Breeding for Aphid Resistance in Rapeseed Mustard

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Abstract

The productivity of oilseed brassicas is severely affected by aphid pests. Among the different aphid species, turnip/mustard aphid, *Lipaphis erysimi* (Kaltenbach), is the key pest of oilseed brassicas in Indian subcontinent inflicting 35.4–91.3% losses under different agroclimatic conditions. The development of an aphid-resistant cultivar offers an effective, economic and eco-friendly method of its management which requires the availability of a crossable source of resistance. Brassica plants employ a plethora of biophysical and biochemical defence mechanisms against insects, which range from surface waxes and trichomes to production of toxic biochemicals such as glucosinolates, isothiocyanates, lectins, volatiles, alkaloids, etc. Such resistant plants can be identified by an effective screening protocol, and the gene(s) of interest can be transferred to the desirable agronomic background by conventional breeding or marker-assisted selection. Not much progress has been made in breeding for resistance in brassicas against aphids primarily due to non-availability of resistant source within the crossable germplasm as well as lack of knowledge on its trait genetics. Though some success has been achieved to introgress the gene of interest to a desirable agronomic background, it has complex and elaborate breeding requirements. An alternate strategy to conventional breeding is the use of insect-resistant transgenes through genetic engineering, but this strategy has its own associated issues. Thus, the

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development of aphid-resistant cultivars requires more research on aphid-plant interactions to identify either an effective aphid resistance gene or a phenomenon that can lead to a new mechanism of resistance.

Keywords
Brassica • Defence • Host plant resistance • *Lipaphis erysimi* • Screening techniques

6.1 Introduction

Crop brassicas belong to the family Brassicaceae. It is a major angiosperm family that includes nearly 375 genera and 3200 species (LeCoz and Ducombs 2006). Members of this family provide major sources of oilseeds, vegetables and condiments. Canola (*Brassica napus*); Indian mustard (*B. juncea*); *B. rapa* ssp. *oleifera*, viz., *toria* and brown *sarson*; and Abyssinian mustard (*B. carinata*) account for almost 13% of the vegetable oil supplies of the world. Besides its economic importance, Brassicaceae are of special significance in the study of insect-plant interactions as all members produce glucosinolates, which have a great influence on such relationships. Further, the genome of the closely related *Arabidopsis thaliana* has been sequenced, which can provide ready access to genetic and genomic resources (Hegedus and Erlandson 2012). *A. thaliana* is ideal as a model system for the study of insect-plant interactions at genetic and molecular level (Mitchell-Olds 2001). This chapter focuses on various aspects of breeding for resistance to mustard aphid in rapeseed-mustard. We also discuss various aspects of aphid biology, host-pest interactions and factors associated with resistance responses of the host.

6.2 The Aphid Complex of Brassicas

Aphids are global pests. Despite forming a small insect group, they inflict serious damage to agricultural crops (Remaudière and Remaudière 1997; Dedryver et al. 2010). They belong to family Aphididae and comprise approximately 5000 species (Smith and Chuang 2014), of which nearly 100 are very damaging for crop plants (Blackman and Eastop 2000, 2007). The main aphids infesting brassica crops are cabbage aphid [*Brevicoryne brassicae* (L.)], turnip/mustard aphid [*Lipaphis erysimi* (Kaltenbach)*/Lipaphis pseudobrassicae* (Davis)], shallot aphid [*Myzus ascalonicus* Doncaster], peach-potato aphid [*Myzus persicae* (Sulzer)], potato aphid [*Macrosiphum euphorbiae* (Thomas)], corn root aphid [*Aphis maidiradicis* Forbes] and root-feeding aphid species, namely, cabbage root aphid/poplar petiole gall aphid [*Pemphigus populitransversus* Riley] and bean root aphid [*Smynthurodes betae* Westwood] (Blackman and Eastop 2000). *B. brassicae*, a native to Europe and worldwide in distribution, is a major pest on vegetable brassicas in most European countries with strong yield reducing impacts. It is a brassica specialist insect that

feeds on phloem sap of its host plants (Cole 1997). Though a primary pest of vegetable brassicas, it also infests other species in genus *Brassica* (Cole 1994a, b, 1997; Kift et al. 2000). *L. erysimi* is a native to eastern Asia (Blackman and Eastop 2000). It is the most serious pest of oilseed brassica, especially in India and other subtropical regions of the world. It may cause 10–90% productivity losses, depending upon the agroclimatic conditions, intensity of population development and crop growth stage (Singh and Sachan 1994; Ahuja et al. 2009). *L. erysimi* is also a vector of ten non-persistent plant viruses like cabbage black ring spot and mosaic diseases of cauliflower, radish and turnip (Blackman and Eastop 1984; Rana 2005). It is a brassica specialist and can develop only on brassicaceous plants. Generally, *B. rapa* and *B. juncea* are better hosts than other *Brassica* species (Rana 2005).

Peach-potato aphid, *Myzus persicae*, is a generalist pest with a host range of more than 400 plant species (Quaglia et al. 1993). It is a major vector of more than 100 plant viruses including potato virus Y and potato leaf roll virus and various mosaic viruses, including western yellows (Ponsen 1972; Eskanderi et al. 1979; Bwye et al. 1997). It is cosmopolitan, polyphagous and an efficient vector of plant viruses. It possesses wide genetic variation for colour, life cycle, host plant relationships and mechanisms of insecticide resistance. Although many consider it to have originated from China, the native place of its primary host *Prunus persica*, others believe it to be a native of Europe (Blackman and Eastop 2007).

6.3 Aphid Biology

Aphids are the specialized phloem sap feeders. Their ability to rapidly exploit the ephemeral habitats makes them serious pests. High reproductive potential and dispersal capacities add to their wide adaptability (Dedryver et al. 2010). Aphids exhibit parthenogenetic viviparity – a process that limits the need for males to fertilize females and obviate egg stage from the life cycle. Thus, aphids reproduce clonally and give birth to young ones. Embryonic development of an aphid begins before its mother's birth leading to telescoping of generations. These attributes permit aphids to efficiently exploit the periods of rapid plant growth, conserve energy and allow rapid generation turnover. Nymphs of certain aphid species can reach maturity in as little as 5 days (Goggin 2007). Parthenogenesis sets them apart from other Hemiptera and has a great influence on their biology. Many species of aphids also exhibit alternation of generations. Evolution of alternating hermaphrodite generations with a series of parthenogenetic, all-female generations dates back to Triassic period (Blackman and Eastop 2007). Coupled with viviparity, this reduces the development period and permits rapid multiplication of aphids. Further, to conserve energy for maximizing their reproduction and survival, aphid colonies exhibit wing dimorphism to produce highly fecund wingless (*apterae*) morphs or less prolific winged (alate) progeny that can disperse to new host plants depending on the resource availability. All these strategies contribute to aphids' success and their abundance in temperate zones. An enormous propagation rate precipitates abnormally high population under favourable conditions (Goggin 2007).

6.3.1 Aphid Life Cycles

Most of the aphid species display relatively complicated life cycles, and each of these life cycles has morphs which specialize in reproduction, dispersal and survival under adverse conditions. Based on how aphids utilize their host plants, life cycle can be of two types: heteroecious or host alternating and monoecious/autoecious or non-host alternating. Heteroecious aphids live on one plant species (primary host) in winter and migrate to another taxonomically unrelated plant species (secondary host) in summer and again migrate to primary host in autumn. On the primary host plant, eggs are laid by females after mating with males. However, on the secondary host plant, they reproduce parthenogenetically. Aphids that interrupt parthenogenetic reproduction with sexual reproduction are termed as holocyclic. In contrast to host-alternating aphids, non-host-alternating aphids remain either on the same or closely related host species throughout the year. They complete both sexual and parthenogenetic life cycle on the same host species. There are also species which do not produce eggs and are known as anholocyclic. Some species can live both holocyclic and anholocyclic lives, simultaneously across wide geographies (Bhatia et al. 2011). However, monoecy and heteroecy can coexist rarely (Williams and Dixon 2007). The presence of both sexual and asexual life cycle ensures that aphids take advantage of both parthenogenesis and genetic recombination that help them to evolve.

Lipaphis erysimi is a holocyclic species with a chromosome number of 2n = 10 (Blackman and Eastop 2000). Although it produces parthenogenetically in warmer climates, a holocyclic reproduction has been reported in western Honshu, Japan, on cruciferous crops (*B. rapa*, *Raphanus sativus*) (Kawada and Murai 1979). A chromosome number of 2n = 8 and differing in karyotype from holocyclic populations have been reported from Northern Europe. Most anholocyclic parthenogenetic populations have 2n = 9, probably derived from eight chromosomes through dissociation of one autosome to produce a small, unpaired element. Though sexual morphs have been reported from North India, populations were mostly anholocyclic (Blackman and Eastop 2007).

Brevicoryne brassicae is a monoecious species that exhibits holocyclic life cycle with parthenogenetic reproduction in warmer climates as well as during warmer periods of temperate climates. However, with the fall in temperature during autumn, males are also produced (Blackman and Eastop 1984), which mate with the females to produce eggs for overwintering. As per Hines and Hutchison (2013), about 15 overlapping generations are passed in a crop season in the United States.

Myzus persicae exhibits holocyclic life cycle, and it overwinters as egg stage on its primary host (peach and related trees). In the subsequent spring or summer season, fundatrix/fundress (the winged stem mother) returns as alate emigrants to secondary host plants and multiplies to apterous and alate viviparae (Moran 1992; Bhatia et al. 2011). The wingless female then gives birth to young ones by parthenogenesis and multiplies at a very fast rate. This results in large aphid populations on different crop plants. When the temperature starts falling late in the season, some of the apterous viviparae turn into apterous oviparae and alate viviparae into alate

males. These males and females start sexual reproduction and lay eggs on the primary host plant (Stern 1995). At the end of winters, females (stem mothers) hatch from the eggs the next spring season and start reproducing parthenogenetically (Bhatia et al. 2011).

6.3.2 Aphid-Host Plant Interactions

Aphids are specialized phloem sap feeders which insert their needle-like stylets in the plant tissue avoiding/counteracting the different plant defences. They withdraw large quantities of phloem sap while keeping the phloem cells alive. In contrast to the insects with biting and chewing mouthparts which tear the host tissues, aphids penetrate their stylets between epidermal and parenchymal cells to finally reach sieve tubes with slight physical damage to the plants, which is hardly perceived by the host plant (Bhatia et al. 2011). The long and flexible stylets move through intercellular spaces in the apoplasm of the cell wall (Giordanengo et al. 2010), although stylets also make intracellular punctures to probe the internal chemistry of a cell (Zust and Agrawal 2016). The high pressure within sieve tubes helps in passive feeding (Bhatia et al. 2011). During the stylet penetration and feeding, aphids produce two types of saliva. The first type is dense and proteinaceous (including phenoloxidases, peroxidases, pectinases, β-glucosidases) that forms an intercellular tunnelled path around the stylet in the form of sheath (Felton and Eichenseer 1999; Zust and Agrawal 2016). In addition to proteins, this gelling saliva also contains phospholipids and conjugated carbohydrates (Urbanska et al. 1998; Miles 1999; Cherqui and Tjallingii 2000; Sharma et al. 2014). This stylet sheath forms a physical barrier and protects the feeding site from plant's immune response (Will et al. 2012, 2013). When the stylets encounter active flow of phloem sap, the feeding aphid releases digestive enzymes in the vascular tissue in the form of second type of 'watery' saliva. The injection of watery saliva (E1) prevents the coagulation of proteins in plant sieve tubes, and during feeding the watery (E2) saliva gets mixed with the ingested sap which prevents clogging of proteins inside the capillary food canal in the insect stylets (Bhatia et al. 2011; Sharma et al. 2014; Zust and Agrawal 2016). Though the actual biochemical mode of action that inhibits protein coagulation is unknown, the calcium-binding proteins of aphid saliva are reported to interact with the calcium of plant tissues. This results in suppression of calcium-dependent occlusion of sieve tubes and subsequent delayed plant response (Will et al. 2007, 2009, 2013). This mechanism of feeding is more specialized and precise, which helps the aphid to avoid different allelochemicals and indigestible compounds found in other plant tissues (Schoonhoven et al. 2007). In addition to this, aphid saliva also contains non-enzymatic reducing compounds, which in the presence of oxidizing enzymes inactivate different defence-related compounds produced by plants in response to the insect attack (Miles 1999).

There are commonalities of events during initial plant reaction to insect feeding or pathogen infection. These include protein phosphorylation, calcium influx, membrane depolarization and release of reactive oxygen species (ROS), such as

hydrogen peroxide (Garcia-Brugger et al. 2006). These lead to activation of phytohormone-dependent pathways. In response to infestation/infection, different phytohormone-dependent pathways are activated. Ethylene (ET) and jasmonate (JA) pathways are activated by different necrotrophic pathogens (Thomma et al. 2001) and grazing insects (Maffei et al. 2007), while salicylate (SA)-dependent responses are induced by biotrophic pathogens (Thomma et al. 2001). These responses lead to the production of various defence-related proteins and secondary metabolites with antixenotic or antibiotic properties. In the event of infestation by aphids, a SA-dependent response was seemingly activated. In contrast, JA-dependent genes were repressed (Zhu-Salzman et al. 2004; Thompson and Goggin 2006; Gao et al. 2007; Walling 2008). All these responses lead to the manipulation of the plant metabolism to ensure compatible aphid-plant interactions.

6.3.3 Aphid Endosymbionts

The phloem sap is a highly unbalanced diet composed principally of sugars and amino acids with high C:N content. The most of the amino acids are present at very low concentrations. Despite their nutritionally poor diet, aphids exhibit high growth and reproduction rates. Since aphids directly feed on the sugars and amino acids, their assimilation efficiency is very high. In addition, essential amino acids required by their growth and development are synthesized by symbiotic bacteria present in their body. These include primary (obligate) symbionts and secondary (facultative) symbionts. *Buchnera aphidicola* (gamma-3 proteobacteria, *Escherichia coli*, is also a member of this group) is the most common vertically transmitted primary symbiont present in most aphid species (Munson et al. 1991; Oliver et al. 2010). Some species of aphids also bear other bacteria, i.e. 'secondary symbionts'. These include several species of gamma-proteobacteria such as *Serratia symbiotica*, *Regiella insecticola* and *Hamiltonella defensa* (Chen et al. 1996; Chen and Purcell 1997; Fukatsu et al. 2000, 2001; Darby et al. 2001; Sandstrom et al. 2001; Haynes et al. 2003; Russell et al. 2003; Moran et al. 2005; Oliver et al. 2010). *B. aphidicola* is a coccoid hosted in the cytoplasm of specialized cells called mycetocytes/bacteriocytes in the haemocoel of insect. These endosymbionts upgrade the aphid diet by converting non-essential amino acids to essential amino acids. The evolution of symbiotic relationship with endosymbionts has enabled aphids to exploit new ecological niches, i.e. to feed on the plant phloem sap which is otherwise the nutritionally poor diet.

6.4 Plant Defence Responses Against Insects

Brassicas possess an array of defence mechanisms against different biotic stressors including insect herbivores. These include surface waxes, trichomes, plant secondary metabolites and different volatiles, which provide varying degree of protection against insects feeding on them. Such defence mechanisms can be constitutive or

inducible and direct or indirect defences. The constitutive defences comprise physical and chemical barriers that exist before insect attack (preformed/innate defences). These may be the ancient defences involving different plant receptors that recognize microbial cell surface molecules, signal transduction pathways that induce transcription of defence-associated genes and antimicrobial effectors, cationic peptides and proteins (Boman 1995; Borregaard et al. 2000; Thomma et al. 2002 as cited from Ahuja et al. 2009). In contrast, inducible defences are induced following invasion of an insect herbivore. This kind of defence is particularly important when the defence is bioenergetically expensive and insect attack is frequent and unpredictable (Haukioja 1999). The defences that show their effect on the herbivore through synthesis of toxins are called direct defences, while the defences that affect herbivores through the attraction of natural enemies of insects are called indirect defences (Dicke 1999). Brassica plants release different volatile compounds to attract natural enemies of insects that feed on them. This release of volatile organic compounds is construed as a 'cry or call' for help by the plant from herbivore predators. The different defence components of brassica plants are discussed in the following subsections.

6.4.1 Biophysical Defences

Many morphological and anatomical characters may influence the suitability of a plant as host to the insect (Southwood 1986). These characters may include epicuticular wax, trichomes, depth of vascular bundles, etc. The epicuticular wax is the first site of interaction between insect and its host plant, and hence, its chemical composition is critical for an insect to feed, probe or oviposit on a plant. The waxes are complex mixtures of very-long-chain lipids substituted with primary alcohols, aldehydes, fatty acids and alkyl esters, all of which primarily occur with even-numbered chain lengths and hydrocarbons, secondary alcohols and ketones with predominance of odd chain lengths (Walton 1990). Waxiness has been found to hinder *L. erysimi* from reaching the undersurface of leaves, where it normally feeds during the vegetative plant stage (Åhman 1990). However, Lamb et al. (1993) reported that elevation of leaf wax did not improve the resistance of *B. napus* or *B. oleracea* (kale and collard) to *L. erysimi*. The neonate larvae of diamondback moth, *Plutella xylostella* L., have been shown to spend more time walking at a faster pace on waxy line of cabbage compared to that on non-waxy one (Eigenbrode et al. 1991). The young larvae of mustard beetle, *Phaedon cochleariae* (Fab.), find it difficult to climb the heavily waxed culm of cabbage on waxy cultivars and failed to reach their feeding site, while they easily walked on the non-waxy cultivars (Stork 1980). Although waxy trait is responsible for resistance to insect pests, glossiness is not a preferred trait in vegetables. Increased resistance to *P. xylostella* was observed in *B. oleracea* and *B. rapa* genotypes having glossy leaves (Ulmer et al. 2002). A significant increase in the feeding by flea beetle, *Phyllotreta cruciferae* (Goeze), was observed after removal of epicuticular wax from leaves of *B. napus* and *B. oleracea* particularly from the area where wax was removed (Bodnaryk 1992) and

most difference in feeding preference was explained by the presence of leaf wax. Reifenrath et al. (2005) observed an increase in *P. cochleariae* activity after removal of leaf wax, suggesting that wax occludes stimulatory signals such as glucosinolates, and they suggested that the resistance was primarily antixenosis. The importance of waxes on leaf surface has received increased attention in the recent years due to their association with polar compounds like glucosinolates, the key host recognition signals for specialist insects (Badenes-Pérez et al. 2010; Städler and Reifenrath 2009). Badenes-Pérez et al. (2010) reported the presence of glucosinolates on leaf surface of three *Barbarea* species but not on the surface of test *B. napus* genotype. The leaf surface wax has been reported to affect even the third trophic level. The aphids' parasitoid host recognition behaviour is influenced by aphid cuticular waxes which in turn are related to the plant surface waxes (Muratori et al. 2006).

Trichomes may also influence leaf herbivory by insects. The trichomes are small, sometimes branched, hair-like structures that are produced from cells of aerial epidermis, produced by most plant species (Werker 2000). Glandular trichomes produce secondary metabolites (e.g. flavonoids, alkaloids, terpenoids) which can either repel or trap insects or can be poisonous (Duffey 1986). The trichome producing morphotype of *Arabidopsis lyrata* was reported to be less damaged by insect herbivores than the glabrous form (Loe et al. 2007). The non-glandular trichomes, unlike glandular trichomes, do not produce secondary metabolites but mainly function as structural defence against small herbivores by interfering with insect movement on the plant surface (Southwood 1986). The insects feeding on trichome-bearing plants show poor weight gain due to poor nutritive value of cellulose-rich trichomes resulting in increased mortality. *B. nigra* lines having high number of trichomes supported less growth of *Pieris rapae* (L.) and increased mortality of *P. cruciferae* (Traw and Dawson 2002). Agrawal (1999) reported an increase in trichome density after insect damage in *Raphanus raphanistrum.* Similarly, Traw (2002) reported an increase in the trichome density as well as glucosinolate level after feeding by *P. rapae* in black mustard. Trichome-bearing pods of *Sinapis alba* were reported to be resistant to flea beetle, while glabrous pods of cultivated *Brassica* species are readily attacked (Lamb 1980).

Expression of *A. thaliana* myb-like transcriptor factor, GLABRA3 (GL3) in *B. napus*, resulted in the production of a dense coat of trichomes on the adaxial leaf surface (Gruber et al. 2006), and *P. xylostella* larvae had difficulty in feeding on these lines and grew slower (Adamson et al. 2008). Despite their negative effects on herbivore insects, trichomes may have their effect at the third trophic level. For example, trichomes on the leaves of trichome-bearing line of *Arabidopsis thaliana* affected the movement of aphid predator, *Episyrphus balteatus* (De Geer), and resulted in reduced performance (Wietsma 2010). Further, trichomes play an important role in the acceptance of host plants for oviposition (Sadeghi 2002), and there was comparatively less oviposition on *A. thaliana* line having higher trichome density (Wietsma 2010).

Before reaching the sieve tubes for feeding, aphid stylets had to pass through different cell layers such as the epidermis, endodermis, cortex and pericycle. The

plants with densely packed cell layers may pose hindrance to the stylets and, hence, may be less preferred (Henning 1966). Moderate resistance to aphids in *B. carinata*, *B. alba* and *Eruca sativa* has been attributed to this factor (Malik 1981). The depth of sieve tubes is an important factor in resistance of a plant to aphids. Aphids must have long stylets to feed on plant tissues with deeply localized vascular bundles (Gibson 1972). Further, such aphids will require more energy to probe deep into the plant tissue, while aphids with short stylets will starve and die (Berlinski 1965).

6.4.2 Biochemical Defences

6.4.2.1 Glucosinolates and Myrosinase-Glucosinolate System

Glucosinolates (GSLs) of brassica plants are a class of secondary metabolites. These amino acid-derived, secondary plant products containing β-D-thioglucose and sulphonated oxime moieties are found almost exclusively in the order Capparales (Halkier and Gershenzon 2006). They are a large group of naturally occurring, non-volatile, sulphur-containing, organic anionic compounds and are reported to be present in 16 plant families (Fahey et al. 2001). GSLs include approximately 140 naturally occurring thioglucosides that mainly differ in their R-group substitutes (Fenwick et al. 1983), and 30 of these are present in *Brassica* species (Bellostas et al. 2007). Although the glucosinolates may confer resistance to insects which feed on them, their breakdown products released after myrosinase hydrolysis can be more toxic. Myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1) catalyses the cleavage of glucosinolates to produce an aglycone moiety (thiohydroxamate-*O*-sulfonate), glucose and sulphate. The aglycone moiety, being unstable, rearranges to form isothiocyanates (ITCs), thiocyanates, nitriles, amines, oxazolidine-thiones and epithionitriles depending upon the glucosinolate being hydrolysed and the reaction conditions (Rask et al. 2000; Sadasivam and Thayumanavan 2003). The concentration of glucosinolates varies widely depending upon different species, plant parts and agronomic and climatic conditions (Font et al. 2005; Tripathi and Mishra 2007). A drastic decline in the concentration of glucosinolates (mainly aliphatic ones) occurs in *B. napus* seeds during the first 7 days of imbibition, while *de novo* synthesis of indolyl glucosinolates and an aromatic glucosinolate (gluconasturtin) takes place concomitantly. Gluconasturtin is not initially present in the seed. During the subsequent growth period, some more glucosinolates also accumulate (Clossais-Besnard and Larher 1991). On the other hand, glucosinolates occur in low concentrations in the fully expanded leaves (Porter et al. 1991). With the start of the reproductive phase of plant, i.e. during flowering, there is a reduction in the concentration of glucosinolates in vegetative plant parts as well as in inflorescence, which otherwise has relatively large amounts of glucosinolates. In contrast to this, during maturation of seeds, glucosinolate synthesis occurs in siliques which are then transported to the seeds through pod shells (Rask et al. 2000). The levels of glucosinolates can also be influenced by environmental conditions. An increase in the concentration of glucosinolates occurs in brassica plants under drought conditions (Bouchereau et al. 1996; Jensen et al. 1996). However, there is no consistent

relationship between glucosinolate concentration and water stress since increased levels of glucosinolates are also observed in plants grown under moist conditions compared to those grown in dry soil (Louda and Mole 1991). In intact plant tissues, glucosinolates and myrosinase are housed separately and individually where these are inactive thus preventing self-toxicity (Jones and Vogt 2001). This intracellular localization of myrosinase has been widely investigated. Lüthy and Matile (1984) propounded 'the mustard oil bomb hypothesis' for this organization. As per this hypothesis, glucosinolates are present in the myrosin grains (vacuoles) of myrosin cells, while the myrosinase is associated with the membranes in the cytoplasm. However, later studies proved that glucosinolates (Kelly et al. 1998) are present in vacuoles of different types of cells, while myrosinases are localized in the myrosin cells (Thangstad et al. 1991; Höglund et al. 1992; Kissen et al. 2009) scattered across the plant tissues. Myrosin cells carry myrosin grains (Bones et al. 1991; Kissen et al. 2009), forming a continuous reticular system or myrosin body (Andreasson et al. 2001; Ahuja et al. 2009). Tissue damage caused by insect feeding brings glucosinolates and myrosinase together, precipitating immediate release of glucosinolate-breakdown products (Bones and Rossiter 2006). Such defensive responses (or 'mustard oil bomb') play multiple roles in plant-insect interactions (Rask et al. 2000; Kissen et al. 2009). These defend the plants against the attacks by generalist feeders (Rask et al. 2000) but at the same time expose them to attack by specialist feeders (Renwick 2002; Bjorkman et al. 2011). Glucosinolates are feeding and oviposition stimulants for more than 25 insect species of the orders Coleoptera, Lepidoptera and Diptera (Hopkins et al. 2009). As a consequence of coevolution, insects like *B. brassicae* and *L. erysimi* (both crucifer specialists) can sequester glucosinolates from host plant to protect themselves from predators. These insects can synthesize their own thioglucosidase endogenously, which is spatially separated in the insect body from sequestered glucosinolates in their non-flight muscles. When an insect is crushed or fed upon by a predator, thioglucosidase hydrolyses the sequestered glucosinolates (glucosinolate concentration in haemolymph is normally 15–20 times more than those in the leaf tissue) to produce toxic products (Bridges et al. 2002; Rossiter et al. 2003). These products taste badly and also release volatiles to alarm other insects in the colony. In comparison, the generalist aphid, *M. persicae*, excretes glucosinolates in its honeydew (Hopkins et al. 2009). Another example of coevolution is the production of a glucosinolate sulfatase enzyme (GSS) by the diamondback moth, *P. xylostella* (specialist) (Ratzka et al. 2002), and desert locust, *Schistocerca gregaria* (Forskål) (generalist) (Falk and Gershenson 2007). GSS desulphonates glucosinolates to produce desulphoglucosinolates which are not amenable to hydrolysis by myrosinase. Thus, the production of toxic isothiocyanates is prevented. This enables the insects to feed on glucosinolate-rich plants (Ratzka et al. 2002; Falk and Gershenson 2007). In contrast, *P. rapae* is able to manipulate glucosinolate hydrolysis reaction in such a way that instead of toxic isothiocyanates, less toxic nitriles are formed (Wittstock et al. 2004). Glucosinolates are also known to stimulate larval feeding and oviposition by adults of the large white butterfly, *Pieris brassicae* (L.), and small white butterfly, *P. rapae* (Renwick et al. 1992; Smallegange et al. 2007). These also stimulate

oviposition by *P. xylostella* (Renwick et al. 2006). Many insects such as *B. brassicae* (Nottingham et al. 1991) and *P. xylostella* (Renwick et al. 2006) carry receptor neurons that can detect isothiocyanates to find host location.

Buxdorf et al. (2013) experimented with *Arabidopsis thaliana* mutants having varying levels of glucosinolates and glucosinolate-breakdown products to study the effects of these phytochemicals on phytopathogenic fungi. It was observed that *Alternaria brassicicola* was more strongly affected by aliphatic glucosinolates and isothiocyanates as decomposition products. *B. cinerea* also induced glucosinolate accumulation at a level higher than that by *A. brassicicola*. For *A. brassicicola*, the type of glucosinolate-breakdown product was more important than the type of glucosinolate from which that product was derived. For example, the sensitivity of the *Ler* background and the sensitivity gained in Col-0 plants expressing epithiospecifier protein depended upon the type of breakdown products, both of which accumulate simple nitrile and epithionitriles, but not isothiocyanates. Correlations between identical compounds in different plant tissues permit (co-)regulation of their biosynthesis or emission. The glucosinolate content seemed positively correlated in leaves and other tissues indicating independent regulation of emission (Sotelo et al. 2014; Gupta et al. 2015). However, none of the leaf or flower volatiles was positively associated with gluconapin, glucobrassicanapin or the sum of all glucosinolates in either leaves or flowers. The lack of consistent positive correlations between VOCs and major defence compounds may indicate that plants avoid eavesdropping by specialist herbivores to locate their host plants. Negative correlations may indicate chemical trade-offs for synthesis of the secondary metabolites.

Although glucosinolates play a defensive role in plants against herbivorous insects, there have been concerns regarding increased insect susceptibility of canola cultivars with exceptionally low level of these compounds. Such concerns may be far-fetched since low glucosinolate levels in such cultivars are confined mainly to the seeds (Milford et al. 1989). Also, high and low glucosinolate cultivars did not differ in their susceptibility to pod midge (*Dasineura brassicae*) (Åhman 1982). Extensive studies in India with both *B. napus* and *B. juncea* canola have shown no reasons to believe that canola quality cultivars were more susceptible than their non-canola counterparts. In fact, the inheritance mechanism of glucosinolates in *B. juncea* seemed to be different in leaves and seeds. Major QTLs accounting for a large variation in seeds or leaves were not co-localized (Gupta et al. 2015). Though there are no supporting references, low glucosinolate plants may be less attractive to specialist insects for which these compounds serve as attractants and feeding stimuli (Gabrys and Tjallingii 2002; Mewis et al. 2002). This is supported by the work of Giamoustaris and Mithen (1995) who reported that increase in the content of glucosinolates in *B. napus* resulted in increased feeding damage by the specialist insects, flea beetles [*Psylliodes chrysocephala* (L.)] and greater incidence of small white butterfly (*P. rapae*), while the damage by generalist pests, i.e. pigeons and slugs, was reduced. Further, glucosinolate-rich flower tissues are preferred more by *P. brassicae* and sustain higher growth compared to leaf tissues (Smallegange et al. 2007) indicating the selective role of glucosinolates to elicit feeding in this specialist insect and the adaptation of the insect to use these compounds to its advantage.

6.4.2.2 Phytoalexins and Phytoanticipins

Phytoalexins are antimicrobial secondary metabolites produced *de novo* by plants in response to biotic or abiotic stresses (Bailey and Mansfield 1982; Pedras and Yaya 2010), while phytoanticipins are constitutive defences already present in the plant irrespective of the stress. Plant secondary metabolites can be phytoalexins in one plant species and phytoanticipins in the other.

Polyphenolics – phenolic acids, flavonoids and lignans, terpenoids, phytosterols and alkaloids – have been associated with plant defences. Phenolics, especially the condensed tannins, are feeding deterrents to several pests on *B. napus* (Meisner and Mitchell 1984; Muir et al. 1999). These act by inactivating digestive enzymes (Nguz et al. 1998) or through antibiotic effects (Duffey and Stout 1996). A sinapic acid – precursor of sinapine – has been found to deter the oviposition by *Delia radicum* (L.) on cauliflower plants (Jones et al. 1988). Flavonoids show both stimulatory and deterrent effects on insects feeding on brassica plants. Quercetin and kaempferol from *Armoracia rusticana* stimulated feeding by *Phyllotreta armoraciae* (Koch) (Nielsen et al. 1979) and *P. xylostella* (van Loon et al. 2002). In contrast, isorhamnetin-3-sophoroside-7-glucoside and kaempferol 3,7-diglucoside found in *B. napus* were deterrent to *Mamestra configurata* (Walker), at levels higher than those found in vegetative tissues (Onyilagha et al. 2004). The phytosterols, strophanthidin and strophantidol, found in *Cheiranthus* and *Erysimum* species, exhibited feeding deterrent action against flea beetle species, *Phyllotreta undulata* (Kutschera), *Phyllotreta tetrastigma* (Comolli) and *P. cochleariae* (Nielsen 1978). Camalexin-deficient *A. thaliana* mutants showed greater susceptibility to the cabbage aphid, *B. brassicae* (Kusnierczyk et al. 2008), suggesting the role of camalexin in insect resistance.

6.4.2.3 Volatile Compounds

Volatile compounds are associated with plant-insect communication, plant-pathogen communication and plant-plant communication (Baldwin et al. 2002). These volatiles can be monoterpenes, sesquiterpenes, indole or 'green leafy volatiles' (Tumlinson et al. 1999). The hydrolysis of glucosinolates leads to the production of volatile thiocyanates, isothiocyanates and nitriles. Cabbage seed weevils, *Ceutorhynchus assimilis* (Paykull), are attracted to 3-butenyl and 4-pentenyl isothiocyanate in *B. napus*, but not to 2-phenylethyl isothiocyanate (Bartlet et al. 1993). Similarly, cabbage root fly, *Delia brassicae* L., was attracted to 4-methylthio-3-butenyl isothiocyanate and 1-cyano-4-methylthio-3-butene produced after glucosinolate hydrolysis in *Raphanus sativus* (Ellis et al. 1980). Though different herbivore insects use these volatile compounds as cues to locate their hosts, these also serve as a means of indirect defence against the herbivores. Plants release volatiles following insect attack to attract natural enemies that keep a check on the herbivore insect population. Volatile z-jasmone not only repels *L. erysimi* but also attracts its parasitoids on brassica plants (Birkett et al. 2000). Blande et al. (2007) have reported the attraction of the aphid parasitoid, *Diaeretiella rapae* (M'Intosh) towards semiochemicals produced by turnip plants after feeding by *L. erysimi* (specialist) and *M. persicae* (generalist). Pope et al. (2008) studied the orientation

response of cabbage aphid, *B. brassicae*, and its parasitioid, *D. rapae*, to alkenyl glucosinolate hydrolysis products. The electroantennogram responses indicated peripheral odour perception in *D. rapae* females to all the 3-butenylglucosinolate hydrolysis products.

6.4.2.4 Lectins

Lectins are found across a range of plant, microbial and animal tissues (Nachbar and Oppenheim 1980; Komath et al. 2006; Michiels et al. 2010; Vandenborre et al. 2011). These are the proteins which selectively bind with carbohydrate moieties of glycoproteins that are located on animal cell surface. Lectins incorporated in artificial diets have been shown to reduce performance of several insect pests (Murdock et al. 1990; Powell et al. 1993; Sauvion et al. 2004a; Vandenborre et al. 2011). Although the actual mechanism of insecticidal action is not clearly known, these are not adequately metabolized by digestive enzymes. These can be lethal due to their affinity to epithelial cells in the insect gut (Vasconcelos and Oliveira 2004). They can bind with gut proteins (e.g. glycosylated proteins) with high affinity (Macedo et al. 2004; Sauvion et al. 2004b). Since, lectins interact with mono- and oligosaccharides, the insecticidal activity may involve a specific carbohydrate-lectin interaction with glycoconjugates on the surface of digestive tract epithelial cells (Macedo et al. 2004), precipitating nausea, vomiting and diarrhoea. They may also cause membrane disruption of epithelial cell microvilli of insects fed upon diet containing lectins (Hart et al. 1988). Lectins show biological activity against a range of sap-sucking insects (Foissac et al. 2000; Powell 2001). *Brassica fruticulosa* – a wild relative of cultivated brassicas – appeared to possess resistance against the cabbage aphid, *B. brassicae* (Cole 1994a, b; Ellis and Farrell 1995; Ellis et al. 2000) as well as to *L. erysimi* (Kumar et al. 2011). A high concentration of lectins appeared responsible for the resistance. Feeding preference/choice tests have shown that *L. erysimi* had maximum feeding preference for *B. rapa* ssp. brown *sarson* cv. BSH 1. Least preference was reported for *B. fruticulosa*. The antixenosis to feeding in *B. fruticulosa* has been reported earlier for cabbage aphid, *B. brassicae*. Monitoring of feeding behaviour of this species by electrical penetration graph (EPG) revealed a significant reduction in the duration of passive phloem uptake on *B. fruticulosa* compared to the susceptible *B. oleracea* var. capitata cv. 'Offenham Compacta'. There was either quick withdrawal of stylets from sieve elements or disrupted phloem uptake (Cole 1994a).

6.5 Host Resistance Against Aphids

Brassica plants are among the oldest cultivated plants known to humans with documented records dating back to ca. 1500 BC (Raymer 2002). The domestication of brassica plants resulted in the narrowing of their genetic base. The breeding efforts in brassica plants were largely focused on high yield and desirable quality traits such as low glucosinolates and erucic acid content, and little attention was paid by plant breeders to maintain adequate level of insect and/or disease resistance. All this

led to loss of genes employed by their ancestors to ward off insect herbivores. It may be possible to remobilize lost defensive genes which requires the screening of a large brassica germplasm for resistance against insects which further requires a quick and efficient screening methodology.

6.5.1 Screening Methodology

Many attempts have been made to identify sources of resistance in primary gene pool of crop *Brassica* species (Brar and Sandhu 1978; Amjad and Peters 1992; Sekhon and Åhman 1992; Bhadoria et al. 1995; Saxena et al. 1995). The literature on the screening techniques for aphid resistance has been reviewed extensively by Bakhetia and Bindra (1977). Available screening techniques are summarized in this section.

6.5.1.1 Screening at Seedling Stage

Screening at seedling stage is always desirable since screening at adult plant stage is often laborious and time consuming. However, no serious attempt has been made to correlate seedling stage resistance with the adult plant resistance. Bakhetia and Bindra (1977) have tried to develop seedling screening methodology which is compatible with adult plant evaluation which is based on the seedling mortality at a defined aphid population level. Population levels of 11, 20, 20 and 30 wingless aphids and 1 ml and 3 ml aphids (1 ml = about 600 nymphs + wingless adults) per plant appeared optimal for resistance screening at cotyledonary, 2-leaf, 4-leaf, 6-leaf, flower bud initiation and flowering stages, respectively (Sekhon and Åhman 1992). The results obtained at all the test stages were comparable when screening was conducted under optimum level of aphid population per plant. The effect on the survival and fecundity was also similar at all the stages studied. Despite its advantages, this screening technique is not widely used for brassica germplasm screening against aphids.

6.5.1.2 Screening at Adult Plant Stage

Adult plant screening is the most widely used method for screening against aphids. Though it is laborious and time consuming, it reflects the resistance shown by plants under actual field conditions. It is based on the different injury symptoms manifested upon aphid feeding such as yellowing, curling, crinkling of leaves, drying of flower buds and flowers and shrivelling of developing pods. Different workers have adopted different grading systems, but the one published by Bakhetia and Sandhu (1973) is generally adopted for screening at adult plant stage. A major limitation of this method is the failure to account for different phenologies of the test genotypes. Late flowering genotypes are sometimes misclassified as resistant as flowering initiations in late genotypes may coincide with season end high temperatures, which are invariably less than congenial for aphid infestation.

Different injury grades are given to the test entries on the basis of degree of insect damage.

Aphid infestation index (AII)	Description
0	Free from aphid infestation. Even if a single wingless aphid is present, the plant is considered infested. Plants showing excellent growth
1	Normal growth, no curling or yellowing of the leaves, except only a few aphids along with little or no symptoms of injury. Good flowering or pod setting on almost all the branches
2	Average growth, curling and yellowing of a few leaves. Average flowering and pod setting on all the branches
3	Growth below average, curling and yellowing of the leaves on some branches. Plants showing some stunting, poor flowering and little pod setting
4	Very poor growth, heavy curling and the yellowing of leaves, stunting of plants, little or no flowering and only a few pods forming. Heavy aphid colonies on plants
5	Heavy stunting of plants, curling, crinkling and yellowing of almost all the leaves. No flowering and pod formation. Plants full of aphids

A specific injury grade is given to every observed plant, and the aphid infestation index (AII) is worked out by multiplying the number of plants falling under each grade with the respective grade number. The AII is calculated at pre-flowering, flowering and pod formation stages as

$$\text{Aphid Infestation Index} = \frac{(0\times a)\pm(1\times b)\pm(2\times c)\pm(3\times d)\pm(4\times e)\pm(5\times f)}{a+b+c+d+e+f}$$

where a, b, c, d, e and f are the number of plants falling under each injury grade.

The different test entries are classified into different resistance categories based on the AII as

Aphid infestation index (AII)	Reaction
0.00–1.50	Resistant
1.51–2.50	Moderately resistant
2.51–3.50	Susceptible
> 3.50	Highly susceptible

The higher the AII, the lower the level of resistance in an entry

6.5.1.3 Other Screening Methods

Only limited attempts have been made to develop a screening technique based on the biology of mustard aphid, despite its significance in identifying sources of resistance. According to Bakhetia and Bindra (1977), it is possible to develop such a criterion for screening since nymphal survival, fecundity, longevity and reproduction are similar at all the plant growth stages. Singh et al. (1965) and Malik (1981) have also reported fecundity to be inversely related to resistance. Aphid population at a particular stage and an increase in the population during a given time interval can also be used in germplasm screening (Bakhetia and Sekhon 1989). More recently, Kloth et al. (2015) have demonstrated the use of automated video tracking for phenotyping of plants for resistance to aphids. Though this method can be used

to screen a large number of accessions at a time, it has the limitation that it uses the leaf discs instead of intact plants and, hence, does not reflect the actual resistance exhibited by plants. The resistance effect was partially lost in the leaf discs. However, this limitation can be overcome by the use of electrical penetration graphs (EPG) (Tjallingii 1988; Trebicki et al. 2012) which uses the intact leaf instead of leaf disc, but this technique has its own high equipment cost limitation.

6.5.2 Breeding for Aphid Resistance

Three different mechanisms are responsible for imparting insect resistance to plants: antixenosis, antibiosis and tolerance. Antixenosis is rarely effective under no-choice conditions since insects can learn to feed on the less preferred plant. In contrast, antibiosis puts a selection pressure on the insects, and there is always a risk of development of insect biotypes, a danger not applicable to tolerance. Tolerance imparts least pressure on the insect to adapt. A sustainable resistance results from amalgamation of all three mechanisms (Smith 1989).

Different breeding methods have been used to develop resistant cultivars. These include intervarietal hybridization, induced mutagenesis or autotetraploidy. *B. napus* strains and colchicine-induced tetraploid *toria* (*B. rapa*) appeared more resistant to mustard aphid in contrast to diploids (Rajan 1961; Singh et al. 1965; Jarvis 1970; Gill and Bakhetia 1985; Kalra et al. 1987), and the resistance was attributed to be due to antibiosis; however, these were cytogenetically unstable. Many workers have also attempted to artificially synthesize alloploids of *B. napus* (Prakash and Raut 1983) and *B. rapa* x *Eruca sativa* (Agnihotri et al. 1990 as cited from Sekhon and Åhman 1992), but these were not resistant to the aphids.

In the past, Lammerink (1968) attempted to develop cabbage aphid-resistant variety of rape after selection in the F_3 generation of the cross (Broad Leaf Essex rape x Colder Swede) x giant rape. He also attempted recurrent selection in the crosses involving purple top white Globe and Sjodin turnip for breeding mustard aphid-resistant variety. Recently Kumar et al. (2011) reported wild *B. fruticulosa* (Plate 6.1) to be resistant to mustard aphid and described attempts at the introgression of resistance gene(s) from *B. fruticulosa* to *B. juncea*. *B. fruticulosa* have been previously reported to possess resistance against the cabbage aphid, *B. brassicae* (Cole 1994a, b, Ellis and Farrel 1995, Ellis et al. 2000). Study of feeding behaviour of *B. brassicae* electronically by electrical penetration graph (EPG) showed a large reduction in the duration of passive phloem uptake from *B. fruticulosa* compared to *B. oleracea* var. capitata cv. 'Offenham Compacta'. There was either quick withdrawal of stylets from sieve elements or disrupted phloem uptake (Cole 1994a). Ellis and Farrel (1995) concluded that resistance of *B. fruticulosa* was due to high levels of both antixenosis and antibiosis. The resistance in *B. fruticulosa* due to antibiosis against *D. radicum* has also been reported by Jenson et al. (2002). *Rorippa indica* is another wild crucifer which is resistant to mustard aphid, and the genes conferring resistance have been recently identified by Bandopadhyay et al. (2013). Sarkar et al. (2016) have cloned, purified and characterized a novel *R. indica*

Plate 6.1 (**a**) *Brassica fruticulosa* – a wild crucifer resistant to mustard aphid (**b**) Susceptible introgression line (**c**) One of the resistant introgression lines

defensin (RiD) which is toxic to *L. erysimi*. This aphid resistance trait can also be successfully introgressed to the cultivated backgrounds as demonstrated by somatic hybrids and their backcross progenies (Mandal 2003; Dutta 2007).

In addition to this, different workers have attempted to induce mutations in *B. juncea* for aphid resistance through chemical (Srinivasachar and Verma 1971) and physical mutagens (Srinivasachar and Malik 1972; Labana 1976), but all these efforts did not yield any result.

6.5.3 Genetic Engineering for Aphid Resistance

An alternative strategy to conventional breeding is the transgenic technology. For phloem-feeding insects, the different strategies can be employed such as expression of protease inhibitors, RNA interference (RNAi), antimicrobial peptides and repellents.

The *Cauliflower mosaic virus* (CaMV) 35S promoter is used to control transgene expression in many transgenic plants (Will and Vilcinskas 2013) which regulates the expression of a β-glucuronidase (GUS) reporter gene for the expression of dsRNA to protect the plants against the coleopterans (Baum et al. 2007) and aphids (Pitino et al. 2011).

The phloem-specific promoters can be used for phloem-specific expression of defence-related compounds against aphids. This would lead to more targeted expression of defence-related compounds with little/no exposure to the nontarget insects. This would also limit GM-associated bioenergetics investment of plant by avoiding the expression of defence-related compounds in plant tissues in the absence of pest attack. The *SUC2* promoter that regulates the *AtSUC2* sucrose-H^+ symporter gene is restricted to the plant phloem which produces aphid toxic proteins. This green florescent protein is transferred through the sieve elements where aphids actually feed (Imlau et al. 1999). Protease inhibitors (PIs) can also be used to confer resistance in plants against different insects including aphids by genetic engineering. These small peptides/proteins reduce or inhibit the activity of proteases required for digestion of proteins. They have been shown to be toxic to a number of pests belonging to order Lepidoptera, Coleoptera and Orthoptera (Boulter et al. 1989). Their potential as insecticidal proteins has also been explored in aphids. PIs ingested with phloem sap may disrupt the digestion of proteins in aphid gut and hence can interfere with normal amino acid assimilation leading to the reduction in growth and subsequent pest damage. The expression of trypsin inhibitors and other PI-like chymotrypsin inhibitors has already been achieved in the phloem of transgenic plants (Dannenhoffer et al. 2001; Kehr 2006). The cysteine protease inhibitor of barley, HvCPI-6, inhibited the performance of *M. persicae* and *Acyrthosiphon pisum* (Harris) in artificial diet (Carrillo et al. 2011). Similarly, cysteine protease inhibitors, oryzacystatin I (OC I), inhibited the growth of *M. persicae*, *A. gossypii* and *A. pisum* (Rahbé et al. 2003). A reduction in adult weight, fecundity and biomass of *M. persicae* fed on transgenic *B. napus* expressing (OC I) was observed in comparison with those fed on control plants. PIs were also shown to defend white cabbage

cultivars and *A. thaliana* against *B. brassicae* (Broekgaarden et al. 2008). PIs, thus, show detrimental effects against aphids, and their use in aphid management, therefore, appears to be an effective strategy for pest management.

Lectins are another class of proteins that have toxic effects on aphids and have the potential to be used for aphid control through genetic engineering. These are the proteins that selectively bind carbohydrates and the carbohydrate moieties of glycoproteins and can be poisonous. Lectins have been reported to show biological activity against a wide range of insects, especially the sap-sucking insects (Foissac et al. 2000; Powell 2001). Genes encoding wheat germ agglutinin from *Triticum* spp. (Kanrar et al. 2002), ACA from *Allium cepa* (Hossain et al. 2006), fusion ASAL from *A. sativum* and ACA from *A. cepa* (Hossain et al. 2006) have been introduced into Indian mustard, *B. juncea*, that provide protection against the mustard aphid, *L. erysimi.* These transgenic plants showed significant toxic effect against *L. erysimi* as evidenced by bioassays under controlled conditions.

Another method of aphid control through transgenic technology is the RNA interference (RNAi), which involves gene suppression at the level of RNA and involves post-translational RNA-mediated gene silencing. The transgenic plants that delivered dsRNA to aphids resulted in inhibition of Rack1 (located in the gut) and C002 (located in the salivary gland) proteins in peach-potato aphid, *M. persicae* (Pitino et al. 2011). The transformed plants of tobacco and *A. thaliana* resulted in reduction in fecundity of aphids with up to 60%t silencing in feeding aphids. Although salivary proteins (Mutti et al. 2006, 2008) and gut proteins (Shakesby et al. 2009) are the most promising RNAi targets for insects with piercing and sucking mouthparts such as aphids, the other targets may include transporters in the bacteriocyte plasma membrane required for nutrients' transport between aphids and their endosymbiont, *Buchnera aphidicola*.

6.6 The Way Forward

Plant resistance to aphids has great potential in managing populations of these important insect pests. Earlier efforts by plant breeders have focused on host plant resistance as a single component of pest management, and hence, greater emphasis was laid on screening for virtual immunity to aphids. Such extremely high level of resistance can result from very high level of toxic (to aphids) substance in the plant, which has many disadvantages such as continuous selection pressure on the insect population to develop resistant biotypes, possible side effects on natural enemies as well as yield drag. Thus, for sustainable pest management, partial resistance to insects has the potential for the future. Such partially resistant cultivars can be integrated with other methods of pest management, which is the main feature of IPM. The effective IPM strategy against aphids infesting rapeseed-mustard could not be developed due to a lack of resistant variety. This is primarily because of lack of in-depth knowledge about the mechanism of resistance. Though transgenics conferring resistance to aphids have been developed, their efficacy in reducing aphid

populations had been evaluated under controlled environments, and field testing of such transgenics is still awaited.

In addition to the inherently or transgenically expressed toxins in plants, other methods to reduce aphid populations on plants can also be developed. Since aphids utilize many secondary plant compounds especially volatiles in host plant recognition, plants can be genetically manipulated to alter their volatile profile, and limited success has been achieved under laboratory conditions (Beale et al. 2006; Schnee et al. 2006). It is a well-known fact that aphids reproduce at exceptionally high rate. A single mother aphid can produce 5.9 billion offspring in 6 weeks (Dixon 2005). Thus, disrupting the host recognition process of a mother aphid can significantly reduce the offspring population. However, this is a theoretical concept, and there is no report highlighting the validity of this strategy. Another potential area of research is the genetic manipulation of induced resistance in plants which is influenced by jasmonic acid (JA), salicylic acid (SA) and ethylene. The associated signalling pathways can be altered genetically to enhance the innate plant resistance level.

An effective and sustainable aphid management requires the adoption of integrated pest management (IPM) strategy. Since host plant resistance forms the core of any IPM programme, there is no effective IPM programme against aphids infesting brassica crops due to the lack of resistant crop cultivars. Rather than complete resistance to aphids, it is the partial resistance that has greater potential for the future, to maintain sustainability of pest management systems.

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