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DOES JASMONIC ACID PREVENT THE GERMINATION OF BARLEY SEEDS?

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Abstract: Effect of jasmonic acid on seed germination and seedling growth of barley (*Hordeum vulgare* L. cv. Bülbül 89) was investigated in the present study. Jasmonic acid concentrations less than 1500 µM have not inhibited the seed germination, while 1500 and 2000 µM jasmonic acid levels caused atypical germination. The germination was completely inhibited at 3000 µM level of jasmonic acid. However, the seedling growth clearly slowed down with increasing concentrations of jasmonic acid. Furthermore, these inhibitions on the seed germination and seedling growth were irreversible.

Key words: *Hordeum vulgare*, jasmonic acid, seed germination, seedling growth

JASMONİK ASİT ARPA TOHUMLARININ ÇİMLENMESİNİ ENGELLER Mİ?

Özet: Bu çalışmada, arpanın (*Hordeum vulgare* L. var. Bülbül 89) tohum çimlenmesi ve fide büyümesi üzerine jasmonik asitin etkisi araştırılmıştır. Jasmonik asidin 1500 µM'dan daha düşük konsantrasyonları tohum çimlenmesini engellemezken, 1500 ve 2000 µM jasmonik asit düzeyleri atipik çimlenmeye neden olmuştur. 3000µM jasmonik asit düzeyinde ise çimlenme tamamen engellenmiştir. Bununla birlikte, fide büyümesi jasmonik asit konsantrasyonlarındaki artışlar ile belirgin bir şekilde yavaşlamıştır. Dahası, tohum çimlenmesi ve fide büyümesi üzerindeki bu engellemelerin geri dönüşümsüz olduğu tespit edilmiştir.

Anahtar kelimeler: *Hordeum vulgare*, jasmonik asit, tohum çimlenmesi, fide büyümesi

INTRODUCTION

Jasmonic acid (JA) and its methyl ester (JA-Me) have been proposed as naturally occurring plant growth regulators because of their wide distribution (PARTHIER 1991). These compounds were isolated from fungi (CROSS & WEBSTER 1970, FERNANDEZ-MACULET & YANG 1992), algae (KRUPINA & DATHE 1991) and many higher plants (MEYER et al. 1984). Jasmonates play an important role in various physiological processes connected with plant growth and development, such as seedling and stem growth (DATHE et al. 1981, KODA et al. 1991), leaf abscission (CURTIS 1984), leaf senescence (UEDA et al. 1981), inhibition chlorophyll synthesis (ABELES

et al. 1989), reduction photosynthesis (MASLENKOVA et al. 1990), callus growth (UEDA & KATO 1982), synthesis of storage proteins (ANDERSON et al. 1989) and respiratory activity (POPOVA et al. 1988).

Exogenous jasmonates seem to have inhibitory effects on germination in many cases of nondormant seeds, for example, sunflower (CORBINEAU et al. 1988), amaranth (KEPCZYNSKI & BIALECKA 1994), lettuce (YAMANE et al. 1981), rapeseed and flax seeds (WILEN et al. 1991). But, jasmonates break the seed dormancy of apple (RANJAN & LEWAK 1992, 1995) and other woody species (DALETSKAYA & SEMBDNER 1989) and promote germination. However, the physiological roles of jasmonates in seed germination still remain unclear.

In view of the conflicting evidence referred to above, the aim of the present work was to check whether JA contributes germination of barley. For similar reason the effect of JA on seedling growth was checked.

MATERIAL AND METHODS

Barley seeds were used in the present study. The seeds were surface sterilized with 1.0 % sodium hypochloride. 25 seeds were placed in 10 cm petri dishes lined by two sheets of Whatman No.1 filter paper moistened with sufficient amount of solutions of 0 (control, C), 1, 3, 5, 10, 20, 30, 40, 50, 100, 200, 400, 500, 1000, 1500, 2000 and 3000 µM JA. Afterwards, they were left in an incubator to germinate at 20 ºC, in continuous dark. The seeds were considered germinated when the radicles reached to 10 mm in length. The germination percentages were recorded after incubation for 7 days. At the end of 7 th day, following the estimation of germination percentage, final percentage of coleoptile emergence, radicle and coleoptile lengths were determined. Furthermore, with special replicas for the experiments fresh weight of the seedlings were recorded.

Seeds kept for 7 days at 20 ºC, in germination media of 1500, 2000 and 3000 µM JA did not germinate. When these seeds were removed from JA solutions and transferred into petri dishes with distilled water, they were germinated as previously described. Each treatment was repeated 4 times. Statistical analyses were done using SPSS program and Duncan's multiple range test.

RESULTS

Table 1 shows the time courses of the germination of barley seeds kept in the various concentrations of JA. The seeds started to germinate easily when incubating in the C medium. Germination percentage of C seeds was 96 % at the 2th day of incubation. JA concentrations under 500 µM have not slowed the germination down. In the other words, these concentrations showed the same effect as C group. 500 and 1000 µM JA partly delayed the germination. Conversely, the seed germination was completely inhibited at 1500 µM JA.

SDÜ FEN EDEBİYAT FAKÜLTESİ
FEN DERGİSİ (E-DERGİ). 2006, 1 (1-2), 35-41

Table 1. The time course of the germination of *Hordeum vulgare* kept in various concentrations of JA

JA concentrations (μM)	Germination, % Days						
	1	**2**	**3**	**4**	**5**	**6**	**7**
C	0.0	100	100	100	100	100	100
1	0.0	100	100	100	100	100	100
3	0.0	100	100	100	100	100	100
5	0.0	100	100	100	100	100	100
10	0.0	100	100	100	100	100	100
20	0.0	100	100	100	100	100	100
30	0.0	100	100	100	100	100	100
40	0.0	100	100	100	100	100	100
50	0.0	100	100	100	100	100	100
100	0.0	100	100	100	100	100	100
200	0.0	100	100	100	100	100	100
400	0.0	0.0	100	100	100	100	100
500	0.0	0.0	100	100	100	100	100
1000	0.0	0.0	0.0	0.0	100	100	100
1500	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2000	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3000	0.0	0.0	0.0	0.0	0.0	0.0	0.0

In parallel with concentration rise, a negative effect of JA has increased all the growth parameters (final percentages of germination and coleoptile emergence, radicle and coleoptile elongation and fresh weight of the seedlings). For instance, germination percentage of C seeds was 100 % at 7th day in distilled water. The concentrations of JA under 1500 μM have not inhibited final germination percentage of barley seeds. These concentrations showed the same effect as C group. But the germination was completely inhibited by JA concentrations more than 1500 μM. Furthermore, 1500 and 2000 μM JA levels caused atypical germination (coleoptile emerges without radicle emergence). Atypical germination percentage was 100 % and 74 % at 1500 and 2000 μM JA, respectively. On the other hand, coleoptile emergence of barley seedlings was completely inhibited at 3000 μM JA level only (Table 2).

Similarly, radicle and coleoptile lengths and fresh weights of the seedlings slowed down with increasing levels of JA. Although, the radicle elongation was completely inhibited by JA concentrations higher than 1000 μM, the coleoptile elongation and fresh weight of seedlings were only inhibited by 3000 μM JA (Table 2).

Table 2. Final percentages of seed germination and coleoptile emergence, radicle and coleoptile lengths, and fresh weight of the seedlings of *Hordeum vulgare* in various concentrations of JA

JA concentrations (µM)	Germination percentage (%)	Coleoptile emergence (%)	Radicle length (mm)	Coleoptile length (mm)	Fresh weight (mg/seedling)
C	*100±0,0 [b]	100±0,0 [c]	121,5±0,8 [j]	99,8±0,8 [l]	258,0±1,2 [m]
1	100±0,0 [b]	100±0,0 [c]	97,9±0,2 [i]	95,5±2,1 [k]	258,0±1,6 [m]
3	100±0,0 [b]	100±0,0 [c]	96,9±1,6 [i]	92,8±1,6 [j]	254,4±0,8 [l]
5	100±0,0 [b]	100±0,0 [c]	92,6±1,2 [i]	91,3±0,8 [j]	245,6±2,1 [k]
10	100±0,0 [b]	100±0,0 [c]	92,8±1,6 [i]	91,2±1,2 [j]	240,4±0,6 [j]
20	100±0,0 [b]	100±0,0 [c]	83,9±1,4 [h]	82,9±1,2 [i]	239,0±1,6 [j]
30	100±0,0 [b]	100±0,0 [c]	68,6±1,6 [g]	80,0±0,2 [i]	226,8±0,2 [i]
40	100±0,0 [b]	100±0,0 [c]	68,5±0,8 [g]	77,5±0,8 [h]	221,2±0,4 [i]
50	100±0,0 [b]	100±0,0 [c]	67,8±0,2 [g]	76,6±1,4 [h]	217,6±1,8 [h]
100	100±0,0 [b]	100±0,0 [c]	64,0±2,1 [f]	76,5±0,6 [h]	216,8±2,1 [h]
200	100±0,0 [b]	100±0,0 [c]	51,3±1,6 [e]	63,3±1,8 [g]	208,8±1,2 [g]
400	100±0,0 [b]	100±0,0 [c]	34,0±1,2 [d]	51,3±0,2 [f]	194,2±0,6 [f]
500	100±0,0 [b]	100±0,0 [c]	31,0±0,8 [c]	45,6±2,1 [e]	185,5±0,8 [e]
1000	100±0,0 [b]	100±0,0 [c]	14,0±1,6 [b]	34,6±0,8 [d]	126,3±1,6 [d]
1500	**0,0±0,0 [a]	**100±0,0 [c]	**0,0±0,0 [a]	**19,2±0,6 [c]	**82,0±0,8 [c]
2000	**0,0±0,0 [a]	**74±2,3 [b]	**0,0±0,0 [a]	**13,2±0,2 [c]	**58,3±1,2 [b]
3000	0,0±0,0 [a]	0,0±0,0 [a]	0,0±0,0 [a]	0,0±0,0 [a]	0,0±0,0 [a]

* Shows values with insignificant difference (< 0.05) for each colomn shown with same letters.
** The seedlings from atypically germinated seeds.

Additionally, these severe inhibitions on the germination and seedling growth were irreversible because they were not relieved by washing the seeds with water after the treatments with 1500, 2000 and 3000µM JA for 7 days (Table 3).

Table 3. Final percentages of germination and coleoptile emergence, radicle and coleoptile lengths, and fresh weights of *Hordeum vulgare* after transfer from JA to distilled water

JA concentrations (µM)	Germination percentage (%)	Coleoptile emergence (%)	Radicle length (mm)	Coleoptile length (mm)	Fresh weight (mg/seedling)
C	100±0,0	100±0,0	121,5±0,8	99,8±0,8	258,0±1,2
1500	*0,0±0,0	*100±0,0	*0,0±0,0	*38,5±1,8	*101,0±0,8
2000	*0,0±0,0	*74±2,3	*0,0±0,0	*20,2±1,2	*96,4±1,8
3000	0,0±0,0	0,0±0,0	0,0±0,0	0,0±0,0	0,0±0,0

* The seedlings from atypically germinated seeds.

DISCUSSION

In this study, effect of JA on seed germination and seedling growth of barley was investigated. Our results showed that JA concentrations under 500 µM did not regard the time courses of the germination of barley seeds, while higher JA levels delayed the germination (Table 1). However, ASGHARI & ISHIZAWA (1998) pointed out that JA-

Me at concentrations over 1 μM delayed the time courses of the germination of cocklebur seeds.

JA_S have been reported to inhibit the seed germination of several plants such as *Amaranthus* (KEPCZYNSKI et al. 1999, BIALECKA & KEPCZYNSKI 2003), *Agrostemma* (SEMBDNER & GROSS 1986), *Lactuca* (YAMANE et al. 1981), *Helianthus* (CORBINEAU et al. 1988) and *Linum* (WILEN et al. 1991). In all of these cases, high concentrations of JA or JA-Me are necessary to inhibit germination. This results are consistent with our findings. Besides, we determined that 1500 and 2000 μM JA levels cause atypical germination (Table 2). However, SEMBDNER & PARTHIER (1993) and ASGHARI & ISHIZAWA (1998) pointed out that a physiological effect of JA_S on seed germination is doubtful. In the case of cocklebur seeds, significant inhibition of the germination was detected even at 1 μM Me-JA.

On the other hand, we observed that the seedling growth slowed down with increasing concentrations of JA (Table 2). Similarly, KODA et al. (1991), PARTHIER (1991), STASWICK et al. (1992) and TSAI et al. (1997) reported that JA_S inhibited root and shoot elongation and fresh weight at some plant species. Furthermore BAZAKANA et al. (1999) and ASGHARI & ISHIZAWA (1998) pointed out that inhibitory effect of JA on germination of *Dioscorea alata* and cocklebur seeds was reversible. Contrarily, TSAI et al. (1997) determined that inhibitory effect of JA-Me on seedling growth of rice was irreversible. Our results showed that this inhibition on both the germination and seedling growth was irreversible (Table 3). Consequently, JA may affect seed germination and seedling growth in different ways depending on plant taxon, applied concentration and treatment styles.

In summary, these results show clearly that JA has dual effect on germination of barley seeds. At low concentrations, JA does not inhibit the germination and at high concentrations JA inhibits the germination. JA_S may inhibit the growth and development by reducing nucleic acid and protein synthesis (ANANIEVA & ANANIEV 1997), or by inhibiting cell division (SWIATEK et al. 2002) and hydrolytic activity (BIALECKA & KEPCZYNSKI 2003). JA may also be effective on the growth and development by causing the reduction of endogenous amounts of stimulating hormones (ASGHARI & ISHIZAWA 1998).

As seen above, effects of JA on seed germination and seedling growth are insufficiently known, hence there is not a consensus. To clarify the effect mechanism(s) dealt chemical further research, fine and detailed, is needed and we believe that our study will contribute to future studies.

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