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Molecules and Methods for the Control of Biotic Stress Especially the Insect Pests – Present Scenario and Future Perspective

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Abstract

Continuous increase in population has unbalanced the demand and supply of agricultural produce. In this scenario, food security in a sustainable manner is being challenged due to several factors. Insect pests are considered as one of the major factors, which accounts for 35–100% crop damage, worldwide. Synthetic insecticides contributed significantly, but several safety concerns are associated with them. Transgenic crops with enhanced biotic or abiotic stress tolerance have shown promising contribution in achieving greater crop productivity. Transgenic cotton expressing Cry toxin of *Bacillus thuringiensis* has tremendously increased the production as well as the societal status of farmers in our country. However, a concomitant increase in the population of minor pests like aphids, whiteflies and others has demanded certain new approaches. Researchers have isolated several other toxic proteins like lectins, protease inhibitors, amylase inhibitors, chitinases, and tried various novel approaches like gene pyramiding, tissue specific expression and modulation in metabolites expression to combat emerging problems of insect pests. Conversely, the emergence of a new type of crop insect pests demands more specific effort for each insect. Besides this, there are several safety and ethical concerns that are associated with the use of genetically modified crops, which also need to be resolved as per demand. Development of a dedicated scientific forum for the proper demonstration of advantages and disadvantages of genetically modified crops to the citizens at ground level might be useful in resolving the societal and ethical concerns in our country.

Keywords: Insecticidal proteins, Cry toxins, Lectins, Protease inhibitors, Chitinase, Transgenic crops

1. Introduction

World population is projected to increase over 1,000 million in the next four decades. An immediate priority for agriculture industry is to achieve maximum production in an environmentally sustainable and cost-effective manner. Food security is on high agenda at the political and social level [1]. Our progeny can face a severe shortage of food supply due to the over demand of continuously increasing population. Jaques Diouf, the Director General FAO, stated (2011) “*The silent hunger crisis, affecting one sixth of all humanity, poses a serious risk for world peace and security*”. The current challenge is to increase primary crop production in agricultural sustainability manner. In order to achieve these goals, it is important to identify and address the major limitations of productivity. Crop damage caused by insect pests is one of the major confinements, which is estimated to be around 35–100%, globally [2]. Synthetic insecticides have made significant contributions in food production, but they are also responsible for environmental and health hazards.

Transgenic crops with enhanced biotic or abiotic stress tolerance have shown promising contribution in achieving greater food security. A milestone was established about 25 years ago with the development of genetically engineered tobacco expressing the entomotoxic Cry protein from the bacterium *Bacillus thuringiensis* (Bt) [3, 4]. Presently, a number of Bt-Cry protein containing products are in the market of the United States (www.aphis.usda.gov) and some other countries. In March 2002, the Government of India permitted the release of transgenic cotton (Bollgard) expressing Bt toxins-Cry1Ac for commercial cultivation, which conferred resistance against bollworms [5]. Subsequently, BollgardII was released, which expresses Cry2Ab toxin along with Cry1Ac. Bt-Cry proteins have increased the productivity of crops substantially by controlling the major insects of order Lepidoptera and Coleoptera [6]. However, a concomitant increase in the population of minor pests (like whiteflies, aphids, leafhoppers and others) has threatened the success of Bt-transgenic crops [7–9]. An alternative strategy is to take advantage of the plant’s own defense mechanisms, either by maneuvering the expression of the endogenous defense proteins or by introduction of an insect toxic gene (like lectins) derived from another plant. Several insecticidal proteins encoding genes have now been isolated from different sources and introduced into crop genomes to combat the issue of various groups of insect pests [10, 11]. Simultaneously, a new approach based on RNA interference is also reported for the control of crop insects [12–14]. Present chapter briefs about the insecticidal proteins and transgenic strategies for the control of crop insects.

2. Insecticidal proteins

2.1. Cry toxins of *Bacillus thuringiensis*

Introduction of Bt-Cry toxins revolutionized the area of insect control through transgenic technology. These are toxic to the insects of orders Lepidoptera, Diptera, Hymenoptera, Coleoptera and also to nematodes. These are produced as parasporal crystalline inclusions in *B. thuringiensis*. More than 500 Cry proteins/genes have been discovered till date, which are

classified into 67 groups (Cry1–Cry67) on the basis of the primary structure [15, 16]. The genes are further divided into four phylogenetically unrelated protein families with different modes of action. These are: (1) three domain Cry toxins (3D) family, (2) mosquitocidal Cry toxins (Mtx) family, (3) binary-like (Bin) family and (4) the Cyt family of toxins [17]. Some Bt strains produce an additional insecticidal toxin called as VIP (vegetative insecticidal proteins) during the vegetative growth phase. Three VIP toxins: VIP1/VIP2, a binary toxin, and VIP3 have been characterized till date [18, 19].

Several insect-resistant transgenic crops have been developed by expressing Bt-Cry proteins, among which corn, cotton, soybean and canola are the most important crops. These transgenic crops are mostly expressing the Cry1Ac and Cry2Ab to control the chewing pests like *H. armigera*, *H. zea* and *Pectinophora gossypiella*, *Heliothis virescens* and *Ostrinia nubilalis* [20]. Some other cry toxin based products are also commercialized, which express Cry1A, Cry1F, Cry1EC, Cry34Ab/Cry35Ab binary toxin, Cry1Ab and Cry3Bb for the control of lepidopteran insect *Spodoptera frugiperda*, *S. litura* and coleopteran insect *Diabrotica virgifera* [6, 20, 21]. Further detail about the commercialized insecticidal crops are given in a later section.

2.2. Lectins

Lectins are carbohydrate-binding proteins, which possess at least one non-catalytic domain for specific and reversible binding to mono- or oligosaccharides [22, 23]. A typical lectin is multivalent in nature, therefore agglutinate cells. Lectins are extensively distributed in nature from prokaryotes to eukaryotes. The specific interaction with glycoconjugates makes them valuable in biomedical sciences and biotechnology [24]. Carbohydrates present in viruses, microorganisms, fungi, nematodes or phytophagous insects interact with plant lectins [25, 26]. In the past decades, many plant lectins are reported to be toxic to several economically important insect pests of various orders [27–29]. To analyze the insecticidal properties under natural conditions, many transgenic plants expressing lectins have been developed. The toxic effects of different lectins have been demonstrated on several insect species; these effects range from a severe delay in development to high mortality in insects [11].

2.2.1. GNA-related lectins

Galanthus nivalis agglutinin (GNA) purified from snowdrop bulbs is the best studied plant lectin for insecticidal properties. The snowdrop lectin specifically binds to terminal mannose residues in high-mannose-N-glycans, which occur very frequently on insect glycoproteins [30]. Toxicity of GNA has been shown for a wide range of insects; but homopteran insects are highly sensitive to GNA. Several GNA-related lectins have been isolated from different *Allium* species which have shown the potential for insect control [11, 29]. Further, accumulation of some lectins like *Allium porrum* agglutinin in the phloem sap in natural situations support the defensive role of lectins against sap-sucking insects [31].

GNA and related lectins have been successfully expressed for resistance against insect pests into a variety of crops [32]. Transgenic rice expressing ASAL caused significant mortality in nymph of hemipteran insect pests [33]. Onion (*Allium cepa*) lectin has shown more potential

against mustard aphid (*Lipaphis erysimi*) in comparison to GNA and ASAL (*Allium sativum* leaf agglutinin) [34]. Transgenic rice expressing ASAL exhibited protection against tungro disease also, after infestation with the *N. virescens* [35]. Vajhala et al. [36] recently demonstrated significant protection in ASAL expressing transgenic cotton against jassid and whitefly. ASAL is also reported to be toxic to chewing insects like *Helicoverpa armigera* and *Spodoptera litura* [27] and several other sucking insects like *Nephotettix virescens* and *Nilaparvata lugens* [37]. Studies related to the mechanism of toxicity showed that ASAL shares the similar receptors with Bt-Cry toxin [28], but both the proteins interact at different positions without steric hindrance and increased the toxicity of each other [29]. Therefore, they can be pyramided together for broad-range insect resistance.

2.2.2. Legume lectins

Legume lectins are purified from seeds and bind to carbohydrate structures like Thomsen-nouveau (Tn) antigen or complex N-glycan with terminal galactose and sialic acid residues. Pea lectin (*Pisum sativum* agglutinin, PSA) expressed in transgenic oilseed rape (*Brassica napus*) shows growth retardation of the pollen beetle larvae (*Meligethes aeneus*) [38] and no effect on the adult beetles [39]. A legume lectin known as Gleheda purified from ground ivy (*Glechoma hederacea*) exhibits high insecticidal activity against the Colorado potato beetle larvae (*Leptinotarsa decem-lineata*) [40]. GS-II lectin isolated from the seed of *Griffonia simplicifolia* shows toxicity to Cowpea weevil (*Callosobruchus maculatus*) [41]. A mannose-binding legume lectin concanavalin A (ConA) from jackbean has shown toxicity to the hemipteran pea aphid (*Acyrtosiphon pisum*) [42, 43] and tara plant hopper (*Tarophagous proserpina*) [44].

2.2.3. Hevein-related lectins

Hevein-related plant lectins exhibit specificity for chitin (chitin forms endo- and exo-arthropods, nematodes and fungi). These are also studied for insecticidal properties [45]. Due to the absence of chitin in mammals, hevein-related lectins are considered safe for the usage in genetically modified crops. Wheat germ agglutinin (WGA) has shown a negative effect on the development of the cowpea weevil (*Callosobruchus maculatus*) larvae and southern corn root worm (*Diabrotica undecimpunctata*) [46, 47]. WGA is active against lepidopteran insect larvae also [47, 48].

2.2.4. Other insecticidal lectins

Several other plant lectins have shown insecticidal property. Transgenic tobacco plants expressing tobacco leaf lectin (NICTABA) is detrimental to the cotton leafworm (*S. littoralis*) and the tobacco hornworm (*M. sexta*) [49]. Another protein, *phloem protein 2* (PP2) belonging to the NICTABA family, also possesses insecticidal activity [50, 51]. The amarantins and the jacalin-related lectins have also shown the potential for insect control, especially against sap-sucking insect pests. Transgenic cotton expressing *Amaranthus caudatus* agglutinin (ACA) under the control of a phloem-specific promoter shows a strong resistance against nymphs of the cotton aphid (*Aphis gossypii*) [52]. Transgenic tobacco expressing Heltuba, a jacalin-related lectin from the *Helianthus tuberosus*, showed reduced development and fecundity of the peach-

potato aphid (*M. persicae*) [53]. Another promising jacalin-related lectin HFR1 is produced in resistant varieties of wheat (*T. aestivum*) during infestation by the Hessian fly larvae. Although HFR1 has not shown any toxicity against Hessian fly, it shows the strong insecticidal activity to the larvae of fruit fly (*D. melanogaster*) [54].

2.3. Proteinase inhibitors

Proteinase inhibitors (PIs) are small molecular weight proteins which affect several metabolic pathways. They are the major components in seeds and storage organs of crops. Mickel and Standish [55] demonstrated the role of PIs in plant defense for the first time and noticed the abnormality in the development of larvae of certain insects fed on soybean products. The feature was attributed to trypsin inhibitors, and it was found to be toxic to the larvae of flour beetle (*Tribolium confusum*) [56].

PIs inhibit the digestion of proteins in midgut and cause mortality of insects due to nutritional imbalance [57, 58]. PIs also interfere with several metabolic processes (like moulting) by blocking the proteolytic activation of enzymes [59]. They affect growth and development, multiplication rate and insect life span [60–62]. PIs have been expressed in several transgenic plants for resistance against insect pests of several classes [63–65]. Pea and soybean trypsin–chymotrypsin inhibitors (PsTI-2, SbBBI) belonging to the Bowman–Birk family [66] and mustard-type trypsin–chymotrypsin variant Chy8 [67] cause significant mortality of pea aphid *A. pisum*. Plant-derived PIs have been used for the development of insect-resistant transgenic plants and projected as an alternative to Bt-Cry proteins [68, 69].

The majority of plant PIs originate from three main families, namely Solanaceae, Leguminosae and Gramineae [70]. Plant PIs can be grouped into four classes: serine, thiol, metallo and aspartyl. Most plant PIs are inhibitors of microbial and animal serine proteases, such as chymotrypsin, trypsin, elastase and subtilisin [71]. Specificity of protease inhibitor families is mainly based on the amino acid residues present in the active site [72].

2.3.1. Serine (Serpine) protease inhibitors

It is found in almost all kingdoms of organisms [73–76]. Several serine PIs have been purified from plants and characterized [77, 78]. Plant serine PIs show inconsistent and varied specificities towards plant proteases [79]. *Hordeum vulgare* serine PI inhibits trypsin, chymotrypsin [80], thrombin, plasma kallikrein, Factor VIIa and Factor Xa [81]. *Triticum aestivum* serine PI inhibits chymotrypsin and cathepsin G [82]. Serine protease inhibitors have been used most commonly for the development of transgenic plants for the control of insect pests [83–85].

2.3.2. Cysteine protease inhibitors

An inhibitor of cysteine proteinases was first described in egg white by Sen and Whitaker [86] and was later named cystatin [87]. Cysteine proteinases inhibitors are widely distributed in plants, animals and microorganisms [88]. Their role in defense has been explored by *in vitro* analysis on inhibition of digestive proteinases from insect pests and nematodes [89–91]. First plant cystatin was isolated from rice seeds and as of now, more than 80 members of different

plant species have been characterized [92, 93]. Barley cystatin in artificial diets hampered the life cycle of two aphid species and also in transgenic *Arabidopsis* [94]. Expression of such inhibitors in maize enhanced the resistance against phytophagous mites [95]. Inhibition of these proteases provides a promising control on insects and therefore PIs can be employed as a potential source of defense in plants against insect pests.

2.3.3. Aspartyl protease inhibitors

It is relatively less studied class, due to the rare occurrence [91]. Potato tubers possess cathepsin D, an aspartic proteinase inhibitor which showed substantial amino acid sequence similarity with the soybean trypsin inhibitor [96]. Aspartic proteases have been found in coleoptera species, such as *Callosobruchus maculatus* [97] and *H. hampei* [98], in which the acidic pH in midgut provides a favourable condition for these proteases [58].

2.3.4. Metallo-proteases inhibitors

The metallo carboxypeptidase inhibitors (MCPIs) have been identified in solanaceous plants tomato and potato [99]. The MCPIs are 38–39 amino acid residues long polypeptide [100, 101]. Plants have evolved at least two families of metalloproteinase inhibitors, the metallo-carboxypeptidase inhibitor family in potato and tomato [102] and a cathepsin D inhibitor family in potato [103]. The inhibitor is produced in potato tubers and accumulates with potato inhibitor I and II families (serine proteinase inhibitors) during tuber development. The inhibitor also accumulates in potato leaf with inhibitor I and II in response to wounding and have the potential to inhibit all the major digestive enzymes (like trypsin, chymotrypsin, elastase, carboxypeptidase A and carboxypeptidase B) of higher animals and many insects [104].

2.4. α -Amylase inhibitors

α -Amylases (α -1,4-glucan-4-glucanohydrolases) are hydrolytic enzymes, which catalyze the hydrolysis of α -1,4-glycosidic bonds in polysaccharides. They are present in microorganisms, animals and plants [105–107]. They are the most important digestive enzymes of many insects which feed exclusively on seed products. Inhibition of α -amylase impairs the digestion in an organism and causes shortage of free sugar for energy. α -Amylase inhibitors (α -AI) are found in many plants as a part of the defense system and abundant in cereals and legumes [108–111].

α -AI of *Phaseolus vulgaris* is the most studied amylase inhibitor and have shown toxic effects to several insect pests [110, 111]. Like lectins, they possess carbohydrate-binding property. There are at least four types of *Phaseolus* amylase inhibitors on the basis of α -AIs: AI-1, AI-2, AI-3 and the null type [112]. AI-1 is present in the most cultivated common bean varieties and inhibits mammalian α -amylases. It also inhibits α -amylases in insects like *C. chinensis*, *C. maculatus* and *B. pisorum* [106]. AI-2 is 78% homologous to AI-1 and found in few wild accessions. It inhibits the *Z. subfasciatus* larval α -amylase and pea bruchid α -amylase [106, 111, 113]. This inhibitor is a good example of co-evolution of insect digestive enzymes and plant defense proteins.

They are potential molecules for the development of insect-resistant transgenic plants [114, 115]. Seeds of transgenic pea and azuki, expressing α -AI-1 inhibitor of *P. vulgaris*, shows resistance against pea weevil (*Bruchus pisorum*), cowpea weevil (*C. maculatus*) and azuki bean weevil (*Callosobruchus chinensis*) [110, 113, 116].

2.5. Chitinase

Chitinases are being employed in plant defense in many ways. It has been used in controlling the growth of fungi and insects. Expression of poplar chitinase in tomato leads to growth inhibition in Colorado potato beetle [117]. Secretome analysis of tobacco cell suspension represents chitinase as the major defense protein [118]. A chitinase-like domain containing 56-kDa defense protein (MLX56) provides strong resistance against cabbage armyworm, *Mamestra brassicae*, and Eri silkworm, *Samia ricini* [119]. Two chitinase like proteins LA-a and LA-b (latex abundant) from Mulberry (*Morus* sp.) latex are found to be toxic against *Drosophila melanogaster* [120].

Chitinases have also been isolated from insects and found to be equally promising in plant defense. Transgenic tobacco plants expressing chitinase of tobacco hornworm (*Manduca sexta*) shows resistance to tobacco budworm *Heliothis virescens* [121]. Hornworm chitinase expressing transgenic plants are also resistant against fungal infection [122]. Further, a recombinant baculovirus expressing chitinase of hard tick (*Haemaphysalis longicornis*) has been shown as bio-acaricide for tick control [123].

3. Insect-resistant transgenic crops

Development of many transgenic crops has been reported for insect resistance. Both private and public sector organizations are involved in the process and they used δ -endotoxins of *Bacillus thuringiensis* to achieve resistance against insects. Among transgenic plants, cotton and maize were the most successful and released for commercial cultivation. These crops are being adopted annually at very high rates. In other words, area under Bt-crops are increasing day-by-day. Successful deployment of these crops has decreased the pesticide usage. However, the sustainability and durability of pest resistance are still a matter of discussion. It is also important to focus on next-generation insect-resistant transgenic crops.

3.1. First-generation insect-resistant transgenic crops

Insect-resistant transgenic crops have not only increased the economy but also the environmental and health benefits [69, 124]. Six transgenic crops (canola, corn, cotton, papaya, squash and soybean) were planted in 2003 in the USA alone. These crops increased farm income by US\$ 1.9 billion by producing an additional 2.4 million tonnes of food and fiber and reduced the use of pesticides by 21,000 tonnes.

In 2009, China government approved the cultivation of Bt-rice (the country has been growing Bt-cotton since 1997). Farm surveys of randomly selected households cultivating Bt-rice

varieties have been performed. The benefit of Bt-rice has been acknowledged to the level of small and poor farmers, it is due to the lesser crop damage by the insects and therefore higher crop yields and less use of pesticides. An improved health has also been observed in Bt-rice cultivating farmers compared to non-Bt rice cultivating farmers [126]. Government of India approved the cultivation of Bt-cotton in 2003, which resulted in a 70% reduction in insecticide applications. This saves up to US\$ 30 per ha in insecticide costs and results 80–87% increase in cotton yield [127]. A spectacular decrease in pesticide usage in Bt cotton fields has also been reported from China. The pesticide poisoning to the farmers reduced from 22% to 4.7% [128].

To assess probable hazards of Bt toxins on non-target insects, field evaluation was performed in Spain [129]. Bt-maize did not show negative impact on non-target pests. Similar numbers of cutworms and wireworms were present in Bt versus non-Bt fields. Surprisingly, higher numbers of aphids and leafhoppers were observed in Bt field.

3.2. Strategies for next-generation insect resistance

3.2.1. Engineering of Cry toxin by domains swapping

Most of the Cry toxins share common three-domain structure in activated form [130]. Domain I gets inserted into the target membrane and forms pore; domain II is associated with receptor binding and thus determines specificity, and domain III is also involved in receptor-binding specificity. It has been demonstrated in a couple of studies that hybrid Cry toxins exhibit higher toxicity. Domain III of Cry1Ac increased the efficacy of various other Cry1 proteins in Cry1–Cry1Ac hybrid [131]. Similarly, Singh et al. (2004) developed a hybrid toxin against *Spodoptera litura*. They replaced a region in domain III of Cry1Ea toxin by 70 amino acid homologous region of Cry1Ca. Transgenic tobacco and cotton expressing hybrid gene are highly effective/toxic to all stages of larvae of *S. litura*. Another hybrid Bt gene was developed by replacing part of domain II of Cry1Ba with that of Cry2a [132]. The transgenic potato expressing the hybrid toxin showed resistance against Colorado potato beetle, potato tuber moth and European corn borer. The strategy provides new opportunities for resistance management as the target receptor recognition of hybrid toxins is expected to be different from currently used Cry toxins.

3.2.2. Plant-derived insecticidal lectins and protease inhibitors

Detail about lectins and protease inhibitors have been discussed in earlier section. Some other insecticidal roles are summarized here. Besides insecticidal potential, GNA and ASAL also serve as a carrier protein for other insecticidal peptides and proteins to the haemolymph of lepidopteran larvae. It has been demonstrated by feeding GNA-allatostatin and GNA-SFI1 fusions to the tomato moth *Lacanobia oleracea* [133–135]. SFI1 is a neurotoxin isolated from the spider *Segestria florentina*. The individual toxin did not cause toxicity through oral delivery; however, the fusion proteins with GNA were toxic.

Lectins are reported to be insecticidal towards sap-sucking insects, where Bt-toxins are not effective. Transgenic tobacco expressing garlic (*Allium sativum*) leaf lectin showed substantial

control over peach potato aphids [136]. Fusion of galactose-binding domain of the non-toxic ricin B-chain with Cry1Ac provides additional binding domains, which increases interactions with the gut receptors in target insects. Transgenic rice and maize expressing the fusion protein show high toxicity in comparison to the Bt-toxin alone [137].

Protease inhibitors (PIs) expressing transgenic plants are not as effective as Bt and insecticidal lectin expressing plants. This is due to the adaptation in gut proteases in phytophagous insects. High genetic diversity in gut proteases and low potency of protease inhibitors is responsible for such adaptation. The combination of inhibitors (potato PI-II and carboxypeptidase) is not enough to avoid the compensatory adaptation [68]. However, inhibitors like barley trypsin inhibitor [65], equistatin from sea anemone [138], other cystatins [139, 140] or use of multiple inhibitors [141] or combination of inhibitors and lectins [142] might also be useful to provide resistance against insects in transgenic plants.

3.2.3. Multiple insecticidal proteins containing transgenic crop

Second-generation Bt transgenic cotton [Bollgard II (Cry1Ac + Cry 2Ab) and Widestrike (Cry1Ac + Cry1F)] are developed to increase the level of resistance against cotton bollworm [143, 144]. It has also been demonstrated that the expression of three insecticidal proteins (Cry1Ac, Cry2A and GNA) into Indica rice control three major pests, rice leaf folder (*Cnaphalocrocis medinalis*), yellow stem borer (*Scirpophaga incertulas*) and the brown plant hopper (*Nilaparvata lugens*) [145]. Cry proteins target the leaf folder and the stem borer, and GNA targets the plant hopper. Comparison of three different Bt-cotton lines (either single Cry1Ac or Cry2Ab, or both genes) for insect damage showed that the lines containing two Bt genes performed better [144]. Broccoli expressing both Cry1Ac and Cry1C exhibited increased resistance to diamondback moths and delayed the resistance development [146, 147]. Similarly, transgenic tobacco expressing Cry1Ac and cowpea trypsin inhibitor (CpT 1) delayed resistance development in *H. armigera* [148]. Recently, Bharathi et al. [149] pyramided two lectin genes ASAL and GNA and showed increased resistance against brown plant hopper, green leaf hopper and white backed plant hopper, as compared to their parental lines expressing single lectin. The performance of transgenic plant pyramided with genes has shown that the insecticidal functions of most of the toxins are non-overlapping and non-competitive.

3.2.4. Tissue-specific or regulated expression

Insecticidal proteins are usually expressed under constitutive promoter for higher accumulation of the proteins. Although the constitutive expression has some advantages, tissue-specific or inducible expression is desirable under certain circumstances. Insect attacks epidermal cells first and therefore the expression of insecticidal proteins under epidermal cell-specific promoters can be a useful strategy. For example, CER6 is an epidermal cell-specific promoter responsible for the expression of an enzyme for cuticular wax production [150]. Similarly, phloem-feeding insects can be targeted by using phloem-specific promoter like PP2 promoter of pumpkin [151], rice sucrose synthase Rss promoter [152] and root phloem-specific promoter AAP3 [153]. Tissue-specific expression of several insecticidal proteins has demonstrated as a good potential for insect control in several studies. Phloem-specific expression of ASAL under

promoter *Asus1* protects tobacco against aphid, *Myzus nicotianae* [154]. Transgenic chickpea expressing ASAL under *rolC* promoter showed effective control over *A. craccivora* [155] and transgenic Indian mustard (*Brassica juncea*) expressing ASAL under *Rss I* promoter showed resistance against aphid *Lipaphis erysimi* [136]. Researchers are also working on sap-sucking pest inducible phloem-specific promoters, which are not only insect-inducible but also insect-specific in nature [156]. Another strategy is temporal expression of insecticidal proteins as some insects infest a crop in a particular phase only. For example, pink bollworm (*Pectinophora gossypiella*) attacks and feeds on the cotton bolls only. At this stage, cotton plants are mature; the expression of Cry toxins goes down and becomes insufficient for effective control.

3.2.5. Strategies to over express secondary metabolites

Secondary metabolites synthesized by the plants participate in a number of physiological and biochemical processes. Our group demonstrated that the over-expression of pectin methyltransferase of *Arabidopsis thaliana* and *Aspergillus niger* in transgenic tobacco plants enhances methanol production, which in turn provided resistance against sap-sucking as well as chewing insect pests [157]. Similarly, transgenic tobacco expressing *AtMYB-12* gene showed enhanced production of rutin in leaves and callus, which confers resistance against *H. armigera* and *S. litura* larvae [158, 159]. *WsSGTL1*, a sterol glycosyltransferases isolated from *Withania somnifera*, was expressed and functionally characterized in transgenic tobacco plants, which showed significant resistance towards *S. litura* [160]. Tobacco plants were transformed by a multigene transfer vector containing three coffee N-methyltransferases genes *CaMXT1*, *CaMXMT1* and *CaDXMT1* responsible for producing caffeine in transgenic plants which showed tolerance to *S. litura* [161]. Dixit et al [162] demonstrated the insect resistance by altering the amino acid composition in sap.

4. Conclusions and perspectives

Transgenic technology (especially Bt crops) has contributed significantly in increasing the crop production worldwide. The crops are protected from being damaged by insect pests. Certainly, this methodology provides an environmentally safe alternative for the synthetic pesticides. Further, it has also been proven to be useful in enhancing nutritional values of crops, improvement of stress tolerance and production of pharmaceutical proteins. Introduction of Bt cotton varieties in India has tremendously increased the yields of cotton and thereby profits to the farmers. Bt proteins are able to control the damage caused by Lepidopteran and Coleopteran insects, but not effective against sap-sucking Homopteran pests [8, 9]. Therefore, an unusual increase in the population of homopteran pests like whiteflies, aphids and leafhoppers on transgenic cotton has been reported [7]. Further, development of resistance in insects against toxins is also going to be a major point of concern, which might ultimately challenge the future of Bt crops. Some defense-related proteins like plant lectins, PIs and chitinases are reported to be toxic to various homopteran insect pests. However, several safety and societal concerns are raised from time to time. Further, there is non-availability of an effective and safe protein against several important and emerging insects, which need an *ab*

initio approach to resolve this issue. A promising and biosafe strategy to defeat the above problem can be: (a) exploration of the plant's own defense mechanisms and manipulation of their expressions or (b) by introducing a gene for insect control derived from other plants, especially derived from non-host plants, and (c) pyramiding of insecticidal proteins for the control of multiple insect pests. Exploration of RNAi mediated insect control by targeting high expressing and/or important vital genes can also be an effective approach (12-14, 163, 164). Besides this, in our country, we need a dedicated forum to popularize the use of genetically modified crops and convince the government as well as citizens at ethical issues.

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