect pest. Defense mechanism before **attack** of insect pests is called **as** constitutive defense and defense mechanism activated after **attacks** of insect pests is known **as** an inducible defense. Resistance or tolerance of plants to insect herbivores and pathogens is mediated via constitutive or induced defense mechanisms. Defense mechanism basically consists of certain steps in which plants firstly detects **an insect** attack by specific recognition signals. Then these signals are transferred to the specific signal transduction pathway which ultimately activates production of defense chemicals.

3.1 Cell sap concentration:

The resistance against insect pests is developed by certain morphological traits, physiological features and biochemical characteristics of the plant and plant make use of these features **to** exert pressure on insect to select plant **as a host**. Some physiological factors are associated with insect resistance. These factors include osmotic concentration of cell sap and leaf exudates. In certain studies, it is indicated that water content and abiotic stresses affects water concentration in cell sap and it is related to resistance or susceptibility to insect pests. Under high water concentration attack of aphids, mites and thrips is increased. Under lower water concentration there is a decrease in jassid and whitefly attack while bollworms are not affected. In cotton, high osmotic concentration of cell sap is associated with jassid resistance [58].

3.2 Defensive compounds/proteins:

In insect-host plant interaction the insect always looks for a host that can provide them proper food. The insect pest is completely depended for its nutrition on **the host plant**. Plants produce a wide range of defense chemicals that are toxic to pests and pathogens. Cotton is equally important worldwide and its production is greatly affected by insects. In cotton, compounds like gossypol, tannin, quercetin, rutin and many other flavonoids contribute insect tolerance. In cotton a phenolic compound, gossypol is present and it is related to resistance against several insect pests. These phenolic compounds had drastic effects on insect's physiology. In cotton, high tannin content is related to bollworm resistance. The accumulation of proline in the tissues of numerous plant species is regarded as a common response to drought as well as other types of stresses [59].

Cotton plants can accumulate secondary metabolites after attack by caterpillars. The defense mechanism is tuned-up by increasing the levels of terpenoids, gossypol, hemi gossypol and hemigossypolone which are stored in sub-epidermal pigment. Phenolics such as cinnamic acid and p-coumaric acid are important compounds, and are toxic to ***Helicoverpa armigera* and *Spodoptera litura***.

As crucial biochemical materials in resistance to arthropod attack, polyphenol oxidases (PPOs) exist in many plants. These PPOs function as defensive enzymes. PPOs are found in nearly all young plant tissues, and their substrates are stored in different organelles like plastids, and vacuoles. When an insect or pathogen attacks, these PPOs produces certain substrate. The interaction between these PPOs and their substrates occurs only after the cell ruptures and is primarily associated with enzymatic browning reactions and the protection against wounding or attacks by insects and pathogens.

3.4 Plant Genetic Engineering and cotton

Nowadays, **the use of** different genes to get desirable characters has become an important tool of plant biotechnology. Scientists have developed various techniques to develop high yielding cotton varieties along with insect pest and herbicide resistance [60].

By Wounding wounding, insect pest or pathogen infestation activates defense mechanism of plants. In response defense related proteins in plants are produced. The genes encoding defense-related proteins can be used **in the** targeted foreign gene expression.

By use of genetic engineering technologies different genes have been incorporated in cotton. Insecticidal (cry) genes **from *Bacillus thuringiensis*** have been utilized. It can effectively control the

cotton bollworm (*Helicoverpa armigera*), thus protecting the ecological environment with the reduced application of chemical insecticides, and exhibited favorable socioeconomic benefits. Cowpea trypsin inhibitor and certain others provide resistance to insect pests and have been commercialized. These genes have resistance against Lepidoptera insects (Cry1Ac+Cry2A). Susceptibility of army-worm larvae to Bt toxin decreases with larval age and toxin concentration that decreases with growth stages and certain other factors.

Protection against targeted insect pests could be achieved in transgenic cotton with an expression of insecticidal genes. The *Bacillus thuringiensis* toxin are produced in different growth stages of the cotton plant. But these toxins should be produced at the appropriate time of the growing season. That's That is why it shows unexpected performance of transgenic Bt cotton against Lepidoptera insect pests. Plant height, main stem node number, and the dry matter accumulation are the same in Bt and non-Bt hybrids up to 89 days after sowing (DAS) [61].

4. Molecular markers associated with insect pest resistance

The breeder looks at the morphology of plant and select desired characteristics with our objective. Usually these characteristics are controlled by many genes and mostly effect by of environment. If quantitative characters are found in the individual component of the DNA associated with one of them and biometry does not identify the locus of that character but effectively manages it. Molecular markers give desirable results quickly and accurately [62]. The common plant breeder have objective to develop from the agronomical point of view better varieties and want to by gathering all the good which are traits present in different lines and wild genotypes. By conventional breeding, transfer all good characters in one genotypes are transferred by using selfing, backcross and hybridization methods which are time consuming and less confirmative. The molecular markers give enable the direct selection of plant at the base of the markers process [63].

In 1983, Tankeley gives five characters that differentiate the molecular markers from phenotypical markers. These properties are include:

- Plant, tissue and cellular levels can be used for the determination of genotypes.
- Mostly loci consists of more number of naturally occurring alleles.

- Morphological neutrality.
- Codominant-Codominance occurs at many loci.
- Less number of epistatic or pleiotropic influence ~~are occurring~~ occur [64].

Molecular markers give the precise result during the screening of nuclei structure of plant groups and results can be used for the variety and breeding program. A few number of marker techniques used for the evaluation of genetic variation e.g. random amplified polymorphic DNA (RAPD) [65], amplified fragment length polymorphism (AFLP) [66] and simple sequence repeats (SSR), [67]. Now simple DNA marker can be formed because of availability of genomic database companies [68]. In plant breeding, markers are very useful in characterization, recognition, genomic fingerprinting, linkage mapping, identification of genetic variations, and marker assisted selection (MAS) [69], in backcrossing linkage drag can be removed ~~now~~ and ~~those the~~ traits ~~can be~~ measured easily ~~that not measure~~ rather than by morphologically [70].

Table 1

Table 1: Location of genes of particular characters can be used for the insect resistant resistance in cotton.

Homeologous chromosome pairs	Characters	Locus	Reference
1,15	Virescent leaf coloration	v^5, v^6	[71]
	Leaf shape	L_1^L, L_2^0	
7,16	Anthocyanin pigmentation	R_1 and R_2	
	Yellow green loci	Yg_1, Yg_2	
12,26	Withering bracts	bw_1, bw_2	
	Nectar less	ne_1, ne_2	
A,18	Flower colour	Y_1, Y_2	

20	Yellow veins	yv	
D	Virescent	v ₈	

In 2009, Iftikhar Ali utilized genotypes FH-634 (glabrous) and Rajhans (pilose) to produce F₂ and F₃ segregating generations. RAPD and SSR markers are PCR (polymerase chain reaction) based that were utilized to identify DNA markers associated with the character of hairiness by utilizing 400 RAPD and 54 SSR primers. During the experiment, PCR condition are specific. In duplicate reaction consistently consistency was analyzed by utilizing eight RAPD primer markers OPO-141200, OPO-11920, OPN-14890, OPH-131100, OPG- 17500, OPG-06980, OPF-11630, OPD-19640. In hairiness in plants having a 150bp DNA segment was enlarged with one SSR primer pair JESPR-154. Linkage map was made of polymorphic DNA markers. Genetic map build-built by RAPD and SSR markers that tells explained heritability leaf hairiness happen. These characters can be utilized to enhance insect resistance in cotton crop [72].

During the early stages of cotton mostly sucking pest, e.g. whitefly (*Bemisia tabaci*), thrips (*Thrips tabaci*), Jassids (*Amrasca biguttula*) and aphid (*Aphis gossypii*) are the main reason of damage and plant cannot stand and at last reduce cotton yield. Through the beginning of monoculture-driven current cultivation, insect resistance is increasing due to the environmental factors. Now, plant breeders used the biotechnological tools for the development of modern insect resistant crops. Breeders are using transgenic approaches instead searching of wild resistant genotype and then cross for the transfer of desirable genes. Such plant breeding easier was easy with the presence of sequence-based molecular approaches. From wild relatives by using wide hybridization to develop two Recombinant recombinant cotton inbred lines (RIL'S) and utilize to get near an iso-genic lines (NIL's) against the sucking pest of cotton [73]. Cotton traits controlled by some specific gene against sucking pest are given in table-Table 2.

Table 2

Table 2: sucking-Sucking insects and sucking insect resistant species in cotton.

Formatted: Subscript

Formatted: Subscript

Insects	Insect resistance species	References
Jassids	<i>G. armourianum</i>	[74]
	<i>G. hirsutum</i> old accessions	[75]
Silver leaf whitefly	<i>G. thurberi</i>	[76]
	<i>G. hirsutum</i> genotypes	[77]
Thrips	<i>G. barbadense</i>	[78]
	<i>G. tomentosum</i>	[78]
	<i>G. darwinii</i>	[78]
Spider mites	<i>G. arboreum</i>	[79]
	<i>G. lobatum</i>	[80]

There is another major issue of nematodes in cotton in some areas. Now researchers are using a molecular methodology to produce nematode resistance in upland cotton cultivars and that's that marks a new era of examination study. For theThe development of nematode resistant cotton, in-by molecular approach to search outwould assist in the identification of the resistant gene and their position on the chromosome. Today, six nematode resistant genes were-have been identified that have different behavior behave like recessive, partially dominant and dominant genes. In *G. longicalyx* species on chromosome 11 dominant genes Ren^{lon} and Ren^{ari} are present and on chromosome 21 of *G. aridum* specie [81, 82]. In *G. barbadense* some partially resistant genes Ren^{barb1}, Ren^{barb2}, and Ren^{barb3} are identified and present on chromosomes 21, 21, and 18 [83].

Bt. cotton developed by the transformation of different genes CryI, CryII, Cry III, Cry IV and Cry in cotton by *B. thuringiensis* bacteria are shown in ~~table-Table No-~~3 [71]. Now Bt. Cotton is used for nematodes and it's-it is seen as "Cry" genes, proteins which are useful against nematodes. "Cry" proteins are insecticide-insecticidal in nature during sporulation phase [84].

Table 3

Table 3: Bt. genes transfer into cotton ~~against in~~ different insect orders.

Genes		Insect orders	Reference s
Cry I	130-140kDa	Lepidopteran specific	[71]
Cry II	65kDa	Lepidopteran and dipteran specific	
Cry III	66-74kDa	Dipteran and coleopteran	
Cry IV	28, 72, 128, 134kDa	Dipteran	
Cry V		Lepidopteran and coleopteran	

4.1 Sources genes under observation:

4.1.1. Cholesterol oxidase:

Cholesterol oxidase taken from a *Streptomyces* fungus can be an effective tool against boll weevil, *Anthonomus grandis* and *H. virescens*.

4.1.2 Iso-pentenyl-transferase (IPT):

It is an enzyme taken from microorganisms. It ~~effects~~ affects the potato aphid *Myzus persicae* and tobacco hornworm *Manduca sexta*. ~~cytokinin~~ Cytokinin-biosynthetic pathway ~~inhibits~~ inhibited by more expression of IPT enzyme.

4.1.3. Lectin genes:

~~It's~~ It is more under investigation because it produces protein and acts on the digestive system ~~through the~~ by binding with blood cells.

4.1.4. Spider and scorpion venom genes:

This gene also expresses and produces protein and have ~~insecticide~~ insecticidal properties.

4.1.5. Stunt virus ~~is a small RNA virus:~~

Stunt virus is a small RNA ~~which has~~ ~~have~~ three genes. It ~~effects midgut of~~ attacks the midgut cell of *Heliothis* species and creates ~~the~~ feeding problem. ~~That~~ ~~The~~ gene can be used against many insects and plant ~~which~~ requires ~~less-small~~ amount of virus which ~~amplified can amplify~~ itself in insects.

4.1.6. Amylases:

Amylase also ~~affects~~ the digestive enzymes and it used in the bean plant for the protection from the bruchid beetles. In cotton it can be used against the lepidopteran insects.

4.1.7. Insect neuropeptides:

Neuropeptide hormones are very small peptide ~~from with~~ 5-50 amino acid. It is easy to work ~~and~~ due to the small size ~~which~~ can be used in cotton by genetic engineering and ~~which~~ 15 hormones were identified

4.1.8. Novel gene:

Novel genes taken from *Photorhabdus luminiscens*. It highly virulent against insects and some other genes [71].

5. Future horizons:

In many countries, cotton is ~~the most~~ important crop, according to the ~~economic and exchange~~ ~~economy~~. Breeders want to produce more yield and better quality ~~of cotton~~. DNA markers ~~have been~~ modified and utilized for the solving many problems. This technique has more degree of automation and directing the ~~Green revolution~~ in agriculture ~~of the~~ world [68].

The present pest problem in cotton can be ~~decreased-reduced~~ by the identification and ~~utilize~~ ~~utilization of~~ the new alleles from wild or wild relatives [85, 86]. ~~The modern~~ molecular technique helps us in the improvement of cotton economical traits, and ~~the use~~ of modern molecular technologies, helping in increasing ~~the~~ genetic gain of economic traits. Now these days ~~it is relies that on the~~ sequencing of *G. raimondii* [87] and *G. arboreum* [88] ~~will which~~ helps us in the identification of new alleles for pest resistant in cotton.

These DNA markers will **be helping us** for observation and introduction of cotton genotypes ~~that having~~ **desired traits**. This technique does not help us for genetic diversity ~~and also will be helping helps~~ in development of linkage map and map all agronomical traits. [89, 90]. In the modern era, now researchers **are developing** more efficient DNA markers, which help ~~the people because it is as~~ an important tool for plant breeders and geneticists for the development of varieties. SNPs marker will be ~~kept large effects on useful as~~ marker assist selection and mapping studies in future. ~~Because because~~ of more abundance and development of **the better** identification system [68].

6. Conclusion:

This study deals with all the ~~methods ways~~ that can be manipulated to develop resistance against cotton pests. The cotton crop **is the world's** most important fiber crop, but it is greatly damaged by insects. Use of sprays and insecticides ~~enhance increases~~ the cost ~~of production to an~~ immensely level ~~that it could not be afforded by the and unaffordable to~~ farmers and industrialists. Many morphological, physiological and molecular markers ~~are discussed that~~ can be used in the breeding strategies and ~~can be~~ the source of natural as well as induced resistance in the crop plants. The plants with greater number of gossypol glands are required so that they are least preferred by the insects, hence **the leaf structure** and color determination is also an important factor. Thickness of ~~leave leaf~~ is also a source of developing resistance. Thin leaves are less succulent so least attracted by the ~~plants insects~~. **In the same** way many other morphological markers can be the source of resistance. Biometrical analysis can be used for the genes, but it does not ~~tell reveal~~ the locus of that particular trait. DNA markers are not time consuming and can **identify** the gene of interest. Molecular markers help us in the identification of some **genes** ~~which have resistant resistant genes~~ against the cotton pest. In some species, these ~~present and genes~~ can be used by genetic engineering. Researchers also identify some other source like ~~fungus of genes fungal genes~~. Researchers are also mapping the genome of cotton genotypes and investigating the genes. ~~Which which~~ can be used in the development of cotton pest resistant genotypes ~~that having for~~ high quality ~~and good~~ yield.

7. References:

- [1] Naqvi RZ, Asif M, Saeed M, Asad S, Khatoon A, Amin I, Mukhtar Z, Bashir A and Mansoor S, Development of a Triple Gene Cry1Ac-Cry2Ab-EPSPS Construct and Its Expression in Nicotiana

- benthamiana for Insect Resistance and Herbicide Tolerance in Plants. *Front. Plant Sci.* 2017;8:55. doi: 10.3389/fpls.2017.00055.
- [2] Singh B and Kaur A. Control of Insect pests in crop plants and stored food grains using plant saponins: A review. *LWT- Food Science and technology* 2018; 87:93-101.
- [3] Khalil H, Raza ABM, Afzal M, Aqueel MA, Khalil MS and MansoorMM. Effects of plant morphology on the incidence of sucking insect pest complex in few genotypes of cotton. *Journal of Saudi Society of agricultural Sciences.*2017; 16:344-349 .
- [4] Keshav A, Shera PS and Singh J. Morphological basis of resistance to spotted bollworm, *Eariasvittella* (Fabricius) in Asiatic cotton. *Phytoparasitica* 2013; 41:235–240. DOI 10.1007/s12600-012-0283-8.
- [5] Abro GH, Syed TS, Tunio GM, Khuhro MA, Performance of transgenic Bt cotton against insect pest infestation. *Biotechnology.* 2004; 3: 75–81.
- [6] Constable GA and Bange MP. The yield potential of cotton (*Gossypiumhirsutum* L.). *Field Crop research* 2015; 182: 98-106.
- [7] Nizamani IA, Talpur MA, Khuhro DR, Nizamani and S.M. Relative resistance of cotton cultivars to sucking complex. *Pak. J. Appl. Sci.* 2002; 2 (6): 686–689.
- [8] Aslam M, Razaq M, Shah SA, Ahmad F, Comparative efficacy of different insecticides against sucking pests of cotton. *J. Res. Sci.* 2004; 15: 53–58.
- [9] Amjad A, Aheer GM, Varietal resistance against sucking insect pests of cotton under Bahawalpur ecological conditions. *J. Agric. Res.* 2007; 45: 205–208.
- [10]Soomro AR, Soomro AW, Soomro K, Mallah GH, Jassid resistant variety CRIS-7A. Central Cotton Research Institute, Sakrand, Sindh, Pakistan. *Pak. J. Biol. Sci.* 2000; 3(2): 332–334.
- [11]Razaq M, Suhail A, Aslam M, Arif MJ, Saleem MA, Khan HA, Patterns of insecticides used on cotton before introduction of genetically modified cotton in Southern Punjab, Pakistan. *Pak J. Zool.* 2013; 45: 574–577.
- [12]Palumbo JC, Horowitz R, Prabhaker N, Overview of insecticidal control and resistance management for Bemisiatabaci. *Crop Prot.* 2001; 20: 739–765.
- [13]Costa DB, Souza B, Carvahlo GA, Carvahlo C, Residual action of insecticides to larvae of *Chrysoperla externa* (Hagen, 1861) (Neuroptera: Chrysopidae) under greenhouse conditions. *Cienc. Agrotec. Lavras.* 2003; 27 (4): 835–839.

- [14]Silva P, Eyraud V, Carre-Pierrat M, Sivignon C, Rahioui I, Royer C, et al. High toxicity and specificity of the saponin 3-GlcA- 28-AraRhaXylmedicagenate, from *Medicago truncatula* seeds, for *Sitophilus oryzae*. *BMC Chemical Biology*, 2012; 12:3.
- [15]Aktar MW, Sengupta D, & Chowdhury A. Impact of pesticides use in agriculture: Their benefits and hazards. *Interdisciplinary Toxicology*, 2009; 2(1): 1-12.
- [16]Kim J, Quaghebeur H and Felton G. Reiterative and interruptive signaling in induced plant resistance to chewing insects. *Phytochemistry*. 2011; 72: 1624-1634.
- [17]Jones JDG. and Dangal JL. The Plant Immune System. *Nature*. 2006;444: 323-329.
- [18]Hua ML, Hua LC, a study on the bollworm resistance of CRI-29 and the target to control the F3 bollworms. *China Cottons*2001; 27: 20–22.
- [19]Khan MT, Naeem M, Akram M, Studies on the varietal resistance of cotton against insect pest complex of cotton. *Sarhad J. Agri*. 2003; 19: 93–96.
- [20]Amjad M, Bashier MH, Afzal M, Comparative resistance of some cotton cultivars against sucking insect pests. *Pak. J. Life Soc. Sci*. 2009; 7 (2): 144–147.
- [21]Silva AX, Jander G, Samaniego H, Ramsey J S, & Figueroa CC. Insecticide resistance mechanisms in the green peach aphid *Myzus persicae* (Hemiptera: Aphididae) I: A transcriptomic survey. *PLoS One*, 2012; 7(6): e36366.
- [22]Michiels K, Van Damme EJM, Smagghe G, Plant-insect interactions: what can we learn from plant lectins? *Arch Insect Biochem. Physiol*. 2010; 73: 193–212.
- [23]VanDamme EJM, Ectopically expressed leaf and bulb lectin from garlic (*Allium sativum* L.) protect transgenic tobacco plants against cotton leafworm (*Spodopteralittoralis*). *Transgenic Res*.2008; 17: 9–18.
- [24]Vandenborre G, Smagghe G and Damme EJM. Plant lectins as defense proteins against phytophagous insects. *Phytochemistry*. 2011; 72:1538-1550.
- [25]De-Maagd RA, Alejandra B, Crickmore N, How *Bacillus thuringiensis* has envolved specific toxins to colonize the insect world. *Trends Genet*. 2001; 17: 193–199.
- [26]Saleem MF, Shakoor A, Shahid M, Cheema MA, Shakeel A, Shahid M, Tahir MU, Bilal MF Removal of early fruit branches as potential regulator of Cry1Ac, antioxidants, senescence and yield in Bt. Cotton. *industrial crops and products*. 2018; 124(2018): 885-898.
- [27]Jun W, Yuan C, Meng-Hao Y, Yuan L, Yu-Jin W, Yuan C, Xiang Z, De-Hua C, The effects of high temperature level on square Bt protein concentration of Bt cotton. *J. Integr. Agric*. 2015; 14: 1971–1979.

- [28]Khan MA, Akram W, Khan HAA, Asghar J, Khan TM, Impact of Bt-cotton on whitefly, Bemisiatabaci (Genn.) population. Pak. J. Agric. Sci. 2010; 47 (4): 327–332.
- [29]Zia K, Ashfaq M, Arif MJ, Sahi ST, Effect of physicomorphic characters on population of whitefly, Bemisiatabaci in transgenic cotton. Pak. J. Agri. Sci. 2011; 48 (1): 63–69.
- [30]Benedict CR, Martin G S, Liu J, Puckhaber L, & Magill CW. Terpenoid aldehyde formation and lysigenous gland storage sites in cotton: Variant with mature glands but suppressed levels of terpenoid aldehydes. Phytochemistry, 2004; 65: 1351–1359.
- [31]Bashir MH, Afzal M, Sabri MA, Raza ABM, Relationship between sucking insect pests and physico-morphic plant characters towards resistance or susceptibility in some new genotypes of cotton. Pak. Entomol. 2001; 23 (1): 75–78.
- [32]Chu CC, Natwick ET, Chen TY, Hennebury TJ, Analysis of cotton leaf characteristic effect on Bemisiatabaci (Homoptera: Aleyrodidae) biotype B colonization on cotton in Arizona and California. Southwestern Entomol. 2003; 28: 235–240.
- [33]Irfan M, Mumtaz I, Raza ABM, Ahmad K, Khan ZI, Arif N, Effect of leaf morphology on the incidence of sucking insect pests in some cotton genotypes. Int. J. Cell Mol. Biol. 2008; 1 (3): 285–291.
- [34]Kamboj HB, Singh R. Effect of morphological variants developed as isolines of cotton on oviposition preference of spotted bollworm. Journal of Cotton Research and Development, 2002;16: 54 –58.
- [35]Ashfaq M, Noor-ul-Ane M, Zia K, Nasreen A, Mansoor-ulHasan. The correlation of abiotic factors and physico morphic characteristics of (*Bacillus thuringiensis*) Bt transgenic cotton with whitefly, Bemisiatabaci (Homoptera: Aleyrodidae) and jassid, *Amrascadevastans* (Homoptera: Jassidae) populations. Afr. J. Agric. Res. 2010; 5 (22): 3102–3107.
- [36]Lanjar, AG, Solangi BK, Khuhro SA and Solangi AW Insect infestation on Bt. and non-Bt. cotton cultivars. Food Sci. Qual. Manag 2014; 27:55-62.
- [37]Madar H, Katti P. Incidence and diversity of leafhopper on sunflower. Karnataka J Agric Sci. 2010; 23(1):149150.
- [38]Butler GDJR, Wilson FD, Fishler G. Cotton leaf trichomes and populations of *Empoasca lybica* and Bemisiatabaci. Crop Protect 1991; 10(6):461-464.
- [39]Murugesan N, Kavitha A. Host plant resistance in cotton accessions to the leafhopper *Amrascadevastans* (Distant). J Biopesti. 2010; 3(3):526-533.

- [40] Kanher FM, Syed TS, Abro GS, Jhangir TM and Tunio SA. Some Physio Morphological Leaf Characters of Gamma Irradiated Cotton Lines to Resistance against Jassid (*AmrascaDevastans* Dist.) *Journal of Entomology and Zoology Studies*, 2016;4(3): 80-85
- [41] Ozgur AF, and Sckeroglu E. Population development of Bemisiatabaci (Homoptera:Aleyrodidae) on various cotton varieties in Cukurova, Turkey. *Agric Eco syst Environ*. 1986; 17:83–88.
- [42] Hasanuzzaman ATM, Islam MN, Zhang Y, Zhang C-Y and Liu TX, Leaf Morphological Characters Can Be a Factor for Intra-Varietal Preference of Whitefly Bemisiatabaci (Hemiptera: Aleyrodidae) among Eggplant Varieties. *PLoSONE* 2016;11(4): e0153880. doi:10.1371/journal.pone.0153880
- [43] Shah MMR and Liu TX. Feeding experience of Bemisiatabaci (Hemiptera:Aleyrodidae) affects their performance on different host plants. *PLoSONE* 2013; 8: e77368. doi:10.1371/journal.pone.0077368 PMID: 24146985
- [44] Halitschke R, Stenberg JA, Kessler D, Kessler A and Baldwin IT, Shared signals—‘alarm calls’ from plants increase appearance to herbivores and their enemies in nature. *EcolLett*.2008; 11:24–34. PMID: 17961175.
- [45] Unsicker SB, Kunert G, and Gershenzon J. Protective perfumes: the role of vegetative volatiles in plant defense against herbivores. *CurrOpinPlantBiol*.2009;12:479–485. doi:10.1016/j.pbi.2009.04.001 PMID:19467919
- [46] Dicke M and Baldwin IT. The evolutionary context for herbivore-induced plant volatiles: beyond the ‘cryforhelp’. *TrendsPlantSci*. 2010; 15:167–175. doi:10.1016/j.tplants.2009;12.002 PMID:20047849.
- [47] Mumm R, and Dicke M. Variation in natural plant products and the attraction of bodyguards for indirect plant defense. *CanJZool*.2010;88:628–667.
- [48] Chu CC, Henneberry T and Cohen AC. Bemisiaargentifolii (Homoptera:Aleyrodidae):host preference and factors affecting oviposition and feedings it epreference.*EnvirolEntomol*.1995; 24:354–360.
- [49] Butter NS and Vir BK. Morphological basis of resistance in cotton to the whitefly Bemisiatabaci. *Phytoparasitica*.1989; 17:251–561.
- [50] Lakshminarayan S, Singh PS and Mishra DS. Relationship between whitefly population, YMV disease and morphological parameters of green gram germplasm. *EnvironEcol*.2008;26:978–982.

- [51]Shibuya T, Hirai N, Sakamoto Y and Komuro J. Effects of morphological characteristics of *Cucumis sativus* seedlings grown at different vapor pressure deficits on initial colonization of *Bemisia tabaci* (Hemiptera:Aleyrodidae). *J Econ Entomol.*1995; 102:2265–2267. PMID:20069856.
- [52]Taggar GK and Gill RS Preference of whitefly, *Bemisia tabaci*, towards black gram genotypes: Role of morphological leaf characteristics. *Phytoparasitica.*2012;40:461–474. doi:10.1007/s12600-012-0247-z
- [53]Frisbie RE, Reynolds HT, Adkisson PL and Smith RF. “Cotton insect pest management”, in *Introduction to Insect Pest Management*, 3rd ed.(Eds.): Metcalf R.L and Luckman W.H. (John Wiley & Sons, Inc. New York) 1994:421–468.
- [54]Maskato Y, Talal S and Gefen T. Red foliage color reliably indicates low host quality and increased metabolic load for development of an herbivorous insect. *Arthropod Plant Interac.*2014; 8: 285–292.
- [55]Petro LO and Redak RA. Host plant preference and performance of *Bemisia argentifolii* (Homoptera: Aleyrodidae) on poinsettia (*Euphorbia pulcherrima*) in relation to variety. *US Dept Agric, Agric Res Serv.* July 2000.
- [56]Petro L, Redak R, Bethke J and Perring TM. Preference and performance of silver leaf whitefly on selected poinsettia varieties. *US Dept Agric, Agric Res Serv.* June 2002.
- [57]Jayraj S, Uthamasamay S. Aspects of insect resistance in crop plants *Proc. Indian Acad. Sci.(Anim. Sci.)*1990;(99)3: 211-224
- [58]Din M, Malik A, Azhar M and Ashraf M. Natural Resistance against Insect Pests in Cotton. *The J. Anim. Plant Sci.* 2016; 26(5): 1346-1353.
- [59]Hasan M, Ma F, Prodhan H, Li F, Shen H, Chen Y and Wang X. Molecular and Physio-Biochemical Characterization of Cotton Species for Assessing Drought Stress Tolerance. *Int. J. Mol. Sci.* 2018; 19:2636.
- [60]Awan MF, Abbas MA, Muzzafar A, Ali A, Tabassum B, Rao AQ, Ahmad I, and Husnain T. Transformation of Insect and Herbicide Resistance Genes in Cotton (*Gossypium hirsutum* L.). *J. Agr. Sci. Tech.* 2015; 17: 275-285.

- [61]Bakhsh A, Anayol E, Khabbazi SD, Karakoç ÖC, Sancak C and ÖzcanS. Development of Insect-resistant Cotton Lines with Targeted Expression of Insecticidal Gene. Arch Biol Sci. (2016) 68(4):773-780.
- [62]Meena AK, Kumar MNV, Katageri IS, Methre R and Kumhar BL. Molecular Markers in Cotton Improvement. Int.J.Curr.Microbiol.App.Sci. 2017;6(5):2627-2644.
- [63]Preetha S and Raveendren TS. Molecular marker technology in cotton.Biotechnologyand Molecular Biology Review. 2008;3(2): 032-045.
- [64]Tanksley, S. D. Molecular markers in plant breeding. Plant Mol. Biol. Rep. 1983;1: 3-8.
- [65]Chaudhary L, Sindhu A, Kumar M, Kumar R and Saini M. Estimation of genetic divergence among some cotton varieties by RAPD analysis. J. Plant Breed. Crop Sci. 2010;2: 39–43.
- [66]Li Z, Wang X, Zhang Y, Zhang G, Wu L, Chi J and Ma Z. Assessment of genetic diversity in glandless cotton germplasm resources by using agronomic traits and molecular markers. Front. Agric. Chin. 2008;2: 245–252.
- [67]Arunita R, Rakshit S, Santhy V, Gotmare VP, Mohan P, Singh VV, Singh S, Singh J,Balyan HS, Gupta PK and Bhat SR. Evaluation of SSR markers for the assessment of genetic diversity and fingerprinting of *Gossypium hirsutum* accessions. J. Plant Biochem. Biotechnol., 2010;19: 153-160.
- [68]Malik W, Ashraf J, Iqbal MZ, Khan AS, Qayyum A, Abid MA, Noor E, Ahmad MQ and Abbasi GH. Molecular Markers and Cotton Genetic Improvement:Current Status and Future Prospects. The Sci. World Jour. 2014: 1-15.
- [69]Kalia RK, Rai MK, Kalia S, Singh R and Dhawan. Microsatellite markers: an overview of the recent progress in plants. Euphytica. 2011;177(3):309–334.
- [70]Appleby N, Edwards D and Batley J. New technologies for ultra-high throughput genotyping in plants. Methods in Molecular Biology. 2009;513;19–39.
- [71]Kranthi KR, Kranthi S, Dongre AB. Cotton biotechnology. Cicc. cicc technical bulletin no: 10.
- [72]Iftikhar A, Abida K, Mehboob UR, Zafar Y, Asif H, Shraf MA, Sana R, Ara Z, Wahid A, Maqsood S, Niaz M and Abbas SQ. Development of genetic linkage map of leaf hairiness in *gossypiumhirsutum* (cotton) using molecular markers. Pak. J. Bot. 2009;41(4):1627-1635.
- [73]Sridhar V, Reddy P Surender, Sunil Reddy S, Mahantesh, SatihalB, Satya Prasad M, Ravi Kumar V and Sateesh Kumar P. Development and validation of molecular markers for sucking pest resistance in Cotton. Canadian Journal of Biotechnology. 2017;1.

- [74]Pushpam R, and Raveendran TS. Production of interspecific hybrids between *Gossypium hirsutum* and Jassid resistant wild species *G. raimondii* and *G. armourianum*. *Cytologia*. 2006; 71:407–418.
- [75]Knutson A, Isaacs S, Campos C, Campos M, and Smith CW. Resistance to cotton fleahopper feeding in primitive and converted race stocks of cotton, *Gossypium hirsutum*. *J. Cotton Sci*. 2014. 18:385–392.
- [76]Walker GP, and Natwick ET. Resistance to silverleaf whitefly, *Bemisia argentifolii* (Hem., Aleyrodidae), in *Gossypium thurberi*, a wild cotton species. *J. Appl. Entomol*. 2006; 130: 429–436.
- [77]Miyazaki J, Stiller W N and Wilson LJ. Identification of host plant resistance to silverleaf whitefly in cotton: implications for breeding. *Field Crops Res*. 2013a; 154:145–152.
- [78]Zhang J, Fang H, Zhou H, Hughs SE, and Jones DC. Inheritance and transfer of Thrips resistance from Pima cotton to Upland cotton. *J. Cotton Sci*. 2013; 169: 163–169.
- [79]Miyazaki J, Stiller WN and Wilso LJ. Novel cotton germplasm with host plant resistance to twospotted spider mite. *Field Crops Res*. 2012; 134:114–121.
- [80]Schuster MF, Maxwell FG and Jenkins JN. Resistance to the two spotted spider mite in certain *Gossypium hirsutum* races, *Gossypium* species, and glanded-glandless counterpart cottons. *J. Econ. Entomol*. 1972; 65:1108–1110.
- [81]Dighe ND, Robinso AF, Bell AA, Menz M, Cantrell RG and Stelly DM. Linkage mapping of resistance to reniform nematode in cotton following introgression from *G. longicalyx* (Hutch. & Lee). *Crop Sci*. 2009; 49:1151-1164.
- [82]Romano GB, Sacks EJ, Stetina SR, Robinson AF, Fang DD, Gutierrez OA, and Scheffler JA. Identification and genomic location of a reniform nematode (*Rotylenchulus reniformis*) resistance locus (Renari) introgressed from *Gossypium aridum* into upland cotton (*G. hirsutum*). *Theor. Appl. Genet*. 2009; 120:139-150.
- [83]Gutierrez, O. A., Robinson, A. F., Jenkins, J. N., McCarty, J. C., Wubben, M. J., Callahan FE, and Nichol RL. Identification of QTL regions and SSR markers associated with resistance to reniform nematode in *Gossypium barbadense* L. accession GB713. *Theor. Appl. Genet*. 2011; 122: 271-280.
- [84]Bravo A, Gill S and Soverom M. Mode of action of bacillus thuringensis Cry and Cyt toxin and their potential for insect control. *Toxicon*. 2007;49:423-435.

- [85]Chee P, Lubbers E, May O, Gannaway J and Paterson AH. Changes in genetic diversity of the U.S. upland cotton. in Proceedings of the Beltwide Cotton Conference, National Cotton Council, San Antonio, Tex, USA, 2004.
- [86]Lubbers E, Chee P, Gannaway J, Wright R, El-Zik E, and Paterson AH. Levels and patterns of genetic diversity in upland cotton. in Proceedings of the Plant and Animal Genome 12th Conference, San Diego, Calif, USA, 2004.
- [87]Wang K, Wang Z, Li F, Yr W, Wang J, Song G, Yue Z, Cong L, Shang H, Zhu S, Zou C, Li Q, Yuan Y, Lu C, Wei H, Gou C, Zheng Z, Yin Y, Zhang X, Liu K, Wang B, Song C, Shi N, kohel RJ, Percy RG, Yu JZ, Zhu YX, Wang J, Yu S. The draft genome of a diploid cotton *Gossypium raimondii*. *Nature Genetics*. 2012;44(10):1098–1103.
- [88]Li F, Fan G, K, Wang Sun F, Yuan Y, Song G, Li Q, Ma Z, Lu C, Zou C, Chen W, Liang X, Shang H, Liu W, Shi C, Xiao G, Gou C, Ye W, Xu X, Zhang X, Wei H, Li Z, zhang G, Wang J, Liu K, Kohel RJ, Percy RG, Yu JZ, Zhu YX, Wang J and Yu S. Genome sequence of the cultivated cotton *Gossypium arboretum*. *Nature Genetics*. 2014;46(6):567–572.
- [89]Chen ZJ, Schefer BE, Dennis E, Triplett BA, Zhang T, Guo W, Chen X, Stelly DM, Rabinowicz PD, Town CD, Arioli T, Brubaker C, Cantrell RG, Lacape JM, Ulloa M, Chee P, Gingle AR, HaiglerCH, Percy R, Saha S, Wilkins T, Wright RJ, Deynze AV, Zhu Y, Yu S, Abdurakhmonov I, Katageri I, Kumar PA, Rahman M, Zafar Y, Yu JZ, Kohel RJ, Wendel JF and Peterson AH. Toward sequencing cotton (*Gossypium*) genomes. *Plant Physiology*. 2007; 145(4):303–1310.
- [90]Zhang HB, Li Y, Wang B and Chee PW. Recent advances in cotton genomics. *International Journal of Plant Genomics*. 2008:20.