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Sorgoleone from *Sorghum bicolor* as a Potent Bioherbicide

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Abstract

Sorgoleone is an allelopathic chemical released from the root exudates of the dryland cereal crop, Sorghum bicolor. It is predominately concentrated in the living root hairs of sorghum. The root hairs of juvenile plants produce higher content of sorgoleone. Its ability to suppress and inhibit the growth of weeds without affecting the crop species offers a promising platform to mark its use as a potential bioherbicide. Weeds with broadleaf and grass weeds were reported to be susceptible to the herbicidal activity of sorgoleone. The pre-emergence and post-emergence applications of sorgoleone strongly inhibited the growth of different weeds in both greenhouse and field conditions. Sorgoleone is a hydrophobic molecule that persists in the soil for a longer duration, thereby adding to its sustainable herbicidal activity. Besides, its allelopathic potential enables its use in crop rotation to protect the soils vulnerable to support the growth of weeds. The mechanisms of its phytotoxic activity focus on the inhibition of photosynthetic apparatus in lower plants by interfering with the uptake of solutes and water molecules. Further it is also a potent inhibitor of electron transport in chloroplast and mitochondria. The effectiveness of the herbicidal activity of sorgoleone is comparable to that of the synthetic herbicides in commercial use.

Keywords: Allelopathy, bioherbicide, root hairs, *Sorghum bicolor*, sorgoleone.

Introduction

Allelopathy is a natural, multi-dimensional phenomenon that involves the interactions between plant species and microorganisms by the synthesis of a variety of allelochemicals referred to as allelopathins or allelopathic compounds[1]. Most allelopathins reported in plants are secondary metabolites exuded by a plant that are phytotoxic to other plant species. They affect the germination and growth of neighboring plants by interfering with various physiological processes like photosynthesis, respiration, water and hormonal balance. However, the basic phytotoxic mechanisms seem to be the inhibition of enzymes involved in these processes. A number of such allelopathins have been characterized in various plants such as juglone from black walnut (*Juglans nigra*), 2-benzoxazolinone from wheat (*Triticum aestivum*), Momilactone from rice (*Oryza sativa*), and sorgoleone from *Sorghum bicolor*[2-4]. The application of these allelopathic traits of crop species for effective weed management in agroecosystems was first suggested by A.R. Putnam in 1980[5,6].

Sorghum bicolor: *Sorghum bicolor* is a cereal and forage crop, preferentially cultivated in arid and semi arid tropical regions due to its greater adaptability to extreme dry climates. Besides its traditional usage in food and fodder, it is also a commercial crop that can be used in the production of ethanol and gluten-free derivatives. Sorghum is the preferred choice of farmers, as a summer cover crop because of its rapid growth and ability to suppress weeds[7]. It is also widely used as a green manure or as a cover crop in the United States[8,9]. The weed suppressive potential of sorghum has been extensively studied and widely adapted for weed management in both horticultural and agronomic cropping systems [9]. The phytotoxic effect on weeds may be attributed to the presence of allelopathins termed as sorgoleone along with several congeners that are commonly exuded from the root hairs of sorghum.

Sorgoleone

An array of lipid quinones and resorcinols are exuded from the root hairs of sorghum[10]. Sorgoleone, one of the major constituent in the exudates (76-99%), is a hydrophobic oily exudate, noted for its allelopathy. Sorgoleone is chemically 2-hydroxy-5-methoxy-3-[(Z,Z)-8′,11′,14′-pentadecatriene]-*p*-benzoquinone (figure-1) with a molecular weight of 358 Daltons[11]. The root hairs of young developing plants exude higher content of sorgoleone. Sorgoleone biosynthesis is initiated only after the root hair development, wherein the nascent secretory cells of root hairs elongate initially and then start to exude sorgoleone from their tips, after they have stopped elongating.

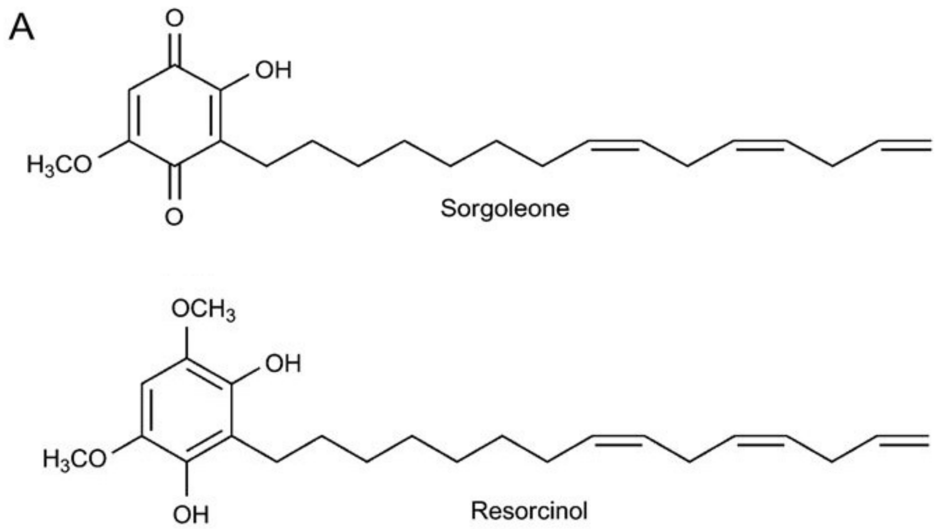

Figure-1
(A) Chemical structure of sorgoleone and a related lipid resorcinol present in the root hairs of sorghum

Sorgoleone biosynthesis in root hairs is constitutive and is proportional to the root biomass[12]. Substantial genetic variability in sorgoleone production capacity was reported in sorghum germplasm[13.] Although sorgoleone production was genetically variable, it was estimated that certain sorghum genotypes could accumulate up to 15 mg sorgoleone/gm of fresh root weight[14]. Sorgoleone can be extracted from the root hairs using methylene chloride and 1% glacial acetic acid[11, 15]. However, methanol and chloroform were also reported to be effective solvents for extracting sorgoleone[16, 17]. *In vitro* studies using purified sorgoleone from *Sorghum bicolor* have demonstrated its effectiveness as a broad-spectrum inhibitor active against many agronomically important monocot and dicot weed species and so has become the allelopathic molecule of interest for its effective use as a natural herbicide [11, 18, 19].

Sorgoleone biosynthesis

Sorgoleone is produced exclusively by the living root hairs of sorghum along with other sorgoleone congeners that differ in the length or degree of saturation of the aliphatic side chain and in the substitution pattern of the quinone ring. Among these congeners, a resorcinolic lipid namely, 4,6-dimethoxy-2-[(Z,Z)-8',11',14'-pentadecatriene] resorcinol (methoxy- dihydrosorgoleone) was identified to be predominant in the root exudates [10, 17, 20, 21]. The various congeners that contribute to the overall allelopathic effect of sorgoleone were isolated using a C8 column chromatography and thin layer chromatography[21]. The biosynthetic pathway for sorgoleone occurs in root hair cells, wherein an atypical 16:3 fatty acyl-CoA starter unit produced by fatty acid desaturase is converted to a pentadecatrienyl resorcinol intermediate by a polyketide synthase. This resorcinolic intermediate is methylated by a *S*-adenosylmethionine-dependent *O*-methyltransferase and subsequent dihydroxylation by a P450 monooxygenase results in the formation of reduced form of sorgoleone[22, 23].

Reports have indicated that the enzymes, fatty acid desaturases[22], polyketide synthases[24,25] and O-methyltransferases[23] play a key role in the biosynthesis of sorgoleone. Typically, a subclass of type III polyketide synthases, referred to as alkylresorcinol synthases, was identified to be involved in the synthesis of the pentadecatrienyl resorcinol intermediate from an atypical 16:3 fatty acyl-CoA starter unit during the initial step of sorgoleone biosynthesis[19]. Molecular studies involving semi-quantitative RT-PCR analysis have revealed that a differentially expressed gene, SOR1 is expressed only in the sorgoleone producing root hairs of sorghum genotypes and it encodes a fatty acid desaturase involved in the formation of a unique double bonding pattern within the aliphatic chain of sorgoleone[26].

Effect of environmental factors on sorgoleone production

Sorgoleone production is influenced by environmental factors, particularly by moisture [27]. It was found that higher levels of water in the rhizosphere can inhibit root hair formation and subsequently sorgoleone production. However, ethylene was found to restore the root hair formation and elongation under such high moisture conditions[28]. Further, sorgoleone production was favored at temperatures between 25 to 35°C and the maximum productivity was attained at 30°C. Seedling development, root growth and sorgoleone levels were greatly reduced at temperatures below 25°C and above 35°C. Additionally, lower pH was found to be beneficial for sorgoleone production, thereby increasing its effectiveness in weed management in acidic soils. In vitro studies have shown that the root hairs secrete increased levels of sorgoleone in the presence of an external stimulus such as velvet leaf root extract, to establish its allelopathic potential[12].

Mechanism of Action

The plant cell receptors that perceive allelopathins operate similarly to those that recognize microbial compounds and hence, molecular and genetic tools that are available for the study of plant–microbe interactions could be used to study the plant–plant interactions that are mediated by allelochemicals[29]. The mechanisms of phytotoxicity have been extensively studied in the allelochemical, sorgoleone. The specificity of the inhibitory effect of sorgoleone on photosynthesis in weeds may be attributed to the fact that it is absorbed through the hypocotyls and cotyledonary tissues of germinating young seedlings and it is not translocated acropetally by xylem in older plants[30]. So in older plants, it acts by inhibiting other molecular binding sites rather than by inhibiting photosynthetic apparatus[17].

The phytotoxic mechanisms induced by sorgoleone involve the inhibition of photosynthesis and oxygen evolution through interactions with components of photosystem II (PS II)[31]. Sorgoleone specifically inhibits the chloroplast electron-transport chain, acting in a similar way as triazine herbicides such as atrazine[13, 31]. The underlying mechanisms seem to be the inhibition of electron transport between plastoquinone compounds in photosystem II (PS II) by binding to the Plastoquinone B (Q_B) electron acceptor at the D1 protein[32,33]. Sorgoleone also disrupts the biosynthesis of carotenoids by inhibiting hydroxyphenylpyruvate dioxygenase (HPPD), resulting in foliar bleaching[34]. Inhibition of H^+-ATPase in plant roots by sorgoleone decreases water uptake by plants and causes ionic imbalance[35]. Further, it also plays a vital role in biological nitrification inhibition, a phenomenon that involves the ability to release nitrification inhibitors from roots that suppress *Nitrosomonas* activity in a dose-specific manner[36].

Sorgoleone as a Bioherbicide

The allelopathic potential of sorghum could be envisaged for the production of modern, natural herbicides that are effective in weed suppression in an eco-friendly manner. Further, the application of biotechnological tools to enhance the production of sorgoleone in the germplasm as well as the modification of structurally similar congeners to improve their weed suppressive potential through cheminformatics and molecular designing tools would offer valuable insights to the use of sorgoleone as a potent bioherbicide.

Sorgoleone exhibited the strongest herbicidal activity on small-seeded weeds and specifically, broadleaf weeds and grass weeds were susceptible to the phytotoxicity of sorgoleone at concentrations as low as 10 μM in hydroponic assays[13,37]. However, broadleaf weeds were more susceptible to the herbicidal activity of sorgoleone than grass weed species[38]. Evidences state that the field applications of phytotoxic water extracts from *Sorghum bicolor* (Sorgaab) as a bioherbicide was effective at 10% (w/v) concentration as a double spray 20/30 and 40/60 days after sowing or after seedling transplantation, yielding higher net productivity in cotton, soybean, wheat and rice. More specifically, Sorgaab was found to be effective in reducing the biomass of barnyard grass (*Echinochloa cruss-galli L.)* by 40%, a prominent weed that is grown in rice fields which in turn subsequently increased the yield of rice by 18%[39-41].

Herbicides are generally used as formulated products that may contain the active ingredient along with other compounds to enhance their dispersion in water, to increase their soil persistence, increased coverage and absorption by weeds[42]. Recent studies have shown that formulated sorgoleone [4.6% wettable powder] greatly inhibited the germination and growth of certain broadleaf weeds such as *Rumex japonicus, Plantago asiatica and Amaranthus retroflexus* without affecting the crop species to a greater extent[43]. Further, a combination of sorgoleone with hairy root extracts of tartary buckwheat (*Fagopyrum tataricum* Gaertn.) exhibited a synergistic effect in suppressing the growth of broadleaf weed species to a significant extent[44].

The bioefficacy of sorgoleone as a natural herbicide was found to be comparable to that of many synthetic herbicides such as diuron, atrazine and metribuzin that inhibit photosynthesis[45]. Further, crop protection enabled by exploiting the allelopathic potential of sorgoleone from sorghum can be used to control the weeds that are already resistant to the commercialized synthetic herbicides with the same mode of action. This in turn, would minimize the use of many environmentally incompatible synthetic herbicides to a greater extent.

Table-1
Common weeds inhibited by Sorgoleone from *Sorghum bicolor*

Allelopathin	Source	Sensitive weeds
Sorgoleone	sorghum (*Sorghum bicolor* L. Moench)	Littleseed canarygrass (*Phalaris minor Retz.),*
		Lesser swinecress (*Coronopus didymus* L.),
		Purple nutsedge (*Cyperus rotundus* L.)
		Black nightshade (*Solanum nigrum* L.)
		Redroot pigweed (*Amaranthus retroflexus* L.)
		Common ragweed ((*Ambrosia artemisiifolia* L.),
		Sicklepod (*Cassia obtusifolia L.)*

Conclusion

The allelopathic nature of sorgoleone contributes to its effective use as a model, natural herbicide thereby providing an attractive alternative to the use of synthetic herbicides in weed management practices, with much less environmental implications. The strong weed suppressive potential of sorgoleone can be adapted to develop effective biorational approaches to weed management with more selectivity and reduced risks. The knowledge of the physiological mechanisms that lead to the production and phytotoxic activity of sorgoleone, provide a scope to enhance its bioavailability and improvise its efficacy in weed control by the application of genetic transformation techniques.

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