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## Mutation Breeding as a Tool for Aphid Resistance in Indian mustard [Brassica juncea (L.) Czern & Coss]

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ABSTRACT: One of the most important oilseeds in India is mustard [*Brassica juncea* (L.) Czern & Coss]. The country's edible oil demands are met in part by its production. Because they have the ability to exploit ephemeral habitats quickly, aphids are specialised sap feeders and dangerous pests. Aphids can be controlled effectively, economically, and environmentally through the creation of an aphid-resistant genotype. As part of their physiochemical defense mechanisms, Brassica plants produce glucosinolates, isothiocyanates, lectins, volatiles, and alkaloids, as well as surface waxes and trichomes to deter infestations of insects. The lack of available resistant sources of intercrossable germplasm in brassicas as well as poor knowledge of trait genetics has hindered aphid resistance breeding in brassicas. The mustard aphid, *Lipaphis erysimi*, is a primary pest in most mustard-growing regions, causing yields to decline by 35.4%-96.0% and oil content to drop by 5-15%. Induced mutagenesis is a reliable method of introducing novel variations within a crop variety in our agricultural development efforts because it has demonstrated its ability to introduce genetic diversity.

Keywords: Indian mustard, mustard aphid, genetic variability, mutagenesis, biophysical, biochemical.

## INTRODUCTION

Crop brassicas are members of the Brassicaceae family. It is a large angiosperm family with about 3200 species distributed in over 375 genera (LeCoz and Ducombs 2006). According to the US Department of Agriculture, India is responsible for 19.8% of the total area and 9.8% of the total production globally. In all mustardgrowing regions of the country, the mustard aphid, L. erysimi, is the main pest. It is a national pest that is major lag in the commercial cultivation, leading to reduction in yield by 35.4-96.0 per cent and in oil content by 5-15 per cent. Scientists in the fields of agronomy and soils have contributed to insecticide use with enhanced protection. The long-term use of these products, however, has had a negative effect on the environment and subsequently on human health, so plant resistance to insects is of increasing importance for protecting both the environment and human health. It is impossible to breed for genetic resistance to aphids

due to limited resistance source within crossable germplasms and an incomplete understanding of the trait's genetics. To achieve this difficult breeding goal, genetic engineering using insect resistance transgenes seems to be the only viable option. Genetic engineering has made some progress toward producing aphidresistant cultivars, but commercialization of such crops is still a long way off. An improvement effort in crop yield must include genetic variability, and induced mutagenesis has been proven as a reliable tool for adding new phenotypic variations to a crop variety.

**Brassica Aphid Complex.** Aphids are a worldwide problem. Although they are a small insect group, they inflict considerable damage on agricultural crops (Remaudière and Remaudière 1997; Dedryver *et al.*, 2010). In the family Aphididae, there are around 5000 species (Smith and Chuang 2014), 100 of which are known to be particularly harmful to crops (Blackman and Eastop 2000, 2007). Aphids that attack brassica crops include the cabbage aphid [*Brevicoryne brassicae*]

(L.)], the turnip/mustard aphid (Lipaphis erysimi (Kaltenbach)/Lipaphis pseudobrassicae (Davis)] as well as root-sap sucking species, such as the cabbage aphid and poplar petiole gall root aphid [Pemphigu Eastern Asia is home to *L*. erysimi (Blackman and Eastop 2000). Oilseed brassica are prone to this pest, a problem that is particularly acute in India and other subtropical locations. It may result in 10-90 percent production losses (Singh and Sachan 1994; Ahuja et al. 2009) depending on the agroclimatic circumstances, population development and crop growth stage. It is a brassica specialist that is able to grow only on brassica species. B. rapa and B. juncea, in general, seem to be better hosts than other Brassica species (Rana 2005). L. erysimi is known to carry 10 non-persistent viruses that affect plants, such as cabbage black ringspot and mosaic disease in cauliflower, radish, and turnip (Blackman and Eastop 1984; Rana 2005). B. brassicae is a European native pest that affects vegetable brassicas in most European nations, reducing yields greatly. Feeding on phloem sap is the pest's specialty. (Cole 1997). It is primarily a pest of vegetable brassicas, although it also infests other Brassica species (Cole 1994a, b, 1997; Kift et al. 2000). Aphid biology. An aphid is a specialist sap feeder that feeds on phloem sap. They are dangerous pests because of their capacity to quickly exploit ephemeral habitats. Their flexibility is enhanced by their high reproductive potential and dispersal abilities (Dedryver et al. 2010). Aphids have parthenogenetic viviparity, which means they don't need males to fertilise females and don't have an egg stage in their life cycle. Aphids reproduce clonally and give birth to offspring in this way. An aphid's embryonic development begins before its mother's birth, resulting in generational telescoping. These characteristics allow aphids to take advantage of moments of high plant development while conserving energy and allowing rapid generation advancement. In some species of aphid, the nymphs mature in just five days (Goggin 2007). The process of parthenogenesis distinguishes them from other Hemiptera and plays an important role in their biology. Aphids tend to have alternate generations, as do many other insects. A series of parthenogenetic, all-female generations evolved during the Triassic period after alternating generations of hermaphrodites (Blackman and Eastop 2007). In conjunction with viviparity, this reduces the development period and speeds up aphid proliferation. Depending on resources, aphid colonies may also produce extremely fertile wingless morphs or less productive winged (alate) progeny that can spread to other host plants. Aphids in temperate zones benefit from all of these tactics. Under ideal conditions, a high propagation rate results in an abnormally large population (Goggin 2007).

Aphid Life Cycles. The life cycles of most aphid species are highly complicated, and each life cycle has

morphs that specialize in reproduction, dispersal, and survival under harsh environmental conditions. Based on how they use their host plants, aphids have heteroecious or host alternate life cycles, or monoecious/autoecious or nonhost alternate life cycles. In heteroecious aphids, the winter is spent on one plant species (the primary host), the summer is spent on a diverse range of plants (the secondary host) and the autumn is spent on the original host. On the major host plant, the females lay eggs after mating with the males. However, they reproduce parthenogenetically on the secondary host plant. Aphids with homocyclic reproduction alternate between parthenogenetic and sexual reproduction. Both sexual and parthenogenetic life cycles are completed on the same host species. The anholocyclic species don't reproduce and don't produce eggs. It is possible for some species to live anholocyclic and holocyclic lives simultaneously in different parts of the world (Bhatia et al. 2011). On rare occasions, monoecy and heteroecy can coexist (Williams and Dixon 2007). The aphid can benefit from parthenogenesis and genetic recombination because it has both sexual and asexual life cycles.

The holocyclic *Lipaphis erysimi* has 2n = 10 chromosomes (Blackman and Eastop, 2000). Despite the fact that it reproduces parthenogenetically in warmer climates, holocyclic reproduction has been observed on cruciferous crops (*B. rapa, Raphanus sativus*) in western Honshu, Japan (Kawada and Murai 1979). The chromosome number of two northern European populations is 8 and the karyotype is different from holocyclic populations. In the majority of anholocyclic parthenogenetic populations, 2n = 9 was most likely created by dissociating one autosome to produce a tiny, unpaired element. North India did have sexual morphs, but the population tended to be anholocyclic (Blackman and Eastop 2007).

The monoecious species Brevicoryne brassicae exhibits a holocyclic life cycle and parthenogenic reproduction in both warm and temperate environments. As the temperature drops in the autumn, males emerge, mate with the females, and produce eggs for overwintering (Blackman and Eastop 1984). According to Hines and Hutchison (2013), there are about 15 overlapping generations in a crop season in the United States.

A holocyclic life cycle suggests that Myzus persicae spends the winter as an adult prodding its primary host (peach trees). In the following spring or summer, fundatrixes or fundresses (the winged stem mother) emigrate to secondary hosts, multiplying into apterous and alate viviparae (Moran 1992; Bhatia *et al.* 2011). By parthenogenesis, or parturition to offspring, the wingless female reproduces rapidly. This causes large populations of aphids to grow on a variety of crop plants. A few apterous vivipares become apterous oviparae later in the season, while alate vivipares become alate males when the temperature drops. They

produce eggs and begin amphimixis on the host plant (Stern 1995). From the eggs laid during the previous winter, the embryonic females (stem moms) hatch and reproduce parthenogenetically the following spring (Bhatia *et al.* 2011).

Aphid-Host Plant Interactions. In order to avoid/overcome plant defenses, aphids inject needlelike stylets into plant tissue to feed on phloem sap. In order to maintain the viability of the phloem cells, they extract a large amount of phloem sap. Aphids are capable of penetrating epidermal and parenchymal cells with their stylets (Bhatia et al. 2011) with little physical harm to their hosts (Bhatia et al. 2011). In addition to making intracellular punctures to observe the chemical chemistry of the cell (Zust and Agrawal 2016), stylets also migrate through intercellular spaces in the apoplasm (Giordanengo et al. 2010). Passive feeding is aided by the high pressure within sieve tubes (Bhatia et al., 2011). In the process of penetrating and feeding, aphids produce two types of saliva. Essentially, the stylet is covered with a thick, proteinaceous coating that forms a tunnelled intercellular channel (containing peroxidases, pectinases. peroxylases. and glucosidases) (Felton and Eichenseer 1999; Zust and Agrawal 2016). As well as proteins, this gelling saliva consists of phospholipids and conjugated carbohydrates (Urbanska and Miles, 1998; Cherqui and Tjallingii, 2000; Sharma and Tjallingii, 2014). The stylet sheath acts as a physical barrier between the feeding site and the plant's immune response according to Will et al. (2012). Feeding aphids release digestive enzymes into the phloem sap flow when they come into contact with the stylets. They do this through the release of a second kind of saliva in the vascular tissue. To prevent protein coagulation, watery saliva (E1) is injected into plant sieve tubes; during feeding, watery saliva (E2) is combined with sap to prevent protein accumulation inside the capillary food canal (Bhatia et al. 2011; Sharma et al. 2014; Zust and Agrawal 2016). Calciumbinding proteins found in aphid saliva interact with calcium in plant tissues, but it is not known how they prevent protein coagulation. Due to the calciumdependent occlusion of sieve tubes being suppressed, the plant response is delayed (Will et al. 2007, 2009, 2013). As a result of this feeding method, the aphid is able to avoid allelochemicals and indigestible substances found in other plant tissues (Schoonhoven et al. 2007). In the presence of oxidizing enzymes, aphid saliva also contains nonenzymatic reducing chemicals that inactivate a number of defense-related molecules produced by plants in response to insect attacks (Miles 1999).

When plants are fed by insects or infected by pathogens, similar events take place. The release of ROS such as hydrogen peroxide is among the effects of protein phosphorylation, calcium influx, membrane depolarization, and other factors (Garcia-Brugger *et al.* 

2006). A result of this is activation of phytohormonedependent pathways. In response to infestation or infection, several phytohormone-dependent mechanisms become active. In contrast, necrotrophic pathogens trigger salicylate-dependent responses (Thomma et al. 2001) and grazing insects trigger ethylene (ET) and jasmonate (JA) pathways (Maffei et al. 2007). Various antixenotic or antibiotic proteins and secondary metabolites are produced as a result of these responses. An SA-dependent response was observed when the plant was infested by aphids. The opposite was true for JA-dependent genes (Zhu-Salzman et al. 2004; Thompson and Goggin 2006; Gao et al. 2007; Walling 2008). The plant metabolism changes in response to all of these reactions, ensuring the compatibility of aphid-plant interactions.

Defence mechanism in mustard. There are many biophysical and biochemical mechanisms by which Brassica plants defend themselves against insects. An insect's preference may be determined by a plant's anatomical and morphological characteristics (Southwood 1986). These characteristics include epicuticulular wax, trichomes, vascular bundle depth, and the production of toxic chemicals such as glucosinolates, isothiocyanates, lectins, volatiles, and alkaloids. An insect's ability to eat, probe, or oviposit on a plant depends on the chemical composition of epicuticular wax since it is the first point of contact between the insect and the plant. It has been discovered that waxiness prevents L. erysimi from feeding on the underside of leaves during the vegetative plant stage (Åhman 1990). Since waxes on leaf surfaces have been shown to interact with polar compounds like glucosinolates, which are important cues for insects to identify their hosts (Badenes-Pérez et al. 2010; Städler and Reifenrath 2009), waxes have attracted a lot of attention over the last few years. Glucosinolates (GSLs) are secondary metabolites found in Brassica plants. Insects may be resistant to glucosinolates, but the breakdown products created by myrosinase hydrolysis can be more toxic. It converts glucosinolates to aglycone molecules (thiohydroxamate-O sulfonate), glucose. and sulfate through thioglucoside glucohydrolase (EC 3.2.3.1). The aglycone molecules can be rearranged to form isothiocyanates (ITCs), thiocyanates, nitriles, amines, oxazolidinethiones, and epithionitriles depending on the condition under which the glucosinolate is hydrolyzed (Rask et al. 2000; Sadasivam and Thayumanavan 2003). There is a substantial variation in plant glucosinolate content depending on species, plant parts, and climatic and agronomic conditions (Font et al. 2005; Tripathi and Mishra 2007). Gluconasturtin is not present in the seed at first. Glucosinolate concentrations increase during subsequent growth stages (Clossais Besnard and Larher, 1991). Glucosinolate concentrations in vegetative plant parts and inflorescences, which contain

relatively large amounts of glucosinolates, have decreased. Siliques produce glucosinolates during seed development, and these siliques are then transferred to the seeds via pod shells (Rask et al., 2000). The inheritance process for glucosinolates in B. juncea leaves and seeds appeared to be different. A significant QTL responsible for a significant difference in seeds or leaves was not found to be colocalized (Gupta et al., 2015). Nitroriles and thiocyanates are produced when glucosinolates are hydrolyzed. Plant-pathogen, plantplant, and plant-insect communication are all connected to volatile chemicals (Baldwin et al., 2002). Indoles, monoterpenes, sesquiterpenes, and 'green leaf volatiles' also represent volatiles (Tumlinson et al., 1999). As a reaction to insect attacks, plants produce volatiles to attract natural enemies that regulate her bivore insect population. The volatile z-jasmone repels L. erysimi and attracts its parasitoids on brassica plants (Birkett et al., 2000).

Trichomes may influence insect herbivory on leaves. A trichome is a branching hair-like structure produced by cells of the aerial epidermis in almost all plant species (Werker 2000). Secondary metabolites (such as flavonoids, alkaloids, and terpenoids) are produced by glandular trichomes. They can be either poisonous or repellent to insects (Duffey 1986). Polyphenolic compounds are thought to be involved in plant defenses, including phenolic acids, flavonoids, lignans, terpenoids, phytosterols, and alkaloids. Several pests on B. napus are deterred by phenolics, particularly condensed tannins (Meisner and Mitchell 1984: Muir et al. 1999). Flavonoids have stimulatory and deterrent effects on insects that feed on brassica plants. They act either by blocking digestive enzymes or by acting as antibiotics (Nguz et al., 1998). (Duffey and Stout 1996). In addition to serving as structural defenses against tiny herbivores, non-glandular trichomes obstruct insect movement on the plant surface (Southwood 1986). More importantly, trichomes are important to host plant acceptability for oviposition (Sadeghi 2002), and there was significantly less oviposition on Arabidopsis thaliana lines with higher trichome density (Wietsma 2010).

The anatomy of sieve tube elements, particularly depth plays key role in a plant's aphid resistance. Aphids require to have long stylets to feed on plant tissues having deeply localised vascular bundles. (Gibson 1972). Furthermore, such aphids need more energy to probe deep into the plant tissue, while aphids with short stylets ought to starve and die (Berlinski, 1965).

Lectins are formed in plant, microbial as well as animal tissues (Nachbar and Oppenheim 1980; Komath *et al.* 2006; Michiels *et al.*, 2010; Vandenborre *et al.* 2011).These are the proteins which bind to the carbohydrate moieties of glycoproteins present on the surface of animal cells. (Murdock *et al.* 1990; Powell *et al.* 1993; Sauvion *et al.* 2004a; Vandenborre *et al.*,

2011). It has been found that lectins in artificial diets reduce the performance of various insect pests. Lectins have been found to have biological activity against a variety of sap-sucking insects (Foissac et al. 2000; Powell 2001). Brassica fruticulosa, a wild relative of farmed brassicas, appeared to be resistant to the cabbage aphid, B. brassicae (Cole 1994a, b; Ellis and Farrell 1995; Ellis et al., 2000), as well as L. erysimi (Kumar et al., 2011). The resistance appears to be caused by a high concentration of lectins. The Indian mustard cultivars cv ashirwad and cv Pusa mahak expressed a considerable number of defence proteins after mustard aphid infection. Aphid resistance in mustard cultivars is connected to the level of expression of such proteins. F26A19.13, a Pentatricopeptide repeat-containing protein, has been found as one of the most essential defence proteins in the plant-insect defence cross-talk because it directly reduces the action of aphid salivary amylase (Ghosh 2021).

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 has slowly narrowed their genetic base. The breeding efforts in brassica have been largely centred around high yield and quality traits such as low glucosinolates and erucic acid content, and little focus has been given to maintain an substantial level of insect and/or disease resistance. All this led to the depletion of genes employed by their ancestors to act against insect herbivores. Remobilization of lost defensive genes which require the screening of large brassica germplasm for resistance against insects is possible but in turn requires a quick and efficient screening methodology. Conventional phenotype-based breeding must be backed up with novel molecular techniques. A pathogen-responsive gene panel was created and is now being utilized in expression-assisted breeding effort to produce aphid-tolerant varieties. SNAP gene which is induced during senescence and pathogen infections (Sun et al. ,2012; Espinoza et al., 2007), MAPK gene which regulate stress responses, developmental programs and innate immunity (Rodriguez et al. 2010; Taj et al., 2011) and LOX gene (Taj et al., 2011) are being explored and incorporated in breeding programmes.

**Nutation breeding in Indian mustard for aphid resistance.** Because of a narrow genetic base, *B. juncea* is sensitive to insect pests, resulting in significant economic losses. Induced mutagenesis has proven to be a reliable means of introducing unique variants within a crop variety, therefore any agricultural development effort must contain genetic variability. The efficiency of this technology has been demonstrated by the release of induced mutants as new varieties in several crops. When no gene, or genes, promoting insect resistance or stress tolerance can be located in the accessible gene pool, plant breeders have no choice but to try mutation induction. Mutagenic effectiveness and efficiency of gamma rays in Indian mustard were recommended, with LD50s starting from 1000 Gy (pollen sterility) to 1200 Gy or higher (survival reduction) being employed for gamma-ray treatment in *brassica juncea*. Based on the percentage of Indian mustard seeds germination of two cultivars Narendra Rai and NDYR 10, EB was the most effective mutagen, followed by MH, Ac, EMS and gamma-radiation.

Usually, mutagenic treatment is applied to seeds, which are then referred to as M1 seeds. As fatty acids are largely under embryogenic control, mutations in the M2 generation can be discovered by examining M2 half-seeds. As a result, mutations can be found after only one year of plant culture.

In the M2 generation, many aphid (*Lipaphis pseudobrassicae*) resistant plants were isolated in two commercial varieties of *Brassica juncea*, Laha 101 and R.L. 18, treated with varying amounts of the mutagens, ethyl methane sulphonate (EMS), maleic hydrazide (MH), ethylene imine (EI), diethyl sulphate (DES) and different doses of gamma rays. EMS and gamma-rays causing more aphid resistant mutations in Laha 101, while HA and EI inducing more aphid resistant mutations in R.L. 18. Because aphid resistant mutations, efficient pest management could be achieved by concentrating the resistant genes in a single strain.

An effective screening technique can identify such resistant plants. There are currently no viable tolerant cultivars in *Brassica juncea* with systemic plant responses in the form of direct or indirect defences against aphid attacks that have been created using traditional methods. One of the primary reasons for the delayed development in generating resistant variants of *Brassica juncea* is the lack of particular procedures for screening large numbers of genotypes required in breeding for the selection of tolerant cultivars in mustard. For prospective genotype selection and cultivar development in *Brassica juncea*, traditional phenotype-based breeding must be supplemented with new molecular techniques.

During the Rabi season, 2016-17 at the Research Farm of the Agricultural Botany Section, College of Agriculture, Nagpur, Maharashtra, India, 138 mutants of the mustard Pusa Bold variety were screened for resistance to the mustard aphid, *Lipaphis erysimi* (Kalt.) in field conditions. The aphid infestation index and aphid population count were used to screen the plants. Aphid infestation indexes ranged from 0.78 (M63 Pusa Bold 1100 gy) to 4.43 (M351 Pusa Bold Control-2). Among all the observed mutants, 14 mutants were classified as extremely resistant (0.78–0.77), 22 mutants as resistant (1.15–1.95 aphid infestation index), 39 mutants as moderately resistant (2.19–2.87 aphid infestation index), and 63 mutants as susceptible (3.11 to 4.43 aphid infestation index). Highly resistant mutants may be employed as donor parents in the development of mustard aphid-resistant cultivars.

Binasarisha-7' and Binasarisha-8' was developed by irradiating seeds from the BARI Sarisha-11 cultivar. M1 was produced in 2004-05 after the seeds were treated to various doses of gamma rays (600, 700, 800, and 900 Gy). In the M3 generation in 2006-07, 32 mutant lines were chosen based on their superior field performance, including seed output per plant and other essential agronomic characteristics, when compared to the mother variety (BARI Sarisha-11). These materials were grown in M4 and M5 generations to investigate their breeding behaviour in terms of seed yield and yield contributing features. The mutant MM-10-4 and MM-08-4 out-yielded the BARI Sarisha-11 in preliminary, advance, and regional yield studies conducted from 2008 to 2011. In addition, the mutant lines MM-10-4and MM-08-4 are found to be somewhat resistant to stem rot, tolerant to Alternaria blight, and had a reduced aphid infestation rate than the control variety. For commercial cultivation, MM-10-4and MM-08-4 was chosen for registration as 'Binasarisha-7' and 'Binasarisha-8' respectively.

## CONCLUSION

In India, the mustard aphid is the most devastating insect in the Brassica family, producing major crop losses. There are currently no viable Brassica juncea tolerant cultivars with systemic plant responses in the form of direct or indirect aphid defences that have been developed using traditional methods. The lack of resistant sources within crossable germplasm, as well as a lack of knowledge about trait genetics and specific procedures for screening large numbers of genotypes required in breeding for the selection of tolerant cultivars in mustard, are the primary reasons for the delay in developing resistant variants of Brassica juncea. Induced mutagenesis has proven to be a reliable means of introducing unique variants within a crop variety, therefore any agricultural development effort must contain genetic variability. The efficiency of this technology has been demonstrated by the release of induced mutants as new varieties in several crops. When no gene, or genes, promoting insect resistance or stress tolerance can be located in the accessible gene pool, plant breeders have no choice but to try mutation induction. These highly resistant mutants developed by mutagenesis can be employed in future breeding programmes to generate aphid resistant/tolerant cultivars, which will result in increased mustard yield while minimising the usage of toxic chemical insecticides.

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