

Review of Literature

Plant phenolic compounds at low concentrations have been widely and more recently been reported to be substances stimulatory to seed germination and plant growth (Ghareib *et al.*, 2010; Hassan and Ghareib, 2009; Hegab *et al.*, 2008; Hegab, 2005). Some phenolics like caffeic, ferulic, and *p*-coumaric acids appear to be more active antioxidants, food flavor precursors and also to be an important part of the general plant defense mechanisms (Floridi *et al.*, 2003). These compounds are found in almost all types of fruits and vegetables, say apples, coffee, beans, grapes, potatoes, prunes, and tea leaves (Lu and Foo, 1999; Griffiths and Bain, 1997; Balentine *et al.*, 1997; Friedman, 1997; Dao and Friedman, 1994; Spanos *et al.*, 1990; Lathia and Frenzen, 1980). Plant phenolics present an interesting class of biological molecules with extremely diverse and important functions (Singh and Kumar, 2014; Singh *et al.*, 2013).

Despite ubiquitous presence of plant phenolics the understanding of their physiological role in plants is scanty. Some investigators believe that phenolics

function as promoters as they have been reported to stimulate IAA, GA₃ and kinetin activity (Buer *et al.*, 2010; Mukharjee and Kumar, 2007; He and Lin, 2001; Leslie and Romani, 1998), increase IAA oxidase, polyphenol oxidase, isoperoxidase, catalase and nitrate reductase activities (Zhang *et al.*, 2011; Batish *et al.*, 2008; Singh *et al.*, 1997), mobilize carbohydrates, proteins and total phenolics (Shuab *et al.*, 2013; Talaat and Balbaa, 2010; Talaat, 2005; He and Lin, 2001), regulate photoperiodism (Turner *et al.*, 1993), floral induction (Sheeja and Mandal, 2003; Ebrahimzadeh and Abrishamchi, 2001), allelopathic substances (Chen *et al.*, 2011; Chobot *et al.*, 2009; Ding *et al.*, 2007; Chon *et al.*, 2005; Weir *et al.*, 2004) and act as phytoalexins (Chen *et al.*, 2011; Mazid *et al.*, 2011; Dixon, 2001; Dixon and Steele, 1999; Grayer and Harborne, 1994). However, others consider these compounds to be inhibitory to growth and developmental phenomena for example suppression of IAA biosynthesis and/or activation of IAA degradation (De Klerk *et al.*, 2011), lowering of the growth stimulating activity of auxins, gibberellins or cytokinins (Kefeli and Kadyrov, 1971), uncoupling of respiration and oxidative phosphorylation (Hatfield *et al.*, 1999; Iiyama *et al.*, 1994), affect seed germination and dormancy (Hussain *et al.*, 2008; Reigosa and Pazos-Malvido, 2007); induce both tap and adventitious rooting and growth (De Klerk *et al.*, 2011). Some groups of workers, however, argue against any basic role of phenolic constituents in plant growth regulation (Bartwal *et al.*, 2013; Firn and Jones, 2009).

Several phenolic compounds like caffeic acid (CFA), *p*-coumaric acid (PC), ferulic acid (FA), chlorogenic acid (CGA), vanillic acid (VA), daidzein (DAI) and scopoletin (SC) are fluorescent compounds (Lang *et al.*, 1991) thereby, act as barrier

against solar UV radiation thus protecting the cells of the mesophyll containing chlorophyll (Yaryura *et al.*, 2013).

Plant growth and development is governed by a mutual interaction among various plant growth regulators (Shuab *et al.*, 2013). Several studies indicate that phenolic compounds show interactions with various growth regulators including auxins, abscisic acid, gibberellins and cytokinins and also with some inorganic ions such as Fe, Mn and PO₄. Besides, various phenolic compounds may even show interactions among themselves in certain growth and developmental phenomena of plants.

Interaction with cytokinins

A few reports are indicative of interaction between coumarin and cytokinins. Thus, Gaspar *et al.*, (1975) observed an interaction between kinetin and coumarin in relation to growth and iso-peroxidases of lentil. It was observed that kinetin, while inhibitory to growth by itself in certain cases, counteracted the coumarin effect. The combined effects of kinetin and coumarin indicated that coumarin antagonized the action of kinetin. An analysis of total peroxidase activity did not provide any explanation for these interactions as coumarin inhibited nearly all iso-peroxidases whose activities were enhanced by kinetin indicating the interaction between these two compounds to be non-specific.

Balasimher *et al.*, (1977) demonstrated interaction between cytokinin and coumarin in the growth and peroxidase activity of mung bean (*Phaseolus radiatus*) seedlings. The cytokinin as well as coumarin inhibited the growth of both hypocotyls and radical while epicotyl growth was unaffected. When coumarin was given in

combination with cytokinin the inhibition was reduced. The growth inhibition was accompanied by a reduction in sulfhydryl (-SH) and chlorophyll contents and enhanced peroxidase activity as a result of coumarin treatment. The effect of coumarin on peroxidase and -SH was reduced by simultaneous treatment with cytokinin. When used singly cytokinin enhanced chlorophyll content and slightly lowered peroxidase activity. Coumarin appeared to counteract the action of cytokinins.

Kinetin and salicylic acid may regulate plant growth and development by enhancing GA₃ metabolism of the plants (Mukharjee and Kumar, 2007). The type and concentration of salicylic acid and cytokinin, either alone or in combination, has been known to strongly influence growth as well as the secondary metabolites in tissue culture (Masoumian, 2011). Cinnamate has been reported to act more independently than having any interactive inhibition or synergism with kinetin as far as certain metabolite mobilization or dry matter accumulation in the isolated cotyledons of cucumber is concerned (Shuab *et al.*, 2013).

Interactions with auxins

In several studies phenolic compounds have been shown to interact with auxins in the growth of intact plants or of excised plant parts. Tayal and Sharma (1983) studied interaction of phenols and IAA on germination and early seedling growth of *Cicer arietinum*. Resorcinol, β -naphthol, phloroglucinol and IAA promoted plumule growth at 10 and 100 mg/L while IAA at all concentrations retarded lateral root development. Each of the above mentioned phenolics at a concentration of 500 mg/L, retarded plumule and radical growth and also lateral root development. IAA at

10 and 100 mg/L synergized the action of phenols at low concentrations but antagonized the effect of all concentrations in case of plumule growth. However, for radicle growth high concentrations of both IAA and phenols had a synergistic effect.

Phenolic compounds have also been linked with auxins in ways other than protecting them from oxidation. Flavonoids may also act as auxin transport inhibitors (Peer and Murphy, 2007; Murphy *et al.*, 2000). Flavonoids interact with PIN2 or affect the distribution of PIN (plant auxin efflux protein) proteins (Buer *et al.*, 2010).

Rekoslavskaya (1974) studied the effects of certain polyphenols on the metabolism and activity of IAA in tobacco tissue culture. When ferulic acid or chlorogenic acid was added to the medium, IAA oxidase was suppressed.

Sharma and Kaushik (1982) demonstrated that both endogenous level of auxin and IAA oxidase activity were affected by certain phenolic acids in cucumber (*Cucumis sativus*). Fortnightly spray application of caffeic acid and protocatechuic acid starting from the two leaf stage increased the endogenous IAA level. At the time of initiation of flowering IAA oxidase activity was however, inhibited. Earlier it was variably thought to act as an auxin-analog (Wong *et al.*, 2005; Yang *et al.*, 1999).

Foliar application of salicylic acid hastened the IAA content in broad bean leaves (Amanullah *et al.*, 2010). *cis*-Cinnamic acid has recently been reported to downregulate several IAA-upregulated genes and also induced early auxin-responsive genes in the *Arabidopsis* roots in a tissue-specific gene analysis (Wasano *et al.*, 2013).

Interaction with gibberellins

There is some evidence to suggest that phenolic compounds interact with gibberellins. Tizio (1976) studied interaction of *p*-coumaric acid and ferulic acid with six different gibberellins (GA's) viz. G₁, G₃, G₅, G₇, G₉ and G₁₃ on tuberization of fragments of shoots of potato (*Solanum tuberosum*) grown *in vitro*. These phenolic acids were found to counteract the retardant action of gibberellins in the tuberization process. Nutbean and Briggs, (1982) demonstrated that gibberellins interacted with phenols in barely presumably through the formation of gibberellin- phenol complexes. Floral bud initiation is hastened and the number of flower buds and flowers per flowering plant (*Impatiens balsamina*) increases in response to even a single treatment with the combination of GA₃ and salicylic acid accompanying a single short day cycle (Sood and Nanda, 2006). Peroxidase assayed using caffeic acid as a hydrogen donor after GA₃ treatment showed inhibition in both cytoplasmic and wall-bound fraction (Patel and Thaker, 2007).

Interaction with abscisic acid

Much evidence suggests an interaction between phenolic compounds and the natural plant growth inhibitor abscisic acid (ABA). Ray *et al.*, (1983) studied the action of phenolic compounds on ABA induced inhibition of hypocotyls growth in seedlings of *Amaranthus caudatus*. ABA strongly inhibited the growth of hypocotyls. The inhibitory effect could, however, be reduced if the seedlings were treated with phenolics such as gallic acid, *trans*-cinnamic acid, ferulic acid, tannin, coumarin and quercetin, indicating an antagonistic action of phenolic compounds with ABA.

Laloraya *et al.*, (1986) also demonstrated reversal of ABA induced stomatal closure by *trans*-cinnamic and *p*-coumaric acids. Employing petiole abscission test

with cotton seedlings, Apte and Laloraya (1982) showed that ABA induced abscission was strongly inhibited by phenolic compounds such as caffeic acid, ferulic acid, rutin and *p*-coumaric acid. The effective concentration of the phenol varied with various phenolic compounds tested.

Cinnamic acid, a derivative of phenylalanine, comprises a relatively large family of organic acid isomers that are extracted from plants or synthesized in the laboratory or chemical factory. Most famous as the phenolic compounds that give oil of cinnamon its characteristic odor and flavor, cinnamic acid appears to have antibacterial, antifungal, and parasite fighting abilities (Andrade-Ochoa *et al.*, 2015; Nowacka *et al.*, 2015; Guzman, 2014; Korosec *et al.*, 2014). In wine, cinnamic acid and its derivatives join benzoic acid derivatives and flavonoids in creating pigments and tannin agents that give each vintage its characteristic bouquet and color.

Cinnamic acid and its derivatives are studied for their various biological activities like antioxidant, anticarcinogenic, hepatoprotective, anxiolytic, insect repellent, antidiabetic and anti-cholesterolemic etc. Different substitutions on basic moiety lead to various pharmacological activities e.g. *m*-hydroxy or *p*-methoxy residue on cinnamic acid (CA) are significantly important functional groups as an effective insulin releasing agent while 3, 4-dihydroxycinnamic acid (caffeic acid, CAF) shows hepatoprotective activity (Catanzaro *et al.*, 2014; Sharma, 2011; Jitareanu *et al.*, 2011; Da Cunha *et al.*, 2004; Narasimhan *et al.*, 2004;).

Also known as phenylacrylic acid, cinnamic acid forms monoclinic crystals, as needles or prisms, with melting point of 133 degree celsius and boiling point of 300 degree celsius. Composed of 9 carbon, 8 hydrogen, and 2 oxygen atoms, cinnamic

acid is classified as a skin, eye and respiratory irritant and is soluble in water or alcohol. Historically, xenobiotic metabolism (oxidation of chemicals foreign to the body) was first postulated and demonstrated by feeding cinnamic acid to people and dogs and then isolating hippuric acid in their urine. This pioneering research was the basis of the newly created synthetic pharmaceutical industry, thus allowing the prediction of the fate of new compounds administered to organisms, and realization that the body had capabilities to perform chemistry that, at the time, was not possible in the laboratory (Zhiqiu *et al.*, 2003).

In nature, cinnamic acid derivatives are important metabolic building blocks in the production of lignins for higher plants (Sim *et al.*, 2015). The diverse flavonoids of the plant world, responsible for the pigments that give flowers their bright colors that attract pollinators and pungent tastes that deter herbivores, are synthesized with cinnamic acid as an intermediate (Tah and Gosset, 2015). An important pharmaceutical for high blood pressure and stroke prevention, known as coumarin or hydroxy-cinnamic acid, is a derivative of cinnamic acid. Known as storax or balsam of Peru to herbalists and early perfumers for centuries is a cinnamate. In the United States, herbalists, native Americans, and early physicians used extractions from the related sweet gum tree to treat coughs, diarrhoea, and dysentery (Hiradate *et al.*, 2005).

Naturally occurring cinnamic acid (CA) exist in both *trans* and *cis* isoforms. UV-light irradiation of *trans*-CA is able to produce *cis*-CA found in both monocots and dicots (Guo *et al.*, 2011). The biological activity of *cis*-cinnamic acid (*cis*-CA) was first reported in 1935 (Hitchcock, 1935; Haagen-Smit *et al.*, 1935) and was

initially thought to act like ethylene. This was alluded to the presence of double bond (HC=CH) in its structure and also due to epinastic response induction in tomato plant (Yang *et al.*, 1999). By employing two mutants of tomato plant, one being deficient in ethylene biosynthesis and the other being deficient in the ethylene perception and after treating these with the vapor of *cis*-CA and ethylene Yang *et al.*, (1999) concluded that the *cis*-CA vapor acts independent of ethylene receptor dependent pathway and also that there are different action sites for *cis*-CA vapor and ethylene.

Later the compound was thought to act like IAA as it has been found to promote growth in the pea split stem curvature test, the pea segment test and the avena straight and curvature tests just as the auxins do (Yang *et al.*, 1999; van Overbeek *et al.*, 1951). Yet, in a study using auxin-insensitive mutants *aux1* and *axr2*, Wong *et al.*, (2005) suggested that the mode of action of *cis*-CA was different from that of auxin. Guo *et al.*, (2011) identified two *cis*-CA-upregulated *Arabidopsis* genes, MLPL1 (AT2G01520) and MLPL2 (AT2G01530). Finally Wasano *et al.*, (2013) using a DNA microarray and Gene ontology enrichment analysis revealed (i) a low percentage of the genes expressed in responds to both *cis*-CA and IAA (ii) several IAA-upregulated genes were downregulated by *cis*-CA and finally (iii), *cis*-CA induced early auxin-responsive genes only in the roots in a tissue-specific gene analysis. All the results confirmed the suggested distinguishable responses of *cis*-CA and IAA (Guo *et al.*, 2011; Wong *et al.*, 2005).

Except a report for *Alpinia malaccensis*, that also in trace amounts insufficient to have any physiological implications, *cis*-CA was hardly reported from any other natural plant source. This therefore, almost established it as a synthetic plant growth

regulator for decades and prompted Zhiqui *et al.*, (2003) to infer that too little an effort was devoted to the study of production and function of this plant growth regulator in higher plants. This according to them was therefore a cause for very few studies available on its physiological roles. Zhiqui *et al.*, (2003) further on showed the presence of natural *cis*-CA in *Brassica parachinensis* also. They reported that the biosynthesis of *cis*-CA is not well understood, but however, suggested possible pathways for *cis*-CA formation viz (i) sunlight-mediated conversion from *trans*-CA, (ii) spontaneous conversion from *trans*-CA in the presence of an electron-transfer facilitator, (iii) isomerase-mediated conversion from *trans*-CA, and (iv) direct enzymatic biosynthesis from L-phenylalanine. Moreover, both *cis*-CA and its glucosides are natural products that could be utilized by various indigenous soil organisms.

Both *cis*-CA and its glucosides are natural products that could be utilized by various indigenous soil organisms. It is thus likely, as per Hiradate *et al.*, (2005), that *cis*-CA and its glycosides are worth considering as plant growth regulators. These they say are inexpensive to synthesize and also possess a low risk of causing environmental toxicity. However, information regarding regulatory effects of *cis*-CA on PAL enzymatic activity remains scarce. *cis*-CA and its immediate derivatives like *cis*-*p*-coumaric acid, *cis*-ferulic acid, and *cis*-caffeic acid (Zhiqui *et al.*, 2003; Wu *et al.*, 2001; Rasmussen and Rudolph, 1997; Locher *et al.*, 1994; Braun and Tevini 1993; Ohashi *et al.*, 1987; Haskins *et al.*, 1964) have been found to be occurring naturally. The biological activities of *cis*-CA have also been demonstrated in plants (Yang *et al.*, 1999; Veldstra 1953). Molecular cloning and subsequent expression of three PAL enzymes (PAL1, PAL2 and PAL4) from *Arabidopsis* have shown that the enzyme

activities of these is affected under *in vitro* conditions by the *cis*-CA isomer (Chen *et al.*, 2005).

Cinnamic acid and its methyl esters and hydroxyl derivatives *p*-coumaric, caffeic, ferulic and sinapic acids constitute important components in prolific manufacturing industries of flavours, perfumes, synthetic dyes and pharmaceuticals (Backes *et al.*, 2015; Adisakwattana *et al.*, 2012; Milkowski and Strack, 2010; Bisogno *et al.*, 2007; Mahesh *et al.*, 2007; Gang, 2005; Sestili *et al.*, 2002; Saija *et al.*, 2000; Schmidt *et al.*, 1999; Natella *et al.*, 1999; Martin-Tanguy, 1997; Dimberg *et al.*, 1993).

Hydroxyl/methyl-cinnamate derivatives being widely distributed throughout the plant kingdom play important role in plant–insect interactions. Their presence has been identified both in aerial and underground tissues of angiosperms as well as ferns (Dias *et al.*, 2003; Odell *et al.*, 1999; Wu *et al.*, 1999; Daayf *et al.*, 1997; Hiraga *et al.*, 1996; Seifert and Unger, 1994; Garcia *et al.*, 1990; Hooper *et al.*, 1984; Williams and Whitten, 1983; Schaefers and Herrmann, 1982). Various orchid species are known to have hydroxyl/methyl-cinnamates, as a component of their floral scent. This therefore acts as an attractant for pollinators like euglossine bees (Eltz and Lunau, 2005; Schiestl and Roubik, 2003; Dodson *et al.*, 1969). Electro-physiological activity of hydroxyl/methyl-cinnamates towards detached bee antennae has been reported by Eltz and Lunau, (2005). Further, leaves of sweet basil (*Ocimum basilicum*) accumulate high levels of methyl substituted derivatives of cinnamate.

Methyl-*p*-coumarate (ferulic acid) shows high levels of insecticidal or insect-deterrent (Wang *et al.*, 2015; Steyn *et al.*, 2002; Winkel-Shirley, 2002; Seifert and

Unger, 1994) and also antifungal properties (Neto *et al.*, 2015; Seifert and Unger, 1994; Bandara *et al.*, 1988). Daayf *et al.*, (1997) suggested that ferulate acts as an elicitor and an elicitor-inducible phytoalexin in *Cucumis sativus*. Lipid peroxidation is a major oxidative process of food spoilage and ferulic acid inhibits spoilage by inhibiting fatty acid peroxidation (Sevgi *et al.*, 2015; Kanski *et al.*, 2002; Graf, 1992). The ferulate esters are substituted cinnamate intermediates synthesized to be transported as cell wall components and also suberin biosynthesis (Wolszleger *et al.*, 2014; Mir Derikvand *et al.*, 2008; Rohde *et al.*, 2004) and of suberins (Rautengarten *et al.*, 2012; Molina *et al.*, 2009; Soler *et al.*, 2007; Bernards *et al.*, 1995). In grasses (monocots) the ferulate-polysaccharide esters have been shown to have well-established roles in polysaccharide-polysaccharide bridging and lignin-polysaccharide crosslinking (Ralph, 2010; Grabber *et al.*, 2000; Hatfield *et al.*, 1999; Ralph *et al.*, 1994a,b). It is, therefore, suggested that ferulate esters may be acting as induction sites for cell wall lignification (Grabber *et al.*, 2002; Ralph *et al.*, 1995) and are therefore, of a common presence in the rice, wheat, oats and sweet corn (Sri *et al.*, 2003).

Sweet basil (*Ocimum basilicum*) has interestingly been shown as an excellent system for investigating methyl-*p*-coumarate (ferulate) production in plants. In basil the glandular trichomes are metabolically super active entities producing large amounts of terpenoids, phenylpropanoids and various fatty acid derivatives (acetate pathway for aromatic compound synthesis) (Iijima *et al.*, 2004a,b). These secretory trichomes synthesize a battery of secondary compounds after diverting intermediates from major primary pathways to secondary pathways; which include the synthesis of abundance of volatile compounds also. Several basil lines, producing differential

quantities of compounds through these pathways, especially volatile and aromatic compounds have been developed (Iijima *et al.*, 2004a,b). One of the lines known as cinnamon basil (line MC) is reported for its production of methylcinnamate in sizable amounts (Iijima *et al.*, 2004a,b) by the activity of a novel carboxyl methyl-transferase designated as *p*-coumaric/cinnamic acid carboxyl methyl-transferase (CCMT).

Caffeic acid is another naturally occurring cinnamate derivative reported in many fruits, vegetables, and other plants, in varying amounts depending upon the plant and its species (Chung *et al.*, 2004). It is also reported in the coffee plant (Chung *et al.*, 2004). Caffeic acid is a 5-*p*-coumarate or 4, 5 dihydroxycinnamate and is implicated prominently in preventing DNA single-strand breakages and cytotoxicity (Meepprom *et al.*, 2015; Sestili *et al.*, 2002). Caffeic acid is a metabolite of the phenylpropanoid pathway found in several plant species, weed residues (Weir *et al.*, 2004) and soils (Siqueira *et al.*, 1991). It has been shown to induce changes in seedling emergence (Miller *et al.*, 1991), root growth (Baleroni *et al.*, 2000; Vaughan and Ord, 1990), photosynthesis (Barkosky *et al.*, 2000), evapotranspiration (Blum and Gerig, 2006), rhizogenesis (Batish *et al.*, 2008). Caffeic acid have been shown to inhibit the root length and fresh and dry weights of different plant species, such as pea (Vaughan and Ord, 1990), canola (Baleroni *et al.*, 2000), *Arabidopsis thaliana* (Reigosa and Pazos-Malvido, 2007), cucumber (Politycka and Mielcarz, 2007), mung bean (Batish *et al.*, 2008) and soybean seedlings (Bubna *et al.*, 2011). Batish *et al.*, (2008) found that caffeic acid affects early growth, and morphogenetic response of hypocotyl cutting of mung bean, also proving its phytotoxicity. In response to caffeic acid, the activities of soluble peroxidase and other antioxidative enzymes (i.e., superoxide dismutase, ascorbate peroxidase, glutathione reductase and catalase) were

reported to increase in mungbean hypocotyls (Singh *et al.*, 2009; Batish *et al.*, 2008). Exogenously applied caffeic acid decreased the PAL activity and hydrogen peroxide content and increased the soluble and cell wall bound peroxidase activities. In conjugation with piperonylic acid (PIP, an inhibitor of the cinnamate 4- hydroxylase, C4H), caffeic acid equalized the inhibitory effect of PIP, whereas the application of methylene dioxycinnamic acid (MDCA, an inhibitor of the 4-coumarate:CoA liogase, 4CL) plus caffeic acid decreased lignin production indicating that exogenously applied caffeic acid can be channelled into the phenylpropanoid pathway via the 4CL reaction, resulting in an increase of lignin monomers that solidify the cell wall and inhibit root growth (Bubna *et al.*, 2011).

Sinapic acid, a phenylpropanoid compound possessing 3,5-dimethoxyl and 4-hydroxyl substitutions in the phenyl group of cinnamic acid has been found in various herbal materials and high-bran cereals. It constitutes over 73% of the free phenolic acids (Kozłowska *et al.*, 1990). It is present in a variety of foods, edible plants, and fruits (Thiyama *et al.*, 2006; Shahidi and Nazck, 2003; Palma *et al.*, 2002; Dabrowski and Sosulski, 1984) particularly in broccoli, leafy brassicas and citrus juices (Stevanovic *et al.*, 2009). It has anxiolytic and anti-inflammatory properties and has been proposed to be an efficient antioxidant (Yun *et al.*, 2008; Yoon *et al.*, 2007). With the exception of its antioxidant activities (Kikuzaki *et al.*, 2002; Niwa *et al.*, 1999) the pharmacological properties of sinapic acid have been rarely reported (Yoon *et al.*, 2007). Sinapate esters (e.g., Sinapoylglucose and sinapoyl malate) are putatively important as UV-protectants in brassicaceae and the genes involved in their biosynthesis are well described in *Arabidopsis* (Fraser *et al.*, 2007; Sinlapadech *et al.*, 2007; Lorenzen *et al.*, 1996). Sinapoyl-malate has been suggested to act as a foliar

UV protectant in *Arabidopsis* (Landry *et al.*, 1995; Sharma and Strack 1985). The biosynthetic pathway leading to sinapoylmalate in the brassicaceae is well characterized biochemically. The *Arabidopsis* genes, encoding the enzymes in both upstream and downstream for those of UDP-glucosyltransferase (UGT) involvement have been identified by mutational analysis (Meyer *et al.*, 1996; Lorenzen *et al.*, 1996). Study of the *fah1*- mutant of *Arabidopsis* which is defective in the accumulation of sinapic acid-derived metabolites, including guaiacyl-syringyl showed that the mutant seedlings were more susceptible than wild type to UV stress (Landry *et al.*, 1995). The *fah1* locus – locus, which is the locus of *Arabidopsis*, encodes the enzyme ferulate-5-hydroxylase (F5H). It catalyzes the rate-limiting step in syringyl lignin biosynthesis and is required for the production of sinapate esters. F5H, is a cytochrome P450-dependent monooxygenase and is responsible for the formation of 5-hydroxyferulic acid; being the precursor for sinapic acid (Ruegger *et al.*, 1999; Chapple *et al.*, 1992). The product of the reaction is 5-hydroxyferulic acid, or metabolites downstream of 5-hydroxyferulic acid, such as sinapic acid and sinapoylmalate. These were involved in UV protection. However, further analysis of *Arabidopsis* for expression of *fah1* have not reported any accumulation of sinapoylmalate (Ruegger *et al.*, 1999), therefore, suggesting that levels of *fah1* do not control flux through this part of the cinnamate pathway. Since the glucose ester is the direct precursor of sinapoylmalate, manipulation of the UGT levels involved in its formation may provide a better tool to investigate the potential link between sinapoylmalate and UV protection (Lim *et al.*, 2001). Zou *et al.*, (2002) and Niwa *et al.*, (1999) have demonstrated that sinapic acid strongly inhibits peroxynitrite mediated oxidation due to its scavenging activity. Sinapic acid isolated from

Brassica juncea has been reported to be a strong inhibitor of the formation of serum protein nitration and low density lipoprotein lipid peroxidation (Zou *et al.*, 2002).

trans-CA is one of the sole precursor for the biosynthesis of all other phenylpropanoids (Dixon, 2001). Cinnamic acid has been demonstrated to have antimicrobial (Beloborodova *et al.*, 2012; Jitareanu *et al.*, 2011; Narasimhan *et al.*, 2004), antifungal (Sharma, 2011; Anslow and Stratford, 2000), antioxidant (Sharma, 2011), insect repellent (Sharma, 2011; Steyn *et al.*, 2002; Winkel-Shirley, 2002; Seifert and Unger, 1994) activities. Horbowicz *et al.*, (2009) reported growth inhibition of the primary root of buckwheat by *trans*-cinnamic acid. The *trans*-CA treatments have been shown to result in an intracellular release of Ca^{2+} from the vacuole to the cytoplasm. Therefore, an increased $[\text{Ca}^{2+}]_{\text{cyt}}$ level accompanied by gradual loss of cell viability in cucumber roots is reported. Taken together, these results were to suggest that $[\text{Ca}^{2+}]_{\text{cyt}}$ homeostatic disturbance is one of the primary triggers for *trans*-CA phytotoxicity in cucumber (Yu *et al.*, 2009).

The compound has also been reported to prevent oxidative protein damages (Adisakwattana *et al.*, 2012), stimulate non-specific membrane permeability thus allowing proton influx across the plasma membrane (Chambel *et al.*, 1999), induce autotoxicity (Chen *et al.*, 2011; Ding *et al.*, 2007), increase oil percent, total oil yield/plant and sugar content (El-Moursi *et al.*, 2012), induce phytotoxicity (Yu *et al.*, 2009), reduce ribulose-1,5-bisphosphate carboxylase (RuBPC) activity and the endogenous polyamine levels (Huang and Bie, 2010), cause reduction in oxidative damage through the induction of ROS scavenging enzymes like superoxide dismutase (SOD) and peroxidase (POD) (Singh *et al.*, 2013). CA was found with the

up regulation ability of ROS scavenging enzymes as well as induction of new proteome biosynthesis in maize plants under saline condition. These findings may be translated into efforts aimed to develop salt tolerant genotypes and maximize the use of CA under saline environment (Singh *et al.*, 2013).

Cinnamic acid and its methylated/hydroxylated derivatives (*p*-coumaric, caffeic, ferulic and sinapic acids) are known allelochemicals that affect the seed germination and root growth of many plant species (Lima *et al.*, 2013; Schoch *et al.*, 2002; Hrubcova *et al.*, 2000). Recent studies have indicated that the reduction of root growth in soyabean by these allelochemicals is associated with premature cell wall lignification (Lima *et al.*, 2013; Yamauchi and Fukushima, 2004). As the influx/channelling of these exogenously applied allelochemicals into the phenylpropanoid pathway increases the total lignin content, by altering the sum and ratios of the *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) lignin monomers (Bubna *et al.*, 2011; Zanardo *et al.*, 2009; Dos Santos *et al.*, 2008). Therefore, as a consequence, the stiffening of the cell wall is enhanced. Due to this premature cell wall lignification the soybean root growth is restricted (Lima *et al.*, 2013).

Jitareanu *et al.*, (2013) have revealed the inhibition of root hair formation and also changes in the structure of the vascular system with phloem elements being most affected in response to cinnamic acid and its various derivatives such as, 4-methoxy-cinnamic acid, 4-(N,N-dimethylamino)-cinnamic acid, ferulic acid, 3,4-dimethoxy-cinnamic acid, *p*-coumaric acid, 4-methyl-cinnamic acid, 4-chloro-cinnamic acid, 3-bromo-cinnamic acid and caffeic acid. The effects of these compounds on the development and histo-anatomy of *Phaseolus vulgaris* therefore, confirmed their

phytotoxic activities. They stress the importance of further toxicological evaluation for these investigated compounds and their analogues. Among a library of *Gloeobacter violaceus* metabolites, Prevost *et al.*, (2013) identified a series of cinnamic acid derivatives, which antagonize the GLIC proton-elicited response. Structure–activity analysis shows a key contribution of the carboxylate moiety to GLIC inhibition. Molecular docking coupled to site-directed mutagenesis support that the binding pocket is located below the classical orthosteric site. Therefore, these antagonists provide new tools to modulate conformation of GLIC, currently used as a prototypic pentameric ligand gated ion channels (pLGICs) and opens new avenues to study the signal transduction mechanism (Prevost *et al.*, 2013).
