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RESPONSE OF ETIOLATED PEA SEEDLINGS AND COTTON TO ETHYLENE PRODUCED FROM L-METHIONINE BY SOIL MICROORGANISMS

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Abstract

The presence of a suitable substrate(s) in soil may release physiologically active concentration of a plant hormone ethylene (C_2H_4) as a result of microbial activity. We isolated three strains of fungi (*Aspergillus clavatus*, *Penicillium commure* and *Thamridium elegans*), from the maize rhizosphere soil, capable of producing C_2H_4 in the soil from L-methionine (10 mmol l^{-1}). The plate and soil inoculation experiments conducted under controlled conditions revealed that the C_2H_4 released as a result of precursor (L-MET)-inoculum (fungi) interaction caused a classical "triple" response in etiolated pea seedlings (a significant reduction in seedling length and increase in stem diameter). The classical "triple" response was also observed in the etiolated pea seedlings grown in non-sterilized soil amended with L-MET (no inoculation). The application of Ag(I), an inhibitor of C_2H_4 action, partially eliminated the classical "triple" response in etiolated pea seedlings. A significant direct correlation (r = 0.910* to 0.997**) was found between classical "triple" response and [L-MET] or [C_2H_4 gas]. The results of pot trial conducted on cotton indicated that L-MET applied at 1.0 and 10.0 mg kg^{-1} soil significantly increased the number of bolls (up to 45.5%), seed cotton weight (up to 35.7%), and root and shoot weight (up to 35.1 and 28.2%) over the unammended control. The results of this study imply that C_2H_4 production is a substrate-dependent biochemical process and application of small quantity of L-MET (1-10 mg kg^{-1} soil) may affect plant growth.

Introduction

Ethylene is one of the most important plant hormones and its effects have been observed practically on almost all the aspects of plant growth, ranging from seed germination to senescence (Arshad & Frankenebrger, 2002; Khalid *et al*., 2006). Both higher plants and soil microflora are known to produce various concentrations of C_2H_4. Several scientists believe that C_2H_4 accumulation in soil is the result of microbial activity (Arshad & Frankenberger, 1998, 2002; Weingart *et al*., 1999). Ethylene concentration in soil may vary depending on the soil properties, nature of the substrate and native microbiota (Zechmeister- Boltenstern & Smith, 1998; Nazli *et al*., 2003; Arshad *et al*., 2004). A suitable concentration of C_2H_4 in soil may create a physiological response in plant (Jia *et al*., 1999).

Although several studies have elucidated the role of exogenously applied C_2H_4 on various physiological processes of plant; however, its use for improving crop production has been limited because of its gaseous nature and therefore difficulty in its direct application to soil. Various compounds including L-MET have been identified which release C_2H_4 and thus their application at a suitable concentration in the presence of soil microflora may affect plant growth (Davies, 1995; Arshad & Frankenberger, 1998; Khalid *et al*., 2006).

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Etiolated pea seedlings show a characteristic "triple" response exclusively to C_2H_4. This so-called "triple" response involves reduction in elongation, swelling of hypocotyl, and a change in the direction of growth (Niljubow, 1901; Goeschi *et al*., 1966). Silver (Ag) is a specific inhibitor of C_2H_4 action and is believed to interfere the binding of C_2H_4 (Beyer, 1976) and thus reduces the impact of C_2H_4 on plant.

This study reports the effects of C_2H_4 produced from L-MET by the rhizosphere soil fungi as well as by the indigenous soil microorganisms on plant growth. The classical "triple" response in etiolated pea seedlings was used as a bioassay for assessing the role of microbially produced C_2H_4 from L-MET.

Materials and Methods

Isolation of rhizosphere fungi: Fungi were isolated from maize rhizosphere soil using the basal salt medium (BSM). The BSM (pH 6.2±0.1) was composed of (g l^{-1}) KH_2PO_4, 1.3; Na_2HPO_4, 2.0; $MgSO_4.7H_2O$, 0.2; $CaCI_2.2H_2O$, 0.5; $FeSO_4.7H_2O$, 0.02; $CuSO_4.5H_2O$, 0.04; $MnSO_4.H_2O$, 0.002; $ZnSO_4$ $7H_2O$, 0.002; $CoCl_2.6H_2O$, 0.001; $Na_2MoO_4.2H_2O$, 0.004. L-MET (10 mmol l^{-1}) obtained from Sigma was used as a sole source of C and N in the medium. Three strains showing the most prolific growth on agar plates were isolated. These strains were identified as *Aspergillus clavatus, Penicillium commune* and *Thamnidium legans* by using slide culture technique (Awan & Rehman, 2002), and were further confirmed by Biolog® identification system (Microlog™ System Release 4.2, Hayward, CA, USA).

The inoculum of each strain was prepared by harvesting the fungal spores in the sterilized distilled water. The plates containing pure fungal cultures were incubated at 30°C until spore formation. Spores were harvested by adding sterilized distilled water in the plates and mixing them in water by gentle shaking with L-shaped glass stick. The procedure was repeated twice for each strain and diluted to yield 2×10^6 CFU ml^{-1} and collected spores were preserved in 20% glycerol at -30°C for subsequent inoculation.

Ethylene production in soil: Preliminary confirmation of C_2H_4 released from L-MET produced by the three fungal strains in sterilized and non-sterilized soil was made by gas chromatography. For this purpose, 50 g soil were added in 460-ml glass bottles, and treated with 100 ml solution of L-MET (10 mmol l^{-1}). The soil was inoculated by adding 1.0 ml of fungal inoculum (2×10^6 CFU ml^{-1}). Each glass bottle was capped with Mininert valves (Pierce, Rock Ford, IL) and incubated in the dark at 30°C for a period of 72 h under shaking at 100 rpm. Similarly, an experiment was conducted with sterilized soil (autoclaved at 121 °C for 1 h) to know the biotic nature of ethylene production from L-MET. Solution of L-MET was sterilized by passing through a 0.22 µm filter (Whatmann). The experiments were run in three replications using a completely randomized design.

After incubation, the C_2H_4 concentrations were determined by gas chromatography (GC-FID, UNICAM-4600) by withdrawing 1-ml gas samples from the headspace air above the soil suspension with a gas-tight glass hypodermic syringe (Nazli *et al*., 2003). The C_2H_4 dissolved in the aqueous phase was not accounted for. The GC with a 2 m Porpak N (particle size, 0.14-0.18 mm) column was operated isothermally at 70°C. The following conditions were used for operating the GC: sample, 1.0 ml; carrier gas (N_2), 13 ml min^{-1}; H_2 flow, 30 ml min^{-1}; air flow, 300 ml min^{-1}; detector temp, 200°C; and injector temp, 120°C.

Classical "triple response in etiolated pea seedlings (plate and soil inoculation experiments): The effects of C_2H_4 released from L-MET by three fungi (*A. clavatus, P. commune* and *T. legans*) and/or indigenous soil microorganisms on etiolated pea seedlings were demonstrated by using the classical "triple" response bioassay which is considered one of the most specific actions of C_2H_4 (Neljubow, 1901). Seeds of pea (*Pisum sativum*, cv. Mateor) were surface sterilized by dipping in 95% ethanol solution for two minutes and 0.02% $HgCl_2$ solution for one minute, which were subsequently washed thoroughly with sterilized distilled water. These seeds were then placed on sterilized filter paper in plates and incubated at 30°C for germination. Pregerminated pea seeds were transferred to100 ml beakers filled with 100 g sterilized soil (autoclaved for 1 h at 121°C) and saturated at 60% of water holding capacity.

In the first experiment, C_2H_4 production by the fungi was achieved by inoculating the plates (15 x 160 mm) containing 15 ml of agar based BSM. Different levels (0-50 mmol l^{-1}) of L-MET (Sigma, St Louis, MO) were used. The BSM was sterilized by autoclaving at 121°C for 15 min and L-MET solution was sterilized by passing it through 0.2 µm pore membrane filter. Inoculation of the agar medium was done by adding 1.0 ml of pre-harvested spores of each fungal strain. The plates were placed at the bottom of the airtight Mason jars wrapped in green foil to provide the "safe" green light. These plates were covered with small tripod stands on which beakers with pregerminated seeds were placed. Incubation was carried out in complete darkness throughout the experiment at 30°C for seven days (168 h). Each treatment was replicated thrice and there were three seedlings in each beaker.

In the second experiment, sterilized soil amended with different concentrations of L-MET was inoculated directly with the fungal strains by adding 1.0 ml of pre-harvested spores. There were three replications per treatment with a total of nine seedlings per treatment.

Similarly, another experiment was conducted in non sterilized soil with different levels of L-MET (0, 10, 20 and 50 mmol l^{-1}) to evaluate the influence of C_2H_4 produced by the indigenous soil microorganisms from L-MET on etiolated pea seedlings. The experiment was repeated with 10 mmol l^{-1} L-MET with or without $AgNO_3$ (240 mg l^{-1}) to see the inhibitory effect of Ag on C_2H_4 production. To compensate the NO_3 effect, the seedling not treated with $AgNO_3$ received foliar application of $NaNO_3$ of the same strength.

Effect of soil applied L-MET on cotton: Pot experiment was conducted in the net house to study the effect of soil applied L-MET on growth and yield of cotton (*Gossypium hirsutum* L.). Each pot (24 cm diam., 26 cm height) was filled with 12 kg sandy clay loam soil collected from the upper soil layer (0-30 cm depth) from a field. The air dried soil (typic Haplocambids) was sieved (40-mesh sieve) and analyzed for physico-chemical properties. The analysis of soil revealed a pH of 7.8; electrical conductivity (ECe), 1.94 dS m^{-1}; cation exchange capacity, 8.2 $cmol_c$ kg^{-1} soil; organic matter, 0.60%; total N, 0.04%; available P, 7.3 mg kg^{-1} and exchangeable K, 118 mg kg^{-1} soil. Nitrogen (60 mg kg^{-1} soil) as urea, phosphorus (20 mg P kg^{-1} soil) as single super phosphate and potassium (10 mg K kg^{-1} soil) as sulfate of potash were applied to each pot including the control. The P and K fertilizers were applied by mixing them uniformly with the soil before filling the pot. Nitrogen was applied in two split doses *i.e.* by mixing half with the soil before filling the pots and adding the remainder two weeks after germination.

Four delinted seeds of cotton (cv. CIM 70) were sown in each pot and plants after germination were thinned to one per pot. Four levels (0, 1, 10 and 50 mg kg^{-1} soil) of L-MET were applied to the soil in solution form. The pots were arranged randomly at

ambient temperature and light in a net house. The pots were kept moist near field capacity (60% WHC) by using good quality canal water [EC = 0.03 dS m^{-1}, sodium adsorption ratio (SAR) = 0.26 (mmol $l^{-1})^{1/2}$, residual Sodium carbonate (RSC) = 0] meeting the irrigation quality criteria for crops (Ayers & Westcot, 1985). Cotton received an equivalent of 50-60 cm of rainfall. Plant protection measures were taken to control the attack of insects and pests. The number of bolls, seed cotton weight, dry root and shoot weight were recorded.

The collected data were subjected to statistical analyses and differences among the treatments were observed by using Duncan's multiple range test (Software MSTATC, Michigan State University, MI, USA). To determine the relationship between substrate [L-MET] and classical "triple" response, the reduction in seedling length and increase in stem diameter of the seedlings were regressed against the [L-MET] or [C_2H_4].

Results

Ethylene biosynthesis and classical "triple" response in etiolated pea seedlings: The study revealed that the selected three fungal strains (*A. clavatus*, *P. commune* and *T. legans*) were capable of producing C_2H_4 (ranging from 342 to 590 nmol kg^{-1} soil) from L-MET in the sterilized soil after 72 h incubation (data not shown). C_2H_4 was not detected in the sterilized soil amended with filter sterilized solution of L-MET. However, a copious amount of C_2H_4 (526 nmol kg^{-1} soil) was observed in the uninoculated non-sterilized soil amended with the sterilized solution of L-MET.

Plate and soil inoculation experiments were conducted to demonstrate the effect of C_2H_4 released as a result of precursor (L-MET)-inoculum interaction on etiolated pea seedlings. The growth of etiolated pea seedlings was markedly influenced by the C_2H_4 released from L-MET produced by the fungi (Table 1). Increasing concentration of L-MET resulted in a stronger classical "triple" response in case of all the three fungi used as inocula. The maximum decrease (77.4%) in the length and increase (170%) in the diameter of etiolated pea seedlings was observed where the BSM with 50 mmol l^{-1} L-MET was inoculated with *A. clavatus*. The combined application of L-MET and fungal inocula to sterilized soil strongly decreased the seedling length (up to 91%) and increased the stem diameter (up to 184%) of etiolated peas compared with the unammended sterilized soil inoculated with the fungi. Overall, the fungal strain *P. commune* in the presence of L-MET exhibited stronger classical "triple" response in etiolated pea seedlings in the sterilized soil.

After demonstrating the role of C_2H_4 (released as a result of precursor-inoculum interaction) in the growth of etiolated pea seedlings, L-MET was applied to non-sterilized soil to evaluate its effectiveness as a substrate in creating classical "triple" response in the presence of soil indigenous microorganisms. Its application to non-sterilized soil resulted in a significant decrease in the seedling length (up to 79.8%) with the increase in stem diameter (up to 91.5%) compared with the unammended soil (Table 2). The effect was more pronounced at higher levels of L-MET than that observed at low levels. Application of $AgNO_3$ (240 mg l^{-1}) partially eliminated the classical "triple" response in the etiolated pea seedlings and thus protected the seedlings against L-MET-dependent C_2H_4 produced by soil indigenous microflora (Fig. 1).

A significant correlation (r = 0.915*-0.996**) was observed between L-MET- [L-MET] and the decreased length of etiolated pea seedlings (Table 3). Similarly, correlation (r = 0.910*-0.997**) was also significant between the [L-MET] and the increase in stem diameter of etiolated pea seedlings.

Table 1. Effect of ethylene produced as a result of precursor (L-MET)-inoculum (fungi) interaction on classical "triple" response in etiolated pea seedlings (The data are average of 3 replications x 3 seedlings).

Fungal strains	L-MET (mmol l^{-1})	Plate inoculation experiment[a]		Soil inoculation experiment[a]	
		Seedling length (cm)	Seedling dia (mm)	Seedling length (cm)	Seedling dia (mm)
Aspergillus clavatus	0	14.93 ± 0.97[b]	0.57 ± 0.06	10.80 ± 0.56	0.85 ± 0.13
	10	4.84 ± 0.32	1.21 ± 0.12	1.93 ± 0.11	1.93 ± 0.31
	20	3.38 ± 0.28	1.38 ± 0.15	1.46 ± 0.11	2.02 ± 0.44
	50	3.38 ± 0.34	1.54 ± 0.16	1.26 ± 0.08	2.07 ± 0.37
Penicillium commune	0	13.96 ± 1.20	0.58 ± 0.08	11.50 ± 0.88	0.72± 0.10
	10	4.84 ± 0.44	1.32 ± 0.20	2.03 ± 0.21	1.76 ± 0.20
	20	4.46 ± 0.34	1.09 ± 0.10	1.26 ± 0.16	1.96 ± 0.32
	50	3.95 ± 0.25	1.46 ± 0.12	1.08 ± 0.10	2.05 ± 0.30
Thamnidium legans	0	14.38 ± 1.06	0.54 ± 0.08	11.42 ± 0.67	0.87 ± 0.13
	10	4.85 ± 0.30	1.16 ± 0.12	1.93 ± 0.23	1.96 ± 0.22
	20	4.47 ± 0.33	1.33 ± 0.19	1.33 ± 0.32	2.10 ± 0.28
	50	3.42 ± 0.19	1.41 ± 0.27	1.24 ± 0.12	2.40 ± 0.36

[a]The data was collected after 168 h of incubation at 30°C
[b]Standard error

Table 2. Effect of L-methionine (L-MET) added to non-sterilized soil on classical "triple" response in etiolated pea seedlings (The data are average of 3 replications x 3 seedlings).

L-MET (mmol l^{-1})	Seedling length (cm)	Seedling dia. (mm)
Unammended control	11.80 a[a]	0.95 c
10	3.70 b	1.41 b
20	3.36 c	1.70 a
50	2.38 d	1.82 a

[a]Values followed by different letter(s) in a column were significantly different at $p \leq 0.05$

Table 3. Correlation between [C_2H_4] or [L-MET] and the growth of etiolated pea seedlings.

[L-MET] or [C_2H_4]	r value	
	Seedling length	Seedling dia.
Plate inoculation	0.915*	0.997**
Soil inoculation	0.953*	0.996**
Soil indigenous microflora	0.996**	0.910*

*Significant at $p \leq 0.05\%$

Table 4. Effect of soil applied L-methionine (L-MET) on growth and yield of cotton in a pot experiment (The data are average of 3 replications).

L-MET (mg kg^{-1} soil)	Number of bolls $plant^{-1}$	Seed cotton (g $plant^{-1}$)	Root weight (g $plant^{-1}$)	Shoot weight (g $plant^{-1}$)
Unammended control	11 c[a]	24.9 c	3.7 b	55.6 c
1	16 a	33.8 a	5.0 a	68.3 ab
10	14 b	30.1 b	4.8 a	71.3 a
50	11 c	26.2 c	3.9 b	65.5 b

[a]Values followed by different letter(s) in a column were significantly different at $p \leq 0.05$

Fig. 1. The growth pattern of etiolated pea seedlings in response to L-methionine derived ethylene produced by indigenous soil microflora in the presence and absence of Ag (I), an inhibitor of ethylene action.

Effect of soil applied L-MET on cotton (pot experiment): The addition of L-MET at lower concentrations (1-10 mg kg^{-1} soil) significantly increased the number of bolls, seed cotton weight, root and shoot weights compared to control (Table 4). The maximum increase in number of bolls (45.5%), and seed cotton (35.7%) and root (35.1%) weights were observed where L-MET was applied at 1.0 mg kg^{-1} soil. The highest shoot weight was recorded with 10 mg L-MET kg^{-1} soil but statistically at par with the shoot weight observed in case of 1.0 mg L-MET kg^{-1} soil. L-MET application at higher concentration (50 mg kg^{-1} soil) resulted in a non-significant increase in seed cotton and root weight over the unammended control; however, the shoot weight was significantly greater with this concentration of L-MET than the control.

Discussion

A series of trials conducted under axenic (controlled) and non-axenic (pot) conditions demonstrated the effectiveness of L-MET in evoking physiological response in plants. The classical "triple" response was observed in etiolated pea seedlings in response to their exposure to C_2H_4 produced from L-MET outside the root zone (plate

inoculation experiment). The degree of the response changed with the concentration of the substrate (L-MET). Similar kind of classical "triple" response was observed in the etiolated pea seedlings exposed to combined application of inocula (fungi) and substrate (L-MET) to the sterilized soil. This premise is supported by the findings that C_2H_4 production is a substrate-dependent biological process and the rhizosphere fungi utilize L-MET for the synthesis of C_2H_4 in soil (data not shown). No such response was observed when substrate was applied alone to the sterilized soil. This implies that the "triple" response in etiolated pea seedlings was evoked by a metabolite (C_2H_4) produced as a result of precursor-inoculum interaction. Moreover, a significant direct correlation was found between [L-MET] and the decrease in seedling length and swelling in the stem diameter of etiolated pea seedlings. The production of a classical "triple" response due to the exogenous application of 1-aminocyclopropane-1-carboxylic acid (an immediate precursor of C_2H_4) to etiolated tomato and *Arabidopsis* seedlings has also been reported (Barry *et al.*, 2001; Ton *et al*., 2001).

The application of L-MET to non-sterile soil also created a classical "triple" response in etiolated pea seedlings similar to that observed in sterile soil inoculated with the fungi, implying that C_2H_4 producing microflora may be ubiquitous in soil, and the availability of a substrate like L-MET could enhance C_2H_4 synthesis in soil. This is further supported from our observations that inoculation of non-sterilized soil with the selected fungi did not affect C_2H_4 production significantly. Previous studies have shown that the addition of C_2H_4 releasing compounds (e.g., calcium carbide, 1-aminocyclopropane-1-carboxylic acid and 2-keto-4-methylthiobutyric acid) to non-sterile soil stimulated C_2H_4 biosynthesis in soil (Nazli *et al*., 2003; Yaseen *et al*., 2006). The application of Ag(I), a known inhibitor of C_2H_4 action, partially eliminated the affect of substrate on etiolated pea seedlings. This implies that indigenous soil microflora produced C_2H_4 from the added substrate, which affected the growth pattern of etiolated pea seedlings.

This study also demonstrated the effectiveness of L-MET for improving the growth and yield of cotton. The results showed that lower concentrations (ranging from 1-10 mg kg^{-1} soil) of L-MET had a significant positive effect on the growth and yield of cotton crop. These positive effects of L-MET could be attributed to physiologically active levels of C_2H_4 at this substrate concentration. It has been postulated that the exogenously supplied phytohormones produce their effects by changing the endogenous level(s)/balance of naturally occurring hormones, allowing the modification of growth depending on age and physiological state of plant, endogenous level of hormones, state of nutrition, and environmental conditions (Abeles *et al*., 1992; Arshad & Frankenberger, 2002).

This study suggested that plant growth could be modified by changing the C_2H_4 levels in the close vicinity of the root through the soil amendment with L-MET like compounds. Since a very small amount of C_2H_4 in the rhizosphere influences the growth of plants, the rate of application could be crop specific.

References

Abeles, F.B., P.W. Morgan and M.E. Saltveit Jr. 1992. *Ethylene in Plant Biology*. Academic Press, San Diego.

Arshad, M. and W.T. Frankenberger Jr. 1998. Plant growth-regulating substances in the rhizosphere: microbial production and functions. *Adv. Agron*., 62: 45-151.

Arshad, M. and W.T. Frankenberger Jr. 2002. *Ethylene: Agricultural Sources and Applications*. Kluwer Academic/Plenum Publishers, New York. 342p.

Arshad, M., Z.H. Nazli, A. Khalid and Z.A. Zahir. 2004. Kinetics and effects of trace elements and electron complexes on 2-keto-4-methylthiobutyric acid-dependent biosynthesis of ethylene in soil. *Lett. Appl. Microbiol*., 39: 306-309.

Awan, J.A. and S.U. Rehman. 2002. *Microbiology Manual*. UNITECH Communications, Faisalabad, Pakistan. 116 p.

Barry, C.S., E.A. Fox, H. Yen, S. Lee, T. Ying, D. Grierson and J.J. Giovannoni. 2001. Analysis of ethylene response in the epinastic mutant of tomato. *Plant Physiol*., 127: 58-66.

Beyer, E.M. Jr. 1976. A potent inhibitor of ethylene action in plants. *Plant Physiol*., 58: 268-271.

Davies, P.J. 1995. The plant hormones: their nature, occurrence, and functions. p. 1-11. In: *Plant Hormones and Their Role in Plant Growth and Development*. (Ed.): P.J. Davies. Martinus Nijhoff, Dordrecht, The Netherlands.

Goeschi, J.D., L. Rappaport and H.K. Pratt. 1966. Ethylene as a factor regulating the growth of pea epicotyle subjected to physical stress. *Plant Physiol*., 41: 877-884.

Jia, Y.J., Y. Kakuta, M. Sugawara, T. Igarashi, N. Oki, M. Kisaki, T. Shoji, Y. Kanetuna, T. Horita, H. Matsui and M. Honma. 1999. Synthesis and degradation of 1-aminocyclopropane 1-carboxylic acid by *Penicillium citrinum*. *Biosci. Biotech. Biochem*., 63: 542-549.

Khalid, A., M. Arshad and Z.A. Zahir. 2006. Phytohormones: microbial production and applications. p. 207-220. In: *Biological Approaches to Sustainable Soil Systems*. (Eds.): N. Uphoff, A.S. Ball, E. Fernandes, H. Herren, O. Husson, M. Laing, C. Palm, J. Pretty, P. Sanchez, N. Sanginga and J. Thies. Taylor & Francis/CRC, Boca Raton, Florida.

Nazli, Z.H., M. Arshad and A. Khalid. 2003. 2-Keto-4-methylthiobutyric acid-dependent biosynthesis of ethylene in soil. *Biol. Fertil. Soil*, 37: 130-135.

Neljubow, D. 1901. U¨ ber die horizontale Nutation der Stengel von *Pisum sativum* und einiger anderer Pflanzen. *Beih. Bot. Zentralbl*., 10: 128-138.

Ton, J., S. Davison, S.C.M. van Wees, L.C. van Loon and C.M.J. Pieterse. 2001. The *Arabidopsis* ISRI locus controlling rhizobacteria-mediated induced systemic resistance is involved in ethylene signaling. *Plant Physiol*., 125: 652-661.

Weingart, H., B. Volksch and M.S. Ullrich. 1999. Comparison of ethylene production by *Pseudomonas syringae* and *Ralstonia solanacearum*. *Phytopathol*., 89: 360-365.

Yaseen, M., M. Arshad and A. Khalid. 2006. Effect of acetylene and ethylene gases released from encapsulated calcium carbide on growth and yield of wheat and cotton. *Pedobiologia*, 50: 405-411.

Zechmeister-Boltenstern, S. and K.A. Smith. 1998. Ethylene production and decomposition in soils. *Biol. Fertil. Soils*, 26: 354-361.

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