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Enhancing Sorgoleone Levels in Grain Sorghum Root Exudates

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Abstract Sorgoleone, found in the root exudates of sorghum [(*Sorghum bicolor* (L.) Moench], has been a subject of continued research. Sorgoleone production in grain sorghum roots was investigated under different growth conditions. Methanol was the most effective solvent for extracting sorgoleone from grain sorghum roots. Sorgoleone production is high in young developing plants. The maximum concentration (μg mg^{-1} root dry weight) was produced in 5-d-old seedlings; beyond this age, production declined. However, considering both root weight and sorgoleone content per seedling, 10-d-old seedlings had the highest total amounts (μg). Compared with the control, sorgoleone content increased 6.1, 8.6, and 14.2 times when sorghum seeds were treated with auxins, Hoagland solution, and a combination of auxins and Hoagland solution, respectively. Among the innate immunity response elicitors, cellulose (an elicitor of plant origin) stimulated higher sorgoleone production than the others, and it produced 6.2 times more sorgoleone than the control. Combined treatment of sorghum seeds with half strength Hoagland solution and 5 μg ml^{-1} of IBA significantly increased both root growth and sorgoleone content in sorghum seedlings.

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Introduction

The allelopathic effect of sorghum was first noticed in crops grown in rotation with sorghum (Breazeale, 1924) and later supported by several studies (Putnam et al., 1983; Forney et al., 1985; Einhellig and Rasmussen, 1989). Several workers have examined the chemical composition of root exudates of sorghum (Guenzi et al., 1967; Lehle and Putnam, 1983; Alsaadawi et al., 1986; Panasiuk et al. 1986). Netzly and Butler (1986) isolated hydrophobic *p*-benzoquinones from grain sorghum roots exudates, which were ultimately identified and named sorgoleone (Netzly et al., 1988). The root hairs of sorghum produce an oily exudate containing the lipid benzoquinone sorgoleone (2-hydroxy-5-methoxy-3-[(8′*Z*, 11′*Z*)-8′, 11′, 14′-pentadecatriene]-*p*-benzoquinone), which is a potent allelochemical (Netzly and Butler, 1986; Inderjit and Duke, 2003). Sorgoleone and its 1 4-hydroquinone form together account for ~50% of the oily exudates from sorghum root hairs (Erickson et al., 2001; Dayan et al., 2009). The remaining percentage consists primarily of alkyl resorcinol analogs, along with small amounts of several sorgoleone congeners that vary in the substitutions in the aromatic ring (Fate and Lynn, 1996; Rimando et al., 1998; Kagan et al., 2003). All these variants of sorgoleone appear to contribute to the overall allelopathic potential of sorghum (Kagan et al., 2003; Rimando et al., 2003). Sorgoleone is phytotoxic to broadleaf and grass weeds at concentrations as low as 10 μM in hydroponic assays (Einhellig and Souza, 1992; Nimbal et al., 1996), and broadleaf weed species are more susceptible than grass

weed species (Uddin et al., 2009). Sorgoleone exerts both pre-emergence and post-emergence effects on different weeds (Czarnota et al., 2001; Weston and Czarnota, 2001).

Factors that affect root hair production and sorgoleone biosynthesis are not well understood. However, sorgoleone biosynthesis is linked intrinsically to the presence of living root hairs (Czarnota et al., 2001; Yang et al., 2004). Furthermore, root hair production is inhibited by excess water (Hess et al., 1992; Yang et al., 2004; Dayan et al., 2007). Sorgoleone levels are sensitive to light; they decreased by nearly 50% upon exposure to blue light (470 nm) and by 23% upon exposure to red light (670 nm) (Dayan, 2006). Sorgoleone levels increase in plants treated with a crude extract of velvetleaf (*Abutilon theophrasti* Medik.) root (Dayan, 2006).

Although the cellular localization and the biosynthetic steps involved in sorgoleone production have been determined (Dayan et al., 2003; Pan et al., 2007; Baerson et al., 2008), limited information is available regarding the factors that affect its biosynthesis. Therefore, this research was conducted to determine the factors that enhance both root growth and sorgoleone content in sorghum roots. Such knowledge may be used to develop a bioherbicide.

Methods and Materials

Plant Material and Growth Conditions Seeds of sorghum (cultivar Chalsusu) were collected from the College of Agriculture & Life Sciences, Gangwon National University, Korea. They were treated with benomyl (a fungicide used for seed disinfection) for 4 h and then rinsed several times in distilled water. For most experiments, 25 seeds along with the treatment material were placed in sterile Petri dishes (100×40 mm) on the surface of sterile Whatman #1 filter paper (diam, 90 mm). The dishes then were placed in a growth chamber at 30°C under standard cool-white fluorescent tubes with a flux rate of 550 μmol s^{-1} m^{-2} and a 16-h photoperiod for 3 wk. However, the seedlings were allowed to grow for 40 d for the experiment to determine the age of maximum seedling growth. Each experiment had four replications and was repeated two to three times.

Extraction Procedure and Sorgoleone Analysis by HPLC Sorgoleone was extracted according to the procedures described by Netzly and Butler (1986); Netzly et al. (1988), and Czarnota et al. (2003a), except that methanol was used as a solvent instead of methylene chloride. Seedling roots were excised and immersed in methanol (1:20 w/v) for 30 sec to extract. The crude extract was filtered and evaporated under vacuum. The dried extract was dissolved in methanol (1 mg ml^{-1}), and the solution was filtered through a poly filter (pore size, 0.45 μm). The filtrate was diluted 4-fold with methanol prior to HPLC analysis. HPLC quantification of sorgoleone was performed using the Futecs NS-4000 HPLC systems (Futecs Co. Ltd.,Daejeon, Korea) with a C_{18} column (250× 4.6 mm, particle size 5 μm; RStech, Daejeon, Korea). The mobile phase was 75% acetonitrile + 25% acidified water. Water was acidified with glacial acetic acid (97.5:2.5 v/v). Sorgoleone was detected at 280 nm with a Waters tunable absorbance detector after injection of 20 μl of the methanol solubilized crude extract sample. The column flow rate was 1 ml min^{-1} with a 40 min total run time for each sample. All samples were run in triplicate. The amount of sorgoleone was calculated on the basis of a standard curve obtained from a purified sample. The sorgoleone standard was provided by Franck Dayan, United States Department of Agriculture-Agricultural Research Service (USDA-ARS), Natural Products Utilization Research Unit.

Optimization of Sorgoleone Extraction Different organic solvents were used, namely methanol, methylene chloride, chloroform, ethanol, butanol, ethyl acetate, hexane, and water, were used to extract sorgoleone from 5-d-old sorghum seedling roots to determine the best solvent for obtaining maximum amounts. The extraction procedure was the same as described above.

Age of Seedling for Maximum Root Growth To determine the optimum age of seedlings for measuring maximum root growth and sorgoleone content, eight sets of Petri dishes (100×40 mm) with four replications were arranged to grow sorghum seedlings. In each dish, 25 seeds were grown. At each 5-d interval, one set of Petri dishes (four dishes) was removed from the growth chamber for measuring root

Table 1 Effect of different organic solvents on sorgoleone extraction from sorghum roots

Organic solvent	Amount of sorgoleone (μg mg^{-1} RDW[a])
Methanol	40.1 a[b]
Methylene chloride	15.7 b
Chloroform	14.0 b
Ethanol	9.8 c
Butanol	0 d
Ethyl acetate	0 d
Hexane	0 d
Water	0 d

[a] *RDW* Root dry weight

[b] Mean values (mean of four replicates with three samples from each replicate) indicated by the same letter in a column do not differ significantly at 5% level (Tukey's studentized range test)

Table 2 Effect of the age of seedlings on root growth and sorgoleone content in sorghum roots

Age of seedlings (d)	RDW[a] (mg)	Sorgoleone concentration (μg mg^{-1} RDW)	Total sorgoleone content (μg)
5	4.0 d[b]	40.1 a	160.2 ab
10	5.9 c	30.1 b	177.3 a
15	6.8 bc	18.8 c	127.7 cd
20	7.7 b	17.3 d	133.4 cd
25	8.8 a	17.0 d	149.4 bc
30	9.6 a	11.9 e	113.9 de
35	9.1 a	11.0 e	100.0 e
40	7.6 b	9.2 f	70.3 f

[a] *RDW* Root dry weight

[b] Mean values (for RDW, mean of 4 replicates with an average of ten seedlings for each replicate; for sorgoleone, mean of four replicates with three samples from each replicate) indicated by the same letter in a column do not differ significantly at 5% level (Tukey's studentized range test)

growth and sorgoleone content, until 40 d after seeding. Water was applied during the entire experimental period.

Auxin Treatment To determine the effect of auxins on root growth and sorgoleone content in roots, three different auxins, namely, indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), and 1-naphthaleneaceti acid (NAA) were tested at different concentrations (0 μg ml^{-1}, 0.1 μg ml^{-1}, 0.5 μg ml^{-1}, 0.75 μg ml^{-1}, 1, 5 μg ml^{-1}, 10 μg ml^{-1}, 25 μg ml^{-1}, and 50 μg ml^{-1}).

Hoagland Solution Treatment Seedlings were grown in Petri dishes as described above, in different strengths Hoagland solution. After seed placement in the dishes, three strengths, i.e., quarter, half, and full Hoagland solution (Hoagland and Arnon, 1950) were applied at three different time points during growth (0 d, 4 d, and 8 d).

Combined Treatment with Auxins and Hoagland Solution Seedlings were grown in Petri dishes in combined solutions of auxins and Hoagland solution (50:50 v/v). Seeds were applied with, quarter, half, or full strengths Hoagland solution along with the optimal concentrations of IAA (1 μg ml^{-1}), IBA (5 μg ml^{-1}), and NAA (1 μg ml^{-1}).

Effect of Elicitors To evaluate the effect of elicitors on growth and sorgoleone content, two elicitors of plant origin (pectin and cellulose) and two of microorganism origin (chitin and chitosan) were tested at different concentrations (0 μg ml^{-1}, 0.1 μg ml^{-1}, 0.5 μg ml^{-1}, 0.75 μg ml^{-1}, 1 μg ml^{-1}, 5 μg ml^{-1}, 10 μg ml^{-1}, and 100 μg ml^{-1}).

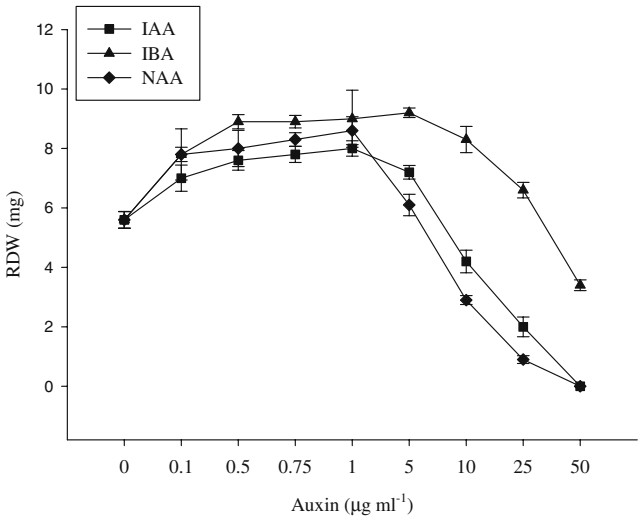

Fig. 1 Effect of different growth hormones on root dry weight of sorghum. Values are presented as mean (SD) (each point is the mean of four replicates with an average of ten seedlings for each replicate). IAA: Indole-3-acetic acid, IBA: Indole-3-butyric acid, NAA: 1-naphthaleneacetic acid, SD: Standard deviation

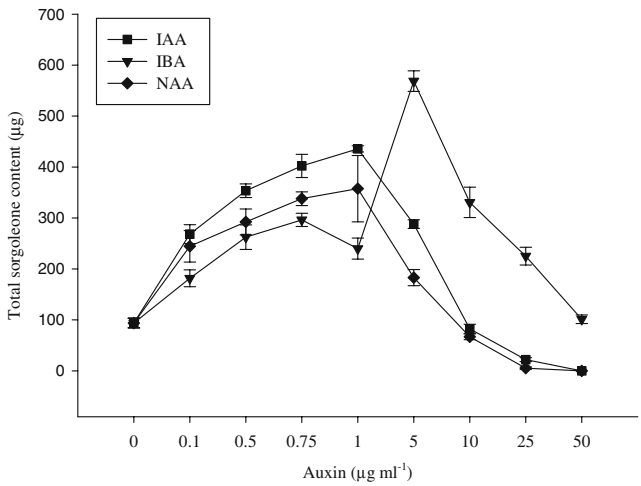

Fig. 2 Effect of different growth hormones on sorgoleone content in sorghum roots. Values are presented as mean (SD) (each point is the mean of four replicates with three samples from each replicate). IAA: Indole-3-acetic acid, IBA: Indole-3-butyric acid, NAA: 1-naphthaleneacetic acid, SD: Standard deviation

Table 3 Effect of different strengths of Hoagland solution, applied at different time points, on root growth and sorgoleone production in sorghum roots

Strength of Hoagland solution	Time of application (DAS)[a]	RDW[b] (mg)	Sorgoleone concentration (μg mg^{-1} RDW)	Total sorgoleone content (μg)
Quarter	0	8.2 cd[c]	47.2 cd	387.3 cd
	4	7.3 e	34.3 e	249.6 ef
	8	6.8 e	25.4 f	173.5 fgh
Half	0	11.1 a	76.2 a	844.3 a
	4	9.4 b	60.9 b	569.6 b
	8	8.3 c	48.7 c	401.8 c
Full	0	9.5 b	12.1 g	115.0 gh
	4	8.0 cd	23.7 f	188.8 fg
	8	7.2 de	41.4 d	299.9 de
Control	–	6.5 e	15.1 g	98.3 h

[a] *DAS* Days after seed placement

[b] *RDW* Root dry weight

[c] Mean values (for RDW, mean of four replicates with an average of ten seedlings for each replicate; for sorgoleone, mean of four replicates with three samples from each replicate) indicated by the same letter in a column do not differ significantly at 5% level (Tukey's studentized range test)

Another experiment was performed using the optimal concentration of each elicitor (chitin, pectin, and cellulose: 1 μg ml^{-1}; chitosan: 5 μg ml^{-1}) applied at 0 d, 3 d, 6 d, and 9 d after seed placement in Petri dish. Distilled water was used before elicitor treatment. Thereafter, seedlings were allowed to grow for 3 wk.

Statistical Analysis All data were analyzed using the SAS 9.1 Software (released in 2006; SAS Institute Inc., Cary, NC, USA). Analysis of variance was performed for each compound concentration, and mean differences were calculated using Tukey's studentized range test. Standard deviations were also provided to indicate the variations associated with the particular mean values.

Results

Optimization of Sorgoleone Extraction The highest amount of sorgoleone was extracted with methanol, followed by methylene chloride and chloroform (Table 1). The amount

Table 4 Combined effect of auxins and Hoagland solution on root growth and sorgoleone production in sorghum roots

Auxin concentration (μg ml^{-1})	Strength of Hoagland solution	RDW[a] (mg)	Sorgoleone concentration (μg mg^{-1} RDW)	Total sorgoleone content (μg)
IAA (1)	Quarter	10.6 cd[b]	46.7 e	495.1 d
	Half	11.9 ab	60.4 c	721.4 b
	Full	11.1 bc	32.5 g	360.3 e
IBA (5)	Quarter	10.5 cd	60.3 c	631.5 c
	Half	13.2 a	84.6 a	1120.5 a
	Full	12.2 ab	40.4 f	492.7 d
NAA (1)	Quarter	9.5 de	53.7 d	511.3 d
	Half	10.5 cd	66.4 b	700.1 bc
	Full	9.1 e	36.0 fg	327.5 e
Control	–	5.0 f	15.8 h	78.1 f

IAA Indole-3-acetic acid, *IBA* Indole-3-butyric acid, *NAA* 1-naphthaleneacetic acid

[a] RDW: Root dry weight

[b] Mean values(for RDW, mean of four replicates with an average of ten seedlings for each replicate; for sorgoleone, mean of four replicates with three samples from each replicate) indicated by the same letter in a column do not differ significantly at 5% level (Tukey's studentized range test)

of sorgoleone in methanol was 2.6 and 2.9 times higher than that in methylene chloride and chloroform, respectively. No sorgoleone was detected upon extraction with butanol, ethyl acetate, hexane, or water.

Change in Sorgoleone Content with Seedling Age Root weight increased with seedling age for up to 30 d, but sorgoleone production decreased with age. Sorgoleone concentration was 40.1 μg mg^{-1} and 30.1 μg mg^{-1} root dry weight (RDW) in 5-and 10-d-old seedlings, respectively (Table 2). The RDW was 4.0 mg and 5.9 mg in 5-and 10-d-old seedling, respectively, and it increased up to 9.6 mg at 30 d. Although sorgoleone concentration decreased with seedling age, root weight increased with time; thus, the total amount of sorgoleone was highest in 10-d-old seedlings. The trend of increase in root growth persisted for up to 30 d, after which root growth declined.

Effect of Auxins on Sorgoleone Production Root growth and sorgoleone content varied widely with different concentrations of auxins (Figs. 1, 2). Considerable RDW and amounts of sorgoleone were observed at auxin concentrations of 0.1–5 μg ml^{-1}, after which both RDW and sorgoleone content decreased drastically. The maximum root growth and sorgoleone were observed by treatment with IBA at 5 μg ml^{-1}, followed by treatment with IAA and NAA at 1 μg ml^{-1}. Compared with the control, 5 μg ml^{-1} IBA, 1 μg ml^{-1} IAA, and 1 μg ml^{-1} NAA produced 6.1, 4.6, and 3.8 times more sorgoleone, respectively.

Effect of Hoagland Solution on Sorgoleone Production Root dry weight was higher when Hoagland solution was applied at 0 d than when the solution was applied at 4 or 8 d after seed placement (delayed application), for all strengths (Table 3). Sorgoleone content showed the same trend as RDW, except when full strength solution was applied. It decreased with delay in the application of both quarter- and half-strength. In the case of the application of full-strength Hoagland solution, sorgoleone content increased with delayed application. Half-strength solution produced maximum root growth and sorgoleone content. Compared with the control, total sorgoleone content and root biomass were 8.6 and 1.7 times higher when half-strength solution was applied at 0 d. Treatment of sorghum seeds with half-strength at 0 d produced 2.8 and 2.2 times more sorgoleone based on RDW, than treatments with full-strength and quarter-strength solution, respectively.

Combined Effect of Auxins and Hoagland Solution on Sorgoleone Production Different strengths of Hoagland solution along with the most optimal concentrations of IAA (1 μg ml^{-1}), IBA (5 μg ml^{-1}), and NAA (1 μg ml^{-1}) were applied to sorghum seeds to increase sorgoleone production. Combined treatment dramatically increased root growth and sorgoleone production (Table 4). In particular, combined application of 5 μg ml^{-1} IBA with half-strength Hoagland solution produced 14.2 times more sorgoleone than the control. Further, this combination increased sorgoleone content by 1.6 and 1.5 times as compared with the combination of half-strength Hoagland

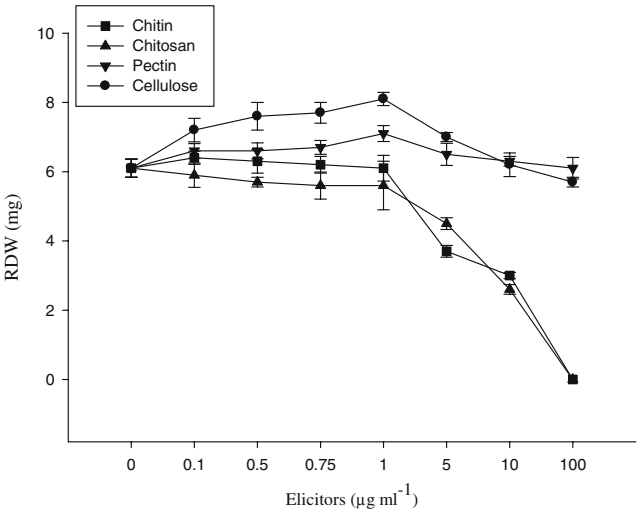

Fig. 3 Effect of elicitors on root growth of sorghum seedlings. Values are presented as mean (SD) (each point is the mean of four replicates with an average of ten seedlings for each replicate). SD: Standard deviation

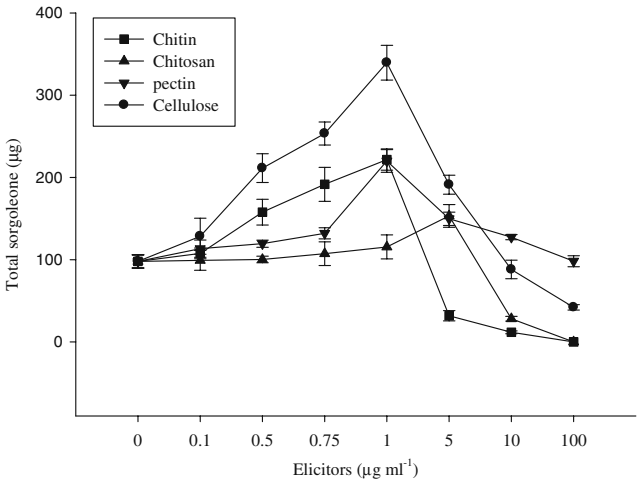

Fig. 4 Effect of different concentrations of elicitors on sorgoleone production in sorghum roots. Values are presented as mean (SD) (each point is the mean of four replicates with three samples from each replicate). SD: Standard deviation

solution with 1 μg ml^{-1} NAA and 1 μg ml^{-1} IAA, respectively.

Effect of Elicitors on Root Growth and Sorgoleone Production Four elicitors—2 of microorganism origin (chitin and chitosan) and 2 of plant origin (pectin and cellulose)—were applied at different concentrations to sorghum seeds. Chitin, pectin and cellulose produced more sorgoleone at 1 μg ml^{-1} than at other concentrations (Figs. 3, 4), while chitosan produced a higher amount at 5 μg ml^{-1} than at other concentrations. With the increase in the concentration of elicitors, sorgoleone content decreased drastically, and in the case of chitin and chitosan, the plants did not survive even at 100 μg ml^{-1} (Fig. 3). Cellulose, an elicitor of plant origin produced the highest root biomass and sorgoleone content at 1 μg ml^{-1}, followed by chitin at 1 μg ml^{-1}. Compared with the control, cellulose at 1 μg ml^{-1} produced 3.5 and 1.3 times more sorgoleone and root biomass, respectively.

Effect of Elicitors, Applied at Different Time Points During Growth, on Sorgoleone Production After seed placement, the optimal concentrations of different elicitors were applied at different time points during growth. Sorghum roots contained more sorgoleone when an elicitor was applied a few days later than when applied in the initial days of growth (Table 5). The highest amount of sorgoleone was obtained when cellulose was applied 6 d after seed placement, followed by chitin, chitosan, and pectin. Compared with the control, cellulose, chitin, chitosan, and pectin produced 6.2, 4.2, 3.3, and 3.2 times more sorgoleone, respectively.

Discussion

Methylene chloride is the most commonly used solvent (Netzly and Butler, 1986; Netzly et al., 1988; Czarnota et al., 2001; Yang et al., 2004) to extract sorgoleone from roots, although chloroform also has been used (Dayan, 2006). In this study, methanol proved to be the best solvent and extracted the maximum amount of sorgoleone, followed by methylene chloride and chloroform. Methanol extracted 2.6 times more sorgoleone than methylene chloride (Table 1).

While root weight increased, sorgoleone production decreased with the age of seedlings. Considering both root weight and sorgoleone content, 10-d-old seedlings were the best, producing the maximum amount of sorgoleone. The trend of increase in root growth persisted until 30 d after which the root growth decreased since the seedlings were

Table 5 Effect of elicitors, applied at different time points, on root growth and sorgoleone production in sorghum roots

Elicitor concentration (μg ml^{-1})	Time of application (DAS)[a]	RDW[b] (mg)	Sorgoleone concentration (μg mg^{-1} RDW)	Total sorgoleone content (μg)
Chitin (1)	0	6.1 e	36.5 g	222.7 hi
	3	6.3 cde	59.7 bc	376.1 cd
	6	6.4 cde	63.7 b	407.7 bc
	9	6.6 cde	19.8 i	130.7 k
Chitosan (5)	0	4.5 f	34.2 gh	153.9 jk
	3	5.9 e	55.2 d	325.7 def
	6	6.1 de	51.7 de	315.4 ef
	9	6.3 cde	31.4 h	197.8 ij
Pectin (1)	0	7.1 bc	30.9 h	219.4 hi
	3	7.0 bcd	42.3 f	296.1 efg
	6	6.4 cde	48.5 e	310.4 ef
	9	6.3 cde	38.3 fg	241.3 ghi
Cellulose (1)	0	8.1 a	41.9 f	339.4 de
	3	8.3 a	55.2 cd	458.2 b
	6	8.7 a	69.2 a	602.0 a
	9	7.9 ab	34.9 gh	275.7 fgh
Control		6.1 e	16.2 i	98.8 k

[a] DAS: Days after seed placement

[b] RDW: Root dry weight

[c] Mean values (for RDW, mean of four replicates with an average of ten seedlings for each replicate; for sorgoleone, mean of four replicates with three samples from each replicate) indicated by the same letter in a column do not differ significantly at 5% level (Tukey's studentized range test)

grown under hypoxic conditions. Hypoxic conditions did not impede root growth until 30 d but growth decreased thereafter; this finding was consistent with that of Yang et al. (2004) that sorghum root development is reduced under hypoxic conditions.

In our study, auxins exerted a positive effect on both root growth and sorgoleone production. Auxins govern many biological processes in plants, such as cell enlargement and division, differentiation of vascular tissue, apical dominance, root initiation, and signaling (Teale et al., 2006). Studies conducted using differentiated tissues to investigate the biochemical relationship between exogenous and endogenous auxin levels have provided interesting findings regarding root-derived biologically active compounds. Researchers investigating the physiological role of exogenously applied auxins in root growth and secondary metabolite production have established that signaling molecules can affect plant tissue stability and secondary product accumulation either individually or through interactions with phytohormones. With regard to the effects of auxins on secondary metabolite production, Bais et al. (2001) noted that high levels of exogenous auxins, specifically IAA and NAA, in the presence of low cytokinin levels, decrease the ability of root cultures of *Cichorium. intybus* to produce coumarin. Lin et al. (2003) showed that coniferin content in *Linum flavum* is increased significantly in the presence of auxins. However, Arroo et al. (1995) showed that IAA addition inhibits secondary metabolite accumulation in the hairy roots of *Tagetes patula*. In contrast, addition of either IBA or NAA stimulates ajmalicine and ajmaline production in *Rauvolfia micrantha* hairy root cultures in a hormone-free medium (Sudha et al., 2003), whereas Rhodes et al. (1994) observed a decrease in nicotine content in the hairy roots of *Nicotiana rustica* when the roots were supplied with auxins together with cytokinins. Luczkiewicz et al. (2002) discovered that the production of the sesquiterpene lactone pulchelin E is enhanced in hairy roots of *Rudbeckia hirta*, compared with that in callus and suspensions cultures, in the presence of auxins.

Czarnota et al. (2003b) confirmed that sorghum root hairs are physiologically active with a complex network of smooth endoplasmic reticulum and possibly Golgi bodies. Small globules of cytoplasmic exudates also were observed to deposit an oily material between the cell wall and the plasma membrane near the root hair tips. Our study confirms that sorgoleone production is related directly to the development of sorghum root hairs. Our positive results from using different auxins for enhanced sorgoleone production, suggests that sorghum roots benefit from developing more sorgoleone-rich root hairs. Sorgoleone production also was stimulated by Hoagland solution treatment, suggesting that sorghum roots benefit from Hoagland solution to develop healthy and vigorous roots.

Among the auxin concentrations and Hoagland solution strengths used, IBA at 5 μg ml^{-1} and half strength Hoagland solution showed the best results with regard to both root growth and sorgoleone production. Hess et al. (1992) indicated that sorgoleone production is quite sensitive to environmental conditions, and it is well documented that sorgoleone production depends mainly on root hair formation (Dolan, 2001; Czarnota et al., 2003b; Yang et al., 2004; Dayan, 2006). Our study shows that sorghum roots develop many branches along with healthy root hairs when IBA and Hoagland solution are applied together.

Stimulation of secondary metabolites by elicitation is one of the few strategies that currently is finding commercial application. Elicitors, compounds of biotic or abiotic origin, upon contact with the cells of higher plants trigger increased production of pigments, flavones, phytoalexins, and other defense related compounds (Flores and Curtis, 1992; Sim et al., 1994; Bhagyalakshmi and Bopanna, 1998; Singh, 1999). Sorgoleone production may be affected by pathogenic infections. It has antifungal properties (Suzuki et al., 1998). Initiation of sorgoleone biosynthesis by eliciting plant defense mechanisms have yielded varying results. Cellulose at 1 μg ml^{-1} significantly increased sorgoleone production, followed by chitin at 1 μg ml^{-1} (Table 5). Treatment with chitin, which is known to induce the expression of systemic acquired resistance (SAR) genes (Hahn, 1996; Zhang et al., 2002), exerted a significant effect on sorgoleone production. Treatment with chitosan and pectin also increased production. Moreover, Savitha et al. (2006) observed a positive effect of different biotic and abiotic elicitors on the production of betalain in the hairy root cultures of *Beta vulgari.*

In summary, sorgoleone production is constitutive to the physiology of mature root hairs of sorghum. Great differences in sorgoleone levels are observed during the early stages of seedling development, and sorgoleone biosynthesis is positively affected by most of the stimuli used in this study. In particular, sorghum seeds treated with half strength Hoagland solution and 5 μg ml^{-1} of IBA significantly increase root growth and sorgoleone content in grain sorghum roots.

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