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Full Length Research Paper

Alleviation of juglone stress by plant growth regulators in germination of cress seeds

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Juglone (5-Hydroxy-1, 4-naphthoquinone) is an allelochemical responsible for walnut allelopathy. The effects of gibberellic acid (GA) and kinetin (KIN) in overcoming juglone stress on seed germination and seedling growth were investigated in cress (*Lepidium sativum* cv. Bandırma). Pretreated seeds with plant growth regulators were used in the study to test their alleviation effect after juglone stress. It was found that seed germination of cress was inhibited by juglone in range of 0.2 - 1 mM concentrations but GA and KIN alleviated this inhibition significantly. Both elongation and fresh and dry weights of the seedlings were also reduced by juglone, and the plant growth regulators alleviated them. GA was found more effective than KIN in alleviation of juglone stress.

Key words: Alleviation, cress, gibberellic acid, juglone stress, kinetin, seed germination, seedling growth.

INTRODUCTION

The chemical interactions that occur among plants are called allelopathy, and the organic compounds playing roles in allelopathy are called allelochemicals. The release of allelochemicals from plants occurs by volatilizetion, 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 (Rice, 1984; Rizvi and Rizvi, 1992; Inderjit et al., 1999; Singh et al., 2002; Batish et al., 2007). Allelochemicals become stressful when they are toxic. Sometimes, a single chemical produced by one organism is harmful to another but beneficial to a third organism. This property of allelochemicals may be used in weed management (Duke et al., 2002; Vyvyan, 2002; Singh et al., 2003; Topal et al., 2006, 2007).

Juglone (5-Hydroxy-1, 4- naphthoquinone) is an allelochemical responsible for walnut allelopathy. The inhibittory effect of black walnut (*Juglans nigra*) on associated plant species is one of the oldest examples of allelopathy (Davis, 1928; Rice, 1984; Rizvi and Rizvi, 1992). Juglone has been isolated from many plants in the walnut family (*Juglandaceae*) including *J. negra* and the 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).

Walnut intercropping system is commonly used in several regions of Turkey, as seen in several countries on the world. However, agronomic yield of some species can be significantly reduced because of deleterious effects of walnut on associated juglone-sensitive species. Although juglone concentration decreased with distance from walnut tree, moderate levels of juglone have been detected at a distance of 4.25 m. Toxicity of juglone is known for a long time and it remains to be an unsolved problem. As a solution, installation of polyethylene root barriers has been suggested to prevent juglone from getting into the alley where associated crop species are planted (Jose and Gillespie, 1998). It is believed that this method can only prevent the effect of juglone exuded from walnut roots; however, it cannot prevent the effect of juglone washed from the leaves.

In previous studies (Kocaçalişkan and Terzi, 2001; Terzi, 2008, 2009; Kocaçalışkan et al., 2009), juglone has been found to inhibit seed germination of several plant species such as cress, tomato, cucumber, alfalfa, radish and watermelon. It has also been shown that the most sensitive species to juglone was cress that juglone was completely inhibited its seed germination. Thus, cress

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Table 1. Effects of PGRs on germination percentage of cress seeds under different juglone stresses at fourth day. Means ± SE, n: 4.

Juglone (mM)	Dist. water (Control)	GA	KIN	GA + KIN
0	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
0.2	0.0 ± 0.0b	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
0.4	0.0 ± 0.0d	87.0 ± 6.4a	11.1 ± 2.3c	56.0 ± 4.7b
0.6	0.0 ± 0.0d	62.2 ± 4.9a	2.2 ± 0.4c	13.3 ± 2.5b
0.8	0.0 ± 0.0d	6.7 ± 0.6a	2.2 ± 0.3c	4.4 ± 0.6b
1.0	0.0 ± 0.0b	6.7 ± 0.5a	0.0 ± 0.0b	0.0 ± 0.0b

Values with different letters within each row are significantly different ($P < 0.05$).

was suggested as a test plant in allelopathic studies (Kocaçalişkan and Terzi, 2001). However, we have not encountered any study to avoid growth inhibitory effect of juglone. Therefore, in this work, we aimed to investigate if pretreatments of cress seeds with gibberellic acid (GA_3) and kinetin (KIN) could overcome juglone's toxic effect because gibberellins and cytokinins are known as common germination agents (Bewley and Black, 1983).

EXPERIMENTAL

Seeds of cress (*Lepidium sativum* cv. Bandırma) were obtained from AGROMAR Company, Bursa, Turkey. Juglone solution was prepared 1 mM dissolved in distilled water by stirring at 40°C for 24 h. From this solution 0.2, 0.4, 0.6 and 0.8 mM juglone solutions were prepared by diluting with distilled water. The concentration range of juglone used in the study is ecologically relevant 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). Juglone chemical and plant growth regulators (GA_3 and KIN) were purchased from SIGMA, USA. Predetermined concentrations of PGRs, 1 mM GA_3 and 0.5 mM KIN and a combination of both substances, were also prepared.

The seeds were surface sterilized with 1% sodium hypochloride. Some of the seeds were soaked in solutions of the PGRs and some others in distilled water for 15 min at room temperature as pretreatments. Thereafter, the solutions were decanted off and the seeds were dried for 1 h. The seeds treated with PGRs were sown in a Petri dish furnished with sheets of filter paper moistened with distilled water or with juglone solution. The seeds non-treated with PGRs but treated with distilled water were also sown in distilled water or juglone solutions as control groups. Then the dishes were left in an incubator at 25°C in continuous dark. Germination percentages of the seeds were determined at intervals of 24 h up to fifty day. Each treatment was replicated four times and 50 seeds were used in each replicate. Standard errors of the means, and significant difference between the treatments were shown on the Tables determined according to Duncan's multiple range tests.

RESULTS

Germination of cress seeds was completely inhibited by juglone in range between 0.2 - 1 mM concentrations. However, pretreatments of cress seeds with GA and KIN significantly alleviated this inhibition (Table 1). As seen in the Table, GA was found the most effective growth regulator in alleviating of the germination inhibition. KIN was found to have lesser effect than GA in this respect. GA and KIN combination did not show any synergistic effect in increasing the alleviation effect. All the treatments alleviated the 0.2 mM juglone inhibition of cress seeds at 100% level. But as juglone concentration increases, alleviation also decreases. KIN and GA+KIN treatments could not alleviate germination inhibition at 1 mM juglone, whereas GA alleviated it by 6.7 (Table 1).

On the basis of 0.4 mM juglone concentration, alleviation effects of GA and KIN depending on time course can be seen in Figure 1. While germination of cress seeds was completely inhibited in 0.4 mM juglone during four days, GA and KIN alleviated this inhibition. However, alleviation effect of GA was higher than KIN during four days. Germination percentage of cress seeds under juglone stress was increased by both GA and KIN, and was relatively higher up to third day and later slowed down. There was no change in the alleviation effect of the PGRs after fourth day. Therefore, these data were not presented.

Root and shoot growth of cress seedlings were elongated, fresh weight and dry weight was also inhibited in 0.4 mM juglone, and were significantly alleviated by GA, KIN and their combination (Table 2). GA was found the most effective PGR in alleviating juglone inhibition related with all growth parameters. However, alleviation effect of GA on dry weights of root and shoot was not significantly different than KIN.

DISCUSSION

Previous works had shown that juglone inhibits germination and seedling growth of several plant species, and especially that seedling growth is much more sensitive to juglone than the seed germination (Rietveld, 1983; Tekintaş et al., 1988; Dornbos and Spencer, 1990; Kocaçalışkan and Terzi, 2001; Terzi et al., 2003). However, no study exists to overcome inhibitory effect of juglone stress on seed germination and seedling growth. It has been indicated that PGRs can overcome some stresses on plants (Hale and Orcutt, 1987). For example, salt (NaCl) stress on seed germination could be overcome by GA and KIN applications (Kabar, 1990).

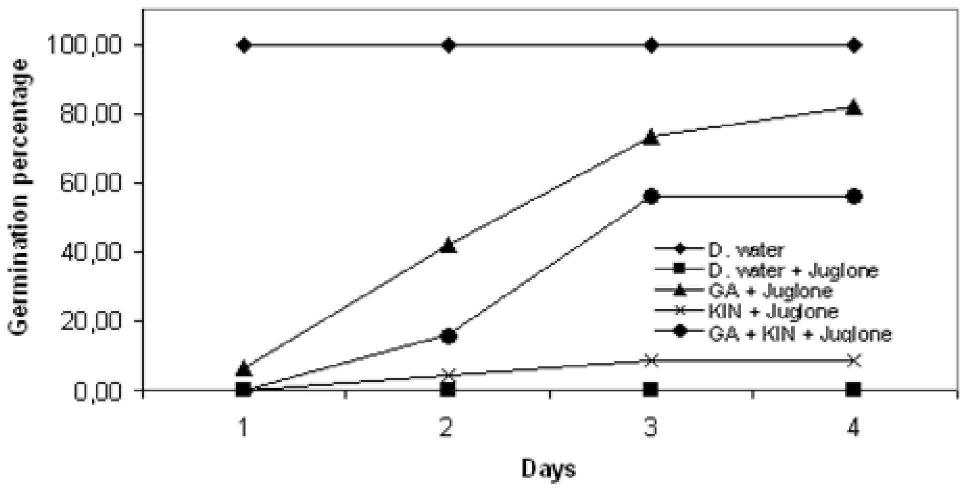

Figure 1. The time course of germination percentage of cress seeds as affected by the PGRs in 0.4 mM juglone medium.

Table 2. Alleviation effect of the PGRs on root and shoot growth of cress seedlings under 0.4 mM juglone stress at fourth day. No growth in 0.4 mM juglone medium was observed. Means ± SE, n: 4.

	GA	KIN	GA + KIN
Root elongation (cm)	0.50± 0.020a	0.40 ± 0.018b	0.50 ± 0.021a
Shoot elongation (cm)	0.70 ± 0.025a	0.60 ± 0.022b	0.50 ± 0.019c
Root fresh weight (mg)	0.82± 0.019a	0.72 ± 0.018b	0.86 ± 0.022a
Shoot fresh weight (mg)	0.32 ± 0.014a	0.28 ± 0.009ab	0.23 ± 0.013b
Root dry weight (mg)	0.39± 0.017a	0.38 ± 0.016a	0.28 ± 0.011b
Shoot dry weight (mg)	0.17 ± 0.011a	0.14 ± 0.010a	0.15 ± 0.009a

Values with different letters within each row are significantly different (P < 0.05).

In the present study, it has been shown that GA and KIN can significantly overcome allelochemical stress of juglone on seed germination and seedling growth (root and shoot) in cress. The physiological action of juglone is not well understood. Juglone inhibits plant growth by reducing photosynthesis and respiration (Hejl et al., 1993; Jose and Gillespie, 1998), increasing oxidative stress (Segura–Aguilar et al., 1992) and blocking protein synthesis in transcription stage by inhibiting RNA polymerases (Chao et al., 2001). In general, allelochemicals is known to inhibit plant growth by reducing chlorophyll and protein contents (Batish et al., 2007: Kocaçalışkan et al., 2009). Overcoming mechanism(s) of GA and KIN on juglone stress is unknown at present. However, it is known that during seed germination the most important event is protein synthesis since it is necessary for embryo growth (Bewley and Black, 1983). Therefore, this implies that GA and KIN may protect protein synthesis against juglone's inhibitory effect. Some allelochemicals such as benzoic acids lower auxin concentration in plants by triggering auxin catabolism, thereby, inhibiting plant growth (Weir et al., 2004). Although no report exist about other PGRs in this respect, but a similar mechanism may occur in juglon's inhibitory effect by decreasing gibberellin and cytokinin concentrations in the seeds during germination. The exogenous supplement of the PGRs in the seeds may result overcoming juglone stress by reversing lowered PGR concentrations as seen in the present study.

As mentioned before, juglone is generally found in the soil of walnut plantation less than 0.1 mM concentration depending on species season and distance from trunk of walnut (it is a weak possibility to reach 1 mM concentration) (Rietveld, 1983; Jose and Gillespie, 1998). Inhibitory effect of a maximum possible concentration of juglone (1 mM) on cress seed germination was seen to be alleviated by GA and KIN or their combination. This may be beneficial practically in arrangement of intercropping system(s) of the juglone sensitive species between walnut trees in the same field.

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