

**EFFECTS OF SOME PLANT GROWTH REGULATORS ON JASMONIC ACID
INDUCED INHIBITION OF SEED GERMINATION AND SEEDLING
GROWTH OF BARLEY**

Kürşat ÇAVUŞOĞLU*, Kudret KABAR, Semra KILIÇ

Süleyman Demirel University, Faculty of Arts and Science, Biology Department,
32260, Isparta, Turkey

*Corresponding author: e-mail:kursat@fef.sdu.edu.tr, fax: 0 246 237 11 06

Received: 20 February 2007 , Accepted: 9 April 2007

Abstract: The effects of gibberellic acid, kinetin, benzyladenine, ethylene, 24-epibrassinolide and polyamines (spermine, spermidine, putrescine, cadaverine) on jasmonic acid inhibition of seed germination and seedling growth of barley were studied. All of the plant growth regulators studied were determined to have a successful performance in reversing of the inhibitory effects of jasmonic acid on the seed germination and seedling growth. Moreover, the above mentioned growth regulators overcame the inhibitory effect of JA on the percentages of germination and coleoptile emergence in the same ratio, while GA₃ was the most successful hormone on the fresh weight and radicle and coleoptile elongation in comparison with the other growth regulators.

Key words: Barley, jasmonic acid, plant growth regulator, seed germination, seedling growth

**ARPANIN TOHUM ÇİMLENMESİ VE FİDE BÜYÜMESİNİN JASMONİK
ASİT TEŞVİKLİ İNHİBİSYONU ÜZERİNE BAZI BİTKİ BÜYÜME
DÜZENLEYİCİLERİNİN ETKİLERİ**

Özet: Arpanın tohum çimlenmesi ve fide büyümesinin jasmonik asit inhibisyonu üzerine gibberellik asit, kinetin, benziladenin, etilen, 24-epibrassinolit ve poliaminlerin (spermin, spermidin, putressin, kadaverin) etkileri araştırılmıştır. Çalışılan bitki büyüme düzenleyicilerinin tümünün tohum çimlenmesi ve fide büyümesi üzerinde jasmonik asitin engelleyici etkisini tersine çevirmede başarılı bir performansla sahip oldukları belirlenmiştir. Dahası, yukarıda sözü edilen büyüme düzenleyicileri çimlenme ve koleoptil çıkış yüzdeleri üzerinde aynı oranda etkili olurken, taze ağırlık ve radikula ve koleoptil uzaması üzerinde diğer büyüme düzenleyicileri ile karşılaştırıldığında en başarılı hormon GA₃ olmuştur.

Anahtar kelimeler: Arpa, jasmonik asit, bitki büyüme düzenleyicisi, tohum çimlenmesi, fide büyümesi

INTRODUCTION

Jasmonic acid (JA) and its derivatives including methyl jasmonate (JA-Me) have been regarded as endogenous plant growth regulators because of their ubiquitous occurrence

in plant kingdom and their pleiotropic effects on plant growth and development (SEMBDNER & PARTHIER 1993). These compounds were isolated from fungi (CROSS & WEBSTER 1970, FERNANDEZ-MACULET & YANG 1992), algae (KRUPINA & DATHE 1991, UEDA et al. 1991) and many higher plants (MEYER et al. 1984). Jasmonates applied exogenously to plants, for example, inhibit stem and root growth (STASWICK et al. 1992, SEMBDNER & PARTHIER 1993), induce pericarp or leaf senescence (YEH et al. 1995), prevent chlorophyll (ABALES et al. 1989) and carotenoid formation (SANIEWSKI & CZAPSKI 1983) and reduce fresh weight (TSAI et al. 1997), RNA and protein synthesis (ANANIEVA & ANANIEV 1997) and respiratory activity (POPOVA et al. 1988).

Moreover, exogenous jasmonates seem to have inhibitory effects on germination in many cases of non-dormant seeds, for instance, cocklebur (ASGHARI & ISHIZAWA 1998), *Amaranthus* (KEPCZYNSKI et al. 1999, BIALECKA & KEPCZYNSKI 2003), sunflower (CORBINEAU et al. 1988) and lettuce (YAMANE et al. 1981) seeds. But jasmonates break the seed dormancy of apple (RANJAN & LEWAK 1992) and other woody species (DALETSKAYA & SEMBDNER 1989, BERESTETZKY et al. 1991) and promote germination.

In the present investigation, we studied reverse the inhibitory effect of JA on seed germination and seedling growth of barley by gibberellic acid, kinetin, benzyladenine, ethylene, 24-epibrassinolide and polyamines.

MATERIAL AND METHODS

Seed and Growth Regulators

In this work, seeds of barley (*Hordeum vulgare* L. cv. Bülbül 89) were used. The seeds were surface sterilized with 1.0 % sodium hypochloride.

As test solutions 900 μM gibberellic acid (GA_3), 100 μM kinetin (Kin), 100 μM benzyladenine (BA), 400 μM ethylene (E), 3 μM 24-epibrassinolide (EBR), 10 μM spermine (Spm), 10 μM spermidine (Spd), 10 μM putrescine (Put) and 10 μM cadaverine (Cad) were used. Concentration of jasmonic acid (JA) was 3000 μM .

Germination of Seed

25 seeds were placed in 10 cm petri dishes lined by two sheets of Whatman No. 1 filter paper and containing sufficient amount of solutions of JA at the concentration preventing completely the germination of the seeds, and of the mixtures of its with GA_3 , Kin, BA, E, EBR, Spm, Spd, Put and Cad alone. The seeds were left in an incubator to germinate at 20 °C, in dark for 7 days. The seeds were considered germinated when the radicles reached to 10 mm in length.

At the end of 7th day, following the estimation of germination percentage, percentages of coleoptile emergence were determined and radicle and coleoptile lengths were measured in mm. Furthermore, with special replicas for the experiments fresh weight of the seedlings were recorded.

Each treatment was repeated 4 times. Statistical analyses were done using SPSS program and Duncan's multiple range test.

RESULTS

Effects of the Plant Growth Regulators on JA-Inhibition of the Germination and Coleoptile Emergence Percentages

3000 μ M JA completely inhibited germination and coleoptile emergence of barley. In removing JA inhibition of the germination and coleoptile emergence percentages, all of the growth regulators studied were effective in the same ratio. The germination and coleoptile emergence percentages reached 100 % when each of the growth regulators were added the medium contained JA. In the other words, the mentioned growth regulators perfectly overcame JA inhibition of the germination and coleoptil emergence (Table 1).

Effects of the Plant Growth Regulators on JA-Inhibition of the Radicle and Coleoptile Elongation

The complete inhibition of radicle and coleoptile elongation by JA was totally reversed by all of the growth regulators. GA₃ was the most effective hormone in reversing JA-induced elongation-inhibition of the radicle and coleoptile from barley seeds in a medium of JA. The other growth regulators were almost effective in the same ratio on these parameters. For example, radicle and coleoptile lengths reached 15.4 and 21.5 mm at GA₃, respectively (Table 1).

Effects of the Plant Growth Regulators on JA-Inhibition of the Fresh Weight Increase

All of the growth regulators studied perfectly overcame the inhibitory effect of JA on fresh weight of barley seedlings. GA₃ was again the most effective hormone in reversing JA-induced fresh weight-reduction. Cytokinins (BA and Kin) were less successful than GA₃. E and Spm were effective in the same ratio. In removing JA-induced fresh weight reduction, Spd, Put and Cad were the most ineffective in comparison with the other growth regulators. To exemplify, fresh weight of the seedlings reached 142.5, 137.6 and 136.4 mg at GA₃, Kin and BA, respectively (Table 1).

Table 1. Percentages of seed germination and coleoptile emergence, radicle and coleoptile lengths, and fresh weights of the seedlings of barley in the media of various growth regulators

Growth regulator	Germination percentage (%)	Coleoptile emergence (%)	Radicle length (mm)	Coleoptile length (mm)	Fresh weight (mg/seedling)
JA	*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
+GA ₃	100±0.0 ^b	100±0.0 ^b	15.4±0.9 ^d	21.5±0.7 ^d	142.5±0.9 ^f
+Kin	100±0.0 ^b	100±0.0 ^b	13.2±0.7 ^c	18.6±0.8 ^c	137.6±0.6 ^e
+BA	100±0.0 ^b	100±0.0 ^b	11.5±0.5 ^b	17.3±0.6 ^b	136.4±0.7 ^e
+E	100±0.0 ^b	100±0.0 ^b	10.8±0.3 ^b	16.8±0.5 ^b	116.5±0.5 ^d
+EBR	100±0.0 ^b	100±0.0 ^b	10.5±0.4 ^b	15.7±0.7 ^b	110.2±0.3 ^c
+Spm	100±0.0 ^b	100±0.0 ^b	11.2±0.7 ^b	19.2±0.9 ^c	118.1±0.6 ^d
+Spd	100±0.0 ^b	100±0.0 ^b	10.8±0.5 ^b	15.8±0.7 ^b	105.4±0.5 ^b
+Put	100±0.0 ^b	100±0.0 ^b	10.1±0.3 ^b	16.1±0.8 ^b	107.2±0.9 ^b
+Cad	100±0.0 ^b	100±0.0 ^b	10.3±0.1 ^b	15.4±0.5 ^b	105.9±0.2 ^b

* Shows values with insignificant difference (P<0.05) for each column shown with same letters

DISCUSSION

The germination of not only barley seeds (CAVUSOGLU & KABAR 2006), but also *Avena* (SATLER & THIMANN 1981), *Amaranthus* (KEPCZYNSKI et al. 1999, BIALECKA & KEPCZYNSKI 2003), *Agrostemma* (SEMBDNER & GROSS 1986), *Lactuca* (YAMANE et al. 1981), sunflower (CORBINEAU et al. 1988) and flax (WILEN et al. 1991) seeds were inhibited by JA-Me or JA. In all of these cases, high concentrations of JA or JA-Me are necessary to inhibit germination. However, SEMBDNER & PARTHIER (1993) and ASGHARI & ISHIZAWA (1998) pointed out that a physiological effects 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 JA-Me.

The complete inhibition of seed germination and coleoptile emergence by 3000 µM JA was totally reversed by all of the growth regulators (Table 1). Many investigators obtained similar results with GA₃ (KEPCZYNSKI & BIALECKA 1994), cytokinins (BIALECKA & KEPCZYNSKI 2003) and E (KEPCZYNSKI & BIALECKA 1997, ASGHARI & ISHIZAWA 1998, KEPCZYNSKI et al. 1999) during the germination of seeds of different species.

Moreover, we observed that JA completely inhibited the seedling growth (fresh weight, radicle and coleoptile elongation) such as the germination and coleoptile emergence (Table 1). Preventive effects of JA on root and stem elongation (YAMANE et al. 1980, KODA et al. 1991, STASWICK et al. 1992) and fresh weight (TSAI et al. 1997) have been shown previously.

On the other hand, all of the growth regulators studied have clearly reversed the inhibitory effect of JA on the seedling growth. Besides, GA₃ was the most effective hormone in reversing JA-induced-inhibition of the radicle and coleoptile elongation and fresh weight of barley seedlings in a medium of JA (Table 1). Similarly, TSAI et al. (1997) pointed out that GA₃ was able to reverse the inhibitory effect of JA-Me on root and shoot growth and fresh weight of rice seedlings and was more effective in preventing the inhibition of shoot growth caused by JA-Me than root.

In summary, the results show clearly that JA inhibits seed germination and seedling growth of barley at high concentrations. The inhibitory effects of JA on the growth and development may act through various ways; by reducing nucleic acid and protein synthesis (ANANIEVA & ANANIEV 1997), or by inhibiting cell division (SWIATEK et al. 2002) and hydrolitic activity (BIALECKA & KEPCZYNSKI 2003). JA also be effective on the growth and development by causing the reduction of endogenous amounts of stimulating hormones (ASGHARI & ISHIZAWA 1998).

Our results also indicate that all of the growth regulators studied have perfectly overcame the inhibitory effect of JA on seed germination and seedling growth of barley. Furthermore, the mentioned growth regulators are known for reversing inhibitory effects of adverse environmental factors on the growth and development by increasing nucleic acid and protein synthesis (WU & ZHAU 1993), amylase activity (KEPCZYNSKI et al. 1999, BIALECKA & KEPCZYNSKI 2003), cell division (KAUR-SAWHNEY et al. 1980) and stabilization of cell membranes (MANSOUR & AL-MUTAWA 1999). These growth regulators may perform their positive effects on the growth and development via one or more the above mentioned mechanisms.

The effects of exogenous GA₃, Kin, BA, E on JA inhibition of seed germination and seedling growth are insufficiently known, while for EBR and polyamines studied there is no extant literature data. To clarify the effect mechanism(s) dealt chemical further research, fine and detailed, is need and we believe that our study will contribute to future studies.

ACKNOWLEDGEMENTS

The authors thank the Scientific & Technological Research Council of Turkey (TUBITAK) and Department of Scientific Research Project Management of Süleyman Demirel University (SDUBAP) for the financial support by the projects TBAG-HD/41 (105T054) and SDUBAP (0835-D-04) respectively. They are also grateful Field Crops Central Research Enstitute of Ankara for supplying seeds of barley.

REFERENCES

- ABALES FB, HERSHBERGER WL, DUNN LJ, 1989. Hormonal regulation and intracellular localization of a 33-kD cationic peroxidase in excised cucumber cotyledons. *Journal of Plant Physiology*, 89, 664-668.
- ANANIEVA K, ANANIEV ED, 1997. Comparative study of the effects of methyl jasmonate and abscisic acid on RNA and protein synthesis in excised cotyledons

- of *Cucurbita pepo* L. (Zucchini). *Bulgarian Journal of Plant Physiology*, 23, 80-90.
- ASGHARI MN, ISHIZAWA K, 1998. Inhibitory effects of methyl jasmonate on the germination and ethylene production in cocklebur seeds. *Journal of Plant Growth Regulation*, 17, 13-18.
- BERESTETZKY V, DATHE W, DALETSKAYA T, MUSATENKO L, SEMBDNER G, 1991. Jasmonic acid in seed dormancy of *Acer tataricum*. *Biochemie und Physiologie Pflanzen*, 187, 13-19.
- BIALECKA B, KEPCZYNSKI J, 2003. Regulation of α -amylase activity in *Amaranthus caudatus* seeds by methyl jasmonate, gibberellin A₃, benzyladenine and ethylene. *Journal of Plant Growth Regulation*, 39, 51-56.
- CORBINEAU F, RUDNICKI RM, COME D, 1988. The effect of methyl jasmonate on sunflower (*Helianthus annuus* L.) seed germination and seedling development. *Journal of Plant Growth Regulation*, 7, 157-169.
- CROSS BE, WEBSTER GRB, 1970. New metabolites of *Gibberella fujikuroi*. N-jasmonoyl- and N-dihydrojasmonoyl-isoleucin. *Journal of the Chemical Society Commun*, 930, 1839-1842.
- CAVUSOGLU K, KABAR K, 2006. Does jasmonic acid prevent the germination of barley seeds? *Süleyman Demirel Üniversitesi Fen Edebiyat Fakültesi Fen Dergisi*, 1(1-2), 35-41.
- DALETSKAYA TV, SEMBDNER G, 1989. Effect of jasmonic acid on germination of nondormant and dormant seeds. *Fiziologija Rastenij*, 36, 1118-1123.
- FERNANDEZ-MACULET J, YANG SF, 1992. Extraction and partial characterisation of the ethylene-forming enzyme from apple fruit. *Journal of Plant Physiology*, 99, 751-754.
- KAUR-SAWHNEY R, FLORES HE, GALSTON AW, 1980. Polyamine-induced DNA synthesis and mitosis in oat leaf protoplast. *Journal of Plant Physiology*, 65, 368-371.
- KEPCZYNSKI J, BIALECKA B, 1994. Stimulatory effect of ethephon, ACC, gibberellin A₃ and A₄₊₇ on germination of methyl jasmonate inhibited *Amaranthus caudatus* L. seeds. *Journal of Plant Growth Regulation*, 14, 211-216.
- KEPCZYNSKI J, BIALECKA B, 1997. The Role of Methyl Jasmonate in Germination of *Amaranthus caudatus* L. Seeds. In: ELLIS RH. BLACK M. MURDOCH AJ. HONG TD. (Eds.) Basic and Applied Aspects of Seed Biology. Kluwer Academic Publishers, Dordrecht, pp. 523-529.
- KEPCZYNSKI J, BIALECKA B, KEPCZYNSKA E, 1999. Ethylene biosynthesis in *Amaranthus caudatus* seeds in response to methyl jasmonate. *Journal of Plant Growth Regulation*, 28, 59-65.
- KODA Y, YOSHIDA K, KIKUTA Y, 1991. Evidence for the involvement of jasmonic acid in the control of the stem-growth habit of soybean plants. *Physiologia Plantarum*, 83, 22-26.
- KRUPINA MV, DATHE W, 1991. Occurrence of jasmonic acid in the red alga *Gelidium latifolium* Z. *Naturforsch*, 46, 1127-1129.
- MANSOUR MMF, AL-MUTAWA MM, 1999. Stabilization of plasma membrane by polyamines against salt stress. *Cytobiosis*, 100, 7-17.

- MEYER A, MIERSCH O, BUTTNER C, DATHE W, SEMBDNER G, 1984. Occurrence of the plant growth regulator jasmonic acid in plants. *Journal of Plant Growth Regulation*, 3, 1-8.
- POPOVA LP, TSONEV TD, VAKLINOVA SG, 1988. Changes in some photosynthetic and photorespiratory properties in barley leaves after treatment with jasmonic acid. *Journal of Plant Physiology*, 132, 257-261.
- RANJAN R, LEWAK S, 1992. Jasmonic acid promotes germination and lipase activity in non-stratified apple embryos. *Physiologia Plantarum*, 86, 335-339.
- SANIEWSKI M, CZAPSKI J, 1983. The effect of methyl jasmonate on lycopene and β -carotene accumulation in ripening red tomatoes. *Experientia*, 39, 1373-1374.
- SATLER SO, THIMANN KV, 1981. *Le Jasmonate de Methyle: Nouveau et Puissant Promoteur de la Senescence de Feuilles*. CR Academical Sciences Paris Series III, 293, pp. 735-740.
- SEMBDNER G, GROSS D, 1986. Plant Growth Substances of Plant and Microbial Origin. In: BOPP M. (Ed.) *Plant Growth Substances*. Springer-Verlag, Berlin, pp. 139-147
- SEMBDNER G, PARTHIER B, 1993. The biochemistry and the physiological and molecular actions of jasmonates. *Annual Review Plant Physiology and Plant Molecular Biology*, 44, 569-589.
- STASWICK PE, SU W, HOMOWELL SH, 1992. Methyl jasmonate inhibition of root growth and induction of a leaf protein are decreased in an *Arabidopsis thaliana* mutant. *Proceedings of the national Academy Sciences*, 89, 6837-6840.
- SWIATEK A, LENJOU M, BOCKSTAELE DV, INZE D, ONCKELEN HV, 2002. Differential effects of jasmonic acid and abscisic acid on cell cycle progression in tobacco BY-2 cell. *Journal of Plant Physiology*, 128, 201-211.
- TSAI FY, LIN CC, KAO CH, 1997. A comparative study of the effects of abscisic acid and methyl jasmonate on seedling growth of rice. *Journal of Plant Growth Regulation*, 21, 37-42.
- UEDA J, MIYAMOTO K, SATO T, MOMOTANI Y, 1991. Identification of jasmonic acid from *Euglena gracilis* Z. as a plant growth regulator. *Agricultural and Biological Chemistry*, 55, 275-276.
- WILEN RW, VAN ROOIJEN JH, PEARCE DW, PHARIS RP, HOLBROOK LA, MOLONEY MM, 1991. Effects of jasmonic acid on embryo specific processes in brassica and linum oil seeds. *Journal of Plant Physiology*, 95, 399-405.
- WU DR, ZHAU YJ, 1993. Effect of brassinosteroids on the metabolism of nucleic acids in epicotyls of bean seedlings. *Acta Physiologica Sinica*, 19, 49-52.
- YAMANE H, SUGAWARA J, SUZUKI Y, SHIMAMURA E, TAKAHASHI N, 1980. Synthesis of jasmonic acid related compounds and their structure-activity relationship on the growth of rice seedlings. *Agricultural and Biological Chemistry*, 44, 2857-2864.
- YAMANE H, TAKAGI H, ABE T, YOKATA T, TAKAHASHI N, 1981. Identification of jasmonic acid in three species of higher plants and its biological activities. *Plant Cell Physiology*, 22, 689-697.
- YEH CC, TSAY HS, YEH JH, TSAI FY, SHIH CY, KAO CH, 1995. A comparative study of the effects of methyl jasmonate and abscisic acid on some rice physiological processes. *Journal of Plant Growth Regulation*, 14, 23-28.