African Journal of Biotechnology Vol. 7 (24), pp. 4446-4449, 17 December, 2008
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2008 Academic Journals

Full Length Research Paper

Juglone effects on seedling growth in intact and coatless seeds of muskmelon

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Accepted 19 November, 2008

Elongation, fresh and dry weights of muskmelon seedlings (*Cucumis melo* cv. Kış kavunu) were enhanced by juglone in intact seeds. In the case of coatless seeds, root elongation decreased but fresh and dry weight of the seedlings were increased by juglone. While protein content was slightly increased by juglone in cotyledons of intact seeds, it did not change significantly in coatless seeds. Catechol oxidizing activity of polyphenol oxidase (PPO) enzyme increased in cotyledons of juglone treated intact seeds whereas it did not change significantly in coatless seeds.

Key words: Juglone, muskmelon, seedling growth, protein content, polyphenol oxidase activity.

INTRODUCTION

The chemical interactions that occur among living organisms including plants, insects and microorganisms are called allelopathy, and the organic compounds involved in allelopathy are called allelochemicals. The release of allelochemicals from plants occur by volatilization, leaching from leaves, exudation from roots and degradation of dead plant parts. All parts have been shown to contain allelochemicals but leaves and roots are the most important sources. Allelochemicals become stressful when they are toxic. Sometimes a single chemical produced by one organism is harmful to another but benifical to a third organism (Whittaker and Feeny, 1971; Rice, 1979; Hale and Orcutt, 1987; Rizvi and Rizvi, 1992). In addition temperature was the most effective agent in enhancing synthesis and exudation of allelochemicals (Pramanik et al., 2000).

The inhibitory effect of black walnut (*Juglans nigra*) on associated plant species is one of the oldest examples of allelopathy. The chemical responsible for walnut allelopathy is juglone (5-hydroxy-1,4-naphthoquinone) (Davis, 1928; Rice, 1984). Juglone has been isolated from many plants in the walnut family (*Juglandaceae)* including *Juglans nigra,Juglans regia* and others (Daglish, 1950; Prataviera et al., 1983). A colourless, nontoxic reduced form called hydrojuglone is abundant, especially in leaves, fruit hulls and roots of walnut. When exposed to the air or to some oxidizing substance hydrojuglone is oxidized to its toxic form, juglone (Lee and Campbell, 1969; Segura–Aguilar et al., 1992). Rain washes juglone from the leaves and carries it into the soil. Thus, neighbour plants of the walnut are affected by absorbing juglone through their roots (Rietveld, 1983). Walnut has been reported to be toxic to both herbaceous and woody plants (Funk et al., 1979; Rietveld, 1983).

Juglone's allelopathic effects on plants are generally toxic but beneficial to some. In the previous study, it was found that seedling growth of tomato, cucumber, garden cress and alfalfa were inhibited strongly by juglone and walnut leaf extracts, but seedling growth of muskmelon was increased by the treatments (Kocaçalışkan and Terzi, 2001). Since positive allelopathic effect is rare in nature, positive effect of juglone on muskmelon was found interesting. Thus, it was investigated later by treating the muskmelon seeds with juglone in pregerminative and postgerminative stages. In this case, pregerminative juglone treatment caused positive effect on muskmelon but postgerminative treatment was contrarily (Terzi et al., 2003). In the same study, it has also been suggested that seed coat of muskmelon may be a barrier to restrict absorption of juglone at the beginning of seed germination (pregerminative stage). Therefore, the objective of this study was to compare juglone's effects on the intact

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and coatless seeds of muskmelon to test the seed coat barrier hypothesis.

MATERIALS AND METHODS

Juglone application and experimentation

Seeds of muskmelon (*Cucumis melo* cv. Kış Kavunu) was obtained from AGROMAR Company, Bursa, Turkey were surface sterilized with 1% sodium hypochloride. Then, they were left in distilled water for 2 h. Next, they were dried on filter papers at room temperature until they reached their previous weight. The chubby, strong and similar sized seeds were selected from these seeds. At least 10 seeds were placed in a Petri dish furnished with sheets of filter paper moistened with distilled water (control) or with juglone solution.

The seeds were divided in two groups. One group intact seeds and the other coatless seeds whose coat was removed after 2 h water imbibition as mentioned above. Two groups of the seeds were treated with both distilled water (control) and juglone. Then the seeeds in dishes were left in a growth chamber for 20 days. Growth conditions of the chamber were “14 h light (20 000 lux) / 24°C / 70% humidity and 10 h dark / 18°C / 80% humidity” (Şeniz, 1993). The experiment was replicated four times.

1 mM juglone solution was prepared by dissolving in distilled water by stirring at 40°C for 24 h (Kocaçalışkan and Terzi, 2001). This concentration of juglone was used since in the soil of walnut plantation, it is generally found in less than 1 mM concentration, depending on walnut species, season and distance from trunk of walnut (Rietveld, 1983; Jose and Gillespie, 1998). Therefore, a maximum possible concentration of juglone (1 mM) was selected. Juglone chemical (5-hydroxy-1,4-naphthoquinone) was purchased from SIGMA.

Determinations

After 20 days of growth in the chamber, seed germination percentage was recorded. Then the seedlings were separated two parts as root and shoot by cutting from the connection point. Seedling growth was determined by measuring root and shoot lenght and fresh and dry weights. For measuring protein content and polyphenol oxidase (PPO) activities 0.5 g of cotyledons was homogenized in 5 ml 0.1 M phosphate buffer of pH 6.5 and centrifuged at 3 500 g for 10 min. The supernatant was used in protein and enzyme activity determinations Protein content was determined by the spectrophotometric method of Bradford (1976). Catecholase and dopa oxidase activities were determined spectrophotometrically by measuring absorbances of reaction products at 430 and 490 nm, respectively (Kabar and Kocaçalışkan, 1990).

RESULTS AND DISCUSSION

In this study, juglone application did not change seed germination. It was 100% in both control and juglone treated seeds. The result therefore was not shown on the Table. However, all the growth parameters as elongation, fresh and dry weights were increased by juglone in intact seeds. Fresh and dry weights were increased by juglone more than elongation of both root and shoot (Table 1). In the previous studies (Kocaçalışkan and Terzi, 2001; Terzi et al., 2003) similar results has also been obtained in muskmelon that seed germination was not effected positively or negatively but seedling growth was increased by juglone.

In the case of coatless seeds of muskmelon, it was partly different, root elongation was decreased by juglone. However, as seen in the Table, fresh and dry weight of the coatless seeds were increased by juglone. This was shown for the first time in the present study that juglone decreases root elongation but increases fresh and dry weight in coatless seeds of muskmelon. This is an ABA similiar effect which is a common inhibitory phytohormone in plants decreasing elongation (Salisbury and Ross, 1985). Thus, in a study, juglone has beeen suggested to have a growth regulator effect (Ranade and David, 1985).

It has generally been assumed that increase in protein content may be a criterion of plant growth and leaves are the most metabolically active organ and rich in protein. Therefore, in this study, protein content was measured in the cotyledonary leaves of muskmelon seedlings. Protein content was increased slightly by juglone in intact seeds. Increase in protein content by juglone is parallel to increase in seedling growth shows positive allelopathic effect of juglone on intact seeds of muskmelon. The increase in protein content by juglone agrees with previous findings (Compton and Preece, 1988; Terzi et al., 2003).

Increase in protein content may stem from increase in some enzymatic proteins such as polyphenol oxidase. Thus, polyphenol oxidase activity was increased by juglone in cotyledons of the seedlings. The enzyme oxidizes mainly diphenolic substates. The main substrates of the enzyme are catechol and dopa in plants, and polyphenol oxidase enzyme oxidizing these substrates are called catecholase and dopa oxidase, respectively (Mayer, 1987). Polyphenol oxidase activity has been shown to increase as a result of juglone treatment in muskmelon (Terzi et al., 2003). In the present study, catecholase activity was increased by juglone but dopa oxidase activity was not changed, significantly. Increase in the activity of catecholase may be a reaction against juglone, since catecholase oxidizes catechol substrate as an oxidative defence mechanism. Thus, juglone was found to induce oxidative stress during seed germination (Segura- Aguilar et al., 1992).

In the present study, juglone has been found to increase all the growth parameters studied in the seedlings of intact seeds but it decreased root elongation in coatless seeds, whereas it did not decrease fresh and dry weight and shoot elongation. This result do not exactly prove the seed coat barrier hypothesis. It may be said that different biochemical mechanism(s) exist in muskmelon seeds to detoxify juglone.

In pea seeds, a relation between PPO activity and seed coat permeability has been suggested. Seed coat of *Pisum elatius* has higher PPO activity but are impermeable, whereas seed coat of *Pisum sativum* has lower PPO activity but are permeable to water. Then, impermeability of *P. elatius* seed coat was attributed to its higher PPO acti-

Table 1. Effect of juglone on growth of root and shoot, protein content and polyphenol oxidase activities of cotyledons in intact and coatless seeds of muskmelon.

Determination	Control (distilled water)	Juglone (1 mM)
Intact seeds		
Root		
Elongation (cm)	5.5 ± 2.2	6.5 ± 3.7*
Fresh weight (mg)	26.8 ± 2.3	45.5 ± 3.8*
Dry weight (mg)	2.2 ± 0.3	4.6 ± 0.4*
Shoot		
Elongation (cm)	2.0 ± 0.3	3.3 ± 0,4*
Fresh weight (mg)	31.2 ± 2.9	53.3 ± 4.1*
Dry weight (mg)	1.5 ± 0.1	3.4 ± 0.2*
Cotyledon		
Protein (mg/g fr. wt.)	1.1 ± 0.1	1.3 ± 0.1
Catecholase (A_{430}/g fr. wt.)	22.1 ± 1.2	27.6 ± 1.4*
Dopa oxidase (A_{490}/g fr. wt.)	26.7 ± 1.1	25.6 ± 1.2
Coatless seeds		
Root		
Elongation (cm)	9.6 ± 0.9	5.2 ± 0.5*
Fresh weight (mg)	56.0 ± 4.1	64.5 ± 4.6*
Dry weight (mg)	3.8 ± 0.4	4.3 ± 0.5
Shoot		
Elongation (cm)	3.3 ± 0.3	3.8 ± 0.4
Fresh weight (mg)	32.7 ± 2.1	66.3 ± 3.3*
Dry weight (mg)	1.3 ± 0.1	3.5 ± 0.3*
Cotyledon		
Protein (mg/g fr. wt.)	1.3 ± 0.2	1.2 ± 0,1
Catecholase (A_{430} /g fr. wt.)	19.6 ± 0.9	19.1 ± 0.7
Dopa oxidase (A_{490} /g fr. wt.)	26.6 ± 1.2	24.9 ± 1.0

* ($P< 0.05$) "t" test, ± SD, n = 4.

vity (Marbach and Mayer, 1974). On the other hand, coat of some fruits was found to have higher PPO activity and this was related to protection of the fruits against viral, bacterial and fungal attacks, since quinones produced from phenolic substrates as a result of PPO activity prevent pathogen invasion (Farkas and Kiraly, 1958; Van Kammen and Brouwer, 1964; Vamos-Vigyazo, 1981; Mayer, 1987). PPO enzyme may play a similar defensive role in muskmelon seed coat against juglone. Further studies are therefore needed to clarify the mechanism (s) and the role of PPO enzyme in this event.

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