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Comparison of antagonisms between abscisic acid and various growth stimulators during germination of barley and radish seeds

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Abstract

In this work, antagonism among abscisic acid preventing seedling growth and germination of barley and radish seeds and gibberellic acid, kinetin, benzyladenine, ethylene, brassinosteroid, triacontanol and polyamines (cadaverine, putrescine, spermidine, spermine) was studied. Although many stimulators mentioned were not able to overcome the radicle elongation-preventive effect of abscisic acid, the seeds reached 100% germination in this hormone medium. In the seedlings of barley, only benzyladenine, kinetin and gibberellic acid alleviated abscisic acid inhibition on coleoptile emergence and elongation. As for radish, all the stimulators except triacontanol could not overcome abscisic acid inhibition of hypocotyl elongation, while they promoted hypocotyl emergence. The barrier of abscisic acid on the fresh weight of the seedlings was passed by all the stimulators at varying levels. But, only benzyladenine was partly successful in the case of radish.

Keywords: Abscisic acid, germination, *Hordeum vulgare*, plant growth regulators, *Raphanus sativus*, seedling growth.

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INTRODUCTION

The plant hormone abscisic acid (ABA) is a universal hormone preventing growth and development in plants. This hormone plays a major role in seed germination, as well as in adaptation to abiotic environmental stresses. It is very well known that it blockes the germination by an increase in its endogenous amount in a seed caused by active endogenous inhibition (Garciarrubio et al. 1997) or by persistent and continuous exogenous inhibitions i.e. environmental stresses such as cold (Chandler and Robertson 1994), high temperature (Gonai et al. 2004), salinity (Jia et al. 2002) and drought (Shinozaki and Yamaguchi-Shinozaki 2000). Actually, ABA mediates some aspects of physiological responses to a lot of environmental stresses (Leung and Giraudat 1998, Rock 2000).

Researchers, in order that plants may survive in stressful environments, have used generally cytokinins (CKs) in alleviation of ABA inhibition caused by stress (Bozcuk and Topçuoğlu 1984, Pospisilova et al. 2000). Although some researchers (Chrispeels and Varner 1967, Khan 1975) suggested that gibberellins (GAs) overcome ABA inhibition on germination only slightly or not at all, the others (Le Page-Degivry 1973, Kabar 1997) suggested that these hormones, contrarily, can successfuly remove this inhibition. Ethylene (E) is also used in relieving environmental stresses in the work of germination (Saini et al. 1986, Corbineau and Come 1995).

It has been reported that brassins (BRs),

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polyamines (PAs) and triacontanols (TRIA) have been investigated for the last two or three decades and are considered plant growth regulators like GAs, CKs, and also induces growth and development in plants. For instance, BRs are reported to promote germination under normal (Steber and McCourt 2001) or environmental stress conditions (Takeuchi 1992, Anuradha and Rao 2001) in some species. There are also similar reports for PAs (Sinska and Lewandowska 1991, Mutlu and Bozcuk 2000). In his work of germination with barley and radish seeds, Çavuşoğlu (2006) observed that these new classes of growth regulators were rather successful in overcoming of high temperature and salinity stresses. However, some works showed BRs (Dhaubhadel et al. 1999), PAs (Mirza and Bagni 1991) and TRIA (Stanislaw and Elbieta 1982) had no effect on seed germination. On the other hand, there have been also researchers that indicate BRs (Bishop and Koncz 2002) and PAs (Gallardo et al. 1992) may be antagonists of ABA in the event of germination.

As a result, the hormonal explanation of germination and understanding of antagonism between ABA and stimulator growth substances seem to be far more difficult at present in comparison with the past.

According to the fact that different stimulator growth substances can remove or alleviate the germination-inhibitive effect of various environmental stresses, the positive property of these stimulators must also include the overcoming of stress-induced ABA inhibition of germination. In order to test this hypothesis in relation to ABA-stimulator hormone antagonism, in the present work, the performances of the growth-stimulator substances mentioned above in relieving the germination-preventive effect of ABA were compared for the seeds of barley and radish, mono-and dicot, respectively.

MATERIAL AND METHODS

The Seeds and plant growth regulators

In this work, seeds of barley (*Hordeum vulgare* L. cv. Bülbül 89) and radish (*Raphanus sativus* L.) 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 brassinosteroid (BR), 10 µM triacontanol (TRIA) and 10 µM polyamine (used polyamines are: cadaverine-Cad, putrescine-Put, spermidine-Spd and spermine-Spm) were used for both species. Concentration of abscisic acid (ABA) was 25 µM for barley and 40 µM for radish. Hormone concentrations were determined in a preliminary investigation.

GA_3 and ABA were dissolved in methanol, Kin in HCl, Cad, Put, Spd, Spm and E in distilled water, BA in 0.3 N KOH, BR in ethanol and TRIA in 0.75 ml $CHCl_3$. Then they were diluted with distilled water to the appropriate concentrations.

Germination of seed

Twenty-Five dry seeds were placed in 10 cm Petri dishes lined with two sheets of Whatman No.1 filter paper and containing sufficient amounts of solutions of ABA (control), and with one of the growth stimulator substances mentioned above. The seeds were left in an incubator to germinate at 20°C, in continuous dark, for 7 days. The seeds of barley and radish were considered germinated when the radicles reached 10 mm and 5 mm in length, respectively (Ungar 1974, Kabar 1990). The germination percentages of the seeds were determined for each 24 h up to the 7th day. At the end of the experiment (7th day), after determining the germination and shoot (coleoptile/hypocotyl) percentages, the lengths of the radicle and shoot in mm, and in addition, the fresh weight of the seedlings (mg/seedling) was determined.

Recovery from ABA inhibition

In order to test whether the inhibitive effect of ABA on germination and seedling growth is permanent or not, the seeds ungerminated even on the 7th day were transferred to distilled water from the ABA medium and their germination percentages were determined for each 24 h for an addition 7 days. At the termination of the 7th day, the percentages of germination and the other growth parameters were recorded again. The same procedures were repeated as "control" in the medium of distilled water not containing ABA.

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

RESULTS

Effects of ABA and stimulator antagonism on Germination Percentage

ABA, as expected, both delayed and inhibited the germination of the seeds (Table 1, 2). The seeds of barley and radish in the medium of ABA were able to begin the germination on the 4th d and, they reached scarcely 20% germination at the end of the experiment.

All the stimulator growth regulators except TRIA not only overcame the preventive effect of ABA on germination but also shortened the time required for germination by alleviating its germination-delaying effect. TRIA had no effect on ABA activity on radish, but was rather effective on barley. While all the stimulators caused 100% final germination in barley, they exhibited a performance between 82% and 100% in radish.

Although all the stimulators except TRIA were effective to a great extent in the breaking of the germination-delaying effect of ABA in both species, GA_3 and BR, with their performances of 92% and 80% on the 2nd day, were more effective in barley, and also BA, with its success of 100% on the 4th day, had more effect, in radish, in comparison with the other stimulators. However, the values of success of all the stimulators in the following days approached each other.

Effects of ABA and stimulator antagonism on the some parameters of growth of the seedlings

The results of the ABA-stimulator antagonism on the radicle elongation, shoot (coleoptile/hypocotyl) percentages and elongations and fresh weight of the seedlings of barley and radish are presented in Table 3 and 4. Although all the stimulators except TRIA considerably removed ABA inhibition on seed germination, their successes did not carry on the mentioned parameters.

Radicle elongation

ABA-induced inhibition of radicle elongation was alleviated largely by GA_3 and partly by Cad in the seedlings of barley. The best effect in radish was carried out by BR and Cad. The other stimulators did not show a serious success.

Shoot percentage

While ABA prevented completely coleoptile emergence in the barley seedlings, there were 20% hypocotyl in radish (Table 3, 4). In the seedlings of radish, all the stimulators, except for TRIA, alleviated ABA-induced inhibition of hypocotyl emergence to varying degrees. Only GA_3, Kin and BA, in barley, with100% coleoptile emergence, showed an important success, but the others had no effect on ABA inhibition.

The most effective antagonists of ABA, in radish, were BA (94%), BR (84%), Kin (82%) and E (76%), respectively. PAs were able to show a performance of 50% and GA_3 a hypocotyl emergence of 60%.

Shoot elongation

Although all the stimulators mostly removed the ABA press on the hypocotyl percentage of emergence from the radish seeds to an important degree, only BA alleviated the ABA inhibition on the longitudinal elongation of hypocotyls to a small extent, the others had no effect in the overcoming of the longitudinal elongation inhibition. As for barley, while Kin and BA were effective to a large extent, GA_3 showed less success than them.

Fresh weight

BA could alleviate the ABA obstacle on the fresh weight increase of radish seedlings, but the other stimulators could not. All the stimulators, in barley, caused increases in the fresh weights of the seedlings in comparison with ones in the medium of ABA alone. However, barley better responded to Kin, BA and GA_3, respectively, than the others.

Recovery from ABA inhibition

The seeds of barley and radish, after being

Table 1. Effects of ABA and stimulator antagonism on the time course of germination of barley seeds.

Growth regulators	Days						
	1	2	3	4	5	6	7
ABA (Control)	0±0,0	0±0,0	0±0,0	12±0,0	16±0,0	20±0,0	20±0,0
ABA+GA_3	0±0,0	92±0,0	92±0,0	100±0,0	100±0,0	100±0,0	100±0,0
ABA+Kin	0±0,0	0±0,0	82±2,3	98±2,3	100±0,0	100±0,0	100±0,0
ABA+BA	0±0,0	0±0,0	90±2,3	100±0,0	100±0,0	100±0,0	100±0,0
ABA+E	0±0,0	0±0,0	40±0,0	52±0,0	86±0,0	100±0,0	100±0,0
ABA+BR	0±0,0	80±0,0	94±2,3	96±0,0	100±0,0	100±0,0	100±0,0
ABA+TRIA	0±0,0	0±0,0	0±0,0	0±0,0	4±0,0	28±0,0	42±2,3
ABA+Spm	0±0,0	0±0,0	44±0,0	44±0,0	78±2,3	100±0,0	100±0,0
ABA+Spd	0±0,0	0±0,0	40±0,0	40±0,0	84±0,0	100±0,0	100±0,0
ABA+Cad	0±0,0	0±0,0	80±0,0	96±0,0	100±0,0	100±0,0	100±0,0
ABA+Put	0±0,0	0±0,0	82±2,3	92±0,0	96±0,0	100±0,0	100±0,0

Table 2. Effects of ABA and stimulator antagonism on the time course of germination of radish seeds.

Growth regulators	Days						
	1	2	3	4	5	6	7
ABA (Control)	0±0,0	0±0,0	0±0,0	16±0,0	20±0,0	20±0,0	20±0,0
ABA+GA_3	0±0,0	28±0,0	60±0,0	84±0,0	90±2,3	100±0,0	100±0,0
ABA+Kin	0±0,0	32±0,0	62±2,3	86±2,3	88±0,0	100±0,0	100±0,0
ABA+BA	0±0,0	52±0,0	66±2,3	100±0,0	100±0,0	100±0,0	100±0,0
ABA+E	0±0,0	44±0,0	68±0,0	76±0,0	88±0,0	88±0,0	90±2,3
ABA+BR	0±0,0	42±2,3	54±2,3	86±2,3	88±0,0	92±0,0	96±0,0
ABA+TRIA	0±0,0	0±0,0	0±0,0	0±0,0	0±0,0	0±0,0	0±0,0
ABA+Spm	0±0,0	52±0,0	60±0,0	68±0,0	78±2,3	80±0,0	82±2,3
ABA+Spd	0±0,0	48±0,0	54±2,3	68±0,0	72±0,0	82±2,3	90±2,3
ABA+Cad	0±0,0	38±2,3	40±0,0	52±0,0	74±2,3	84±0,0	84±0,0
ABA+Put	0±0,0	40±0,0	48±0,0	52±0,0	80±0,0	84±0,0	84±0,0

removed from the ABA presence which was preventing their germinations during the 7 day experiment, were transferred to the medium of distilled water and were kept to germinate in an incubator at 20°C for 7 additional days. The results of the germination are presented in Table 5 and 6. Barley seeds being transferred from the ABA medium to water reached 100% of germination within 1 day, but radish on the 5th day.

With the delaying and inhibitive effect of ABA on germination of barley completely removed, its inhibitive effect was observed to be reversible, but in radish its delaying effect permanent.

The values of the growth parameters of the seedlings after transfer to distilled water are seen in Table 7 and 8.

The preventive effect of ABA on shoot emergence (%) disappeared in the seedlings of barley and radish after transfer to water. However, although ABA inhibition on radicle elongation and fresh weight in radish seedlings, and on coleoptile elongation in addition to these parameters in barley seedlings were excellently removed in comparison with the ABA medium before transfer to distilled water, we can say that this inhibition, when compared to the seedlings from the seeds germinated only in the medium of distilled water (in ABA absence), was removed to a great extent, but not completely, and partly continues. The inhibition on the hypocotyl elongation of the seedlings of radish had been exactly overcome.

DISCUSSION

As known, exogenous ABA is a potent inhibitor of seed germination and seedling growth in many species (Khan and Downing 1968, Kabar 1997). The seedling growth and germination of barley and radish, as expected, were inhibited in a medium of ABA alone (Table 3, 4). ABA can perform this preventive effect in many ways. ABA may interfere with seed germination by changing the water

Table 3. Effects of ABA and stimulator antagonism on the some parameters of growth of barley seedlings.

Growth regulators	Growth parameters				
	Germination percentage	Radicle length (mm)	Coleoptilep ercentage	Coleoptile length (mm)	Fresh weight (mg/seedling)
ABA (Control)	*20±0,0 ^{a}	10,0±0,0 ^{a}	0±0,0 ^{a}	0,0±0,0 ^{a}	91,0±0,8 ^{a}
ABA+GA_3	100±0,0 ^{c}	16,9±0,2 ^{f}	100±0,0 ^{b}	20,3±0,1 ^{b}	144,0±0,7 ^{g}
ABA+Kin	100±0,0 ^{c}	10,0±0,0 ^{a}	100±0,0 ^{b}	52,0±0,4 ^{d}	189,2±0,6 ^{i}
ABA+BA	100±0,0 ^{c}	10,1±0,1 ^{ab}	100±0,0 ^{b}	51,3±0,6 ^{c}	176,0±0,7 ^{h}
ABA+E	100±0,0 ^{c}	10,7±0,5 ^{d}	0±0,0 ^{a}	0,0±0,0 ^{a}	107,4±0,5 ^{e}
ABA+BR	100±0,0 ^{c}	10,5±0,0 ^{bcd}	0±0,0 ^{a}	0,0±0,0 ^{a}	111,6±0,9 ^{f}
ABA+TRIA	42±2,3 ^{b}	10,0±0,0 ^{a}	0±0,0 ^{a}	0,0±0,0 ^{a}	100,9±0,2 ^{b}
ABA+Spm	100±0,0 ^{c}	10,1±0,0 ^{ab}	0±0,0 ^{a}	0,0±0,0 ^{a}	105,4±0,6 ^{d}
ABA+Spd	100±0,0 ^{c}	10,3±0,1 ^{abc}	0±0,0 ^{a}	0,0±0,0 ^{a}	102,6±0,9 ^{c}
ABA+Cad	100±0,0 ^{c}	11,2±0,5 ^{e}	0±0,0 ^{a}	0,0±0,0 ^{a}	105,4±0,5 ^{d}
ABA+Put	100±0,0 ^{c}	10,6±0,0 ^{cd}	0±0,0 ^{a}	0,0±0,0 ^{a}	105,0±0,5 ^{d}

*The difference between values with the same letter in each column is not significant at the level 0.05 (± Standard deviation)

Table 4. Effects of ABA and stimulator antagonism on the some parameters of growth of radish seedlings.

Growth regulators	Growth parameters				
	Germination percentage	Radicle length (mm)	Hypocotyl percentage	Hypocotyl length (mm)	Fresh weight (mg/seedling)
ABA (Control)	*20±0,0 ^{b}	8,2±0,1 ^{de}	20±0,0 ^{b}	6,9±0,2 ^{e}	54,0±1,8 ^{f}
ABA+GA_3	100±0,0 ^{g}	8,2±0,2 ^{de}	62±2,3 ^{e}	5,9±0,1 ^{cd}	44,0±0,8 ^{d}
ABA+Kin	100±0,0 ^{g}	5,1±0,1 ^{b}	82±2,3 ^{g}	6,5±0,5 ^{de}	49,6±0,5 ^{e}
ABA+BA	100±0,0 ^{g}	5,7±0,0 ^{b}	94±2,3 ^{h}	8,1±0,3 ^{f}	64,2±1,1 ^{g}
ABA+E	90±2,3 ^{e}	7,0±0,6 ^{c}	76±0,0 ^{f}	5,8±0,3 ^{cd}	49,0±0,8 ^{e}
ABA+BR	96±0,0 ^{f}	10,9±0,2 ^{f}	84±0,0 ^{g}	5,9±0,2 ^{cd}	42,0±0,7 ^{c}
ABA+TRIA	0±0,0 ^{a}	0,0±0,0 ^{a}	0±0,0 ^{a}	0,0±0,0 ^{a}	0,0±0,0 ^{a}
ABA+Spm	82±2,3 ^{c}	6,9±0,1 ^{c}	50±2,3 ^{cd}	5,2±0,2 ^{b}	44,0±0,8 ^{d}
ABA+Spd	90±2,3 ^{e}	7,7±0,6 ^{d}	50±2,3 ^{cd}	6,2±0,2 ^{d}	42,2±1,1 ^{c}
ABA+Cad	84±0,0 ^{d}	10,9±0,8 ^{f}	52±0,0 ^{d}	5,7±0,1 ^{cd}	39,0±0,2 ^{b}
ABA+Put	84±0,0 ^{d}	8,8±0,2 ^{e}	48±0,0 ^{c}	5,4±0,0 ^{bc}	41,5±0,5 ^{c}

*The difference between values with the same letter in each column is not significant at the level 0.05 (± Standard deviation)

status of the seed so that water uptake is inhibited (Schopfer and Plachy 1984). All the growth regulators lead to increases in imbibition in various levels in the presence of ABA in barley (Table 3). The most effective ones were Kin, BA and GA_3, respectively. It may be said that the used stimulators overcame ABA-inhibition of germination by counteracting ABA on imbibition of barley seeds. TRIA did not show a performance to ABA in radish seeds. The fact that the other regulators beside BA succeed in alleviating the germination-preventive effect of ABA on a large scale, although they were not able to overcome the water uptake reductive effect of this inhibitor (Table 4), and this is very suprising. The regulators used may mediate the operation of the basic processes required for germination and mentioned below in the presence of water in the minimum amount up taken. In other words, they might have reduced the need of water in radish seeds for germination.

On the other hand, the used stimulators reached the final germination of the seeds of both species until 100s% and also succeed in alleviating the germination-delaying effect of ABA (Table 5, 6), i.e. shortened the time required for germination (Table 1, 2), although they were not able to remove the inhibitive effect of ABA on the elongation of the radicle and shoot (hypocotyl or coleoptile). Moreover, in comparison with the ABA medium, all the stimulators except TRIA caused 2,5 to 4,5 fold increase in the percentages of hypocotyl emergence of radish. Whereas, only BA had been effective on hypocotyl elongation, and also BR and Cad on radicle elongation. As for barley, while GA_3, Kin and BA had effect on the percentage of coleoptile emergence, with

Table 5. Time course of germination of barley seeds after their transfer from ABA medium to distilled water.

Germination medium	Days						
	1	2	3	4	5	6	7
Distilled water (control)	0±0,0	90±2,3	98±2,3	100±0,0	100±0,0	100±0,0	100±0,0
ABA	0±0,0	0±0,0	0±0,0	12±0,0	16±0,0	20±0,0	20±0,0
Transfer from ABA to distilled water	100±0,0	100±0,0	100±0,0	100±0,0	100±0,0	100±0,0	100±0,0

Table 6. Time course of germination of radish seeds after their transfer from ABA medium to distilled water.

Germination medium	Days						
	1	2	3	4	5	6	7
Distilled water (control)	0±0,0	98±2,3	100±0,0	100±0,0	100±0,0	100±0,0	100±0,0
ABA	0±0,0	0±0,0	0±0,0	16±0,0	20±0,0	20±0,0	20±0,0
Transfer from ABA to distilled water	80±0,0	86±2,3	90±2,3	94±2,3	100±0,0	100±0,0	100±0,0

Table 7. Some growth parameters of the seedlings from barley seeds germinated for 7 d in the medium of water by recovering from ABA.

Germination medium	Growth parameters			
	Coleoptile percentage	Radicle length (mm)	Coleoptile length (mm)	Fresh weight (mg/seedling)
Distilled water (control) first 7th d	100±0,0	86,0±1,1	105,7±0,7	311,6±0,9
ABA first 7th d	0±0,0	10,0±0,0	0,0±0,0	91,0±0,8
7th d after transfer from ABA medium to distilled water	100±0,0	72,3±0,5	82,0±0,4	264,0±0,5

Table 8. Some growth parameters of the seedlings from radish seeds germinated for 7 d in the medium of water by recovering from ABA.

Germination medium	Growth parameters			
	Hypocotyl percentage	Radicle length (mm)	Hypocotyl length (mm)	Fresh weight (mg/seedling)
Distilled water (Control) first 7th d	100±0,0	76,7±0,3	42,0±0,6	125,6±0,5
ABA first 7th d	20±0,0	8,2±0,1	6,9±0,2	54,0±1,8
7th d after transfer from ABA medium to distilled water	100±0,0	46,5±0,6	44,3±0,6	108,8±0,9

100%, the other stimulators had no effect on these parameters. Also, only GA_3 and Cad had the stimulative effect on radicle elongation, and only BA, Kin and GA_3, respectively, on coleoptile elongation.

As seen, the used stimulators, in relation to ABA-stimulator antagonism on the mentioned parameters of growth, exhibited different modes of effectiveness in barley and radish. One of the best-characterized effects of ABA is that it can inhibit the germination of seeds by limiting the availability of energy and nutrients as a result of the fact that it delays and prevents the syntheses and/or activities of hydrolases inducing mobilization and breakdown of food reserves in seed (Sodkiewicz and Sodkiewicz 2003). Concequently, the germination may have occured both very slowly and at a low ratio (Table 1, 2). In addition to GAs (Jacobsen and Chandler 1987), E (Jacobsen 1973), CKs (Khan 1969) and BRs (Altmann 1999) may also ameliorate the germination of seed in an ABA medium by inducing hydrolytic activity. That also PAs stimulate hydrolytic activity is not far from probability.

The most common response of cells to ABA, is, as known, growth inhibition which results from limitation of cell extensibility (Kutschera and Schopfer 1986) and inhibition of cell division (Liu et al. 1994). ABA is well known to prevent radicle and shoot elongation (Karssen 1976) by these and the other possible modes of action. Although the amounts of used stimulators in barley and radish seeds were not able to overcome ABA-induced-inhibition of radicle elongation, they reached the final germination of the seeds until 100s% (Table 3, 4). This case indicates that these stimulators overcame the mitotic activity-preventive effect of ABA, and so they lead the seeds to germinate by causing radicle protrusion but that they may have not be able to overcome ABA-induced growth inhibition of daughter cells occured by cell division. GA_3 had also promoted markedly radicle elongation of barley seedlings. As for radish, BR and Cad were effective.

Similarly, the fact that BA, Kin and GA_3 alleviated the emergence and elongation inhibition of coleoptile of ABA can suggest that they relieved ABA-induced inhibition of both cell division and coleoptile elongation. In the case of radish, all the stimulators added to the ABA medium, beside TRIA, stimulated hypocotyl emergence and, except BA, could not overcome ABA inhibition on hypocotyl elongation may be a sign for that these growth substances could overcome the cell inhibition of ABA, but not its inhibition of elongation.

Actually, GA_3 (Liu and Loy 1976), CKs (Werner et al. 2001), BRs (Guadinova et al. 1995) and PAs (Costa and Bagni 1983) might make a counterattack ABA by promoting cell division. E, as the other stimulators, overcame the germination-inhibition of ABA. Although E antagonistically responded to ABA in germination, both inhibit root (Beaudoin et al. 2000) and shoot growth (Salisbury and Ross 1992) is not surprising and is a well known event.

On the other hand, BRs (Steber and McCourt 2001) may also promote longitudinal elongation, just like GAs (Ono et al. 2000). Moreover, it has been reported that PAs (Chattopadhayay et al. 2002) and CKs (Kaur et al. 1998) may be effective on longitudinal elongation. However, BRs have been also suggested to inhibit root elongation (Guan and Roddick 1988) due to induced ethylene synthesis (Arteca and Bachman 1987). Whereas, we saw BR to stimulate radicle elongation of radish in the medium of ABA.

ABA may also play a preventive role in seed germination by inhibiting synthesis of protein (Ho 1982) and nucleic acid (Ananieva and Ananiev 1997). Alleviation of ABA inhibition of germination by used growth stimulators in this work may be accompanied by increases in RNA and protein syntheses. In fact, CKs (Ohya and Suzuki 1988), GAs (Fincher 1989), PAs (Palavan and Galston 1982) and BRs (Bajguz 2000) have been proven to stimulate the syntheses of protein and nucleic acid.

The function of ABA in controlling dormancy and inhibition of germination might be to prevent these basic metabolic processes which start very soon after the beginning of imbibition. Thus, germination will be prevented in its early stages and dormancy will then be marked by lowered metabolic activity. ABA, depending on its concentration, might have delayed and/or prevented the germination of the seeds for the reasons mentioned, and in addition, might have blocked growth of the seedlings. Promoters growth substances used can also overcome these inhibitions by counteracting ABA.

The similarity of some functions of promoters used in this work seems like insurance for the life of the plant so that if one of them is more effective in a plant, it will succeed these similar functions. It has been emphasized before that seeds of the same or different species may contain different levels of GAs (Taylor and Wareing 1979), CKs (Khan 1971), E (Zapata et al. 2004), PAs (Basu et al. 1988), BRs (Schmidt et al. 1997) and ABA (Bozcuk and Topçuoğlu 1984) leading to various depths of dormancy, from no apparent dormancy to absolute dormancy. The seeds, therefore, should not be expected to give the same response to application of any growth regulator.

The findings we obtained suggest that any stimulator used in this work, one of GA_3, Kin, BA, BR and PAs, is sufficient in alleviating ABA inhibition in the germination of barley and radish and that the permissive action of a CK is not necessary. All these stimulators could be regarded as the antagonists of ABA in seed germination. Moreover, we can say these promoters act on the common denominator (s), i.e. in a very similar fashion, in response to ABA.

In addition to the well-characterized antagonisms between ABA and GAs or CKs, resent studies also began to demonstrate interactions between ABA and E (Gazzarrini and McCourt 2001), BRs (Finkelstein et al. 2002), PAs (Gallardo et al. 1992). It is not probable that germination and dormancy in nature are governed by absolute presence or absence of a hormone. Perhaps, it is more appropriate to say that individual hormones in a seed, at any one time, are at a physiologically effective or ineffective concentration. These concentrations must depend on many metabolic and environmental factors. Thus, it may be more accurate to think of a common pool of antagonists against ABA.

We propose that BRs, PAs, GAs, CKs and E or one or a few of these promoters may be needed to antagonize seed dormancy and stimulate germination. The obtained data and the others may serve to provide a new conceptual tool for designing a hypotheses of seed dormancy and germination and for reinterpreting old concepts and findings.

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REFERENCES

Altmann T (1999) Molecular physiology of brassinosteroids revealed by the analysis of mutants. Planta 208, 1-11.

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.

Anuradha S, Rao SSR (2001) Effect of brassinosteroid on salinity stress induced inhibition of seed germination and seedling growth of rice (*Oryza sativa* L.). Plant Growth Regulation 33, 151-153.

Arteca RN, Bachman JM (1987) Light inhibition of brassinosteroid-induced ethylene production. Plant Physiology 129, 13-18.

Bajguz A (2000) Effect of brassinosteroids on nucleic acids and protein content in cultured cells of *Chrorella vulgaris*. Plant Physiology and Biochemistry 38, 209-215.

Basu R, Maitra N, Ghosh B (1988) Salinity results in polyamine accumulation in early rice (*Oryza sativa* L.) seedlings. Australian Journal of Plant Physiology 15, 777-786.

Beaudoin N, Serizet C, Gosti F, Giraudat J (2000) Interactions between abscisic acid and ethylene signaling cascades. Plant Cell 12, 1103-1115.

Bishop GJ, Koncz C (2002) Brassinosteroids and plant steroid hormone signaling. The Plant Cell 14, 97-110.

Bozcuk S, Topcuoglu SF (1984) Degisik su stresi kosullarinda bitkilerde absisik asit (ABA) miktarinin degisimi ve bunun fizyolojik olaylar üzerine etkileri. Doga Bilim Dergisi 8, 265-272.

Cavusoglu K (2006) Geleneksel hormonlarla son yillarda bulunan bazi hormonlarin ve büyüme düzenleyicilerinin yüksek sicaklik ve tuz (NaCl) stresleri altindaki arpa ve turp tohumlarinin çimlenmesi üzerindeki etkilerinin karsilastirilmasi. Doktora Tezi, Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü, Isparta.

Chandler PM, Robertson M (1994) Gene expression regulated by abscisic acid and its relation to stress tolerance. Annual Review of Plant Physiology and Plant Molecular Biology 45, 113-141.

Chattopadhayay MK, Tiwari BS, Chattopadhayay G, Bose A, Sengupta DN, Ghosh B (2002) Protective role of exogenous polyamines on salinity-stressed rice (*Oryza sativa* L.) plants. Physiologia Plantarum 116, 192-199.

Chrispeels MJ, Varner JE (1967) Hormonal control of enzyme synthesis on the mode of action of gibberellic acid and abscisin in aleurone layers of barley. Plant Physiology 42, 1008-1016.

Corbineau F, Come D (1995) Control of Seed Germination and Dormancy by the Gaseous Environment. In: Kigel J, Galili G (eds), Seed Development and Germination, Marcel Dakker, New York, 397-424.

Costa G, Bagni N (1983) Effects of polyamines on fruit-set of apple. Horticultural Science 18, 59-61.

Dhaubhadel S, Chaudhary S, Dobinson KF, Krishna P (1999) Treatment with 24-epibrassinolide, a brassinosteroid, increases the basic thermotolerance of *Brassica napus* and tomato seedlings. Plant Molecular Biology 40, 333-342.

Fincher GB (1989) Molecular and cellular biology associated with endosperm mobilization in germinating cereal grains. Annual Review of Plant Physiology and Plant Molecular Biology 40, 305-346.

Finkelstein RR, Gampala SSL, Rock CD (2002) Abscisic acid signaling in seed and seedlings. The Plant Cell 14, 15-45.

Gallardo M, Matilla A, Sanchez-Calle IM (1992) Effects of spermine, abscisic acid and temperature upon ethylene production in *Cicer arietinum* seeds. Plant Physiology and Biochemistry 30, 19-27.

Garciarrubio A, Legaria JP, Covarrubias AA (1997) Abscisic acid inhibits germination of mature Arabidopsis seeds by limiting the availability of energy and nutrients. Planta 203, 182-187.

Garciarrubio A, Legaria JP, Covarrubias AA (1997) Abscisic acid inhibits germination of mature Arabidopsis seeds by limiting the availability of energy and nutrients. Planta 203, 182-187.

Gazzarrini S, McCourt P (2001) Genetic interactions between ABA, ethylene and sugar signaling pathways. Current Opinion Plant Biology 4, 387-391.

Gonai T, Kawahara S, Tougou M, Satoh S, Hashiba T, Hirai N, Kawaide H, Kamiya Y, Yoshioka T (2004) Abscisic acid in the thermoinhibition of lettuce seed germination and enhancement of its catabolism by gibberellin. Journal of Experimental Botany 55, 111-118.

Guadinova A, Sussenbekova H, Vojtechnova M, Kaminek M, Eder J, Kohout L (1995) Different effects of two brassinosteroids on growth, auxin and cytokinin concentrations in tobacco callus tissue. Plant Growth Regulation 17, 121-126.

Guan M, Roddick JC (1988) Epibrassinolise-inhibition of development of excised, adventitious and intact roots of tomato (*Lycopersicon esculentum*): comparison with the effects of steroidal estrogens. Physiologia Plantarum 74, 720-726.

Ho THD (1982) The Mechanism of Abscisic Acid During Seed Germination. In: Khan AA (ed), The Physiology and Biochemistry of Seed Development, Dormancy and Germination, Elsevier, Amsterdam, 299-321.

Jacobsen JV (1973) Interactions between gibberellic acid, ethylene and abscisic acid in control of amylase synthesis in barley aleurone layers. Plant Physiology 51, 198-202.

Jacobsen JV, Chandler PM (1987) Gibberellin and Abscisic Acid in Germinating Cereals. In: Davies PJ (ed), Plant Hormones and Their Role in Plant Growth and Development, Martinus Nijhoff, Dordrecht, 164-193.

Jia W, Wang Y, Zhang S, Zhang J (2002) Salt-stress-induced ABA accumulation is more sensitively triggered in roots than in shoots. Journal of Experimental Botany 53, 2201-2206.

Kabar K (1990) Comparison of kinetin and gibberellic acid effect on seed germination under saline conditions. Phyton 30, 291-298.

Kabar K (1997) Comparison of reversal of abscisic acid-induced inhibition of seed germination and seedling growth of some Gramineae and Liliaceae members by kinetin and gibberellic acid. Turkish Journal of Botany 21, 203-210.

Karssen CM (1976) Two sites of hormonal action during germination of *Chenopodium album* seeds. Physiologia Plantarum 36, 264-270.

Kaur S, Gupta AK, Kaur N (1998) Gibberellin A_3 reverses the effect of salt stress in chickpea (*Cicer arietinum* L.) seedlings by enhancing amylase activity and mobilization of starch in cotyledons. Plant Growth Regulation 26, 85-90.

Khan AA (1969) Cytokinin-inhibitor antagonism in the hormonal control of alpha-amylase synthesis and growth in barley seed. Physiologia Plantarum 22, 94-103.

Khan AA (1971) Cytokinins: permissive roles in seed germination. Science 171, 853-859. α

Khan AA (1975) Primary, preventive and permissive roles of hormones in plant systems. Botanical Review, 41, 391-420.

Khan AA, Downing RD (1968) Cytokinin reversal of dormin (abscisin II) inhibition of growth and -amylase synthesis in barley seed. Physiologia Plantarum 21, 1301-1307.

Kutschera U, Schopfer P (1986) Effects of auxin and abscisic acid on cell wall extensibility in maize coleoptiles. Planta 167, 527-535.

Le Page-Degivry T (1973) Influence de l'acide abscissique sur de developpement des embryous de *Taxus baccata* L. cultives in vitro. *Z*. Pflanzenphysiology 70, 406-413.

Leung J, Giraudat J (1998) Abscisic acid signal transduction. Annual Review of Plant Physiology and Plant Molecular Biology 49, 199-122.

Liu PBW, Loy JB (1976) Action of gibberellic acid on cell proliferation in the subapical shoot meristem of watermelon seedlings. American Journal of Botany 63, 700-704.

Liu Y, Bergervoet JHW, Ric de Vos CH, Hilhorst HWM, Kraak HL, Karssen CM, Bino RJ (1994) Nuclear replication activities during imbibition of abscisic acid-and gibberellin-deficient tomato (*Lycopersicon esculentum* Mill.) seeds. Planta 194, 368-373.

Mirza JI, Bagni N (1991) Effect of exogenous polyamines and difluoromethylornithine on seed germination and root growth of *Arabidopsis thaliana*. Plant Growth Regulation 10, 163-168.

Mutlu F, Bozcuk S (2000) Tuzlu koşullarda aycicegi tohumlarinin çimlenmesi ve erken büyüme üzerine dissal spermin'in etkileri. Turkish Journal of Biology 24, 635-643.

Ohya T, Suziki H (1988) Cytokinin-promoted polyribosome formation in excised cucumber cotyledons. Journal of Plant Physiology 133, 295-298.

Ono EO, Nakamura T, Machado RSR, Rodriques JD (2000) Application of brassinosteroid to *Tabebuia alba* (Bignoniaceae) plants. Revista Brasileira Fisiologia Vegetal 12, 187-194.

Palavan N, Galston AW (1982) Polyamine biosynthesis and titer during developmental stages of *Phaseolus vulgaris*. Physiologia Plantarum 55, 438-444.

Pospisilova J, Synkova H, Rulcova J (2000) Cytokinins and water stress. Biologia Plantarum 43, 321-328.

Rock C (2000) Patways to abscisic acid-regulated gene expression. New Phytologist 148, 357-396.

Saini HS, Bassi PK, Consolacion ED, Spencer MS (1986) Interactions among plant hormones, carbon dioxide, and light in the relief of thermoinhibition of lettuce seed germination: studies in a flow-through gaseous system. Canadian Journal of Botany 64, 2322-2326.

Salisbury FB, Ross CW (1992) Plant Physiology. Wadsworth Publishing Company Belmont, California.

Schmidt J, Altmann T, Adam G (1997) Brassinosteroids from seeds of *Arabidopsis thaliana*. Phytochemistry 45, 1325-1327.

Schopfer P, Plachy C (1984) Control of seed germination by abscisic acid. II. Effect on embryo water uptake in *Brassica napus* L. Plant Physiology 76, 155-160.

Shinozaki K, Yamaguchi-Shinozaki K (2000) Molecular responses to dehydration and low temperature: differences and cross-talk between two stress signaling pathways. Current Opinion Plant Biology 3, 217-23.

Sinska I, Lewandowska U (1991) Polyamines and ethylene in the removal of embryonal dormancy in apple seeds. Physiologia Plantarum 81, 59-64.

Sodkiewicz W, Sodkiewicz T (2003) Inhibition of alpha-amylase acting in hexaploid triticale lines by exogenous abscisic acid. Biologia Plantarum 46, 419-422.

Stanislaw L, Elbieta S (1982) Interaction of triacontanol with gibberellin in control of lettuce seed germination. Physiologia Plantarum 55, 183-187.

Steber CM, McCourt P (2001) A role for brassinosteroids in germination in Arabidopsis. Plant

Physiology 125, 763-769.

Takeuchi Y (1992) Studies on physiology and applications of brassinosteroids. Shokubutsu no Kogaku Chosetsu 27, 1-10.

Taylor JS, Wareing PF (1979) The effect of stratification on the endogenous levels of gibberellins and cytokinins in seed of drauglas-fir (*Pseudotsuga menziesii*) (Mirb.) Franco) and sugar pine (*Pinus lambertiana* Dougl.). Plant Cell Environment 2, 165-172.

Ungar IA (1974) The effect of salinity and temperature on seed germination and growth of Hordeum jubatum. Canadian Journal of Botany 52, 1357-1362.

Werner T, Motyka V, Strnad M, Schmülling T (2001) Regulation of plant growth by cytokinin. Proceedings of the National Academy of Sciences 98, 10478-10492.

Zapata PJ, Maria Serrano M, Teresa Pretel M, Asuncian Amaros M, Botella A (2004) Polyamines and ethylene changes during germination of different plant species under salinity. Plant Science 167, 781-788.

Zeevaart JAD, Creelman RA (1988) Metabolism and physiology of abscisic acid. Annual Review of Plant Physiology and Plant Molecular Biology 39, 439-473.

Arpa ve Turp Tohumlarinin Cimlenmesi Sirasinda Cesitli Buyume Tesvik Edicileri ve Absisik Asit Arasindaki Antagonizmalarin Karsilastirilmasi

Ozet

Bu calismada arpa ve turp tohumlarinin cimlenme ve fide buyumesini engelleyen absisik asit ile gibberellik asit, kinetin, benziladenin, etilen, brassinosteroit, triakontanol ve poliaminler (kadaverin, putressin, spermidin, spermin) arasindaki antagonizma incelenmistir. Soz konusu tesvik edicilerin bircogu absisik asitin radikula uzamasi uzerindeki engelleyici etkisini ortadan kaldirmada basarisiz olmalarina ragmen, bu hormon ortaminda tohumlar %100 cimlenme gostermislerdir. Arpa fidelerinde sadece benziladenin, kinetin ve gibberellik asit koleoptil cikisi ve uzamasi uzerindeki absisik asit engellemesini hafifletmistir. Turpta ise, triakontanol disindaki tum tesvik ediciler hipokotil uzamasi uzerindeki absisik asit engellemesini ortadan kaldiramamalarina ragmen, hipokotil cikisini tesvik etmislerdir. Fidelerin taze agirligi uzerindeki absisik asit engellemesi tum tesvik ediciler tarafindan cesitli duzeylerde hafifletilmistir. Fakat turpta sadece benziladenin kismen basarili olmustur.

Anahtar Kelimeler: Absisik asit, cimlenme, *Hordeum vulgare*, bitki buyume duzenleyicileri, *Raphanus sativus*, fide buyumesi.