Chapter

Innovative Strategies to Develop Abiotic and Biotic Stress Tolerance in Mustard (Brassicaceae)

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Abstract

Mustard crop is the third important source of vegetable oil randomly below soybean *L*. and palm, all over the world. Brassica crop is extremely susceptible to some biotic and abiotic stresses and they significantly influence the quality and quantity of the crop. In the past generally breeding techniques are used to develop resistance in mustard to avoid diseases though various pathogens are soon able to overcome that resistance by modifying their metabolic cycles. To bear the challenge there is an urgent need to develop abiotic as well as biotic stress tolerant plants using advanced techniques by understanding metabolic and biochemical pathways of plants and pathogens. Several techniques such selection of stress tolerance microbes, metabolite, enzymes, and genes are very important to avoid stresses. Whereas several techniques such as deployment of molecular markers for breeding, identification of Quantitative trait loci (QTL), *in vitro* tissue culture etc. can be more useful to improve biotic and abiotic stress tolerance in mustard. To develop healthy and high yield varieties, the mix of these techniques is needs to be implemented.

Keywords: stress tolerance, mustard, molecular approaches, biological approaches

1. Introduction

Rapeseed-mustard is the third most important oilseed crop after soybean and groundnut, contributing nearly about 20–25% of the total oilseed production in the country. In India, rapeseed-mustard occupy approximately 22.2% of total oilseeds cultivated area and approximately 32% of the country's total oilseed production, with an area of 6.00mha, production of 8.04mt and yield of 1339 kg/ha during 2017–2018 [1]. Brassica oilseed crops play a vital role in the diversification in cropping system and also in providing the quality food by meeting the fat requirement to same extent. Mustard is considered to be of high economic importance in local as well as international trade and it is one of the major contributors to Yellow Revolution. Many biotic and abiotic stresses are liable for reducing the production and productivity of rapeseed-mustard. Biotic stresses are diseases and pests and abiotic stresses are due to environmental factors like temperature, salinity, drought, frost and Water logging stress. Diseases play a pivotal role in reducing the quality and quantity of mustard crop [2, 3]. Unfavorable environmental conditions severely affect growth, productivity and genome stability of the crop. These unsuitable ecological factors are a hazardous for plants that avert them from reaching their full genetic potential and decrease the crop productivity worldwide. Various abiotic Stresses, such as extreme temperature, salinity and heavy metal toxicity cause massive crop yield loss. Pattern of climate is becoming more erratic globally with increased occurrence of drought, flood, storms, heat waves, and seawater intrusion.

According to Howe and Jander [4], array of morphological, genetic, biochemical and molecular processes has been targeted to develop resistance in plants. These mechanisms may be expressed constitutively as preformed resistance, or they may be inducible and deployed only after attack. The latest studies indicate that the plant mechanisms of disease resistance or susceptibility are related to mechanistic response [5]. Various plant pattern recognition receptors (PRRs) that sense pathogens or conserved molecules termed pathogen-associated molecular patterns (PAMPs) and then induce PAMP triggered immunity (PTI), in case of biotic stress. While in case of abiotic stress plants respond to various stress factors such as salinity, heat, cold, drought, excess water, heavy metal toxicity, nutrient loss and pass information through multifaceted molecular signaling pathways leading to expression of stress-related genes. These responses at the molecular, cellular, physiological and biochemical levels enable the plants to survive [6].

The new biological and molecular tools have been opened up new perspectives in stress biology and can be applied in Mustard to develop biotic and abiotic stress tolerance. The omics approaches such as genomics, proteomics, metabolomics and transcriptomics have direct potential for improving stress tolerance in plants. The use of PGPRs, beneficial metabolites and enzymes produced by microorganisms are found to effective in biotic and abiotic stress management in mustard.

2. Abiotic stresses in mustard and strategies to develop tolerance

Productivity of brassica crop is affected by a various abiotic stresses. These may include deficit or excess water availability, salinity levels in soil as well as in irrigation water and extreme temperatures. In addition, mineral deficiency or toxicity and excessive chemical content in soil are frequently faced by plants. Sometimes various abiotic stresses occurs in combination and affect the plants severely. For example, scarcity of water and high temperature are commonly occurs in the period of drought and can be induced by mineral toxicities that restrict root growth. Further, plants are also exposed to salinity, drought and frost-like conditions in combination in many cases. Abiotic stresses are primarily unavoidable and are the most harmful factor concerning the growth and productivity of brassica crops.

2.1 Strategies to develop salinity stress tolerance in mustard

Salt stress is one of the major limiting factor that disturb the yield and other agronomic important characters of mustard. Vital [7] observed that, soil texture and composition adversely affected by one of the major environmental factor that is salt stress. High salt concentration leads to imbalance of nutrients and ions, it reduces the normal morpho-physiological and other biological processes of mustard [8]. High salt concentration negatively affects the seed germination in many Brassica species, also showed retardation in plant growth and development, resulting in reduced crop yield and even death of plant under severe conditions [9]. Due to high concentration of salt content, the osmotic pressure of soil is higher than the root cells, thereby root cells instead of absorbing water from soil lose water leading to water and nutrition imbalance in plants, thus adversely affects plant growth [10]. Plants use different resources to sense, counter and acclimatize to altering the saline environment based by making modifications in morphological, physiological traits and molecular metabolism which may further be enhanced by thiourea TU induction. Recently published reports have thoroughly explained the function of TU in inducing the salt tolerance and primary mechanisms in many plants, including Indian mustard (*Brassica juncea*) [11]. Evidence suggests that TU treatment (6.5 mM) improved salt tolerance in *Brassica juncea* by enhancing the translocation of sucrose from source to sink [12]. Recently, it has been discovered that mitochondria play a critical role in plant protection again salinity stress [13]. This is an important mechanism by which TU maintains mitochondrial homeostasis and ATPases (FoF1-ATP synthase) plays an important role in TU-induced salt tolerance in *Brassica juncea* [14]. TU application can also alleviate the adverse effects of salt stress by inducing changes in transcription through the modulation of microRNA and hormone production [15]. A prolific root system is important to improve stress tolerance and final yield [16]. Endophytic *P. indica* induces salt tolerance in mustard by increasing the levels of antioxidants. The continous exposure of 500 mM NaCl solution nonsymbiotic plants *Leymus mollis* (dunegrass) cause severe wilting and desiccation in 7 days and the plants were dead after 14 days. Contrary to this the symbiotic plants infected with *Fusarium culmorum* did not show signs of wilting even exposed to 500 mM NaCl solution for 14 days [17]. Salt tolerant varieties like CS52, CS54, CS56, CS58, CS 234–4 and Narendra Rai have better tolerance potential can be grown in such condition.

2.2 Strategies to develop Drought stress tolerance in mustard

Drought can severely affect seed traits such as seed germination, seed yield and seed quality as well as plant vegetative growth. Shekari et al. reported that the most sensitive stage for drought injury was flowering resulting in high loss in seed as well as oil yield by 29.5% and 31.7%, respectively [18]. Hasanuzzaman et al. examined that *Brassica napus* may be more resistant to drought stress than that of *Brassica rapa* [19].

The challenge is even greater for developing drought tolerant trait in plants for water-limited environments where occurrence, timing and severity of drought may fluctuate from one zone to the next and also over the years. Furthermore, it induces large impacts on emergence, growth, quantity and quality of produce production through phenological, physiological and biochemical pathways [20]. Physiological changes in water potential and relative water content of the water-stressed leaves through osmoregulation and osmotic adjustment have been observed in Brassica crops. In the process of physiological adaptation, maintenance of turgor pressure appears to be the central process. In crop production, different techniques are used to conserve water and increase water use efficiency in order to tackle water scarcity. One of them is planting method which affects the plant population and nutrient availability. Most commonly used cheapest method for water conservation are drill sowing raised bed planting and furrow planting. Seedling establishment is a phonological stage at which drought stress could be damaging. Seed broadcasting technique of Brassica crops results in uneven distribution and leads to imbalances availability of water, space and nutrients and poor seedling establishment and ultimately lower yield. Plant growth regulators (PGRs) regulate the germination, formation and distortion of roots, leaves and stem elongation and ripening etc. exogenous application of salicylic acid, gibbereallic acid and cytokinins also improves stress tolerance in mustard. Potassium (K) is one of the key plant nutrients and is involved in drought mitigation by regulating turgor pressure, photosynthesis, translocation of assimilates to various organs and enzyme activation [21].

Other than traditional techniques the Transcription factors (TFs) are emerging as useful resources for genetic engineering to induce drought tolerance in mustard plants, because they act as mjors regulators of various stress-regulatory pathways. Many TFs belonging to families AP2/EREBP, MYB, WRKY, NAC, bZIP have been involved in drought stress tolerance and some TF genes have also been engineered to develop stress resistance in plants. TFs are very important regulators, as they function as terminal transducers and comprehensively regulate the expression of group of downstream genes by combination of specific cis elements in their promoter region [22]. Over-expression of a constitutively active form of AtDREB2A from Arabidopsis has been reported to improve the tolerance to drought and osmotic stresses [23].

2.3 Strategies to develop cold stress tolerance in mustard

Frost is a sudden crop killer with devastating threat, especially in the north and northeastern parts of India where temperature unexpectedly drops below 0°C. Low-temperature stress not only decreases grain yield but also affects crop grain quality [24]. Shah et al. reported that whole plant death in mustard if frost stress affects the seedling stages. The injury rate of frost stress depends on many important components such as duration and amount of cold stress, different stages of plant growth and moisture content. It has direct effect on the flowering and siliqua development and prevents seed formation, thereby affecting crop productivity, causing considerable yield loss [25].

Cold stress tolerance mechanism in plants is regulated via transcriptional activation or repression. The majority of stress associated proteins such as heat shock, chemical shock and late embryogenesis abundant proteins (HSPs, CSPs and LEA) are accumulate upon extreme temperature stress. They act as molecular chaperones, which are responsible for protecting the cellular machinery in a broad range of cellular processes. Evidence suggests that cold tolerance is linked with the increased expression of genes involved in transcriptional regulation, osmotic adjustment, antioxidant defense and metabolite biosynthesis [26]. Varieties like RGN-48, RK-9001, RH-8816, RGN-13, RH-819, Swaranjyoti, RH-781 have been reported to have good frost tolerance. Mustard crop can be also protected from frost by chemical spray of dimethyl sulphoxide, dithane or 0.15% of H_2SO_4.

2.4 Strategies to develop heavy metal toxicity stress tolerance in mustard

Plants largely depend on soil solution to acquire nutrients for their growth and developmental cycle. The recent increase in contamination of arable lands with heavy metals is one of the most important causes of loss in crop productivity [27]. Extensive exposure to heavy metal contamination threatens the sustainability of environmental and agricultural systems. Crops are routinely subjected to metal toxicity due to improper irrigation methods and the addition of excessive quantities of chemical fertilizers, and other synthetic nutrients [28]. Some (potentially toxic) heavy metals, such as Cu, Zn, Ni, Co, Se, and Fe, are also essential elements required for the optimal performance of plants and become toxic when accumulated in excess in soil solution [29, 30]. On the other hand, non-essential elements, such as arsenate (As), cesium (Cs), lead (Pb), and cadmium (Cd), can hamper crop productivity when accumulated in the soil even in trace amounts [31]. Soil contamination with heavy metals causes accumulation of these toxic metals in plant parts, resulting in decreased crop productivity and increased risk to animal and human health [32].

The root-associated dark septate endophyte (DSE), *Exophiala pisciphila* isolated from *Zea mays* showed enhanced antioxidant enzyme activity under increased soil Cadmimium (Cd) stress [33]. Three important genes involved in uptake, detoxification and transport of Cd were recognized as downregulation of ZIP, upregulation of PCS and MTP upon inoculation with DSE and exposed to high concentration of Cd. The *Pseudomonas* and *Gigaspora* are reported to alter level of 1-aminocyclopropane-1-carboxylate (ACC) which further increase the tolerance of heavy metals by directly manipulating the ethylene levels in plants [34].

2.5 Identification of quantitative trait loci (QTL) to develop abiotic stress tolerance in mustard

Quantitative trait locus (QTL) is a statistical method developed to analyse and correlate the phenotypic and genotypic data to estimate the genetic variations in complex traits. This technique is less time consuming and gives better mapping resolution by exploring and utilizing each event of recombination that occurs in the evolutionary history. In various crops, QTLs were recognized for a several beneficial agronomic traits, such as enhancing abiotic and biotic stress tolerance, yield and yield contributing factors of the crop, flowering time, root development and uptake of nutrients and nitrogen fixation. Molecular markers linked with various agronomic traits derived from association mapping are reported in crops including soybean [35] and brassica [36, 37]. Lu et al. reported that resequencing of 588 *Brassica napus* accessions from 21 countries has generated 5,294,158 (single nucleotide polymorphisms) SNPsand 1,307,151 indels. The genome-wide association study (GWAS) find 60 loci considerably associated with agronomic traits such as stress tolerance, seed quality etc., which may be proven as a valuable resource for genetic improvement [38].

All these factors such as salt stress, drought and frost decline the crop quality and quantity. These abiotic stresses can be managed by exploring genetic resources material and agronomic factors. Donor lines have been identified for different abiotic stresses and are being used in the breeding programmes for developing tolerant varieties against abiotic stresses. A number of improved cultivars or hybrids have been developed which perform better under different type of stresses resulting in lower reduction upon exposure to stress compared to high yielding varieties. Conventional techniques of plant breeding have not been proved that much successful in addressing abiotic stresses mitigation so far. Therefore reason and need for adoption of new molecular approaches.

3. Biotic stresses in mustard and strategies to develop tolerance

Besides environmental stresses, biotic stresses are diseases and insect-pests. The fungal diseases are considered as an important biotic constraint in mustard, which leads to significant yield losses of crop world-wide. More than thirty diseases are known to occur on brassica crops in India [39]. However, only few of them are considered as major diseases on the basis of economic yield losses and according to their distribution in the country. Major biotic stresses of rapeseed-mustard in India are Alternaria blight [*Alternaria brassicae* (Berk.) Sacc.], white rust [*Albugo candida* (lev.) Kuntze], Powdery mildew (*Erysiphe cruciferarum*), Downy mildew (Hyaloperonospora parasitica) and Sclerotinia rot (*Sclerotinia sclerotiorum*) which influence the quality and quantity of seed [40–42]. Alternaria blight [*A. brassicae* (Berk.) Sacc.] and White rust [*A. candida* (lev.) Kuntze] have been reported to be most wide spread and destructive fungus diseases of mustard all over the world [43]. The details of these diseases and their causal organism which are affecting rapeseed-mustard crop in India are mentioned in **Table 1** below.

3.1 Conventional approaches for disease management

The following strategies are helpful for disease management.

- Optimization of sowing time is very important as it significantly affects the disease incidence and severity.
- Use of certified seeds, chemicals, disease resistant and tolerant varieties.
- Seed treatment with various biocontrol agents viz., *T. viride*, *G. virens, Pseudomonas* or botanicals like *Allium sativum* etc. Use of bio-control agents (BCA) is advantageous as they are often effective against a wide range of soil-borne pathogens. Moreover, they are ecofriendly, cost effective and their use avoids the risk of development of resistance in the pathogen towards the control agent.
- Use recommended doses of N, P and K fertilizers, maintaining optimum plant population with recommended crop spacing.

Application of more use of pesticides leads to the development of resistance in the target pests, and has negative impacts on biodiversity. More importantly, plants will have increased susceptibility to pests due to the implications of changes of climate. Under such conditions, gaining better knowledge on physiology of plants could lead to sustainable control of biotic stresses. Several plant breeding techniques has been used in extensively to develop biotic stress tolerance in mustard. The stress responses in plants is showed high levels of complexity and redundancy at the sensitivity, response and expression levels with interconnection between stress pathways and over lapping functions between stress metabolites and stress proteins in different stresses. In the case of stress proteins, there are limits on genes of known function that are available but perhaps more importantly the issue of whether single or multiple gene transformations will confer stable resistance. Regular upgradation of technology is required to develop better solution of biotic stresses. Now-a-days, several molecular techniques are considered for a better crop disease management. Lack of disease resistance sources of plant breeding is serious problem and difficult challenge for crop improvement and this problem can be solved through using biotechnology approaches. This is one of the best option and

Disease	Causal organism	Yield losses
Alternaria blight	*Alternaria. brassicae* (Berk.) Sacc.	10–70
White rust	*Albugo candida* (lev.) Kuntze	Upto 47
Sclerotinia rot	*Sclerotinia sclerotiorum*	Upto 35
Downy mildew	*Hyaloperonospora parasitica*	17—37
Powdery mildew	*Erysiphe cruciferarum*	Upto 18

Source: Directorate of Rapeseed-Mustard Research (DRMR).

Table 1.
Economically important biotic stresses (diseases) of mustard and losses caused by them.

opportunity to develop strategies for biotic stress tolerance in crop [44]. Several methods has been employed to develop biotic stress tolerance are discussed below.

3.2 Molecular approaches for disease management

3.2.1 In-vitro tissue culture to develop biotic stress tolerance

The conventional breeding techniques are used for the incorporation of genes of interest from inter-crossing species into the crop for the development of biotic stress tolerance; however, these methods proved less effective with undesirable results [45]. Moreover, biotechnological techniques can be effective for the development of stress-tolerant plants. The genetic transformation involves the transfer of stress tolerance gene from gene pools in various plant species for establishment of stress tolerant crops. Genetic engineering could be most effective for the improvement of crop varieties; however, the major trouble associated with this approach is the low transformation competence, silencing of transgene and low gene expression [46]. Recently, tissue culture has proved to be an appropriate and less costly technique for development of stress-tolerant plants. The tissue culture plants are grown in controlled lab conditions requires limited time and space with potential to develop of stress-tolerant plants and leads to the better understanding of biochemical and metabolic pathways of plants growing in harsh environmental conditions [47]. Using partially purified culture filtrates, *B. napus* showing resistance to *Alternaria brassicicola* has been obtained [48] and *B. napus* showing resistance to *Phoma lingam* has been obtained through embryonic culture [49].

In vitro selection through enhanced expression of pathogenesis-related (PR) proteins, antifungal peptides or biosynthesis of phytoalexins is an important tool for desirable plant selection [50, 51]. This technology is having an upper hand over transgenic approach for developing improved disease-tolerant crops [52]. Developing pathogen resistance through in vitro selection can be carried out using organogenic or embryogenic calli, shoots, somatic embryos or cell suspensions. By exposing these cultures to different toxins produced by various plant pathogens, tolerant plants can be raised [51]. "Pusa Jaikisan" is the first high yielding variety of mustard though tissue culture and suited for nontraditional areas.

3.2.2 RNAi-mediated plant defence to develop biotic stress tolerance

RNA interference (RNAi), is a powerful technology for discovering the functional genetic sequences and harness the down regulation of expression of gene(s) specifically. To accomplish the modified gene expression for a particular trait, gene silencing viz. cosuppression, post transcriptional gene silencing, virus-induced gene silencing etc. can be used. This molecular phenomenon has become a focal point of modern plant biology research across the globe. Thus it has been remarkably used in crop improvement likewise has become a valuable tool for functional genomics in Brassica (Brassica sp.). The rapid adoption of RNAi has replaced previous antisense technology. RNAi has aided in identification of different functions and biological roles of various mustard genes, which are involved in fertility and somatic embryogenesis, resistance to biotic and abiotic stresses and qualitative improvements in oil seed as well as it also have major role in yield and maturity traits.

3.2.3 Use of microorganisms in biotic stress tolerance

The symbiotic interactions between plant and microorganisms may result in several outcomes as defined by fitness benefits by each of the partners [53].

Interaction to host plants can be positive, neutral or negative. Variations in the outside environment put the plant metabolism out of homeostasis, which creates necessity for the plant to harbour some advanced genetic and metabolic mechanisms within its cellular system [54]. The priming of host response against pathogen is termed as induced systemic resistance, in this case the host response is activated by nonpathogenic plant-associated microorganisms. The ISR induce plant defense mechanisms and protects unexposed part of plants against a future attack by pathogenic microbes and insect pests. Plant hormones ethylene and jasmonic acid plays a regulatory function in the network of interrelated signaling pathways involved in ISR induction [55].

Many studies have been dedicated to the induced systemic resistance SR mediated by free-living rhizobacterial strains [56] the resistance in Mustard (*Brassica juncea*) Induced against Alternaria Black Spot using a virulent *Alternaria brassicae* Isolate-D whereas the strains of *Alternaria alternata* failed to induce resistance against *Alternaria brassicae* [57]. Subsequently attention was drawn to ISR mediated by several other species of genus *Pseudomonas* and the effect was characterized in different plant–pathogen systems. *Pseudomonas* sp. Strain-1 was shown to suppress Sclerotinia stem rot incited by *Sclerotinia sclerotiorum* on stem of mustard. Configuring the functions of endophytes there role in stress tolerance increases immensely. Endophytic microbes improves the plant health by deterring herbivory and pathogenesis while also facilitating plant growth through nutrient uptake, water use efficiency and curtailing of environmental stresses. The endophytic bacteria *Pseudomonas syringae* bacterium was able to induce disease resistance via defense priming [58].

4. Conclusion

Based on the foregoing chapter, there is no doubt biotic and abiotic stresses are the major barriers in enhancing the productivity of rapeseed-mustard crop. Conventional plant breeding techniques has not provided us full proof protection against abiotic and biotic stresses so far. So, there is an urgent need to minimize the adverse effects of these stresses on the brassica crops to enhance the productivity and production to meet the ever-growing demand of oil in the country. The use of genetic and genomic analysis which helps to identify DNA regions tightly linked to agronomical traits in mustard. Molecular markers for the indirect selection of improved crops speeds up the selection process by alleviating time-consuming approaches direct screening under screen house and field conditions. Use of PGPRs in mustard can also be highly effective as these microorganisms are able to reduce abiotic as well as biotic stress in plants by producing beneficial enzymes, proteins, hormones etc. Tissue culture is another advantageous technique in mustard as this crop is highly susceptible stresses at initial stages. Therefore the use of one particular technique or the mix of these techniques has a potential to improve the stress tolerance in rapeseed mustard.

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