

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

### **Abstract**

Cotton is a very important fiber and oilseed crop of many countries. The economy of most countries is 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. In-built resistance in plants is offered by various mechanisms that can be of morphological, physiological and molecular origin; these mechanisms can be exploited for the purpose of ameliorating the current scenario. The use of morphological, physiological and molecular markers for the ideotype plant development is 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 used widely and with great ease. Physiological markers used at the protein level evaluate its expression in cotton against insects. The plant produces a 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 may evaluate the presence of genes that are involved in producing structures and chemicals to prevent the invasion of foreign pests. These have the ability to express and play important role in cotton against 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 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 declined to a greater extent over the years. At present 60% of foreign exchange is 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 ranked 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 deterioration in the productivity of 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 resulting in the plant wilting and dropping its leaves [5]. Cotton is most susceptible to the insect pests such as *Helicoverpa* species which can reduce the production by 60%, irrespective of all the efforts to control them [6]. Among sucking insects causing critical damage to cotton and 40-50% reduction in yield include *Aphis gossypi*, *Bemisia tabaci*, *Thrips tabaci* and *Amrascibiguttella* [7, 8,9]. The most common way of controlling the pests by the farmers is by use of insecticidal sprays that are quick in eliminating these pests [10, 11]. The immense use of such chemicals poses serious harm to the human health and resistance in these insects 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 to some beneficial insects, fishes and humans [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]. There is the need to develop a strategy that controls these pests without the use of such detrimental chemicals [3].

There are many other ways to control these pests that could cause decline in cotton production. Plants contain various mechanisms that can control the pest population. 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 involving gene- for -gene interaction [17]. These mechanisms could be either of morphological or physiological 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 serve as bio pesticide without any adverse effects to the environment [21]. Plants produce a class of special chemicals (proteins) called secondary metabolites that can avert the pests action. Some of these proteins include lectins or agglutinins [22, 23,24]. This group has a major characteristic of identifying the special type of pest and then bind their internal carbohydrate structures [24]. Advancements in molecular techniques led to the development of Bt cotton that showed significant resistance levels without damaging environment. It can kill the pests that suck 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 was reported that the level of toxin produced was not sufficient to kill the insect pests [27]. In this review the morphological, physiological and molecular markers involved in developing resistance against cotton insect pests are discussed.

## 2. Morphological Markers associated with resistance against insect pests

Many characteristics in different plant 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 causes decrease in **the insect** population especially; whitesfly. Hence, the plants with higher gossypol content are required that may 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 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 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 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 the insect population **of** the plant [35, 3]. In addition to these the lower bract sizes is also desirable as it harbours 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, thus injecting some poisonous substances inside it [36].As a result, the plant starts wilting and drying and gradually becomes weak; and curls its leaves thereby deceasing yield 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 low number of trichomes on the lower sides of the leaves were preferable to jassids while the plants with high number of trichomes harboured 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- and super-okra- leaved plants usually exhibit higher resistance. Broad leaf laminas 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 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 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 was considered a low quality plant [54]. Light green leaves 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 a 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 *Helicoverpaarmigera* and *Spodopteralitura*.

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, 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 (*Helicoverpaarmigera*), 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 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 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 by gathering all the good traits present in different lines and wild genotypes. By **conventional** breeding, 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 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 include;

- Plant, tissue and cellular levels used for the determination of genotypes.
- Mostly loci **consists of** more number of naturally occurring alleles.
- Morphological neutrality.
- Codominance occurs at many loci.
- Less number of epistatic or pleiotropic influence 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 and the traits measured easily rather than morphologically [70].

**Table 1**

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

Homeologus chromosome pairs	Characters	Locus	Reference
1,15	Virescent leaf coloration	$v^5, v^6$	[71]
	Leaf shape	$L_1^L, L_2^O$	
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  $F_2$  and  $F_3$  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 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 built by RAPD and SSR markers explained heritability leaf hairiness. 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 was easy with the presence of sequence-based molecular approaches. From wild relatives by using wide hybridization to develop two 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 2.

**Table 2**

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

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 method to produce nematode resistance in upland cotton cultivars and that marks a new era of study. The development of nematode resistant cotton by molecular approach would assist in the identification of the resistant gene and their position on the chromosome. Today, six nematode resistant genes have been identified that behave like recessive, partially dominant and dominant genes. In *G. longicalyx* species on chromosome 11 dominant genes Ren<sup>lon</sup> and Ren<sup>ari</sup> are present and on chromosome 21 of *G. aridum* specie [81, 82]. In *G. barbadense* some partially resistant genes Ren<sup>barb1</sup>, Ren<sup>barb2</sup>, and Ren<sup>barb3</sup> 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 V in cotton by *B. thuringiensis* bacteria are shown in Table 3 [71]. Now Bt. Cotton is used for nematodes and it is seen as “Cry” genes, proteins which are useful against nematodes. “Cry” proteins are insecticidal in nature during sporulation phase [84].

**Table 3**

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

Genes		Insect orders	Reference
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 affects the potato aphids *Myzus persicae* and tobacco hornworm *Manduca sexta*. Cytokinin-biosynthetic pathway inhibited by more expression of IPT enzyme.

### **4.1.3. Lectin genes:**

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

### **4.1.4. Spider and scorpion venom genes:**

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

### **4.1.5. Stunt virus**

Stunt virus is a small RNA which has three genes. It attacks the midgut cell of *Heliothis* species and creates feeding problem. The gene can be used against many insects and plant which require small amount of virus which 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 with 5-50 amino acid. It is easy to work 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 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 reduced by the identification and 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 on the sequencing of *G. raimondii* [87] and *G. arboreum* [88] 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 having desired traits. This technique does not help us for genetic diversity 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 as an important tool for plant breeders and geneticists for the development of varieties. SNPs marker will be useful as marker assist selection and mapping studies in future because of more abundance and development of better identification system [68].

## 6. Conclusion:

This study deals with all the methods 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 increases the cost of production immensely and unaffordable to farmers and industrialists. Many morphological, physiological and molecular markers can be used in the breeding strategies and 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 leaf is also a source

of developing resistance. Thin leaves are less succulent so least attracted by the 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 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 resistant genes against the cotton pest. In some species, these genes can be used by genetic engineering. Researchers also identify some other source like fungal genes. Researchers are also mapping the genome of cotton genotypes and investigating the genes which can be used in the development of cotton pest resistant genotypes for high quality yield.

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