

REVIEW

Phytoalexins from Poaceae : their types, biosynthesis, role in biotic and abiotic stress adaptation and application in human health

PUJA SASHANKAR¹ AND BISHWANATH CHAKRABORTY^{2*}

¹Department of Botany, Gorubathan Govt. College, P.O. Fagu, Pin -735231, Kalimpong, West Bengal

²Department of Botany, University of North Bengal, Siliguri 734013, West Bengal

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Plants synthesize low molecular weight antimicrobial compounds known as phytoalexins in response to biotic and abiotic stresses, which take part in intricate defence system and thereby enable plants to control invading micro-organisms. Extensive work has been done on diverse chemically active phytoalexins produced by a number of plant families. In this review attempts have been made to highlight the developments of phytoalexin research on cereal crops – rice, sorghum, wheat and maize belonging to family Poaceae with special emphasis on their types, biological activity, mechanisms of their synthesis, induction and their role in stress adaptation. Prospects of these phytoalexins in food preservation and their role in stabilizing economic status and food security have also been discussed.

Keywords: Elicitor, induction, momilactones, oryzalexin, phytocassanes, rice phytoalexins, sakuranetin, sakuratenin,

INTRODUCTION

Phytoalexins were first conceived by Muller and Borger during 1940 as antifungal substances produced by cells of potato tubers as they develop hypersensitive response following penetration and infection by an incompatible races as of *Phytophthora infestans* (Mont.) de Bary. The hypothetical phytoalexins were suggested to prevent further growth of the hyphae in these cells and also to confer cross-protection against infection by compatible races inoculated at the same time or some hours later. Although no chemical entities were isolated from potato at this time, and they suggested that phytoalexins were non-specific in their effect on fungi, and that a major difference between resistant and susceptible varieties of plant was the speed of formation of phytoalexins in response to infection. Phytoalexins are a diverse group of low molecular weight secondary metabolites that exhibit antimicrobial activity, and are momentarily generated by either endogenous or

exogenous signal molecules (biotic and abiotic stress) called elicitors. They are considered as molecular markers of disease resistance (Pedras *et al.* 2011; Schmelz *et al.* 2011) and their antimicrobial effect is targeted to a variety of plant pathogens.

Phytoalexins have been found in at least 75 host plants, including cruciferous vegetables, soybean, garlic, tomato, beans, potatoes, and cereals, suggesting that a wide variety of plants may be rich sources of these compounds. The most important phytoalexins are currently thought to be derived from the shikimate (phenylpropanoid), isoprenoid (terpenoid) and alkaloid forming pathways, exemplified by the stilbenes, sesquiterpenes and camalexins, respectively (Großkinsky *et al.* 2012). Phytoalexin research has evolved over the years not only to cover defense against plant pathogens and pests but also extends to human health promotion (Bouee *et al.* 2009; Jahangir *et al.* 2009; Huffaker *et al.*, 2011; Nwachukwu *et al.* 2013). Induced resistance in plants against pathogen attack occurs following a

*Correspondence: bncnbu@gmail.com

series of biochemical events (Jeandet *et al.*, 2013) such as elicitor-receptor recognition, formation of signal molecules, activation of gene leading to *de-novo* synthesis of defense enzymes mainly chalcone synthase (CHS), chalcone isomerase (CHI) and phenylalanine ammonia lyase (PAL) involved in phytoalexin biosynthesis. Chemical diversity of phytoalexins and their antimicrobial and biological activities, different biosynthetic pathways and their role in plant defence mechanisms, molecular events for induced immunity in plants, transport and metabolism in fungi as well as their role in human health have been extensively reviewed (Jeandet *et al.*, 2014). Various phytoalexin synthesis in different families during incompatible interaction with fungal pathogens has been elucidated (Chakraborty, 2018). Recently expression of phytoalexin biosynthesis pathway genes in sugarcane following interaction with red rot pathogen has been explored (Nandakumar *et al.*, 2021). In this review attempts have been made to highlight the important developments of phytoalexin research exceptionally on those crop plants viz. rice, wheat, sorghum and maize belonging to family Poaceae with special emphasis on their types, biosynthesis, elicitation, mode of action.

PHYTOALEXINS FROM POACEAE

The Poaceae or grass family covers substantial surface area of the earth's land mass. Cereal crop plants, the most important members of the Poaceae, provide the bulk of the world's caloric intake. Agricultural automation can play an important role towards achieving the Sustainable Development Goals (SDGs), not least SDG 1 (No Poverty) and SDG 2 (Zero Hunger) and those relating to environmental sustainability and climate change, by building resilience, raising productivity and resource-use efficiency, and improving food quality and safety (FAO, 2022) World rice production is forecast to rise by 1.3 percent in 2023/24 to 523.5 million tonnes, while international trade is expected to drop by 4.3 percent in volume terms to 53.6 million tonnes (FAO, 2023). Rice and wheat are the two most important cereals for direct human consumption; however maize, wheat and rice together accounted for 87% of all grain production worldwide and 43% of all food calories over a

decade ago. Barley is grown largely in high altitudes, mainly as animal feed, while sorghum is a sturdy pro-poor cereal that is grown in warm ecological zones.

Our discussions will be focused on prospects of phytoalexins in these most important cereal crops. Members of the Poaceae produce largely diterpenoid phytoalexins. The committed step in labdane-related diterpenoid biosynthesis is catalysed by labdadienyl/copalyl diphosphate synthase, CPS (Wu *et al.* 2012). Molecular phylogenetic analyses based on characterization of maize, barley, wheat and rice CPS suggest that the expansion and functional diversification of the CPS gene to more specialized metabolism may have occurred early in cereals (Harris *et al.* 2005; Peters, 2006; Spielmeier *et al.* 2004). Thus, it appears plausible that more specialized labdane-related diterpenoid metabolism will be widespread throughout the Poaceae. Some members of the family however produce flavonoid phytoalexins.

RICE PHYTOALEXIN

Plants respond to pathogen infection through a variety of defense responses that include the production of phytoalexins, antifungal secondary metabolites. Rice (*Oryza sativa* L.) is cultivated in warm climate areas and is an important food source. It provides about 20% of the total direct human food-energy intake worldwide and is also the predominant staple food in many developing countries especially in Asia. Rice production, in milled terms, increased steadily over the years and will reach 523.5 million tonnes during 2023-24 (FAO, 2023). Furthermore, the relatively small size of the rice genome, approximately 430 Mb, the ease of its transformation, the extensive genetic sequence information available for it, coupled with its importance as a food crop make rice a model system and most studied member of the Poaceae. Rice has been studied extensively, especially with respect to its metabolism including the production of phytoalexins in response to extrinsic stress (Kikuchi *et al.* 2003; Peters, 2006). In rice, diterpenoid phytoalexins and one flavonoid phytoalexin have been identified following treatment with elicitors such as chitin

Table 1: Phytoalexins from Poaceae

| Crop plants | Phytoalexin | Compound | References |
|-------------|---------------------|---|-------------------------------|
| Rice | Diterpenoids | Momilactones Phytocassanes Oryzalexin | Schmelz <i>et al.</i> (2014) |
| | Flavanones | Sakuranetin | Jeandet <i>et al.</i> (2013) |
| Maize | Sesquiterpenoid | Zealexins Kauralexins HDMBOA-glucose | Huffakar <i>et al.</i> (2011) |
| Sorghum | Deoxyanthocyanidins | Luteolinidin Apigeninidin | Poloni and Schirawski (2014) |

oligosaccharide (biotic) and in leaves infected with the blast fungus *Magnaporthe grisea* (biotic) or irradiated with UV light (abiotic). Other known elicitors of phytoalexin production in rice are cerebrosides and xylanase protein from *Trichoderma viride*, and ethylene-inducing xylanase in rice-cultured cells. These elicitors are known to bring about a variety of defense responses in rice (Okada *et al.* 2009; Kurusu *et al.* 2010). From the prism of their hydrocarbon precursors, rice phytoalexins can be classified into different groups namely oryzalexins, phytocassanes, momilactones and sakuranetin (Okada *et al.* 2009). The major intermediates in the biosynthesis of the four major groups of diterpene phytoalexins oryzalexins A–F, phytocassanes A–E, momilactones A and B and oryzalexin S are ent-sandaracopimaradiene, entcassa-12,15-diene, 9 α -pimara-7,15-diene and stemar-13-ene, respectively (Toyomasu *et al.* 2008) whereas sakuranetin belongs to the flavonoids. Different types of phytoalexins produced by rice have been presented in Fig.1 and their antimicrobial activities have been presented below.

Momilactones

Momilactones are potent phytoalexins and allelochemicals, and have been found only in rice and the moss *Hypnum plumaeforme* (Kato-Noguchi, 2011). Momilactones A (3-oxo-9 α -pimara-7,15-dien-19,6 α -olide) and B (3,20-epoxy-3 α -hydroxy-9 α -pimara-7,15-dien-19,6 α -olide) were first isolated from rice husks as growth inhibitors. They were later found as phytoalexins in the leaves

and straw of rice treated with 2,2-dichloro-3,3-dimethylcyclopropane carboxylic acid before infection by *Magnaporthe grisea*. In addition to biotic (*Magnaporthe grisea*) and abiotic (physical and chemical treatments) elicitors, several other elicitors of these diterpenoid phytoalexins have been identified including cerebroside, α -glucosyloligosaccharides chitin, N-acetylchitoheptaose and chitosan (Koga *et al.* 2003; Okada *et al.* 2009; Shimizu *et al.* 2012). Momilactones A and B exert antifungal activity toward *Magnaporthe oryzae* which causes rice blast, *Botrytis cinerae*, *Fusarium solani*, *Colletotrichum gloeosporides* and *Fusarium oxysporum* although the antifungal activity of momilactone B is thought to be better than that of momilactone A (Fukuta *et al.* 2007). This discrepancy could be due to the additional six-membered ring or the hydroxyl group, which seem to be the only additional features in the structure of momilactone B. Moreover, both phytoalexins also possess antibacterial activity against *Escherichia coli*, *Pseudomonas ovalis*, *Bacillus pumilus* and *Bacillus cereus* (Fukuta *et al.* 2007)

Phytocassanes

Phytocassane A to D are produced upon elicitation by fungal pathogen *Magnaporthe grisea* and isolated from rice stems infected with *Rhizoctonia solani*. Phytocassane E, which is induced by the potato pathogen *Phytophthora infestans* also shows antifungal property against *Magnaporthe grisea*. These studies indicated that the antifungal activities of phytocassane B, C and E (EC50

values, 4–7 $\mu\text{g}/\text{mL}$) are about four-folds stronger than the activities of phytocassane A and D. This higher antifungal activity was attributed to the β -hydroxyl group in C-1 position of phytocassane B, C and E, which can form intramolecular hydrogen bond with the carbonyl group in position C-11.

Oryzalexin

Like momilactones, oryzalexins A, B and C were initially isolated from *Magnaporthe grisea* infected leaf tissues of rice. However, the biosynthesis and accumulation of oryzalexin A, B and C have also been shown to be elicited by UV radiation and jasmonic acid. When tested for antifungal activity against *Magnaporthe grisea* spore germination, the oryzalexins were shown to be weaker (EC_{50} values, 20–136 $\mu\text{g}/\text{mL}$) than momilactones A and B with EC_{50} values of 15 and 3 $\mu\text{g}/\text{mL}$, respectively, and the additional lactone moiety in the momilactones was suggested to be the active functionality (Koga, 2003). Among the oryzalexins, oryzalexin D seems to have the best antifungal activity and this could possibly be due to the hydroxyl group at C-3 and C-7 positions in its structure.

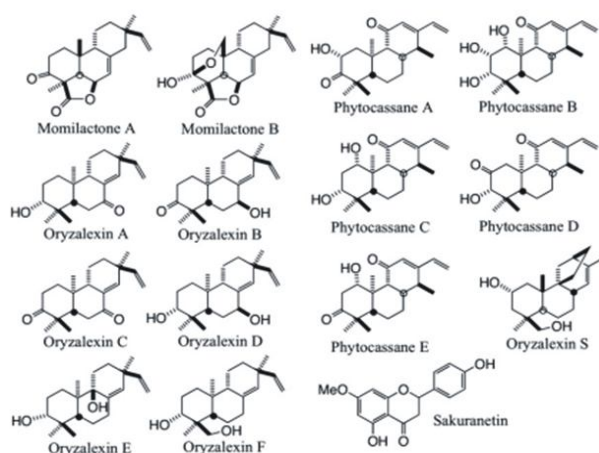


Fig. 1 : Rice phytoalexins (Source: Peters, 2006)

Sakuranetin

Sakuranetin (5,42 -dihydroxy-7-methoxyflavone or 7-O-methylnaringenin) is a flavone phytoalexin that was first identified as a phytoalexin produced in rice leaves. Sakuranetin accumulates in response to UV light treatment or other elicitors

such as attack by pathogens and chemical (copper chloride and jasmonic acid) treatment. Other known elicitors are methionine, coronatine and chisotanologomers (Nakazato *et al.*, 2000; Tamogami and Kodama, 2000; Obara *et al.*, 2002). The ability of sakuranetin to inhibit *Magnaporthe grisea* spore germination (EC_{50} value, 15 $\mu\text{g}/\text{mL}$) is thought to be due to the methyl group in the C-7 position (Koga, 2003). Moreover, the flavonoid phytoalexin has been recently pursued as a potential antibacterial agent due to its inhibition of *Helicobacter pylori* β -hydroxyacyl-acyl carrier protein dehydratase, an enzyme required for cellular fatty acid synthesis, by preventing the binding of substrate to the enzyme's active site (Zhang *et al.* 2008).

BIOSYNTHESIS

Rice (*Oryza sativa*) is among the most important staple foods in the world. Cultivated rice produces a variety of labdane-related diterpenoids; not only phytohormone gibberellins (GAs) but also phytoalexins for defense including phytocassanes, momilactones and oryzalexins. Their carbon skeleton diterpenes are constructed from geranylgeranyl diphosphate *via* ent-copalyl diphosphate (ent-CDP) or its diastereomer syn-CDP. These two-step reactions are successively catalyzed by homologs of the two diterpene synthases, ent-CDP synthase (ent-CPS) and ent-kaurene synthase (KS) that are responsible for the biosynthesis of GAs; e.g. *OsCPS4* and *OsKSL8* that are involved in the biosynthesis of oryzalexin S, a rice phytoalexin. Identification of the *O* diterpene synthase genes uncovered two diterpenoid biosynthesis gene clusters, termed as *Os02g* cluster and *Os04g* cluster. It has been shown that the *Os02g* cluster and *Os04g* cluster are mainly responsible for biosynthesis of phytocassanes and momilactones, respectively.

Extensive functional characterization of diterpenoid phytoalexin biosynthetic genes in rice, revealed that the genes for the biosynthesis of the major diterpenoid phytoalexins, phytocassanes and momilactones, are clustered on chromosomes 2 and 4 and that their expression is coordinately induced in rice cells after elicitation. Isopentenyl

diphosphate, an early precursor of diterpenoid phytoalexins, was found to be synthesized through the plastidic methylerythritol phosphate pathway. It was found that chitin elicitor receptor kinase *OsCERK1* and a mitogen-activated protein kinase cascade, the *OsMKK4-OsMPK6* cascade, play essential roles in the elicitor-induced production of diterpenoid phytoalexins. In addition, a basic leucine zipper transcription factor, *OsTGAPI*, was identified as a key regulator of the coordinated expression of the clustered genes and the methylerythritol phosphate pathway genes. Naringenin 7-O-methyltransferase (*OsNOMT*) was also identified as a key enzyme in the biosynthesis of another major rice phytoalexin, sakuranetin.

Fifteen phytoalexins have been identified in rice plants infected by the blast fungus *Magnaporthe oryzae* and rice leaves irradiated with ultraviolet (UV) light. Although one of these compounds, sakuranetin, is a flavonoid, the remaining 14 are diterpenoids that can be classified into four groups based on basic carbon skeleton: phytocassanes A to E, oryzalexins A to F, momilactones A and B, and oryzalexin S. In rice plants, the production of phytoalexins, including sakuranetin, is induced by pathogen infection, UV irradiation, jasmonates, and CuCl_2 . In the biosynthetic pathways of diterpenoid phytoalexins common precursor geranylgeranyl diphosphate (GGDP) is postulated to be sequentially cyclized via ent-copalyl diphosphate (ent-CDP) to entcassa-12, 15-diene and ent-sandaracopimaradiene, producing phytocassanes A to E and oryzalexins A to F respectively. GGDP is also probably cyclized via syn- CDP, to 9H-pimara-7, 15-diene and temar-13-ene, producing momilactones A and B and to oryzalexin S respectively. In fact, it was demonstrated that enzyme preparations from chitin-elicited suspension- cultured rice cells converted ent-CDP to ent-sandaracopimaradiene, a putative diterpene hydrocarbon precursor of oryzalexins A to F. Similarly syn-CDP was converted to 9H-pimara-7,15-diene and temar-13-ene, putative diterpene hydrocarbon precursors of momilactones A and B and oryzalexin S respectively (Fig.2). It has also been suggested that microsomal cytochrome P⁴⁵⁰ monooxygenases and a dehydrogenase are involved in the downstream oxidation of diterpene

hydrocarbons, producing the bioactive phytoalexins. Sakuranetin is biosynthesized from naringenin by S- adenosyl-L-methionine-dependent naringenin 7-Omethyltransferase (NOMT). Because naringenin is a biosynthetic intermediate to a variety of flavonoids, NOMT plays a key role in sakuranetin biosynthesis at a branch point from the common flavonoid biosynthetic pathway (Peters, 2006).

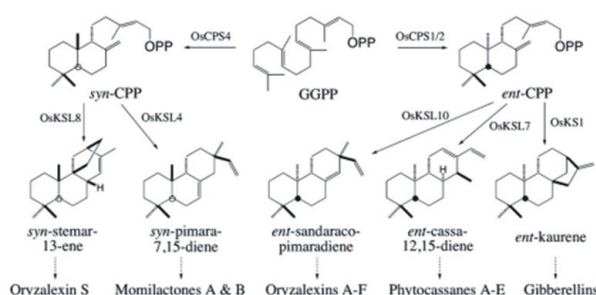


Fig. 2. Rice phytoalexin biosynthesis. The corresponding enzymes are indicated along with the derived products. Dashed arrows indicate multiple enzymatic steps. (Source: Peters, 2006)

Biosynthetic pathway of diterpene phytoalexin has been confirmed by the observed synchronous accumulation of seven methyl erythritol phosphate (MEP) pathway gene transcripts (*OsDXS3*, *OsDXR*, *OsCMS*, *OsCMK*, *OsMCS*, *OsHDS* and *OsHDR*) in elicitor-induced rice (*Oryza sativa*) cells and the next steps of this biosynthesis are predicted to occur in plastids (Jeandet *et al.* 2014). Diterpenoids result from the subsequent action of diverse enzymes using GGDP as the starting block. Class II diterpene cyclases named copalyldiphosphate synthases (CPS) are the first to act on GGDP catalyzing the initial cyclization of the latter to copalyldiphosphate (CDP). CDP is the required substrate for class I diterpene synthases named kaurene synthase like (KSL). Sequential action of CPS and KSL produces the olefin precursors of the main diterpene phytoalexin families. Stereochemically differentiated isomers are used subsequently by KSL: the *ent*-CDP in the biosynthesis of phytocassanes A-E and oryzalexins A-F and the *syn*-CDP in the construction of momilactones A and B. Further additions of oxygen in the formation of oryzalexins, momilactones and phytocassanes require a series of cytochrome P450 (CYPs) (Fig.3).

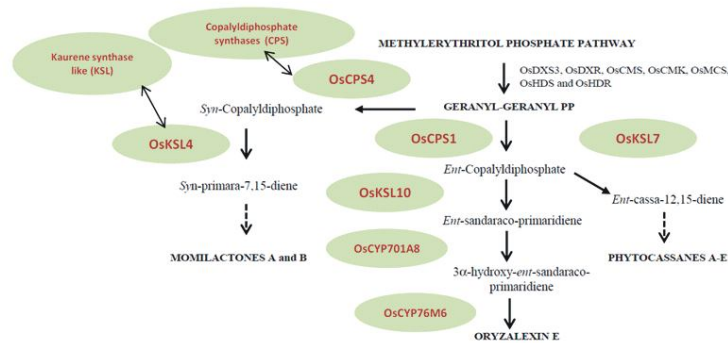


Fig. 3 : Biosynthetic pathway of diterpenoid phytoalexins (Source : Jeandet *et al.* 2014)

In rice plants, diterpenoid phytoalexins and the flavonoid phytoalexin sakuranetin are induced not only by biotic and abiotic stresses such as the pathogen *Magnaporthe oryzae*, CuCl_2 and UV irradiation, but also by the plant hormone jasmonate. Furthermore, treatment with *M. oryzae* and CuCl_2 induces the biosynthesis of jasmonates in rice plants. Recent studies have indicated that endogenous jasmonyl-L-isoleucine (JA-Ile) is not necessarily required for the production of diterpenoid phytoalexins in blast-infected or CuCl_2 -treated rice leaves. However, JA-Ile is required for the accumulation of the flavonoid phytoalexin, sakuranetin. The roles of JA-Ile in UV-induced phytoalexin production were investigated and found that UV-irradiation induces the biosynthesis of JA-Ile and its precursor jasmonic acid. Further, it was observed that rice jasmonate biosynthesis mutants produced diterpenoid phytoalexins but not sakuranetin in response to UV, indicating that JA-Ile is required for the production of sakuranetin but not diterpenoid phytoalexins in UV-irradiated rice leaves. Elucidating the molecular mechanisms of the modulation of phytoalexin biosynthesis finds applications in plant engineering for disease resistance.

SORGHUM PHYTOALEXINS

Sorghum produces two distinct phytoalexins belonging to the 3-deoxyanthocyanidin chemical group, known as apigeninidin[2-(4-hydroxyphenyl)benzopyrylium chloride] and luteolinidin[2-(3,4-dihydroxyphenyl)chromenylium-5,7-diol] (Fig. 4A), in addition to a variety of derivatives, like 5-methoxy-luteolinidin, caffeic acid ester of

arabinosyl-5-O-apigeninidin, and 7-methoxyapigeninidin. A mixture of these characteristic reddish- and orange-colored compounds are known to be synthesized in the cytoplasm of epidermal sorghum cells infected with *Colletotrichum sublineolum* where they accumulate in initially colorless inclusion bodies. These inclusion bodies migrate to the infection zone, where they first accumulate and become pigmented, then lose their spherical shape and release their red contents at the infection site. Accumulation of the 3-deoxyanthocyanidins occurs much faster in pathogen-challenged cells of resistant cultivars than of susceptible ones, suggesting that early phytoalexin accumulation is important to prevent proliferation and spread of fungal hyphae (Basavaraju *et al.* 2009)

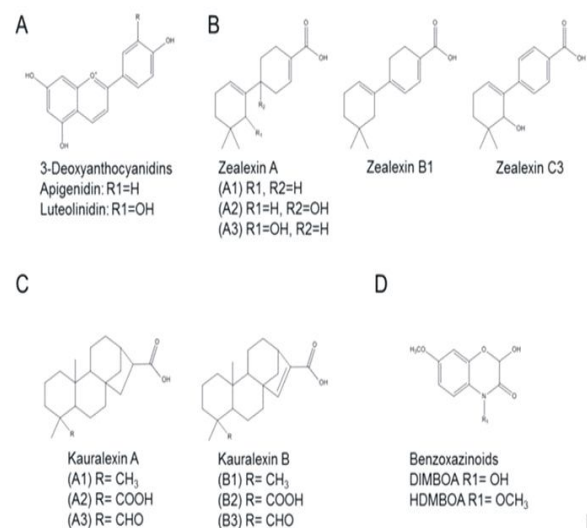


Fig. 4 : Structures of phytoalexins produced by (A) Sorghum and (B - D) Maize (Source: Poloni and Schirawski, 2014)

Increase levels in apigeninidin and luteolinidin was accompanied by increased concentrations of peroxidases, β -1,3-glucanases and chitinases in sorghum seedlings following inoculation with *Fusarium proliferatum* and *Fusarium thapsinum*, while in addition to phytoalexin, a fast and coordinated accumulation of *PR-10* and chalcone synthase transcripts was also observed following inoculation with *Cochliobolus heterostrophus* (Huang and Backhouse, 2004).

MAIZE PHYTOALEXIN

In maize, phytoalexins are represented by members of the terpenoid class, including zealexins and kauralexins on the one hand and benzoxazinoids on the other hand (Fig 4 B-D), the biosynthesis of which are fully described.

In response to stem attack by the European corn borer (*Ostriniana bilalis*) and fungi, Schmelz *et al*, (2011) have observed the induced accumulation of six ent-kaurane-related diterpenoids, collectively termed kauralexins. Isolation and identification of the predominant *Rhizopus microsporus*-induced metabolites revealed ent-kaur-19-al-17-oic acid and the unique analog ent-kaur-15-en-19-al-17-oic acid, assigned as kauralexins A3 and B3, respectively. Encoding an entcopalyl diphosphate synthase, fungal-induced An2 transcript accumulation precedes highly localized kauralexin production, which can eventually exceed 100 $\mu\text{g g}^{-1}$ fresh weight. Pharmacological applications of jasmonic acid and ethylene also synergize the induced accumulation of kauralexins. Occurring at elevated levels in the scutella of all inbred lines examined, kauralexins appear ubiquitous in maize. At concentrations as low as 10 $\mu\text{g mL}^{-1}$, kauralexin B3 significantly inhibited the growth of the opportunistic necrotroph *R. microsporus* and the causal agent of anthracnose stalk rot, *Colletotrichum graminicola*. Kauralexins also exhibited significant *O. nubilalis* antifeedant activity. Presence of diterpenoid defenses in maize has been established which enables a more detailed analysis of their biosynthetic pathways, regulation, and crop defense function.

Nonvolatile terpenoid phytoalexins occur throughout the plant kingdom, but until recently

were not known constituents of chemical defense in maize (*Zea mays*). Huffakar *et al.* (2011) have described a novel family of ubiquitous maize sesquiterpenoid phytoalexins, termed zealexins, which were discovered through characterization of *Fusarium graminearum*-induced responses. Zealexins accumulate to levels greater than 800 $\mu\text{g g}^{-1}$ fresh weight in *F. graminearum*-infected tissue. Their production is also elicited by a wide variety of fungi, *Ostriniana nubilalis* herbivory, and the synergistic action of jasmonic acid and ethylene. Zealexins exhibit antifungal activity against numerous phytopathogenic fungi at physiologically relevant concentrations. Structural elucidation of four members of this complex family revealed that all are acidic sesquiterpenoids containing a hydrocarbon skeleton that resembles b-macrocarpene. Induced zealexin accumulation is preceded by increased expression of the genes encoding Terpene synthase 6 (TPS6) and TPS11, which catalyze b-macrocarpene production. Furthermore, zealexin accumulation displays direct positive relationships with the transcript levels of both genes. Microarray analysis of *F. graminearum*-infected tissue revealed that *Tps6/Tps11* were among the most highly up-regulated genes, as was An2, an ent-copalyl diphosphate synthase associated with production of kauralexins. Transcript profiling suggests that zealexins cooccur with a number of antimicrobial proteins, including chitinases and pathogenesis-related proteins. In addition to zealexins, kauralexins and the benzoxazinoid 2-hydroxy-4,7-dimethoxy-1,4-benzoxazin-3-one-glucose (HDMBOA-glucose) were produced in fungal-infected tissue. HDMBOA-glucose accumulation occurred in both wild-type and benzoxazine-deficient1 (*bx1*) mutant lines, indicating that *Bx1* gene activity is not required for HDMBOA biosynthesis. Together these results indicate an important cooperative role of terpenoid phytoalexins in maize biochemical defense. Biosynthesis of the 3-deoxyanthocyanidin phytoalexins from sorghum is reported in transgenic maize lines expressing the MYB transcription factor *yellow seed1 (y1)*, an orthologue of the maize gene *pericarp color1 (p1)* in the work of Ibraheem *et al.*(2015). Expression of this transcription factor leads to the production of chemically modified 3-deoxyanthocyanidins and

a resistance response of Y1-maize plants to leaf blight (*Colletotrichum graminicola*).

BIOSYNTHESIS

Biosynthesis of the 3-deoxyanthocyanidin phytoalexins is independent of light and occurs in the dark, in contrast to the biosynthesis of anthocyanins that is light dependent. Biosynthesis of the 3-deoxyanthocyanidins luteolinidin and apigeninidin, of the flavones luteolin and apigenin, and the leucoanthocyanidins and anthocyanins occurs via common and specific pathway steps (Liu *et al.*, 2010). Common steps include the formation of *p*-coumaroyl CoA, that is generated from phenylalanine via the action of the enzymes phenylalanine ammonia lyase (PAL) to synthesize cinnamic acid, cinnamate-4-hydroxylase (C4H) to synthesize *p*-coumaric acid, and coumaroyl CoA ligase (CCL) for generation of *p*-coumaroyl CoA. *p*-Coumaroyl CoA is the substrate of the enzyme chalcone synthase (CHS), which catalyzes the condensation of *p*-coumaroyl CoA and three molecules of malonyl CoA form naringenin chalcone that is converted to naringenin by a chalcone isomerase (CHI). Genes of the 3-deoxyanthocyanidin biosynthesis phenylalanine ammonia lyase, chalcone synthase, and dihydroflavonol 4-reductase were induced when sorghum was challenged with the maize pathogens *Bipolaris maydis* or *Sporisorium reilianum* f. sp. *zeae* but not when challenged with the sorghum pathogen *S. reilianum* f. sp. *reilianum* (Zuther *et al.* 2012). Whole transcriptome of sorghum inoculated with the necrotroph *Bipolaris sorghicola* revealed that in addition to the up-regulation of genes encoding key enzymes for phytoalexin biosynthesis, many other plant genes with a suspected role in defense were up-regulated, which includes genes encoding plant receptors, genes involved in MAPK cascades and Calcium signaling, transcription factors and genes involved in downstream responses (peroxidases, PR proteins and genes implicated in biosynthesis of lignin (Mizuno *et al.*, 2012; Yazawa *et al.*, 2013). Biosynthetic pathways of phytoalexins from sorghum has been discussed by Poloni and Schirawski (2014) and presented in Fig. 5.

ELICITORS OF PHYTOALEXINS

The molecules that signal plants to begin the process of phytoalexin synthesis are called elicitors. Elicitors of biotic origin may be involved in the interaction of plants and potential pathogens, whereas abiotic elicitors are not involved in normal host-pathogen interactions. In natural conditions, the stimulus is provided by the presence of the micro-organism and its perception by the host initiates the chain of events leading to phytoalexin synthesis. Biotic elicitors may originate in the invading organism, in which case they are referred to as “exogenous”, whereas “endogenous” elicitors are of plant origin and are generated by the interaction between micro-organism and plant. Molecules with elicitor activity have been identified across a wide range of structural types including polysaccharides, glycoproteins, lipids, lipopolysaccharides, oligosaccharides and even enzymes, though their activity can be attributed to their effect in releasing elicitor-active components from the cell walls of the pathogen or host. Abiotic elicitors form a diverse collection of molecules that are not derived from natural sources, such as the tissues of the pathogen or host. Under normal circumstances, they would not be encountered by the plant. The group includes compounds such as fungicides, salts of heavy metals, for example Cu²⁺ and Hg²⁺; the detergents, basic molecules such as polylysine and histone; reagents that are intercalated DNA. Treatment of plant tissues with factors that cause stress, for example repeated freezing and thawing, wounding or exposure to UV light can also induce phytoalexin synthesis. It is well known that treatment of plants with various biotic or abiotic agents, the so-called elicitors, can activate complex mechanisms in the cells by altering primary and secondary metabolisms in a coordinate fashion. Elicitors are also recognized as efficient inducers of phytoalexins.

INDUCTION OF PHYTOALEXINS

Alternatives to fungicides in plant protection have arisen with the discovery of disease resistance inducers of biotic and abiotic origins that induce a localized or systemic resistance in susceptible plants, which become resistant to subsequent

infections. Depending on their efficacy, these compounds can be used either alone or in combination with fungicides. Many compounds have been commercially released in some countries as a plant health promoter of annual crops under the name Bion or Actigard (Peng and Zhenqi, 2006). SA-dependent defense pathway can be activated by treatment of plants with chemical inducers such as benzo (1,2,3)-thiadiazole-7- carbothioic acid-S-methyl ester (acibenzolar-S-methyl, ASM or BTH, Bion) developed as a potent SAR activators which do not only possess antimicrobial properties, but instead increase the crop resistance to diseases by activating SAR signal transduction pathways in several plant species. BTH is a chemical analogue of SA and has been used successfully to induce resistance to a wide range of diseases on held crops. The non protein amino acid, β -aminobutyric acid (BABA) protects numerous plants against various pathogens. Several products have also been used as inducers of resistance in plants against pathogens, including chitosan, salicylic acid analogues living or processed fungal products (Hjeljord *et al.* 2000) and seaweed extracts. Certain synthetic compounds with no direct antimicrobial effect such as 2,6- dichloroisonicotinic acid (INA) and potassium salts has been reported to induce SAR in plants (Oostendorp *et al.* 2001). Seed bacterization as well as foliar application of *Bacillus altitudinis* could markedly reduced the natural occurrence of Brown spot disease of rice plants caused by *Drechslera oryzae*. HPLC analysis of treated rice plants showed highest level of phytoalexin namely Phytocassanes suggesting induction of resistance in rice plants (cultivar Black nuniya) against *D. oryzae* (Khatai *et al.* 2016).

ROLE OF RICE PHYTOALEXINS IN STRESS ADAPTATION

Biotic Stress

Antimicrobial activities of rice phytoalexins have been investigated with rice pathogens, in particular, blast fungus. Rice diterpenoid phytoalexins inhibit spore germination and germ tube growth of *Magnaporthe oryzae* with half-inhibition concentration (IC₅₀) values of 1-35 and 2-103g/ml respectively. Sakuranetin was found to exhibit

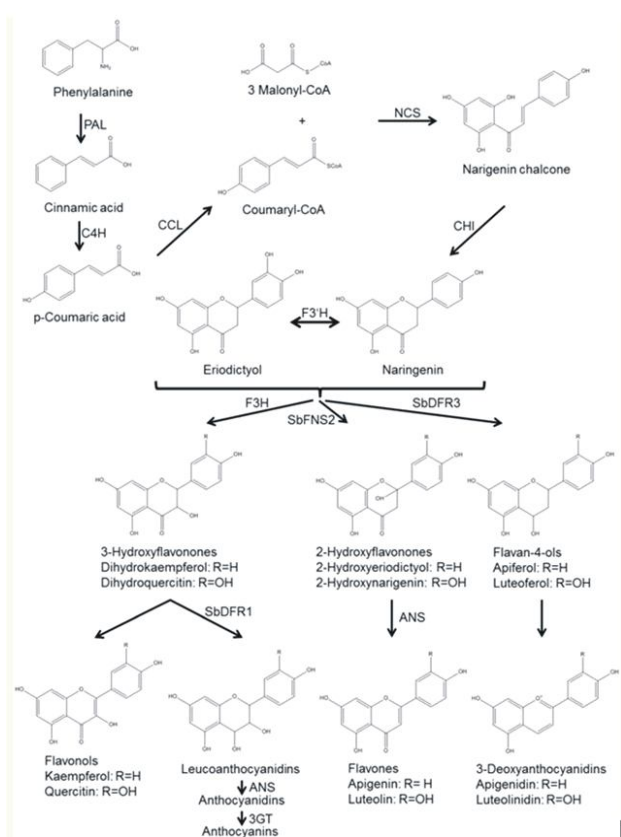


Fig. 5 :Biosynthetic pathway of 3-deoxyanthocyanidins in Sorghum. [ANS – anthocyanidin synthase, C4H- cinnamate-4-hydroxylase, CCL – coumaryl-CoA ligase, CHI – chalcone isomerase, F3'H – flavanone-3'-hydroxylase, F3H – flavanone-3-hydroxylase, NCS- Naringenin chalcone synthase, PAL – phenylalanine ammonia lyase, SbDFR1 – dihydroflavonol-4-reductase 1, SbDFR3 – dihydroflavonol 4-reductase 3, SbFNS2 – flavones synthase 2] (Source: Poloni and Schirawski ,2014)

strong inhibitory activity against germ tube growth of blast fungus with an IC₅₀ of 5 g/mL, which is more potent than diterpenoid phytoalexins . In addition to anti-blast fungal activity, a recent study determined that sakuranetin has broad antimicrobial activity against diverse rice fungal and bacterial pathogens. Bacterial blight is one of the most destructive rice diseases, which caused by *Xanthomonas oryzae* pv. *oryzae*. The characterization of the cytochrome P450gene Oscyp7I22, which belongs to the CYP71Z subfamily, revealed that the over expression of Oscyp7I22 in rice enhances resistance to *Xanthomonas oryzae* pv. *oryzae* at the booting stage (Li *et.al.*, 2012). It was also reported that the ectopic expression of hrf1 in rice enhanced resistance to bacterial blight (Akagi *et.al.*, 2014). Recently, several amide compounds, N- benzoyltryptamine, N- cinnamoyltryptamine and N- cinnamoyltyramine,

reported from UV-irradiated rice leaves shown antimicrobial activity against *Bipolaris oryzae* and *X. oryzae* pv. *oryzae*. Plant activators such as Benzothiadiazole (BTH) protect plants against diseases by priming the salicylic acid (SA) signaling pathway. In rice, the transcription factor *WRKY45* plays a central role in this process. The mechanism involved in defense-priming by BTH and the role of *WRKY45* was investigated during the rice-*M. oryzae* interaction. The diterpenoid phytoalexins biosynthetic genes were barely up regulated in BTH-treated rice plants, but were induced rapidly after *M. oryzae* infection in a *WRKY45*-dependent manner. These results indicate that the DP biosynthetic genes were primed by BTH through *WRKY45*. It was reported that phytocassanes A, B, C and D are produced by induction mediated by infection with the pathogens *M. oryzae* and *Rhizoctonia solani*. However, the phytocassanes E are induced by the pathogen *Phytophthora infestans* and have antifungal action against *M. oryzae*. Furthermore, phytocassane accumulation was most abundant at the edges of necrotic lesions, indicating that the phytoalexins prevent subsequent spread of the fungus from the infected site. The Momilactones A and B exert antifungal action against *M. oryzae*, *Botrytis cinerea*, *Fusarium solani*, *F. oxysporum*, *Colletotrichum gloeosporioides*. These phytoalexins possess antibacterial activity against *Escherichia coli*, *Pseudomonas ovalis*, *Bacillus pumilus* and *B. cereus*. Sakuranetin exhibits antibiotic activity to *Helicobacter pylori* and allergy preventive activity. Phenylamide phytoalexins, such as CinTrp and CouSer, are reported to have antimicrobial properties against pathogenic bacteria, as well as anti-inflammatory and anti-atherogenic effect. Hasegawa *et.al.*, (2014) analysed blast fungus-responsive characters of a flavonoid phytoalexin sakuranetin with special emphasis on its accumulation in infected rice leaves, their antifungal activity as well as detoxification by fungus. .

Abiotic stress

The accumulation of sakuranetin and phenylamide phytoalexins in UV- irradiated rice leaves suggests a defensive role of rice phytoalexins in UV- induced

oxidative stress (Park *et.al.*, 2014). Momilactones A (MA) and B (MB) have been known to play an important role in rice allelopathy. At concentrations higher than 100 μ M and 300 μ M of MB and MA, showed strong inhibition on paddy weeds. Moreover, MA and MB have been documented as phytoalexins involved in the defense mechanism of rice against weeds and plant pathogens. Although MA presented in greater quantity than MB, phytotoxic levels of MB were greater than those of MA. It was reported that the development of bioactive reagents derived from momilactones A and B is more potent to reduce salinity and drought stresses than weed tolerance in rice. Two rice cultivars including a drought tolerant (Q8) and a drought susceptible (Q2) were foliar applied with exogenous vanillic acid (VA) and p-hydroxybenzoic acid (PHBA) to examine their effectiveness on drought- tolerant levels and induction of pigments, antioxidants, phenolics, flavonoids and phytoalexin momilactones A (MA) and B (MB). The results revealed that the application of VA and PHBA not only increased the antioxidant activity and total pigments, phenolics, and flavonoids but also induced phytoalexins MA and MB to enhance rice drought tolerance, of which MB may play a greater role than MA. The introduction of rice cultivars with high quantity MA and MB, especially the use of molecular markers relevant to MA and MB synthesis genes, including the dehydrogenase gene (AKI03462) and two P-450 genes (CYP99A2 and CYP99A3) form a chitin oligosaccharide elicitor and UV- inducible gene cluster, together with OsKS4 and OsCycl, the diterpene cyclase genes, on the chromosome 4, may be effective to enhance the drought tolerance by rice breeding. The root treatment of protocatechuic acid and vanillic acid was effective to enhance submergence tolerance of rice, highlighting that either the accumulation of phenolic acids may be useful to promote tolerance of rice against environmental stresses. Recent studies have shown that phenolic compounds (phenylamides and sakuranetin) play an important role in disease resistance of rice. The phenolic phytoalexins are also implicated in rice defense against biotic and abiotic stresses through the reinforcement of cell walls and scavenging ROS, as well as exhibiting an allelopathic property. In addition to their biological functions in plants,

rice phytoalexins show a diverse range of health beneficial properties. Thus, more research is required to clearly understand the biological roles and biosynthetic routes of phenolic phytoalexins in rice, as well as for their biotechnological applications.

PROSPECTS OF PHYTOALEXINS IN FOOD PRESERVATION

Since the advent of phytoalexins, a lot of attention has been devoted to their use in providing resistance to plant diseases and pests in plants. Progress has been made in transferring biosynthetic genes of known phytoalexins to plants that do not produce such defense compounds, thereby conferring resistance to the new hosts against pathogenic fungi. Moreover, some phytoalexins have attracted particular interest as modulators of abnormal physiological processes relevant in human health promotion (Boue *et al.* 2009; Jahangir *et al.* 2009, Nwachukwu *et al.* 2013). However, despite the fact that phytoalexins are synthesized in plants to combat microbial infection, there is a dearth of literature information on the use of the inducible plant compounds as antimicrobial food preservatives for reducing food losses and wastages due to spoilage microbes, or against food-borne pathogens. The prospects are particularly strong with the abundance of natural structurally diverse phytoalexins in the cereal food Poaceae and other sources, which will ensure sustainability and possible safety for use in the food system when compared to synthetic antimicrobial agents. Moreover, pigmented phytoalexins such as the 3-deoxyanthocyanidins can potentially play dual roles in food product development as new antimicrobial agents. The stability of 3-deoxyanthocyanidins relative to anthocyanin also means that their use may forest all the degradation of the latter in the presence of ascorbic acid, a traditional food additive, a phenomenon that causes fading in fruit juices. This stability of 3-deoxyanthocyanidins has been demonstrated in extracts from sorghum bran where they retained 31% of their colour even at low acidic pH values (Ojwang and Awika, 2008). The prospective food application of phytoalexins at the commercial scale depends on their accumulated amounts in the

plants after elicitation, which varies depending on the Poacea food, phytoalexin type and duration of exposure to elicitors. In maize, kauralexins have been reported to exceed $100 \mu\text{g g}^{-1}$ fresh weight in 48 h and up to $167 \mu\text{g g}^{-1}$ fresh weight in 10 days (Schmelz *et al.* 2011). Moreover, a recent study reported that zealexins accumulate to levels greater than $800 \mu\text{g g}^{-1}$ fresh weight in maize (Huffaker *et al.* 2011). The production of rice phytoalexins sakuranetin was reported to exceed 500 ng/wound (Tamogami and Kodama, 2000) while momilactone A was found to accumulate to levels up to $10.4 \mu\text{g g}^{-1}$ fresh wt. in rice. Likewise, the 3-deoxyanthocyanidin, luteolinidin was produced in sorghum at concentrations exceeding 150 ng/mg tissues after 120 h of elicitation. Given the advances in identifying eliciting factors and understanding phytoalexin biosynthesis, increased accumulation of desired phytoalexins can be readily achieved, thus making available sufficient quantities of the compounds for diverse food applications. The abundance of these phytoalexins in the Poaceae, the large-scale production of their cereal crop sources, and the large data available on their chemistry and genetics provide a promising platform for the development of novel natural food preservatives.

APPLICATION OF PHYTOALEXIN IN HUMAN HEALTH AND CONTROL OF DISEASES

Recently it has been demonstrated that phytoalexins may also display health-promoting effects in human. The sorghum (*Sorghum bicolor*) phytoalexins, 3-deoxyanthocyanins, have been proposed as medical agents against proliferation of several human cancer cell lines (Shihet *al.* 2007) and have been shown to induce apoptosis, inhibit cell proliferation, metastasis, and angiogenesis and sensitize tumor cells to therapeutic-induced cytotoxicity which might be useful in helping to reduce incidence of gastrointestinal cancer (Yang *et al.* 2009). The flavone luteolin is well characterized for its antioxidant and anti-inflammatory activities both *in vitro* and *in vivo* (Seelinger *et al.*, 2008). Luteolinidin in a concentration of $200 \mu\text{M}$ reduced the viability of HL-60 cells by 90% and HepG2 by 50%. Experiments using 3-deoxyanthocyanins against

colon cancer stem cells showed a reduction of proliferation and apoptosis in these cells, in which luteolinidin was more effective than apigeninidin. The compounds also presented effect against breast cancer MCF 7 cells (Suganyadevi *et al.* 2013).

Besides, one of the extensively studied phytoalexins –resveratrol produced by grapevine has been acclaimed for its effects and its wide range of purported healing and protective powers as a cardio-protective, antitumour, neuroprotective and antioxidant agent in addition to antibacterial and antifungal compounds (Jeandet *et al.* 2014). Increased myocardial cell apoptosis, mitochondrial dysfunction and caspase-independent apoptosis pathway were found to be involved in the mediation of cardio-toxicity. There is evidence that resveratrol (stilbenes phytoalexin) produced in Vitaceae (grapevine) could act as an anti-apoptotic agent to provide cardio-protection through inhibition of caspase-3 expression and activity. Similarly, the series of metabolic events leading to hyperglycaemia and eventually to diabetes due to reduced number of glucose transporters, down regulation in the number of insulin receptors as well as defects of tissue insulin signal transduction were improved following resveratrol exposure to diabetic rats, suggesting that this phytoalexin could induce blood glucose homeostasis in order to prevent hyperglycaemia. On the other hand, chronic administration of resveratrol in rats significantly increased nitric oxide production leading to increased cerebral blood flow in order to protect animal from ischemia-induced neuron loss. While both resveratrol and camalexin also inhibited the inflammatory response of colon cancer cell lines *in vitro* and induced apoptosis in prostate cancer cells. Thus, phytoalexins synthesized and usually released by plants in response to microbial attack and infections have also ameliorative potential on diabetes mellitus, ischemia, cardiomyocytes apoptosis, cancer, tumour, microbial pathogens, hyperglycaemia and oxidative stress, which will have significant impact in control of human diseases (Chakraborty, 2019).

CHALLENGES AHEAD

Great efforts have resulted in a wealth of information on the identity, inducing conditions, and

the biosynthesis genes of the major phytoalexins in rice, sorghum and maize. Elucidation of the biosynthesis of numerous phytoalexins also permitted the use of molecular biology tools for the exploration of the genes encoding enzymes of their synthesis pathways and their regulators. Treatment of plants with phytoalexins may represent an interesting alternative to synthetic fungicides. This has led to potential applications for increasing plant resistance to diseases. As such, scientists have attempted to increase their accessibility by inventing diverse *in vitro* and *in vivo* approaches. Among these approaches, bioengineering plant transcription factors that directly regulate phytoalexin biosynthesis genes may be the most promising. Recent research has identified conserved transcription factors that directly regulate distinct phytoalexin biosynthesis pathways in different plant species. The intriguing results provide new insight into how conserved defense signaling pathways in plants result in lineage-specific biochemical defences, which also suggest that a common transcription factor network could be engineered to enhance the biosynthesis of different phytoalexins in plants. However, the picture is far from complete since one or more transcription factors required to fully activate phytoalexin biosynthesis remain unidentified, and the exact mechanism of how the conserved factors regulate diverse phytoalexin pathways remains to be clarified. We need to learn more about the mechanism of toxicity towards fungi, bacteria or insects, in order to be able to use phytoalexins for the creation of resistant crop plants. Phytoalexins including modulation of their biosynthesis, molecular engineering in plants, biological activities, structure/activity relationships and phytoalexin metabolism by micro-organisms, phytoalexin gene transfer in plants as well as their involvement in human health have opened up avenues for further investigations. An increased knowledge of the mode of action of phytoalexins and related compounds will also help to explore their potential for use in human health. Finally, future research on these cereal crops will help to preserve world nutrition and improve world economy.

CONCLUSION

Phytoalexins are compounds synthesized by plants in response to extrinsic stress such as microbial

attack and physical injury. Some members of the Poaceae, including cereal crops rice, maize and sorghum, are known to produce substantial amounts of structurally diverse groups of phytoalexins. Phytoalexins identified in the cereal food Poaceae include the momilactones, oryzalexins, phytocassanes and sakuranetin from rice; the kauralexins and zealexins from maize; and the 3-deoxyanthocyanidins from sorghum. These phytoalexins are known to exhibit considerable antimicrobial activities against a wide array of pathogenic fungi and bacteria. Since many genes encoding the enzymes involved in their biosynthetic pathway are now well studied, the expression of these genes can be manipulated to engineer future crops for better resistance toward plant pathogens. Despite their prospects for use as naturally derived antimicrobial agents, there is scarcity of information on the application of these inducible compounds in the food system. Since food wastage due to spoilage microorganisms constitutes a challenge in global food security, these phytoalexins can potentially be utilized as sustainable natural antimicrobial food preservatives considering the abundance of their sources. Besides their key role in plant defense, phytoalexins are also helpful in promoting human health. The sorghum (*Sorghum bicolor*) phytoalexins, 3-deoxyanthocyanins, might be useful in helping to reduce incidence of gastrointestinal cancer. Some other phytoalexins are known to possess various bioactive properties such as antioxidant, anticancer, antidiabetic, antiparasitic, cardioprotective, neuroprotective, and growth-stimulating. This paper reviews the chemistry, biosynthesis and antimicrobial activities of phytoalexins from the cereal food Poaceae, and highlights their role in plant defense, prospects application in food preservation as well as their application in promoting human health.

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