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REVIEW

# Engineering plants for aphid resistance: current status and future perspectives

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**Abstract**
***Key message*** **The current status of development of transgenic plants for improved aphid resistance, and the pros and cons of different strategies are reviewed and future perspectives are proposed.**
*Abstract* Aphids are major agricultural pests that cause significant yield losses of crop plants each year. Excessive dependence on insecticides for aphid control is undesirable because of the development of insecticide resistance, the potential negative effects on non-target organisms and environmental pollution. Transgenic plants engineered for resistance to aphids via a non-toxic mode of action could be an efficient alternative strategy. In this review, the distribution of major aphid species and their damages on crop plants, the so far isolated aphid-resistance genes and their applications in developments of transgenic plants for improved aphid resistance, and the pros and cons of these strategies are reviewed and future perspectives are proposed. Although the transgenic plants developed through expressing aphid-resistant genes, manipulating plant secondary metabolism and plant-mediated RNAi strategy have been demonstrated to confer improved aphid resistance to some degree. So far, no aphid-resistant transgenic crop plants have ever been commercialized. This commentary is intended to be a helpful insight into the generation and future commercialization of aphid-resistant transgenic crops in a global context.

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## Introduction

Aphids (Aphididae) are major agricultural pests that cause significant yield losses of crop plants each year by inflicting damage both through the direct effects of feeding and by vectoring harmful plant viruses (Miles 1989; Tagu et al. 2008; International Aphid Genomics Consortium 2010). Annual worldwide crop losses due to aphids are estimated at hundreds of millions of dollars (Blackman and Eastop 1984; Morrison and Peairs 1998). Along with the application of nitrogen fertilizer and elevation of atmospheric $CO_2$ concentration, aphid infestation becomes more serious (Awmack and Harrington 2000; Aqueel and Leather 2011). For many crops, insecticides provide a simple and effective strategy for aphid control. However, the application of such chemicals is not desirable in the long term, because of the development of insecticide resistance (Sabater-Muñoz et al. 2006) and the potential negative effects on non-target organisms, and the need for more sustainable agricultural practices with fewer external chemical inputs (Yu et al. 2012b). This is further accentuated due to a very limited germplasm available for breeding against aphid resistance (Bhatia et al. 2012). As a result, conventional breeding efforts for developing aphid-resistant cultivars for minimizing the use of insecticides have not met with any success (Bhatia et al. 2012), outbreak of aphids causing substantial losses is reported regularly. For example, in 2010–2011

crop seasons, approximately 62.5, 14, 90, 32 and 23 % of the total growth area of wheat, maize, cotton, oilseed and soybean suffered severe aphid infestations in China, respectively (Xia et al. 2012; Yu et al. 2012b). Up to now, breeders are still struggling to find an efficient strategy for aphid control in major crop plants. Development of aphid-resistant plants through genetic engineering would be a good alternative strategy (Yu et al. 2012b).

Compared with conventional crop breeding programs, genetic engineering of plants could not only widen the potential pool of useful genes but also permit the introduction of several different desirable genes in a single event. Ever since the first report of transgenic plants in 1984 (Horsch et al. 1984), some efforts have been undertaken toward developing aphid-resistant transgenic plants. In this review, we briefly introduce the distribution of major aphid species and their damages on crop plants. Furthermore, the explored aphid-resistance genes/strategies and their applications in developments of transgenic plants for enhanced aphid resistance are reviewed, and future perspectives in this area are proposed.

## The distribution of major aphid species and their damages on crop plants

Aphids are distributed worldwide, but are most common and serious in temperate zones. For example, sub-Saharan Africa and Australia have a very poor aphid fauna with only 219 and 180 species, respectively. By contrast, 1,416 species are found in North America, about 1,500 species in Europe and more than 1,000 species in China (Coeur d'acier et al. 2010). Of the major aphid species infesting wheat in China, the grain aphid (*Sitobion avenae* F.) is the most dominant and destructive one, affecting the wheat production areas in Yellow Huai and the Northern China Plain, the Southwest, Northwest and the Middle Yangtze River regions (Zhang et al. 2009). The grain aphid also occurs widely in Europe, West Africa, America and other Asian countries. Many other aphid species infested on crop plants, including Greenbug (*Schizaphis ramentum*), Russian wheat aphid (*Diuraphis noxia*), Corn leaf aphid (*Rhopalosiphum maidis*), Cotton aphid (*Aphis gossypii*), Pea aphid (*Acyrthosiphon pisum*), Green peach aphid (*Myzus persicae*) and so on, also with a nearly worldwide distribution (Table 1). Aphids migrate great distances and cause serious crop losses world widely. For example, the native distribution of Russian wheat aphid (RWA) is believed to centre on the Iranian–Turkestanian mountain range and gradually spread to southern Russia, most Europe, Central Asia, North and South America, North and South Africa (Kovalev et al. 1991; Zhang et al. 2012). It was during the 1970s and 1980s that RWAs began to cause severe crop damage in major grain producing areas in Europe, Africa and the Americas (Kovalev et al. 1991; Stary 1999; Smith et al. 2004). The soybean aphid (*Aphis glycines*) is native to and widespread in Asia and has been recognized, since the year 2000, as the single most important arthropod pest of soybeans in North America (Ragsdale et al. 2011). While the potato aphid (*Macrosiphum euphorbiae*) originated in North America, it has spread to the temperate regions of Europe and Asia and is found in all areas in which potatoes are grown (http://www.extento.hawaii.edu/kbase/crop/type/macrosip.htm).

Aphids elicit multitude-damaging effects on hundreds of plant species, including agriculturally important crops such as wheat, maize, cotton, soybean, pea, potato and etc. (Table 1). They not only cause direct damage by feeding from the phloem, but also the indirect damage by excreting honeydew and vectoring viruses (Miles 1989). Sieve diversion by aphid nymphs and adults depletes the plant from photoassimilates, and thus devitalizes the plant completely (Bhatia et al. 2012). The photosynthesis process of the infested plants is impaired due to the growth of saprophytic sooty mold on aphid honeydew, a sugar-rich aphid secretion (Rabbinge and Vereyken 1980). Some aphid species even inject toxins into plants, which further distorts plant growth (Burd and Burton 1992). Whilst feeding, many aphid species can also acquire and spread plant viruses. Aphid spreads luteoviruses accounting for about 45 % insect-borne viruses, which represents the greatest threat to agricultural crops (Nault 1997; Bhatia et al. 2012).

Most aphid species attack the aerial parts of plants during all developmental stages, and the plants infected can have a variety of symptoms (Table 1). The nature and extent of damages caused by aphid infestation vary widely depending on the variety of the respective aphid and host plant species. For instance, compared with the bird cherry-oat aphid (*Rhopalosiphum padi*) which causes no obvious symptoms except reduced plant growth at high population densities (Delp et al. 2009), grain aphid is a more destructive one by causing reduction in the number of spikes, the number of grains per spike, and reduced grain weight, wheat yields can thus be reduced by up to 30 % during outbreaks (Kolbe and Linke 1974). Green peach aphid infests hundreds of species from 40 plant families (Blackman and Eastop 1984) and is commonly found on potato plants. Green peach aphid can attain very high densities on young plant tissues, causing water stress, wilting and reduced growth rate of the plant. Prolonged aphid infestation can cause appreciable reduction in the yield of potato (Saljoqi 2009). Moreover, as one of the most versatile viral vectors, peach aphids are capable of transmitting more than 120 plant viruses including potato leaf-roll virus, which

**Table 1** Distribution of major aphid species and their damages on crop plants

| Crop plants | Aphid species | Distribution[a] | Damages caused by aphids |
|---|---|---|---|
| Wheat | Grain aphid *Sitobion avenae* | Europe, Asia, West Africa and America | Prefer to feed on wheat ears; causing a reduction in the number of heads, the number of gains per head, and a reduced seed weight; produce the high volume of honeydew; a vector of barley yellow dwarf virus (http://www.rothamsted.ac.uk/insect-survey/STSitobion_avenae.html) |
| | Greenbug *Schizaphis graminum* | South Europe, Asia, North and South America, West and South Africa | Prefer to feed on the underside of lower leaves; causing young leaves to turn yellow and older leaves to develop orange–red spots and eventually die from the injection of toxic saliva, reduction in root and shoot biomass; a vector of barley yellow dwarf virus (http://iwheat.org/book/greenbug) |
| | Russian wheat aphid *Diuraphis noxia* | West, Central and South Europe, Central Asia, North and South America, North and South Africa | Prefer to feed on new wheat leaves; causing white or yellow streaking and leaf rolling, later in the season infested leaves can trap emerging heads, preventing good grain fill; a vector of barley yellow dwarf virus (http://iwheat.org/book/russian-wheat-aphid) |
| Maize | Corn leaf aphid *Rhopalosiphum-maidis* | South Europe, Middle Asia, North America | Cause mottling and discoloration of leaves, damage is most severe between the late-whorl and pollination stages, aphids feeding at those stages cause stunting, shriveled and shrunken ears, and possibly barrenness; produce the high volume of honeydew; a vector of barley yellow dwarf virus (http://ipm.illinois.edu/fieldcrops/insects/corn_leaf_aphid/) |
| Soybean | Soybean aphid *Aphis glycines* | Asia, North America | Prefer to feed on young leaves of soybean; causing distorted and yellowed leaves, stunted plants with reduced pod and seed counts; produce the high volume of honeydew; a vector of several virus, including alfalfa mosaic virus, soybean mosaic virus, bean yellow mosaic virus (http://eol.org/pages/3689195/overview) |
| Cotton | Cotton aphid *Aphis gossypii* | Europe, Asia, North and South America, Africa and Australia | Prefer to feed on the underside of leaves or on the growing tips of shoots; infested leaves often become cupped downwards and may appear wrinkled, heavy infestations result in wilting; produce the high volume of honeydew; a vector of several virus, including cucumber mosaic virus and watermelon mosaic virus (http://en.wikipedia.org/wiki/Aphis_gossypii) |
| Pea | Pea aphid *Acyrthosiphon pisum* | Europe, Asia, Africa, North and South America | Cause stunting, and subsequent distortion and yellowing of leaves and pods; produce the high volume of honeydew; transmitting more than 30 plant viruses, including pea leaf-roll virus, pea enation mosaic virus, pea mosaic virus and pea seed borne mosaic virus (http://www.rothamsted.ac.uk/insect-survey/STAcyrthosiphon_pisum.html) |
| Potato | Green peach aphid *Myzus persicae* | Europe, Asia, North and West America, Africa | Prefer to feed on young plant tissue, causing water stress, wilting, and reduced growth rate of the plant; produce the low volume of honeydew; transmitting more than 120 plant viruses, including potato leaf roll virus (http://www.rothamsted.ac.uk/insect-survey/STMyzus_persicae.html) |
| | Potato aphid *Macrosiphum euphorbiae* | Europe, Asia, North America | Prefer to feed on lower parts of the plant, causing physical damage to foliage, early large infestations cause the upper leaves of some potato varieties to roll upward; produce the high volume of honeydew; transmitting more than 50 plant viruses, mainly of the non- persistent variety, but with less efficiency than *Myzus persicae* (http://www.rothamsted.ac.uk/insect-survey/STMacrosiphum_euphorbiae.html) |

[a] Cited from http://www.agroatlas.ru/en/content/pests/#S

could lead to yield reductions of 40–70 % in the infected fields (Mowry 2005; Ramsey et al. 2007). For soybean aphid, its feeding can reduce soybean photosynthetic rates by up to 50 % in infested leaflets (Catangui et al. 2009), causing distorted and yellowed leaves, stunted plants with reduced pod and seed counts (http://eol.org/pages/3689195/overview). It seems that the plant stage, when aphid starts infestation, will not affect the population growth and subsequent reproduction dynamics of the soybean aphid (Li et al. 2004; Rutledge and O'Neil 2006); however, different plant stages do show differential susceptibility to aphid infestation, and younger soybean plants seem to be more susceptible (Rutledge and O'Neil 2006). On average, the calculated maximum possible yield loss was 75 % for soybean aphid infestations starting at the five-node (V5) stage versus 48 % at the full bloom (R2) stage (Catangui et al. 2009).

Meanwhile, symptoms and damages caused by aphids can be highly variable and were also dependent in a large part on aphid density and infestation duration. For example, low levels of soybean aphid infestation have little or no influence on soybean growth or reproductive output. However, larger aphid populations exceeding the economic threshold can significantly affect soybean yield and the oil content of the seeds, which usually decline linearly as the peak aphid numbers per plant and maximum cumulative aphid-days per plant increased (Ragsdale et al. 2007; Beckendorf et al. 2008).

## Current status of transgenic plants engineered for aphid resistance

Engineering of transgenic plants to fight insect pests has been established for more than 20 years with most commercialized insect-resistant crops expressing *Bacillus thuringiensis* (*Bt*) toxins (Gatehouse et al. 2011). Although these toxins are powerful and effective agents against lepidopteran and coleopteran pest species, they do not affect hemipteran pests such as aphids. Therefore, alternative genes/strategies are required for aphid control. Here, we will mainly focus on these genes/strategies with demonstrated effects on aphid control in transgenic plants, including the expression of aphid-resistant genes, genes involved in metabolic pathway and aphid-derived double-stranded RNA (dsRNA) strategies.

### Transgenic plants expressing aphid-resistance genes

Up to now, the aphid-resistance genes transferred into plants to date mainly target the insect digestive system. Most were lectin genes derived from a range of higher plants, whereas some protease inhibitors, toxins and plant-derived resistance (*R*) genes have also been engineered into a variety of plant species for aphid control.

#### *Plant lectins*

Lectins are ubiquitous in plants showing carbohydrate specificity for glycoconjugates present in organisms (such as viruses, microorganisms, fungi, nematodes or phytophagous insects) outside the plant kingdom, whereas these glycoconjugates (e.g., galactose, sialic acid) have low abundance or are absent in plants (Vandenborre et al. 2011). Genome or transcriptome analyses revealed that plant lectins can be classified into 12 distinct families with evolutionary and structurally related lectin domains (Van Damme et al. 2008). Among these 12 families, *Galanthus nivalis* agglutinin (GNA)-related lectins, legume lectins, hevein-related lectins, amaranthin family, ricin-related lectins, jacalins and *Nicotiana tabacum* agglutinin (NICTABA)-related lectins exhibit toxicity to insects (Van Damme et al. 2008). It is presumed that after ingestion by phytophagous insects, the plant lectins are released from the disrupted cellular structures and come into contact with carbohydrate structures present in the midgut of insects, causing disruption of the digestion system and thus lead to the developmental stunting and/or mortality of insects (Vandenborre et al. 2011).

Genetically engineered plants for enhanced aphid resistance with plant-derived lectins have achieved successes to some degree. So far, more than 13 lectin genes belonging to seven families have been isolated and transferred into plants (Table 2). Among these lectins, GNA-related lectins, also known as monocot mannose-binding lectins, targeting specifically to high mannose or oligo-mannose N-glycans in glycol proteins, have been widely used in engineering plants for the improvement of aphid resistance such as tobacco (Hilder et al. 1995), potato (Down et al. 1996; Gatehouse et al. 1996), wheat (Stöger et al. 1999) and maize (Wang et al. 2005). Transgenic wheat plants expressing GNA at levels greater than 0.04 % of total soluble protein decreased the fecundity, but not the survival of grain aphids (Stöger et al. 1999). Transgenic maize plants with a phloem-specific GNA expression demonstrated enhanced resistance against corn aphid in the field trials (Wang et al. 2005). Another well-exploited lectin gene is the *Pinellia ternate* agglutinin (PTA), which is isolated from a traditional Chinese medicinal plant. Aphid bioassay studies showed that PTA had significant insecticidal activities against cotton and green peach aphids when incorporated into the artificial diets (Huang et al. 1997; Pan et al. 1998). Transgenic tobacco, wheat and isatis root plants exhibited negative effects on the growth of green peach aphid or greenbug, although the expression level of PTA gene was lower than expected (Yao et al. 2003; Yu and Wei 2008; Xiao et al. 2012). It is worth to note that the insecticidal activities against aphids are positively correlated with lectin concentration in transgenic plants. When expressed

**Table 2** Lectin genes engineered into plants for aphid control

| Lectin family | Lectins | Plants | Targeted aphids | References |
|---|---|---|---|---|
| GNA-related lectin | GNA | tobacco | *Myzus persicae* | Hilder et al. (1995) |
| | | aize | *Rhopalosiphum maidis* | Wang et al. (2005) |
| | | wheat | *Sitobion avenae* | Stöger et al. (1999) |
| | | potato | *Myzus persicae* | Gatehouse et al. (1996) |
| | | | *Aulacorthum solani* | Down et al. (1996) |
| | ASAL | mustard | *Lipaphis erysimi* | Dutta et al. (2005a) |
| | | tobacco | *Myzus persicae* | Dutta et al. (2005b) |
| | | | *Myzus nicotianae* | Sadeghi et al. (2007) |
| | ACA | mustard | *Lipaphis erysimi* | Hossain et al. (2006) |
| | PTA | tobacco | *Myzus persicae* | Yao et al. (2003); Jin et al. (2012) |
| | | wheat | *Schizaphis graminum* | Yu and Wei (2008) |
| | | isatis root | *Myzus persicae* | Xiao et al. (2012) |
| | DB1 | tobacco | *Myzus persicae* | Kato et al. (2010) |
| | ZGA | tobacco | *Myzus nicotianae* | Ye et al. (2009); Zhou et al. (2011) |
| | PPA | tobacco | *Myzus nicotianae* | Wu et al. (2012) |
| Legume lectins | ConA | potato | *Myzus persicae* | Gatehouse et al. (1999) |
| Hevein-related lectins | WGA | mustard | *Lipaphis erysimi* | Kanrar et al. (2002) |
| Ricin-related lectins | SNA-I′ | tobacco | *Myzus nicotianae* | Shahidi-Noghabi et al. (2009) |
| Amaranthins | Amaranthin | cotton | *Aphis gossypii* | Wu et al. (2006a) |
| Jacalins | HTA | tobacco | *Myzus persicae* | Chang et al. (2003) |
| NICTABA-related lectin | AtPP2 | *Arabidopsis* | *Myzus persicae* | Zhang et al. (2011) |

PTA in tobacco chloroplasts, the content of PTA could accumulate up to 9.2 % of total soluble protein in mature leaves, resulting in the aphid population on transgenic plants reduced by 89–92 % (Jin et al. 2012). Other GNA-related lectins, including *Zephyranthes grandiflora* agglutinin (ZGA), *Allium sativum* leaf lectin (ASAL), *Allium cepa* agglutinin (ACA), *Dioscorea batatas* tuber lectin 1 (DB1) and *Pinellia pedatisecta* agglutinin (PPA), also show insecticidal activity against aphids (Table 2). In planta bioassay conducted with mustard aphid (*Lipaphis erysimi*) nymphs, transgenic mustard ectopically expressing ACA was found to be more toxic than ASAL and GNA (Hossain et al. 2006). This indicates that different lectins may have different levels of toxicity toward different aphid species. Therefore, it would be better to compare the insecticidal activities of different kinds of lectins in an artificial feeding assay before choosing the respective lectin gene for the genetic engineering of crop plants.

Besides, another two lectin family members, legume lectins and hevein-related lectins, also exhibited toxicity to aphids in transgenic plants. Legume lectins have been purified especially from seeds and several legume lectins were shown to bind to carbohydrate structures that are not present in plants such as the Thomsen-nouveau antigen (Tn) antigen or complex N-glycan structures with terminal galactose and sialic acid residues (Vandenborre et al. 2011). A well-studied member of this family is ConA, a mannose-binding legume lectin from jackbean. ConA-expressing potatoes decreased the fecundity of green peach aphids by up to 45 % (Gatehouse et al. 1999). Most of hevein-related lectins exhibit specificity for chitin, the main constituent of the peritrophic matrix in the insect guts (Hakim et al. 2010). Transgenic mustard-expressing wheat germ agglutinin (WGA; belonging to Hevein-related lectins) showed significant insecticidal activity against mustard aphid (Kanrar et al. 2002).

Except for the lectins described above, several plant-derived lectins belonging to other lectin families, such as *Amaranthus caudatus* agglutinin (amaranthin), *Sambucus nigra* agglutinin (SNA-I′), *Helianthus tuberosus* agglutinin (HTA) and phloem proteins 2 (PP2), were also shown to have insecticidal properties against aphids when transferred into plants (Table 2). When the Amaranthin was ectopically expressed in cotton under the control of a phloem-specific promoter, enhanced resistance was observed against the nymphs of the cotton aphid *Aphis gossypii* (Wu et al. 2006a). SNA-I′ expressed in seeds and/or vegetative tissues of the Elderberry bark *Sambucus nigra* was found to be toxic to the tobacco aphids (*Myzus nicotianae*) (Shahidi-Noghabi et al. 2009). Transgenic tobacco plants expressing HTA, a lectin from the Jerusalem artichoke *Helianthus tuberosus* belonging to the family of jacalin-related lectins, caused developmental stunting and decreased fecundity of the green peach aphid (Chang et al. 2003). PP2, a member

of NICTABA-related lectins conserved in plants, which was found to involve in phloem-based defense mechanism (Dinant et al. 2003; Beneteau et al. 2010). Overexpression of an *Arabidopsis AtPP2-A1* repressed phloem feeding of green peach aphid (Zhang et al. 2011).

On the whole, so far, a number of lectin genes have been successfully engineered into plants to improve aphid resistance (Table 2). The effects of lectin genes transgenic plants on biological parameters of aphids are usually feeding inhibition, decreased larval weight, delayed development, lower fecundity and higher mortality. However, some plant lectins may be toxic to non-target organisms. For example, GNA could cause adverse effects on predatory 2-spots ladybirds (*Adalia bipunctata*) and parasitoids (*Aphidius ervi*) via aphids in the food chain (Birch et al. 1999; Hogervorst et al. 2009). Indeed the report that genetic-modified (GM) potatoes expressing GNA exhibited toxicity to rats and compromised their immune systems contributed to the 'Pusztai affair', which became a major trigger for the subsequent mistrust of biotech companies and cause of public concern over GM foods (Ewen and Pusztai 1999). Similarly, the ricin, a potent cytotoxic lectin derived from castor bean (*Ricinus communis*), consists of a neutral A-Chain (32 kDa) bound by a disulfide bond to an acidic B-Chain (34 kDa). The B-subunit binds to glycoproteins on the surface of epithelial cells, enabling the A-subunit to enter the cell via receptor-mediated endocytosis. This subunit inactivates eukaryotic ribosomal RNA by depurinating a specific ribosomal residue, thereby inhibiting protein synthesis (Lord et al. 1994; Olsnes 2004). The lethal dose in humans is estimated as little as 500 μg (Musshoff and Madea 2009). Therefore, the risk of unintended cross-species agglutination would be a major concern over the biosafety of application of these lectin genes in agriculturally important crops for aphid control because humans and animals may consume these crops as food or feed, especially when the constitutive promoter was used to derive the expression of these lectin genes in the genetically engineered crop plants.

### *Protease inhibitors*

As one of the major plant defense strategies against herbivores, protease inhibitors (PIs) are small proteins, currently described in more than one hundred plants (Ryan 1990; Ceci et al. 2003). The dominant concept explaining their modes of action against insects is that PIs expressed in plants could inhibit protein digestion in insect. This results in amino acid deficiencies, and thereby leads to serious developmental stunting or mortality of larvae (Ceci et al. 2003).

Many insects, particularly Lepidoptera, depend on serine proteases (including trypsin, chymotrypsin and elastase endoproteases) as their primary protein digestive enzymes (Hilder and Boulter 1999). The effects of serine PIs are well described in Lepidoptera and Coleoptera, but remain poorly documented in Hemiptera. Potato plants transformed with the mustard trypsin inhibitor *mti-2* gene did not show any insecticidal effect on aphids, some transgenic lines even exhibited probiotic effects on the fitness of aphids, increased their daily fecundity and nymph survival (Saguez et al. 2010). The insect species in Hemiptera, mainly rely on cysteine proteases as their primary digestive protease, suggesting that aphids can be targeted with such cysteine PIs for aphid control. When administered in artificial diets, the cysteine PIs oryzacystatin I (OC-I) exhibited significant growth inhibition on the pea aphid, the cotton/melon aphid, the peach aphid and the potato aphid (Rahbé et al. 2003; Azzouz et al. 2005). Stable expression of the *OC-I* gene in oilseed rape and eggplant had negative impact on aphid population growth (Rahbé et al. 2003; Ribeiro et al. 2006). Likewise, cysteine PIs *HvCPI-6* from barley showed toxicity to pea aphid nymphs in artificial diets, and a significant delay of aphid development was observed when reared on *Arabidopsis* plants ectopically expressing *HvCPI-6* (Carrillo et al. 2011).

However, a major disturbing problem in PIs transgenic plants for pest control is the rapid adaptations of pests to protease inhibitors by synthesizing proteinases that are either insensitive to PIs or have the capacity to degrade them (Bown et al. 1997; Jongsma and Bolter 1997; Harsulkar et al. 1999). Some PIs are even toxic to beneficial insects such as honey bees (*Apis mellifera*) (Malone et al. 1995; Burgess et al. 1996). Therefore, the practical applications of PIs in plant protection still remain to be established in more detail. The ability of target pests to overcome the effects of the introduced PIs or the possible negative effects of this inhibitor against non-target organisms at the ecosystem level is the issues that should be addressed (Carlini and Grossi-de-Sá 2002).

### *Toxins*

Transgenic crop plants expressing *Bt* toxins have been used successfully for the management of Lepidopteran and Coleopteran pest species. Commercial *Bt* cotton, maize and soybean planted in a global context had a significant beneficial impact on global agriculture (Gatehouse et al. 2011). However, relatively low toxicity of *Bt* toxins against Hemipteran pests has, thus, far prevented their application for the management of these sap-sucking pests. Binding of a *Bt* toxin to the gut of a target insect is an important step for toxicity (Soberón et al. 2007). In the case of aphids, the physiological factors contributing to low levels of toxicity are probably due to toxin instability in the aphid gut and low levels of binding (Li et al. 2011; Chougule and

Bonning 2012). To overcome this limitation, Chougule et al. (2013) inserted a 12-aa pea aphid gut-binding peptide by adding to or replacing amino acids in one of three loops of the Cyt2Aa, resulted in enhanced binding and toxicity against both the pea aphid and the green peach aphid in an artificial diet assay. Similarly, when the ricin B-chain (a Gal-binding domain of the castor oil plant lectin) was fused to C terminal of domain III of Cry1Ac, the fused protein could bind to Gal residues in potential receptors in the Hemipteran pest. Transgenic rice plants engineered to express this fusion protein were toxic to a Hemipteran pest leafhopper (*Cicadulina mbila*), which is not susceptible to the native Cry1Ac (Mehlo et al. 2005).

Another promising aspect of employing insect-specific toxin for the development of aphid- resistant plants is the delivery of this kind of toxins to aphids by the coat protein of an aphid-vector plant virus. By fusing to a coat protein of a lutevirus which is an aphid-vectored plant virus, a spider-derived insect-specific toxin (peptide ω-hexatoxin-Hv1a) could be delivered into insect and acts within the hemocoel, resulting in the mortality of four aphid species such as the pea aphid, bird cherry-oat aphid, soybean aphid and green peach aphid either in an experimental membrane sachet or in transgenic *Arabidopsis* plants (Bonning et al. 2014). Insect-specific toxins that act only within the hemocoel of the insect constitute an untapped resource for the development of insect-resistant transgenic plants (Bonning et al. 2014). The range of aphid species that can be targeted using this approach may be large because luteovirid virions also could enter the hemocoel of non-vector aphids such as the bird cherry-oat aphid (Gray and Banerjee 1999). Hence, a transgene consisting of a lutevirus coat protein–toxin (CP-P) fusion is expected to be effective against multiple aphid species. Given that only aphids transmit luteovirids, the luteovirid CP-P fusion strategy for toxin uptake is expected to be specific to aphids, without harming non-target organisms (Bonning et al. 2014).

### *Plant-derived R genes*

In agricultural practice, breeding for aphid-resistant cultivars is usually achieved by transfering the *R* gene from the donor aphid-resistant germplasm to an adapted cultivar. Notable examples of aphid *R* genes bred into crop cultivars include barley and wheat cultivars resistant to RWA (Mornhinweg et al. 1995; Bregitzer et al. 2005; Collins et al. 2005; Qureshi et al. 2006; Murugan et al. 2010), melon cultivars resistant to cotton/melon aphid (McCreight et al. 1984; Lecoq et al. 1998), lettuce cultivars resistant to lettuce aphid (McCreight 2008), and soybean cultivars resistant to the soybean aphid (McCarville et al. 2012). For example, most RWA-resistant wheat cultivars planted commercially in the United States have relied on the *Dn4* gene from PI 372129 (Collins et al. 2005; Qureshi et al. 2006). The commercialized RWA-resistant barley 'burton' was developed from STARS-9301B, which contained RWA resistance controlled by *Rdn1* and *Rdn2* genes (Mornhinweg et al. 1995; Bregitzer et al. 2005).

Up to now, two aphid *R* genes, *Mi-1.2* from wild tomato confers resistance to potato aphid and *Vat* gene from melon controls resistance to the cotton/melon aphid, have been cloned (Rossi et al. 1998; Dogimont et al. 2009). Both isolated *Vat* and *Mi-1* are members of the nucleotide-binding site and leucine-rich repeat region (NBS-LRR) family of resistance genes, to which belong the majority of the genes, isolated to date, conferring resistance to bacteria, viruses, fungi and nematodes (Dogimont et al. 2010). This may suggest that plant–aphid resistance is mediated by the specific recognition of aphid-effector proteins that trigger signaling cascades to rapidly activate plant defenses against aphids in a similar scheme that was widely described for most plant–pathogen interactions (Dogimont et al. 2010). Several other predicted aphid NBS-LRR *R* genes have been well reviewed recently (Dogimont et al. 2010; Smith and Chuang 2014), including the lettuce *Ra* gene resistant to lettuce root aphid (Wroblewski et al. 2007) and soybean *Rag1–Rag3* gene resistant to soybean aphid (Kim et al. 2010; Zhang et al. 2010; Jun et al. 2012). In *Medicago truncatula*, *AKR* gene resistant to blue green aphid (Klingler et al. 2005), *TTR* gene resistant to spotted alfalfa aphid (Klingler et al. 2007), and *AIN* gene resistant to blue green aphid and pea aphid (Klingler et al. 2009) are predicted to reside in a cluster of NBS-LRR genes on chromosome 3. We can anticipate the isolation of additional *R* genes for aphid resistance following a deeper understanding mechanism underlying the plant–aphid molecular interactions.

Transgenic plants for aphid control using *R* genes have been documented that susceptible tomato transformed with *Mi-1.2* showed enhanced resistance against potato aphid (Rossi et al. 1998). However, NBS-LRR *R* genes usually confer a species- and biotype-specific aphid resistance, aphids may rapidly generate new virulent biotypes to breakdown this type of resistance, and transgenic plants engineered with these *R* genes may encounter a mixed aphid species in a natural ecological agro-system as well. For example, breeding for durable resistance to RWA in cereals is challenged by the fast emergence of new damaging biotypes (Jyoti and Michaud 2005; Smith et al. 2004). In addition, most aphid species consisting of biotypes can infest different plant species with a family or subfamily, e.g., the pea aphid consists of several biotypes living on distinct legume hosts (Peccoud et al. 2009), resulting in the transfer of *R* genes from one plant species to another may not work at all. For example, when *Mi-1.2* was transferred to eggplant, it could not confer resistance to potato aphids (Goggin et al. 2006).

## Manipulation of diverse plant-derived genes involved in metabolic pathway

Many secondary plant metabolites such as alkaloids, steroids, foliar phenolic esters (rutin, cholorogenic acid, etc.), terpenoids, cyanogenic glycosides, glucosinolates, saponins, flavonoids andpyrethrinsmay also act as potent protective chemicals against aphids (Sharma et al. 2000). Among a wide range of secondary metabolites, 2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIMBOA), camalexin, benzoxazinoids, $N^{\delta}$-acetylornithine, luteolin and genistein had been proved to exhibit detrimental effects on aphid in artificial diet assay (Escobar et al. 1999; Adio et al. 2011; Ahmad et al. 2011; Goławska and Łukasik 2012; Kettles et al. 2013). Some enzymes involved in plant secondary metabolites, such as phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO) and peroxidase (POD), are considered important biochemical markers in breeding cereal plants for resistance against aphids (Han et al. 2009). The availability of the genes encoding the biosynthetic enzymes of secondary metabolism has made engineering plants for aphid control feasible. When simultaneously expressing three *N*-methyl transferases in chrysanthemum, transgenic plants producing caffeine exhibited strong resistance against cotton aphids (Kim et al. 2011). Trichomes located on the plant leaf surface could serve as physical barriers to prevent insect feeding, and secrete secondary metabolite sucrose esters which play a major role in deterring the settling and probing of aphids (Neal et al. 1990). Chloroplast expression of β-Glucosidase in tobacco produced higher trichome density and more sucrose esters, resulting in aphid population on transgenic plants reduced more than 15 times compared with control (Jin et al. 2011).

Terpenoids (including mono-, sesqui- and diterpenes) are major components of plant volatile blends and play a predominant role in repellence of aphids and attraction of enemies or predators of the herbivores (Köpke et al. 2008). Terpenoids are either derived from the mevalonate pathway (MVA) or the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway, which lead to the formation of the C5 units isopentenyl diphosphate (IPP) and its allelic isomer dimethylallyl diphosphate (DMADP), the basic terpenoid biosynthesis building blocks (Mahmoud and Croteau 2002). The sequential head-to-tail addition of IPP units to DMAPP in condensation reactions initially yields geranyl diphosphate (GPP, C10), farnesyl diphosphate (FPP, C15) and geranyl geranyl diphosphate (GGDP, C20), which are the precursors of monoterpene, sesquiterpene and diterpene, respectively. In most cases, biosynthesis of sesquiterpenes is assumed to take place at the cytosol/endoplasmic reticulum boundary, whereas monoterpene and diterpene biosynthesis are compartmentalized in plastids (Bohlmann et al. 1998) (Fig. 1). Some plant-derived terpenoids, including *p*-benzoquinone, farnesol, bisabolene, β-citronellol, linalool and geraniol, have been shown to act as aphid-feeding deterrents and are often toxic at higher levels (Gutiérrez et al. 1997; Burgueño-Tapia et al. 2008; Halbert et al. 2009). Apart from acting as feeding deterrents to insects, volatile terpenoids released from different plant tissues also act as host location cues for insect natural enemies (Kappers et al. 2005).

Genetic manipulation of terpenoids in plants could provide an alternative tool for aphid control. *P450*-suppressed transgenic tobacco plants producing higher levels of diterpene cembratriene-ol could greatly diminish aphid colonization (Wang et al. 2001). Recombinant linalool/nerolidol synthase (FaNES1) catalyzes the biosynthesis of the monoterpene alcohol linalool and its sesquiterpene counterpart nerolidol. In dual-choice assays with green peach aphid, the *FaNES1* transgenic *Arabidopsis* plants significantly repelled the aphids (Aharoni et al. 2003). Aphids release alarm pheromone from the cornicles on their abdomen when attacked by their natural enemies (Bowers et al. 1972; Pickett and Griffiths 1980). (*E*)-β-Farnesene (EβF) as the main, and generally the only component of the aphid alarm pheromone, that can interrupt aphid feeding and cause other aphids in the vicinity to become agitated or disperse from their host plant (Bowers et al. 1972; Pickett and Griffiths 1980; Wohlers 1981; Francis et al. 2005). Certain plants can also synthesize EβF. The genes encoding EβF synthases that convert farnesyl diphosphate (FPP) to the acyclic sesquiterpene EβF, have been isolated and characterized from Douglas fir (Huber et al. 2005), Yuzu (Maruyama et al. 2001), sweet wormwood (Picaud et al. 2005; Yu et al. 2012a) and peppermint (Crock et al. 1997; Prosser et al. 2006; Yu et al. 2013). Overexpression of a black peppermint EβF synthase gene in *Arabidopsis* elicited potent effects on the behavior of the green peach aphid (alarm and repellent responses) and its natural enemy, the parasitoid *Diaeretiella rapae* (an arrestant response) (Beale et al. 2006). In our previous studies, we engineered tobacco plants with the EβF synthase genes from sweet wormwood and Asian peppermint, transgenic plants also were capable of synthesizing and emitting pure EβF. Behavioral studies involving the green peach aphids, and predatory lacewings (*Chrysopa septempunctata*) demonstrated that the transgenic tobacco could repel aphids and attract lacewings, thus minimizing aphid infestation (Yu et al. 2012a; Yu et al. 2013). Therefore, the EβF-emitting transgenic plants may have practical applications in agriculture as a result of not only repellence to aphids and reducing aphids population growth, but also recruiting the natural enemies of aphids by increasing predation on habituated aphids for aphid control (de Vos et al. 2010; Yu et al. 2012b).

However, secondary plant metabolites are usually the products of a set of complex multi-enzyme pathways. The

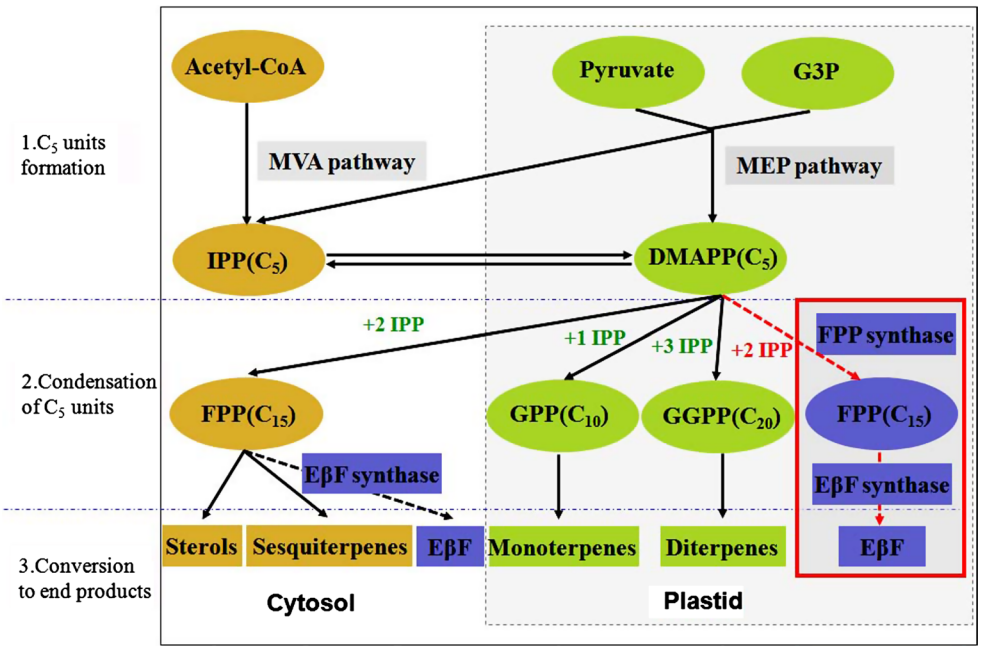


**Fig. 1** Metabolic engineering of terpene biosynthesis pathway using plant-derived (E)-β-farnesene synthase genes to generate a novel type of aphid-resistant genetically modified crop plants. *MVA* the mevalonate pathway, *MEP* 2-*C*-methyl-D-erythritol 4-phosphate pathway, *G3P* glyceraldehyde-3-phosphate, *IPP* isopentenyl diphosphate, *DMADP* dimethylallyl diphosphate, *GPP* geranyl diphosphate, *FPP* farnesyl diphosphate, *GGDP* geranyl geranyl diphosphate. The EβF synthase gene could convert FPP to the acyclic sesquiterpene EβF. EβF, as the major or only component of alarm pheromone of most aphid species, could cause repellence of aphids and also the attraction of natural enemies, and thus minimize aphid infestation. Certain plants have the genes encoding the EβF synthase and can release EβF, and thus have the natural capacity of repellence to aphids. There is only FPP synthase, but no EβF synthase, available in tobacco, wheat, cotton, soybeans and other agronomic important crops, therefore, expression of plant-derived EβF synthase genes in these plants, could be a potential strategy for aphid control (in *blue context*) (Yu et al. 2012b). *Red box* indicated the potential strategies to increase the amount of EβF synthesized in transgenic plants. One strategy is targeting EβF synthase to a suitable subcellular compartment. For example, a chloroplast form of FPP synthase exists in rice, wheat and tobacco (Sanmiya et al. 1999), therefore the chloroplast could be an ideal compartment for EβF engineering in these plants. The other is simultaneously overexpressing the exogenous FPP synthase and EβF synthase in the plastid of plants

manipulation of some metabolic pathways by the introduction of single enzyme encoding sequence may pose very considerable technical difficulties (Hilder and Boulter 1999). For instance, low sesquiterpene production of transgenic plants overexpressing sesquiterpene synthase genes were observed in some studies (Hohn and Ohlrogge 1991; Wallaart et al. 2001; Aharoni et al. 2006). Our previous studies also demonstrated that transgenic tobacco engineered with EβF synthase genes from sweet wormwood or Asian peppermint, emitting EβF ranged from approximately 1.55 to 4.85 ng/day/g fresh weight, much lower than expected (Yu et al. 2012a, 2013). To increase the amount of sesquiterpene synthesized, the following strategies may be considered (Fig. 1). Firstly, foreign sesquiterpene synthases could be targeted to a suitable subcellular compartment. A FPP synthase isoform existed in the mitochondria of *Arabidopsis* (Cunillera et al. 1997), therefore Kappers et al. (2005) presumed that FPP should be available in this cell compartment and generated higher levels of nerolidol by targeting the strawberry *FaNES1* gene to mitochondria. A chloroplast form of FPP synthase exists in rice, wheat and tobacco (Sanmiya et al. 1999), therefore the chloroplast could also be an ideal compartment for sesquiterpene engineering in these plants. Secondly, the sesquiterpene biosynthetic pathway could be redirected from its natural cytosolic location to the plastids (Fig. 1). Wu et al. (2006b) generated transgenic tobacco plants producing high levels of sesquiterpene by overexpressing both an avian FPP synthase and an appropriate sesquiterpene synthase in the chloroplast, the transgenic plants could increase the synthesis of the sesquiterpenes patchoulol and amorpha-4, 11-diene more than 1000-fold.

## Plant-mediated RNAi strategy

Expression in transgenic plants of double strand (dsRNA) designed against insect target genes has been shown to give protection against pests through RNA interference (RNAi), opening the way for a new generation of insect-resistant crops (Baum et al. 2007; Mao et al. 2007; Price

and Gatehouse 2008). In the case of plant-mediated RNAi for insect control, both cell autonomous and non-cell autonomous RNAi are required for the persistence of RNAi effect. For cell-autonomous RNAi referring local RNAi effects, the silencing process is limited to the cell in which the dsRNA is introduced, expressed and encompasses the RNAi process within individual cells (Meister and Tuschl 2004; Jinek and Doudna 2008; Siomi and Siomi 2009). The interfering effect of non-cell-autonomous RNAi, can take place in tissues/cells different from the location of application or production of the dsRNA. There are two different kinds of non-cell-autonomous RNAi: environmental RNAi and systemic RNAi. Environmental RNAi describes all processes in which dsRNA is taken up by a cell from the environment (Whangbo and Hunter 2008). Systemic RNAi refers to all processes in which the silencing signal is transported from the cell in which the dsRNA is applied or expressed to other cells and tissues in which the silencing could take place (van Roessel and Brand 2004; Jose and Hunter 2007). In multicellular organisms, systemic RNAi can follow environmental RNAi and cell-autonomous RNAi will always followed non-cell-autonomous RNAi (Huvenne and Smagghe 2010). At least two mechanisms underlying RNAi in insects have been described so far, i.e., the transmembrane channel-mediated uptake mechanism and an alternative endocytosis-mediated uptake mechanism (Huvenne and Smagghe 2010). In aphid, except for the existence of SID-1, which is a multispan transmembrane protein mediating a systemic RNAi effect, the uptake mechanism of dsRNA remains to be determined (Fig. 2) (Xu and Han 2008; Huvenne and Smagghe 2010). In our previous study, when dCTP labeled with Cyt 3 was added during the synthesis of dsRNA in a dsRNA artificial diet-feeding assay, the fluorescence signal were observed first in the grain aphid mouthparts, and then centralized in the midgut and finally it spread through the whole body (Zhang et al. 2013). This implies that long-lasting systemic RNAi effects may exist in aphid species, although the mechanisms underpinning the spread of fluorescence signal still need to be further investigated; for example, the spread of fluorescence signal is through the aphid's circulatory system or the in vivo amplification of siRNA, in which cells or tissues the target genes were silenced, and whether the proposed receptor-mediated endocytosis or the transmembrane channel-mediated uptake were the mechanisms leading to the persistence of RNAi effect (Zhang et al. 2013). Nevertheless, RNAi-mediated knockdown of *C002*, a gene strongly expressed in the salivary glands of pea aphids (*Acyrthosiphon pisum*) have led to the death of aphids through direct injection of siRNA into aphid hemolymph (Mutti et al. 2006). V-ATPase is a membrane-bound protein that acts as a proton pump to establish the pH gradient within the gut lumen of many insects. Knockdown of *vATPase* transcripts following feeding on *vATPase* dsRNAs also led to significant mortality of *Acyrthosiphon pisum* (Whyard et al. 2009). Furthermore, injection of dsRNA-targeting genes encoding a calcium-binding protein calreticulin and a gut cathepsin, and feeding dsRNA of a water-specific aquaporin gene in artificial diet assay led to the downregulation and malfunction of these targeted genes in *Acyrthosiphon pisum*, although the target gene expression knockdown did not exceed 50 % and was transient, persisting for less than a week (Jaubert-Possamai et al. 2007; Shakesby et al. 2009).

So far, four cases of plant-mediated RNAi for aphid control have also been reported. Silencing *C002* gene and a gut-specific gene *Rack-1* in peach aphid resulted in the knockdown of these two genes by up to 60 % after feeding on transgenic tobacco and *Arabidopsis* plants, with affected aphids producing less progeny (Pitino et al. 2011). Host-generated siRNAs attenuated the expression of a serine proteinase gene in peach aphid, leading to a significant reduction in their fecundity and parthenogenetic population upon feeding on transgenic *Arabidopsis* plants (Bhatia et al. 2012). Similarly, continuous feeding on transgenic tobacco-expressing dsRNA of a gap gene *Mphb* inhibited green peach aphid reproduction, thus minimized aphid infestation (Mao and Zeng 2014). Carboxylesterases (CbEs) can hydrolyze the esters of carbamates and pyrethroids and are widely distributed in microorganisms, plants and animals (Sogorb and Vilanova 2002). Silencing of this carboxylesterase (*CbE E4*) gene by use of plant-mediated RNAi impairs grain aphid's tolerance of Phoxim insecticides (Xu et al. 2014). These lines of evidences exemplify the feasibility of plant-mediated RNAi approach for aphid control in agricultural practice.

However, a potential risk for application of this technology in agricultural practice is the off-target silencing effect on non-target organism since specific functional domains of certain genes are highly conserved across different organisms. If off-targeting can unexpectedly silence genes in plant or other non-target organisms (e.g., beneficial insects, other herbivores), such unintended effects will raise biosafety concerns not only about the pleiotropic phenotypes of the plants to be engineered but also the environmental consequences for the herbivores or beneficial insects (Xu et al. 2006; Auer and Frederick 2009; Senthil-Kumar and Mysore 2011).

## Future perspectives

To date, different strategies and/or genes have been endeavored for engineering plants' aphid resistance. For aphid control, it does not necessarily mean to kill all the aphids in the field; strategies that reduce infestation below an

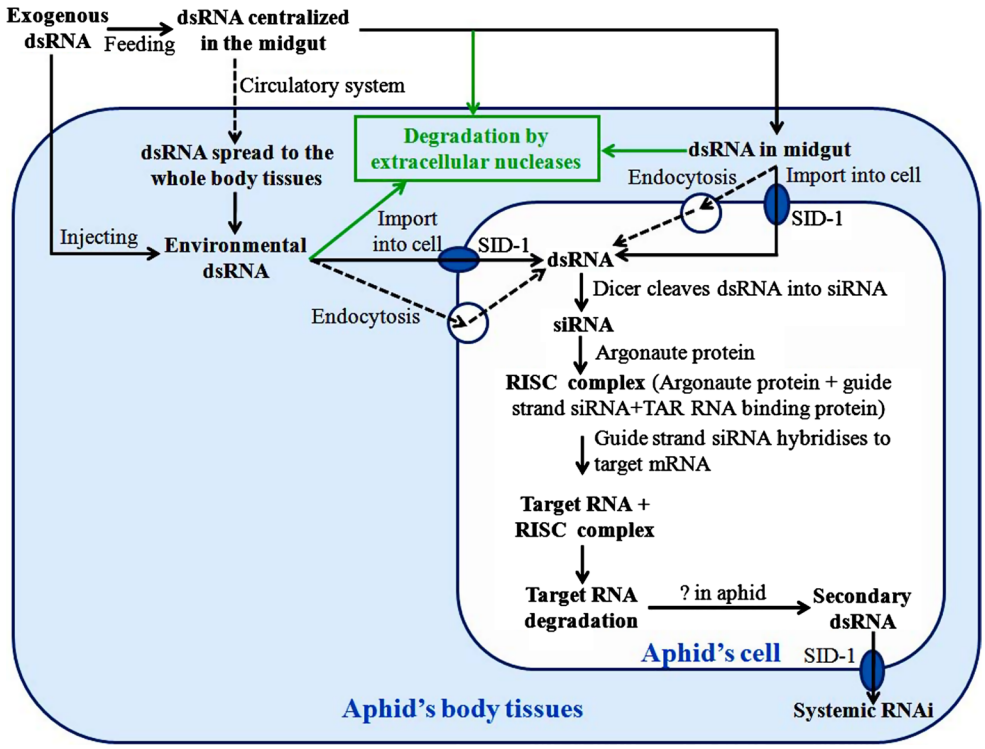


**Fig. 2** Schematic show of the possible RNAi mechanism induced by dsRNA in aphid. For the artificial feeding assay, the midgut is the primary target organ, causing environmental RNAi. Environmental RNAi can also be induced by directly injecting dsRNA into aphid's body tissues. In case of environmental RNAi, the dsRNA is taken up from the environment of the cell, processed by dicer into small interfering RNA (siRNA) and assembled with the Argonaute protein into the RNA-induced silencing complex (RISC). The RISC complex targets and degrades specific mRNAs based on the siRNA sequence. For cell-autonomous RNAi refers to local RNAi effects, the silencing process is limited to the cell in which the dsRNAs are taken up. In contrast, systemic RNAi effects are mediated through the production of new dsRNAs by RNA-dependent RNA polymerase (RdRP), which has not been identified in aphid so far. The secondary dsRNAs are further exported from one cell to spread the RNAi effect to other cells or tissues (Price and Gatehouse 2008). However, the mechanisms underpinning the spread of silencing signal still need to be further investigated; for example, the spread of silencing signal is through the aphid's circulatory system or the in vivo amplification of siRNA, in which cells or tissues the target genes are silenced. Meanwhile, the transport protein SID-1 has been identified in aphids, whether the proposed receptor-mediated endocytosis is also responsible for the dsRNA uptake remains to be determined

economically relevant level are also of interest according to the concept of integrated pest management. In this context, the effects of transgenic plants on both aphid infestation and the behavior of their predatory and parasitoids enemies need to be evaluated prior to large-scale field test or commercialization.

A number of lectin genes have been successfully engineered into plants to improve aphid resistance including agriculturally important crop plants such as wheat, maize, cotton and, etc. (Table 2). However, some lectins, such as GNA could cause adverse effects on predatory ladybirds and parasitoids *Aphidius ervi* or even animals (Ewen and Pusztai 1999; Birch et al. 1999; Hogervorst et al. 2009), resulting in major concerns of biosafety issues for the application of these lectin genes for aphid control. Therefore, taking advantages of these lectins for aphid control, issues such as the non-target organism toxicity of introduced lectins need to be evaluated in the future.

Low level toxicity of *Bt* toxins against aphids has been reported due to toxin instability in the aphid gut and low levels of binding (Li et al. 2011; Chougule and Bonning 2012). Different strategies to enhance the binding activity of *Bt* toxin to the guts of aphid species such as insertion of a 12-aa aphid gut-binding peptide and/or using the fusions of different *Bt* toxins may share the benefits of these toxin genes in aphid control in agricultural practice (Mehlo et al. 2005; Chougule et al. 2013). Similarly, a transgene consisting of a lutevirus coat protein–toxin fusion is expected to be effective against multiple aphid species (Bonning et al. 2014). These strategies may broaden the options for transgenic plant-mediated suppression of aphids with modified toxins.

Plants engineered with plant-derived EβF synthase genes could cause repulsion of aphids and also the attraction of natural enemies, thus minimizing aphid infestation in *Arabidopsis* and tobacco plants (Beale et al. 2006; Yu

et al. 2012a, 2013). EβF is a color and odor-free volatile component that is presumed to have no adverse effect on human or animal consumption. Genetic engineering of crop plants to biosynthesize and emit EβF for aphid control could be a good alternative strategy. Current work undergoing both at Rothamsted Research (UK) and our ICS, CAAS lab has been focused on the generation of aphid-resistant wheat using different promoters to drive the exogenous EβF synthase gene expression in transgenic wheat plants. Whereas Rothamsted scientists successfully got transgenic wheat-emitting EβF with a constitutive ubiquitin promoter through bombardment transformation and field trials of this GM wheat plants with the EβF synthase gene are ongoing at Rothamsted and have been widely advertised (http://www.rothamsted.ac.uk/our-science/rothamsted-gm-wheat-trial). By using the rice *Rubisco* small subunit promoter (*rbc*S), which has been reported to direct exogenous gene expression specifically to leaves and other green tissues (Huang and Lin 2007), we also successfully generated *MaβFS1* (an EβF synthase gene from Asian peppermint) transgenic wheat plants by *Agrobacterium*-mediated transformation method. GC–MS and dual-choice assay demonstrated that the transgenic wheat plants could emit EβF and show repellence to grain aphids compared with the wild-type plants (unpublished data by Rothamsted Research, UK and ICS, CAAS lab). These facts indicate that plant-derived EβF synthase genes are good candidates for manipulating crop plants for aphid resistance. However, availability of the precursor (FPP) supply might be a major limiting factor in the biosynthesis of EβF in crop plants (Yu et al. 2012b). Therefore, the availability of FPP precursor needs to be investigated in the target crop plants before being engineered using this strategy. Furthermore, to exploit EβF synthase genes for effective aphid control, it is necessary to develop new strategies to increase the EβF production in transgenic plants, such as targeting EβF synthase to a suitable subcellular compartment or redirecting EβF biosynthetic pathway from its natural cytosolic location to plastids as indicated in Fig. 1. At last, it is worth to note that continuous growth of the aphid colonies on transgenic *Arabidopsis* plants that produce EβF led to habituation within three generations, and the habituated aphids showed no avoidance response and produced more progeny (de Vos et al. 2010). Although this might result in the increased predation of these habituated aphids and the individual aphids from the habituated colony could revert back to being EβF sensitive in three generations (de Vos et al. 2010), it would be necessary to address whether the transgenic crop plants engineered with EβF synthase gene affect the behavior and fitness of both aphid and aphid's parasitoids and predators in diverse agro-ecosystems.

Plant-mediated RNA interference (RNAi) could be considered another efficient alternative strategy in generation of transgenic wheat resistant to aphids via a non-toxic mode of action. Up to date, plant-mediated RNAi for insect control has been reported in Lepidopteran and Coleopteran plant pests (Baum et al. 2007; Mao et al. 2007), and recently in phloem-sucking Hemipteran pests, rice brown planthoppers (Zha et al. 2011). Plant-mediated RNAi has also exhibited potential effects for aphid control in model *Arabidopsis* and tobacco plants (Pitino et al. 2011; Bhatia et al. 2012; Mao and Zeng 2014) and one case in wheat for impairing grain aphids' resistance to Phoxim insecticides by silencing *CbE4* gene (Xu et al. 2014). The basis for generation of this new type of transgenic crop plants is the availability of more biological information and genomic or transcriptomic sequences from different aphids species to facilitate RNAi target selection. Genome sequence of the pea aphid, whose host range is predominantly restricted to leguminous species, provided a foundation for post-genomic studies of fundamental biological questions both in pea aphid and other aphid species. The assembled genome sequence data of the pea aphid, along with ESTs and full length cDNAs are accessible at the AphidsBase web portal (http://www.aphidbase.com) (Legeai et al. 2010). It revealed the presence of more coding sequences than previously reported in other insect genomes and also identified genes with no orthologs in other insects (International Aphid Genomics Consortium 2010). This provides an efficient way for the selection of aphid-specific potential RNAi targets without off-target effects on other insects. With the accessibility of next-generation sequencing technologies and the release of the first aphid genome, sequencing other aphid species in addition to pea aphid will greatly facilitate the selection of species-specific RNAi targets. In our recent study, transcriptome profiling of grain aphid and comparative transcriptomic analysis between grain aphid and pea aphid revealed that more than 4,800 unigenes were grain aphid-specific unigenes (unpublished data by ICS, CAAS lab). Furthermore, we performed de novo transcriptome assembly and gene expression analyses of the alimentary canals of grain aphids upon their feeding on wheat plants using Illumina RNA sequencing. The transcriptome profiling generated 30,427 unigenes with an average length of 664 bp. Comparison of the two transcriptomes of the alimentary canals of pre- and post-feeding grain aphids indicated that 5,490 unigenes were differentially expressed, among which, diverse genes and/or pathways involved were identified and annotated. Among these unigenes, 16 that significantly up- or downregulated upon feeding were selected for dsRNA artificial feeding assay. Of these, five unigenes led to higher mortality and developmental stunting in artificial feeding assay due to the downregulation of the target gene expression. Transcriptome profile analysis indicated that highly expressed genes involved in ingestion and digestion might achieve more effective knockdown or

silencing of the target genes and higher mortality of aphid (Zhang et al. 2013). This further augments that for the organisms without sufficient genomic information, transcriptome profiling through RNA-seq could provide massive candidate genes for screening RNAi targets in large scale for potential application in insect pest control (Wang et al. 2011).

For potential RNAi target selection in plant-mediated RNAi for aphid control, we need to keep in mind that the sequence-specific degradation of the target genes mediated by siRNA both to the target insect pest and its natural enemies such as ladybird beetles and parasitoid wasps, and even to high animal such as human being, as the risk of cross-species silencing would be a major biosafety concern for the future application of RNAi-mediated transgenic plant resistance against aphid infestation. It is therefore recommended that it is better to select the aphid-specific genes or sequences with no orthologs with the non-target organisms such as the donor plants for engineering, natural enemy of aphid and human being (Zhang et al. 2013). The sequence specificity of the endogenous RNA interference pathway allows targeted suppression of genes essential for insect survival and enables the development of durable and efficacious insecticidal products having a low likelihood to adversely impact non-target organisms. The spectrum of insecticidal activity of a 240 nucleotide (nt) dsRNA targeting the Snf7ortholog in western corn rootworm (WCR; *Diabrotica virgifera virgifera*) was characterized by selecting and testing insects based upon their phylogenetic relatedness to WCR. Evaluating the relationship between minimized shared nt sequence length and insecticidal activity. A shared sequence length of 21 nt was required for efficacy against WCR (containing 221 potential 21-nt matches) and all active orthologs contained at least three 21-nt matches (Bachman et al. 2013). This laid the basis for the selection of RNAi targets and design of dsRNAs avoiding the off-target effects on other organisms or selection of RNAi targets effective for several aphid species using the conserved sequences among them provided that the sequences of target genes are available in the respective species. At last, it is worth to mention that the enhanced effectiveness of RNAi strategies might be achieved by pyramiding multiple RNAi targets. Although this strategy has not yet applied to the management of aphid infestation, the feasibility has been demonstrated in *Drosophila* (Schmid et al. 2002).

In generation of aphid-resistant transgenic plants, constitutive promoters that are used for expression control of defense compounds such as dsRNAs may be regarded as inefficient because they produce siRNA in the absence of infestation. The feeding behavior of aphids suggests that phloem-specific promoters would be more useful because they can drive the exogenous gene highly expressed in the phloem. This could enhance the resistance of transgenic plants against phloem-feeding aphid pests by increasing the content of defense compounds in phloem sap while reducing the exposure of non-target insects to the same compounds. Furthermore, this approach would also reduce the GM-associated resource investment by the plant by avoiding the expression of defense compounds in cells/tissues where they would never encounter the pest (Will and Vilcinskas 2013). However, it should be also noted that aphids puncture other cells on the paths to the phloem and acquire nutrients from the xylem. Moreover, aphid-mediated transmission of the potyviruses can occur without phloem feeding. Thus, it would be effective to also drive the production of compounds deterrent to aphids in the other cell types. An ideal promoter should be inactive prior to aphid infestation and/or wounding. Several promoters have been identified to be inactive when tissues are intact but are activated by wounding, including the mannopine synthase (mas) promoter (Langridge et al. 1989), the potato proteinase inhibitor II (pinII) promoter (Godard et al. 2007), and the PR1-a promoter (Tiwari et al., 2011). The inducible PR1-a promoter is activated by salicylic acid, a chemical involved in wound-induced signaling pathway in plants (Tiwari et al. 2011), and its production is triggered upon aphid feeding (Zhu-Salzman et al. 2004). The ideal promoter for the control of aphid resistance genes expression would therefore be chimeric, combining the functional elements of wound-inducible promoters (e.g., PR1-a). This would allow the development of transgenic plants with defense mechanisms triggered only by aphid feeding (Will and Vilcinskas 2013). Furthermore, as a matter of fact, all the strategies described or documented so far mainly focus on the development of transgenic aphid-resistant plants that addressing different levels of aphid–plant interactions. With a common goal to disturb host plant acceptance by aphids and to disrupt their ability to take nutrition from plants, a combined strategy could be adopted by gene pyramiding. For example, generation of the bivalent transgenic crop plants either by co-transformation or by crossing the EβF-emitting transgenic lines with dsRNA transgenic lines could achieve lines both repellent to aphids and reducing aphid infestation by reducing its fecundity or population growth, even the mortality of aphids.

To develop aphid-resistant crops for commercialization, it is equally important to assess the efficacy of the transgenic plants on aphid control at trophic level. Aphid fitness parameters such as development, body size, reproduction, and survival rate need to be thoroughly investigated. Furthermore, aphid behavior provides additional insights into the interaction between host plants and pests, and this will enable us to determine if the aphids that are repelled by a plant, unable to access the plant, or have disrupted nutrition uptake. Moreover, whether transgenic plants affected the behavior and fitness of aphid's parasitoids and predators is

also an important issue. Because a large guild of parasitoids and predators in agro-ecosystems are recognized as important sources of bio-control for invasive aphids, which can significantly affect aphid population growth, especially during aphids' early colonization stages (Edwards et al. 1979; Chiverton 1986). In a large-scale field test or commercialization, transgenic plants will encounter a mixed aphid species in an opening ecological agro-system, thus the plant performances in the field tend to be very different to those in controlled conditions. Therefore, physiological processes and basic modes of intra-specific and inter-specific interactions among aphids, their symbionts and their hosts need to be addressed in detail. This is the basis for the development of tailor-made transgenic crop plants that can withstand one or several dominant aphid species in a respective habitat (Will and Vilcinskas 2013). At last, aphid infestation is not only a direct cause of crop losses, but also leads further losses by virus transmission. Thus, the consequences of transgenic plants on the probing behavior, and subsequent uptake and transmission of the viruses by aphids, should also have to be investigated (Gatehouse et al. 1996).

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