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Glucosinolates and Its Role in Mitigating Abiotic and Biotic Stress in *Brassicaceae*

Parul Chowdhury

Abstract

Abiotic stresses such as increase in daily mean temperature, changed patterns of precipitation, increase in episodes of drought and floods in future are faced by the plants and pose threats to crop production and food security. Induction of secondary metabolites by several abiotic stress conditions can be helpful in the crop protection against biotic stress and can be a major link between biotic and abiotic stress. Plants also face threats by injury caused by herbivores and insects that chew on plants. Plant develops, coordinates and combines defence mechanism to cope with the challenges caused by the injuries. The plant family *Brassicaceae* (or *Cruciferae*) includes some of the world's most economically important crops; especially members of the genera *Brassica L.* *Brassicaceae* vegetables are a good source of secondary metabolite that is Glucosinolates. Which are responsible for characteristic flavour and odour, when degraded. Glucosinolates and their degradation products play important roles in stress tolerance, plants respond to abiotic and abiotic stress by systematically accumulating higher levels primary and secondary metabolites for increasing their resistance. Glucosinolates play important role and have a relation with biotic and abiotic stress in *Brassica* plant family, as they can act as a signalling molecules and affect the physiology of plant.

Keywords: abiotic stresses, biotic stress, *Brassicaceae*, glucosinolates, stress tolerance, human health

1. Introduction

Plants are attached to the ground by the roots for water and nutrient supply and fixed at one place, so they have to face various abiotic stresses such as temperature, water availability, salt concentrations, etc. This is a common environmental stress which impacts productivity of the plants worldwide. Along with abiotic stresses, plants also face injuries by herbivory and insects that chew on plants. Once the plant faces mechanical damage by insects, a lot of changes occur in the plant [1]. In case of biotic stress, immediately after wounding by pest changes occur in the damaged area, which can be systematic (in the non-wounded areas) or local (in the tissues directly damaged). Once the injury occurs, metabolic changes, physiological changes and induction of gene expression started taking place [2]. The response to stress is not simple, rather involves different mechanisms and molecules. The strength and type of response depend upon plant species, developmental stage, nature of stress

and environmental conditions. Plant develops, coordinates and combines all the defence mechanisms to cope with the challenges caused by the injuries [1, 3, 4].

The plant family *Brassicaceae* (or *Cruciferae*) includes some of the world's most economically important crops, especially members of the genera *Brassica* L. (Coles, mustards, oilseed rapes, turnips, etc.). These cultivated species include a large variety of leaf and root vegetables, oilseed crops which are consumed all over the world [5]. *Brassicaceae* vegetables are a good source of antioxidants because of their high phenolics and sulphur-containing glucosinolate content. These glucosinolates are responsible for characteristic flavour and odour of these vegetables. Myrosinase (thioglucoside glucohydrolase; EC3.2.1.1), an enzyme present in *Cruciferae*, is activated when cells are damaged by herbivory, cutting and chewing of insects. These Myrosinases hydrolyse glucosinolates into a variety of products, which generally include isothiocyanates, nitriles, epithionitriles and oxazolidine-2-thiones [6]. Glucosinolates and their degradation products play important roles in pathogen and insect interactions, stress tolerance as well as in human health. These compounds have chemoprotective properties against chemical carcinogens. Their role in prevention of cardiovascular disease is due to properties such as inhibiting the enzyme activation, modifying the steroid hormone metabolism and protecting against oxidative damage [7, 8]. Isothiocyanate and indole products formed from glucosinolates hydrolysis by Myrosinase may regulate cancer cell development by regulating target enzymes, controlling apoptosis and blocking the cell cycle [9].

Various biotic and abiotic factors activate the defence system in plants, and these result in significant variation in the plant metabolome. These factors result in enhanced production of certain metabolites, e.g., amino acids, sugars, indoles, phenolics including glucosinolates. Glucosinolates production in stressed plants is seen to be enhanced, and they have a crucial role to play in mitigating the negative effect of stress. The concentration and type of glucosinolates and their hydrolysis in plants are affected by many factors, such as age, abiotic stress such as heavy metal, water availability, postharvest storage signalling molecules [10, 11]. These play an important role in abiotic stress. Their levels change in response to a variety of abiotic stress tolerances [12]. They also play an important role in plant-insect interactions. Their levels also change in response to herbivory in plants [13].

2. Glucosinolates and abiotic stress

Plants synthesise a variety of secondary metabolites that do not seem to have any essential role in plants, but presence of these compounds plays a very important role in plants and has many advantages, especially in defence. Glucosinolates, a class of secondary metabolites mainly found in Brassica family, are nitrogen and sulphur-containing compounds derived from amino acids such as methionine, alanine, valine or leucine (aliphatic); phenylalanine or tyrosine (aromatic) and tryptophan (indolic glucosinolates). These glucosinolates are usually increased in different biotic or abiotic stress conditions and play an important role in plant defence in these unfavourable conditions [14].

During growth, plants are exposed to various biotic (herbivory, fungal, bacterial and/or viral infection) and abiotic (metals, UV, temperature) stresses. It leads to upregulation of various gene and biochemical changes, which finally results in an enhancement of the synthesis of primary and secondary metabolites. Plants have acquired various stress tolerance mechanisms, involving physiological and biochemical changes that result in adaptive or morphological

changes [15]. It was also found that plants respond to abiotic stress by accumulating higher or lower levels of Glucosinolates. The intensity and duration of the abiotic stress, as well as the developmental stage of the plant at the moment of the imposed stress, are important factors in the accumulation of each specific Glucosinolates [16, 17].

The mechanism by which the plant resists these stresses with help of metabolites is unclear as their regulation is a complex process because glucosinolates synthesis pathways are linked to various other pathways making it more complex to understand its direct role. There are studies in elucidating a relationship between the metabolites and stress, but there is a strong need to study underground mechanisms as well.

2.1 Salt stress

One of the major abiotic stresses affecting plant growth and development is salt stress [18]. There are various ways by which plants respond to this stress conditions either by osmotic adjustments or by accumulating secondary metabolites, such as Glucosinolates [19].

In one of the studies, Broccoli was grown in greenhouse condition and was treated with NaCl (0, 40, 80, 100 mM) to study the effect of Glucosinolates in salt-stressed Broccoli. Glucosinolates were determined at three different time points (1, 3, 6 days upon treatment), and it was found that the total aliphatic and indoles were lower at 40, 80 and 100 mM NaCl applications compared with control plants determined at three different time points [20]. A study was conducted with two Broccoli cultivars *cv Parthenon* and *cv. Naxos* grown in controlled conditions in Hoagland solutions with five salt treatments for 15 days. To avoid osmotic shock, 30 mM salt solution was added every hour until the final salt concentrations of 0, 30, 60 and 90 mM were reached. It was found that *cv Naxos* was more sensitive to salt as compared with *cv Parthenon*, and the indole glucosinolates were not affected by salinity in *Parthenon* while it decreased significantly in *cv Naxo*, whereas *Naxos cv* accumulated more aliphatic GSLs under salt stress than *Parthenon cv* suggesting that each broccoli cultivar adopted distinct strategy, which can be dependent on various factors [16].

Greater accumulation of proline and glucosinolates was found with increased salinity level in Canola or oilseed rape (*Brassica napus* L.), but it was dependent on the age and stage of plant growth—seedling, vegetative and reproductive. 131 *Brassica napus* genotypes were evaluated at these stages for glucosinolates with 0, 50, 100, 150, 200, 250, 300 and 350 mM NaCl concentration. It was concluded in this study that salt stress genotype should be selected at different stages as it is a developmental phenomenon for improvement of canola genotype productivity [21].

Six types of Brassica rocket genotypes used for salad belonging to the *Brassicaceae* family were tested at 0, 65 and 130 mM NaCl treatment for morphological parameter as well as different Glucosinolates concentrations. Morphological parameters were different for different genotypes, but it was observed that Glucosinolates content increased in moderate saline treatment, indicating that these rocket salads can be improved for good Glucosinolates content by irrigating them with salt water [22]. Increased level of Glucosinolates were shown to be increased in Radish, Kale and other *Brassicaceae* family as demonstrated by work done [23–25]. So it is clear from the above studies that at moderate salt stress, Glucosinolates content of the plant is increased and which in turn helps the plant in fighting stress tolerance along with other factors such as morphological other secondary metabolites and accumulating compatible solutes such as proline.

2.2 Water stress

Drought is unavailability of adequate amount of water to a plant, required for its proper growth and development. Drought in agriculture field can occur due to a variety of reasons majorly including low precipitation, inadequate ground water. When *Brassica* family is tested for Glucosinolates content in drought conditions, it was found out that it increases with drought in different varieties of *Brassica*. One such study was done on molecular mechanism in understanding the gene upregulated in response to drought conditions in Chinese cabbage, and it was found that glucosinolates content also increased with the drought stress indicating its possible role in drought [26, 27]. Even when table drought was given to *Arabidopsis* by PEG 6000, they showed an increase in glucosinolates concentration indicating their role in drought stress response, but this response was short-term response. When the drought stress increases, glucosinolates content decreases [28].

2.3 Heat stress

Abnormally high temperature during hot weather is again one kind of abiotic stress. Effect of high temperature depends on the duration and its intensity. Heat stress may cause various physical, physiological and molecular damage to the plants and in turn affects its productivity. In a study on *Arabidopsis* mutant TU8, (Glucosinolate-deficient and heat-sensitive mutant) [29], it was found from research that due to ineffective expression of heat shock protein (HSP), these mutants were unable to survive in high temperature and were glucosinolate-deficient too [30]. When overexpressing a protein phosphate gene in *Brassica rapa* *BrPP5.2* and thereby developing a transgenic line, it was observed that these plants develop a heat tolerance and high glucosinolates indicating role of glucosinolates in stress tolerance [31]. In other studies by different research groups, it was found that a variety of different glucosinolates content increased upon long-term and short-term heat shock in *Brassica* plants [32, 33]. As is a major part of the human diet and considering the health-benefiting glucosinolates in sprouts, this was increased by giving heat stress to developing sprouts. Heat shock at 60 degree C, in developing sprouts of cabbage, improves the isothiocyanates production and in turn the glucosinolates [34].

2.4 Heavy metal stress

Use and need of metals in plants are important as they act as cofactors in various enzymes such as Mg^{2+} , Mn^{2+} , Co^{2+} , Zn^{2+} , but with increase in industrialisation use of heavy metals such as Hg, Pb, Cd, Ni Ba Cr which are highly toxic even at a very low concentration. There are micronutrient essential metals such as Mn, Mo, Zn, Cu, Co which are required by plants in low amounts, but they also become toxic at high concentrations. Effect of cadmium toxicity was evaluated on cabbage and kale, and it was found that glucosinolates accumulate in stems as compared with other organs such as root or leaves, which indicate that glucosinolates can play role in heavy metal stress tolerance as plants have shown no metal toxicity physically [35]. In another study, *Brassica oleracea* plants were treated with solutions of $ZnSO_4$ and $CdSO_4$ by mixing them in soil for treatment of plants with the heavy metals Zn and Cd, and it was found that glucosinolates content increased as the concentration of Zn and Cu increased [36]. Increased concentration of cadmium was studied on *Brassica napus*, and it was found that glucosinolates concentration increased with increase in concentration of heavy metal [37]. Chinese cabbage when exposed to elevated Zn concentration was shown to overexpress the genes, APS reductase

(APR), associated with the biosynthesis of primary sulphur compounds. There was also an increase in aliphatic glucosinolates with an increase in gene expression of key biosynthetic enzymes and regulators such as CYP79B3, CYP83B1, MYB34 [38]. Brassica plants were also used for phytoremediation in heavy metal-contaminated soil, and it was also found as mentioned in above studies that glucosinolates concentration increases with increase in concentration of heavy metal. It can also be stated that accumulation of glucosinolates can be one of the strategies for heavy metal tolerance in *Brassica* plants.

2.5 UV stress

Plant growth and development are mainly determined by sunlight. Sunlight is used in germination, development, photomorphogenesis and various other important functions in plants. But as sunlight is composed of UV-A, UV-B and UV-C, these radiations cause stress in plants. As a result of these radiation stresses, DNA damage, generation of reactive oxygen species (ROS) and many other cellular changes take place in plants. Glucosinolates also get affected by stress caused by UV light, as proven with the studies done on broccoli sprouts which were exposed to various low- and high-intensity UVA and UVB lamps for 120 min which resulted in high levels of glucosinolates in broccoli sprouts [39]. Even the combined effects of Methyl Jasmonate (MJ), UVA or UVB lights enhance the glucosinolates in broccoli sprouts [40]. As it is known from the studies that glucosinolates levels are upregulated during the UV stress [41], and it was also associated with increased biotic stress tolerance too. Low-to-medium UV-B doses were applied to sprouts of broccoli, and it was found that there was an increase in glucosinolate levels of 4-methylsulfinyl butyl GS and 4-methoxy-indol-3-ylmethyl GS. These glucosinolates in turn affect the genes related to jasmonic acid and salicylic acid signalling pathway, which ultimately leads to overexpression of bacterial and fungal pathogen-responsive genes [42].

3. Glucosinolates and biotic stress

Biotic stress in plants is caused by any living organisms, such as insects, pests, viruses, bacteria, fungi, nematodes, etc. Microorganisms damage plants by causing symptoms such as plant wilt, leaf spots, root rot, etc. Insects can cause physical damage to plants as they either chew or suck on the plants, including the leaves, stem, bark and flowers. They can also act as a vector of viruses and bacteria from infected plants to healthy plants. As plants do not have any immune systems to deal with this kind of stress, they respond to this stress by developing many changes at morphological, chemical and protein levels. The main aim of these changes is to protect the plants from foreign invaders. One such chemical is glucosinolates which get elevated in response to biotic stress for the purpose of defence in plants.

Chemically, glucosinolates are composed of thiohydroximate-O-sulfonate group linked to glucose and an alkyl, aralkyl or indolyl side chain (R). More than 200 glucosinolates have been identified in Brassica crops, and they are characterised mainly by the variable R group, which can be aromatic, indolic or aliphatic derivatives of the amino acid precursors methionine, tryptophan and phenylalanine, respectively [43, 44].

Glucosinolates play a variety of biological functions in plants [45] and get converted into various degradation products (isothiocyanates, thiocyanates, indoles, etc.), when vegetables containing them are cut or chewed because during this process, they come in contact with the enzyme myrosinase which hydrolyses

them, as discussed earlier in this chapter. A sudden outbreak of these insecticidal compounds in field is called as 'mustard oil bomb' [46]. These degradation products have both harmful and beneficial roles in plants as well as in human health. These compounds protect plants against various pathogens and weed but are also responsible for the bitter flavour of the crucifers because of the presence of some glucosinolates such as sinigrin and progoitrin [47]. Glucosinolates can act as potent anticarcinogens too [48, 49].

Studies have determined the effect of insect pest and glucosinolates levels in *Brassica* [50, 51]. These kinds of studies were done directly on the glucosinolates level in plant tissue and with the study of expression of gene family which is associated with the glucosinolates and its associated enzymes. To study the biotic stress, signalling molecules such as plant hormones, salicylic acid (SA), jasmonic acid (JA) and ethylene play very important roles. In case of insects and pest, mechanical injury is mimicked with the help of damage through forceps and needles to plants. It has been proved that plant signalling molecules such as methyl jasmonate, salicylic acid and glucose as well as mechanical injury affect glucosinolates levels in *Arabidopsis* and *Brassica* species [52–55]. Therefore, these signalling molecules are used for the study of biotic stress in plant in laboratories.

Several reports exist on the toxicity of Glucosinolates hydrolysis products to bacteria and fungi. The activity of Glucosinolates and their degraded products against various strains of microorganisms has been documented by many investigators being present in the leaves of *Brassica* spp. at concentrations that can prevent the development of pathogens [56].

3.1 Glucosinolates and pest

When generalist herbivores feed on *Brassica*, degradation product of Glucosinolates in them, the isothiocyanates have toxic effects and function as repellents. However, specialist herbivores use Glucosinolates as attractants, and they can survive on plants containing them. For example, *Brevicoryne brassicae* aphids, flea beetles, *Psylliodes chrysocephala* and the Lepidopteran pest, *Pieris rapae* are some examples of specialist herbivores [57].

It was also found that plants respond to herbivore or insect damage by systematically accumulating higher levels of glucosinolates and thus presumably increasing their resistance. Usually it is the indole glucosinolates which become induced [58, 59]. Sometimes volatiles produced by Glucosinolates provide indirect protection to plants by attracting natural enemies of herbivores such as parasitoids [60].

Lepidopteran that feeds almost exclusively on *Brassicaceae* plants is *Plutella xylostella* (Diamond Moth Back). A study done by [61] compares two lepidopteran larvae, one specialist and one generalist, namely *Mamestra brassicae* (Lepidoptera, Noctuidae) and the specialist *Pieris rapae* (Lepidoptera, Pieridae) respectively with respect to six different genotypes of *Brassica oleracea*. They found that two aliphatic (sinigrin and glucoiberin) and one indole (glucobrassicin) glucosinolates affect the larvae growth which in turn is affected by the age of the *Brassica oleracea* plants. Mature plants produce more Glucosinolates and are protected from the pest as compared with the young plants which is determined by larval development time, body weight, mortality and feeding rate of two larvae selected for study.

Genetic engineering of glucosinolates biosynthesis pathway for the production of glucosinolates in non-Glucosinolates producing plants was successfully employed by [62]. Genetic transformation of *Nicotiana tabacum* (tobacco) for the benzyl glucosinolate pathway was done from *A. thaliana* which results in successful production of benzyl glucosinolate in tobacco, which attracted the female pest *Plutella xylostella* (diamondback moth). Larvae hatched that could

not survive on tobacco plants demonstrate that this strategy can be used as the trap crop for many important crucifer crops.

It was found that *Brassica* plants were used for the purpose of Biofumigation in fields which include suppression of pests and pathogens through release of volatile substances from degradation of biomass into the soil. *Brassica* plant contains higher amount of Glucosinolates and its breakdown product which are released after tissue damage, such as isothiocyanates which are toxic, and also they have the fumigant property which is comparable with metham-sodium [63–65].

4. Conclusion

Abiotic stress such as heat, drought and high salt and biotic stress including various attacks on plant from insects, pest or other bacterial viral disease can affect the plant in terms of physical, physiological and many other biochemical and molecular aspects, which can retard its growth and productivity.


Brassicaceae family includes important crop due its medicinal and nutritional properties. This family has plants which are valuable source of oil and food containing medicinal values including anti-cinereous property for humans. These crop plants like other crop plant face abiotic and biotic stress both, and they use various mechanisms to overcome these stresses. Glucosinolates, which are responsible for the pungent characteristic smell of *Brassica*, are responsible for various biotic and abiotic stress tolerances in Brassica. They have particular pathway from which they are synthesised and regulated by various factors. MYB group of transcriptional factors are involved in glucosinolates synthesised pathway [66]. Environment, signalling molecules, herbivores and pathogens also regulate the glucosinolates biosynthesis which can help in their stress tolerance by upregulating different transcription factors and genes. In case of biotic stress, glucosinolates in the form of ‘mustard oil bomb’ can provide the defence against generalist and specialist herbivore. In spite of so many studies and research done, there are many gaps yet to be identified and many conclusions need to be explored in depth. Role of glucosinolates can be much explored as nutrition, medicinal and biocontrol agents in field. Glucosinolates also play important role in stress tolerance in Brassica, understanding their deeper role in abiotic and biotic stress can help in increasing their productivity worldwide.

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