Review Paper

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

Abstract

Cotton is most important fiber and oil seed crop of many countries. commerce of countries depends 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. Farmer is continuously using chemical control method to control all these pests which increases cost of production to many folds. In addition to this it also causes many environmental as well as health hazards. Built in resistance in plants is offered by various mechanisms they can be of morphological, physiological and molecular origin these mechanisms can be exploited for the purpose of ameliorating the current scenario. They use morphological, physiological and molecular marker for the ideotype plant development is something 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 and evaluate its expression in cotton against insects. Plant produce special group of proteins as lecitins and other phenolic compounds that reduce the insect population to great extent. Molecular markers used at gene level and evaluate the presence of genes that are involved in producing structures and chemicals to prevent the invasion of foreign pests. This has ability to express and play important role in cotton against insect pest. 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

Introduction:

Cotton is the one of most important non-food and fiber crop around the globe. Due to its importance in economic sector it is also called white gold. Pakistan had a great era of cotton production in the past that has been declined to greater extent. Even at present 60% of foreign exchange is through 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 four 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 production of agricultural products [2]. Insect pests are the main factor for the deteriorating the productivity of Cotton by decreasing quality as well as yield [3]. Cotton accommodates about 1326 insect species in the world that damage it from sowing 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 plant wilts and drops its leaves [5]. Cotton is the favorite crop of the insect pests Helicoverpa species can reduce the production by 60% even with all the efforts to control them [6]. Among sucking insects following critically damage the cotton and cause 40-50% reduction in yield *Aphis gossypi, Bemisia tabaci, Thrips tabaci* and *Amrasca bigutella* [7, 8, 9]. The most common way of controlling the pests by the farmers is 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 insects become resistant to these chemicals. In addition to this environment is also polluted by them [12], [13]. These chemicals are non-degradable and very much poisonous for the beneficial insects, fishes and humans also [14]. 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 to develop a strategy that controls these pests without the use of such detrimental chemicals [3].

Many other ways can be there for controlling these pests that decline the cotton production. Plants can contain various mechanisms that can control 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 first step is to identify the pathogen and then regulate some action due to some gene for gene interaction [17]. These mechanisms could be of morphological physiological and of 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 pests' population [20]. The plant derived chemicals which act as repellent can be served as bio pesticide having no ill effects on environment [21]. Plants produce a class of special chemicals (proteins) called secondary metabolites that can avert the pests action. One of these proteins are lectins or agglutinins [22, 23, 24]. This group has an eminent characteristic that it can identify special type of pest and can bind their internal carbohydrate structures [24]. Advancements in molecular techniques lead 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 reported that the level of toxin produced is not adequate to kill the insect pests [27]. In this review the markers related to morphological, physiological and molecular basis are discussed that are involved in developing resistance against cotton insect pests.

Morphological Markers associated with resistance against insect pests

Many characters in different plant can 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 decreasing insect population specially whitefly. Hence the plants with higher gossypol content are required that may support lesser or no 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 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 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 with low insect population [28, 29]. Hair density is related with insect population also, higher the hair density 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 lesser hair showed lower 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 good way than smooth leaves [34, 4]. Leaf lamina thickness is also an important factor higher the thickness of leaf lamina lower will be the insect population on the plant [35, 3]. In addition to these the lower bract size is also desirable as it is involved in less 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 suck sap from it, in addition it inserts some poisonous substances inside it [36]. As a result, the plant starts wilting and drying and gradually becomes week and turns its leaves as it can have been seen clearly from the heavily affected cotton by jassids and yield is decreased 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 important factor for controlling jassids. Plants having lowest number of trichomes on the lower sides of the leaves were favorite to jassids and the plants with high number of trichomes show usually less insect population [40].

Leaf size and shape also determine the insect pests attack. 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 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 attraction of insects. Insect preference is for special traits of its choice for example whitefly use to lay 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 insect. It has been observed that plants with thin leaf lamina possess the lesser 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 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 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 considered low quality plant [54]. Light green leaves are 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 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].

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 attack of insect pest. Defense mechanism before attack of insect pests is called as constitutive defense and defense mechanism activated after attack of insect pests is known as inducible defense. Resistance or tolerance of plants to insect herbivores and pathogens is mediated via constitutive or induced defense mechanisms. Defense mechanism basically consist of certain steps in which plants firstly detects insect attack by specific recognition signals. Then these signals are transferred to the specific signal transduction pathway which ultimately activates production of defense chemicals.

- **Cell sap concentration**:

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 exert pressure on insect to select plant as 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].

- **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 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 tune-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 functions 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.

- **Plant Genetic Engineering and cotton**

Now a days, 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].

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 to 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 by 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 armyworm 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 expression of insecticidal genes. The Bacillus thuringiensis toxin are produced at different growth stages of the cotton plant. But these toxins should be produced at appropriate time of growing season. That’s 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 same in Bt and non-Bt hybrids up to 89 days after sowing(DAS) [61].

Molecular markers associated with insect pest resistance

The breed looks the morphology of plant and select desired characteristics with our objective. Usually these characteristics are controlled by many genes and mostly effected by environment. If quantitative characters are found in the individual component of DNA associate with one of them and biometry do not identify the locus of that character but effectively manage it. Molecular markers give desirable results quickly and accurately [62]. Commonly plant breeder have objective to develop the agronomical point of view better varieties and want to gather all good which are present in different lines and wild genotypes. By convention breeding, transfer all good character in one genotypes by using selfing, backcross and hybridization are time consuming and less confirmative. Molecular markers give direct selection of plant on the base of markers process [63].

In 1983, Tankeley gives five character that differ the molecular markers from phenotypical markers. These properties are

- plant, tissue and cellular level can be used for the determination of genotypes.
- Mostly loci consist on the more number of naturally occurring alleles.
- Morphological neutrality.
- Codominant occur at many loci.
- Less number of epistatic or pleiotropic influence are occurring [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. 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 sequences repeats (SSR), [67]. Now simple DNA marker can be formed because of availability of genomic databases 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 traits can be measured easily that not measure by morphologically [70].

Table 1

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

Homeologus chromosome pairs	**Characters**	**Locus**	**reference**
1,15	Virescent leaf coloration	v^5,v^6	[71]
	Leaf shape	L_1^L, L_2^0	
7,16	Anthocyanin pigmentation	R_I and R_2	
	Yellow green loci	yg_I, yg_2	
12,26	Withering bracts	bw_I, bw_2	
	Nectar less	ne_I, ne_2	
A,18	Flower colour	Y_I, 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 associate with the character of hairiness by utilizing 400 RAPD and 54 SSR primers. During experiment, PCR condition are specific. In duplicate reaction consistently 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 150bp DNA segment was enlarged with one SSR primer pair JESPR-154. Linkage map were made by polymorphic DNA markers. Genetic map build by RAPD and SSR markers that tells heritability leaf hairiness happen. These character can be utilized to enhance insect resistance in cotton crop [72].

During 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 environment 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 gene. Such plant breeding easier 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 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	*G. armourianum*	[74]
	G. hirsutum old accessions	[75]
Silver leaf whitefly	*G. thurberi*	[76]
	G. hirsutum genotypes	[77]
Thrips	*G. barbadense*	[78]
	G. tomentosum	[78]
	G. darwinii	[78]

Spider mites	*G. arboreum*	[79]
	G. lobatum	[80]

There is another major issue of nematodes in cotton in some areas. Now researcher using molecular methodology to produce nematode resistance in upland cotton cultivars and that's new era of examination. For the development of nematode resistant cotton, in molecular approach to search out the resistant gene and their position on the chromosome. Today six nematode resistant genes were identified that have different behavior like recessive, partial dominant and dominant. 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 partial resistant genes Ren^{barb1}, Ren^{barb2}, and Ren^{barb3} are identified and present on chromosomes 21, 21, and 18 [83].

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

Table 3

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

Genes		**Insect orders**	**References**
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	

Sources genes under observation:

- **Cholesterol oxidase:**

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

- **Iso-pentenyl-transferase (IPT):**

  It is an enzyme takes from microorganism. It effects the potato aphids *Myzus persicae* and tobacco homworm *Manduca sexta*. cytokinin-biosynthetic pathway inhibits by more expression of IPT enzyme.

- **Lectin genes:**

  It more under investigation because it produces protein and act on the digestive system through bind with blood cells.

- **Spider and scorpion venom genes**:

  This gene also express and produce protein and have insecticide properties.

- **Stunt virus is a small RNA virus:**

  Stunt virus is a small RNA have three genes. It effects midgut of attacks the midgut cell of *Heliothis* species and create feeding problem. That gene cane be used against many insect and plant require less amount of virus which amplified itself in insect.

- **Amylases**:

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

- **Insect neuropeptides**:

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

- **Novel gene:**

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

Future horizons:

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

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

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

Conclusion:

This study deals with all the ways that can be manipulated to develop resistance against cotton pests. The cotton crop is world's most important fiber crop but it is greatly damaged by insects. Use of sprays and insecticides enhance the cost to an immense level that it could not be afforded by the 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 are least preferred by the insects hence leaf structure and color determination is also an important factor. Thickness of leave is also a source of developing resistance. Thin leaves are less succulent so least attracted by the plants. In 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 the locus of that particular trait. DNA markers not time consuming and can be identify the gene of interest. Molecular markers help us in the identification of some genes which have resistant against the cotton pest. In some species these present and can be used by genetic engineering. Researchers also identifying some other source like fungus of genes. Researchers also mapping the genome of cotton genotypes and investigating the genes. Which can be used in the development of cotton pest resistant genotypes that having high quality and good yield.

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