Phyton (Horn, Austria)	Vol. 30	Fasc. 2	291–298	20. 12. 1990

Comparison of Kinetin and Gibberellic Acid Effects on Seed Germination under Saline Conditions

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Received November 17, 1989

Key words: Germination, salt stress, gibberellic acid, kinetin, *Gramineae*, dicotyledons.

Summary

KABAR K. 1990. Comparison of kinetin and gibberellic acid effects on seed germination under saline conditions. – Phyton (Horn, Austria) 30 (2): 291–298. – English with German summary.

The germination of 6 species of each *Gramineae* (monocots) and dicots on saline media after pretreatment of the dry seeds with kinetin and GA_3 was tested. In dicots investigated, kinetin was found to be more effective in alleviation salt stress on germination, but GA_3 in *Gramineae*. Kinetin + GA_3 in combination did not exceed the effect of kinetin in dicots, in some of the *Gramineae* tested an additive effect was observed. The effect of the both growth regulators similarly appeared in emergence of shoot (coleoptile or hypocotyl) after germination as well. In addition, the shoot of control seedlings was more sensitive to salt than the radicle. It is deduced from the obtained findings that more than one mechanism act in the germination of both Gramineae and dicots under saline conditions.

Zusammenfassung

KABAR K. 1990. Vergleich der Wirkung von Kinetin und Gibberellinsäure auf die Samenkeimung unter salinen Bedingungen. – Phyton (Horn, Austria) 30 (2): 291–298. – Englisch mit deutscher Zusammenfassung.

Je sechs Vertreter der Gramineen und von Dikotylen wurden nach Vorbehandlung der trockenen Samen mit Kinetin bzw. GA_3 auf ihr Keimverhalten auf salzhältigen Substraten untersucht. Kinetin vermochte den Salzstreß bei der Keimung der untersuchten Dikotylen zu mindern, GA_3 hingegen bei den Gramineen. Kinetin + GA_3 wirkte bei Dikotylen gleich wie Kinetin allein, bei einigen Gramineen war eine additive Wirkung zu beobachten. Beide Wachstumsregulatoren wirkten gleicher-

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weise auf das Erscheinen der Sproßorganne (Coleoptile bzw. Hypocotyl). In den Kontrollen war der Sproß salzempfindlicher als die Radikula. Aus den Ergebnissen wird geschlossen, daß in den Gramineen und Dikotylen mehr als ein Mechanismus das Keimverhalten bestimmt.

Introduction

Probable osmotic effect of salinity on seed germination has been well known (UNGAR 1974, YENTÜR 1982, KURTH & al. 1986, KABAR & BALTEPE 1987). The usefulness of treatments of plant growth regulators, one of methods used in relieving this negative effect, has been shown rather well. In the most of studies with growth regulators, however, only kinetin alone (ODEGBARO & SMITH 1969, KAUFMANN & ROSS 1970, BOZCUK 1978) or GA_3 (KAHN & al. 1957, MERT & YÜREKLI 1978), to a lesser extent these two regulators singly or in combination (BRAUN & KHAN 1976, KABAR & BALTEPE 1987), or other bioactive chemicals such as fusicoccin, ethephon (BRAUN & KHAN 1976) have been used.

In their study with lettuce, BRAUN & KHAN 1976, and KABAR & BALTEPE 1990, in their work with barley and lettuce, reported that kinetin was more effective in lettuce, but GA_3 in barley. According to these data, it has been thought a hypothesis that kinetin may play a more important role in alleviating osmotic stress on the germination of dicots, but GA_3 in the *Gramineae* (monocots). Therefore, the basic objective of the present work with various seeds of the Gramineae and dicots is not to overcome salt stress on germination by growth regulators, kinetin and GA_3, but to examine the responses of the two groups seeds to exogenous kinetin and GA_3 treatments.

Materials and Methods

The seeds used and the pretreatments

Seven species of *Gramineae* used in this work are the following: three cultivars of wheat (*Triticum aestivum* L. cvs. Yayla 305, Lancer and Haymana 79), two cultivars of barley (*Hordeum vulgare* L. cvs. Hudson and Krusevac-1), rye (*Secale cereale* L.), and oat (*Avena sativa* L. cv. Ankara 76). As dicots, seeds of cabbage (*Brassica oleracea* L. var. *capitata* cv. Erzurum), cotton (*Gossypium hirsutum* L. cv. Deltapine), sunflower (*Helianthus annuus* L. cv. TR-83), red clover (*Trifolium pratense* L. cv. Resident), alfalfa (*Medicago sativa* L. cv. Kayseri), and radish (*Raphanus sativus* L. Spring and Winter radishes) were used.

The seeds were surface sterilized with 1.0% sodium hypochlorite. Before sowing,, they were soaked in distilled water (control, C) or in aqueous solutions of growth regulators in predetermined concentrations, 0.5 mM kinetin, 1.0 mM GA_3, and a combination of both substances, for 2 h at room temperature. Thereafter, the solutions were decanted off and the seeds were vacuum dried for 1 h. Each treatment was replicated at least twice.

Seed germination

25 seeds from each treatment were placed in Petri dishes furnished with 2 sheets of Whatman No. 1 filter paper moistened with sufficient amounts of NaCl solutions

having predetermined water potentials (-bars, according to LANG 1967 for 25° C), sufficient for inhibition of the germinations of seeds to a great extent, Salt concentrations used for each seed are presented as water potential in Table 1 and 2. 12 cm Petri dishes for sunflower and cotton, and 9 cm dishes for other seeds were used. After sowing, Petri dishes were kept in an incubator at 25° C, in continuous dark for 7 d. In a preliminary investigation it was observed that all the seeds germinated about 100% in distilled water and that none of them were light-sensitive.

At the end of the experiment, the final germination percentages of the seeds germinated in saline medium were determined. The seeds of the *Gramineae* were considered to be germinated when the radicle reached 5 mm in length, and those of the dicots become visible. In addition, shoot (coleoptile or hypocotyl) percentages of both the groups were determined. Percent data were evaluated with analysis of variance.

Results

The results are presented in Table 1 and 2. Sunflower, cabbage, and cotton from dicot seeds untreated with growth regulators (control, C) responded to salt about similarly. Spring and winter radishes were more salt tolerant. The least tolerant ones were alfalfa and red clover. Also, Yayla 305 and Haymana 79 cultivars of wheat and rye seeds from the Gramineae members used in this study were the most resistant ones to salt. Krusevac-l of barley and oat seeds were the least salt tolerant. The salt thresholds, however, which enabled the least salt tolerant seeds to germinate, are at considerable high levels.

In general, GA_3 dramatically had more effect than kinetin in alleviating salt stress on the germination of all the *Gramineae* seeds used (Table 1). For example, GA_3 treatment supplied about 2 fold more increase in the germination percentages of all the three cultivars of wheat at -20.5 bars of salinity than that by kinetin. Also GA_3, according to control, increased the germination percentages of the wheat cvs. Yayla 305, Haymana 79, and Lancer 6, 5 and 14 fold, whereas, kinetin caused 3, 2, and 4.5 fold increase in the germination of these seeds in comparison to control, respectively. Even though kinetin failed the germinations of cv. Krusevac-l of barley and oat at -15.9 and -18.2 bars, in turn, GA_3 enabled 14% germination at these salt levels. Synergistic or additive effect of Kinetin + GA_3 combination was not observed in each seed. For example, although this combination had more effect in cvs. Yayla 305 and Lancer of wheat and in cvs. Krusevac-l and Hudson of barley than GA_3, there was no similar effect in cv. Haymana 79 (wheat), rye, and oat. However, this combination was much more effective in all the seeds than kinetin.

GA_3 enormously maintained its stimulative effect observed on the germination percentages on the coleoptile emergence as well. For example, in all the three cultivars of wheat there was coleoptile emergence neither in control seedlings nor in those treated with kinetin, except 10% coleoptile emergence by kinetin at -20.5 bars NaCl for cv. Yayla 305. Whereas GA_3

Table 1.

Percentages of germination and emergence of coleoptiles of seeds of some *Gramineae* treated with the plant growth regulators kinetin (K) and gibberellic acid (G) at varied salinities (-bar). C = control. Means in lines with the same letters are not significantly different at 5% level.

Species Cultivar	Water potential (NaCl, bars)	Germination (%) C	K	G	K+G	Emergence of coleoptile (%) C	K	G	K+G
Triticum aestivum									
cv. YAYLA 305	−20.5	10^{a}	34^{b}	64^{c}	62^{c}	0^{a}	10^{b}	60^{d}	56^{c}
	−22.8	2^{a}	16^{a}	36^{b}	52^{c}	0^{a}	0^{a}	34^{b}	48^{c}
	−25.1	0^{a}	0^{a}	20^{b}	46^{c}	0^{a}	0^{a}	18^{b}	30^{c}
cv. Haymana 79	−20.5	12^{a}	26^{b}	64^{c}	72^{c}	0^{a}	0^{a}	38^{b}	66^{c}
	−22.8	2^{a}	10^{a}	36^{b}	44^{b}	0^{a}	0^{a}	32^{b}	32^{b}
	−25.1	0^{a}	0^{a}	14^{b}	18^{b}	0^{a}	0^{a}	10^{a}	10^{a}
cv. Lancer	−18.2	26^{a}	44^{b}	76^{c}	90^{d}	0^{a}	0^{a}	72^{b}	84^{c}
	−20.5	4^{a}	18^{b}	56^{c}	80^{d}	0^{a}	0^{a}	42^{b}	72^{c}
	−22.8	0^{a}	6^{a}	46^{b}	54^{b}	0^{a}	0^{a}	22^{b}	24^{b}
	−25.1	0^{a}	0^{a}	16^{b}	28^{b}	0^{a}	0^{a}	12^{b}	14^{b}
Hordeum vulgare									
cv. Hudson	−15.9	20^{a}	38^{b}	48^{bc}	60^{c}	2^{a}	36^{b}	44^{bc}	54^{c}
	−18.2	4^{a}	4^{a}	20^{b}	36^{c}	0^{a}	4^{a}	10^{a}	34^{b}
cv. Krusevac-l	−13.7	22^{a}	18^{a}	38^{a}	74^{b}	0^{a}	10^{a}	24^{b}	68^{c}
	−15.9	6^{a}	0^{a}	14^{ab}	26^{b}	0^{a}	0^{a}	0^{a}	18^{b}
Avena sativa									
cv. Ankara 76	−13.7	20^{a}	18^{a}	32^{b}	36^{b}	0^{a}	6^{a}	24^{b}	26^{b}
	−15.9	8^{a}	16^{b}	24^{c}	26^{c}	0^{a}	4^{a}	16^{b}	20^{b}
	−18.2	0^{a}	0^{a}	14^{b}	18^{b}	0^{a}	0^{a}	4^{a}	6^{a}
Secale cereale									
	−22.8	20^{a}	22^{a}	40^{b}	44^{b}	0^{a}	14^{b}	30^{c}	36^{c}
	−25.1	0^{a}	12^{b}	24^{c}	26^{c}	0^{a}	10^{b}	18^{c}	20^{c}

stimulated coleoptile emergence between 10% to 72% in these wheat cultivars. Kinetin + GA_3 in combination was more effective in all cases than kinetin alone. This combination generally had an important effect, according to GA_3, on coleoptile emergence of the other seedlings except oat and rye.

In the case of dicots, the effects of kinetin and GA_3 applications on the germination and hypocotyl percentages were surprisingly contrary to those in the *Gramineae* (Table 2). That is, kinetin had much more effect than GA_3. Kinetin +GA_3 combined had no more effect than kinetin on both germination and hypocotyl percentages in all the seeds, except percent germination

Table 2.

Percentages of germination and emergence of hypocotyles of seeds of some dicots treated with the plant growth regulators kinetin and gibberellic acid at varied salinities. Symbols see Table 1.

Species Cultivar	Water potential (NaCl, bars)	Germination (%)				Emergence of hypocotyle (%)			
		C	K	G	K+G	C	K	G	K+G
Gossypium hirsutum									
cv. Deltapine	−11.4	28^a	62^c	40^b	28^a	22^a	52^c	34^b	18^a
	−13.7	6^a	50^c	24^b	12^a	2^a	40^c	16^b	4^a
	−15.9	0^a	20^b	8^a	6^a	0^a	12^b	2^a	0^a
Trifolium pratense									
cv. Resident	−6.9	14^a	52^c	30^b	58^c	12^a	42^c	24^b	44^c
	−9.1	8^a	34^b	16^a	38^b	4^a	30^b	8^a	32^b
	−11.4	0^a	26^b	8^a	30^b	0^a	16^b	0^a	20^b
Raphanus sativus									
cv. Winter radish	−15.9	24^a	58^b	34^a	68^b	0^a	28^c	18^b	30^c
	−18.2	6^a	36^b	14^a	42^b	0^a	18^b	2^a	18^b
	−20.5	0^a	12^a	0^a	18^a	0^a	0^a	0^a	0^a
cv. Spring radish	−15.9	22^a	64^c	28^a	44^b	12^a	46^c	20^a	26^{ab}
	−18.2	2^a	26^b	8^a	18^b	0^a	10^b	0^a	12^b
Brassica oleracea var. *capitata*,									
cv. Erzurum	−11.4	30^a	52^b	36^a	54^b	26^a	52^b	30^a	52^b
	−13.7	10^a	36^b	14^a	40^b	0^a	24^b	0^a	28^b
Helianthus annuus									
cv. TR-83	−9.1	50^a	86^b	50^a	84^b	34^b	76^d	24^a	48^c
	−11.4	22^a	36^b	18^a	26^a	8^a	26^b	8^a	8^a
Medicago sativa									
cv. Kayseri	−9.1	14^a	36^b	18^a	48^c	4^a	36^c	16^b	44^c
	−11.4	4^a	22^b	4^a	24^b	0^a	20^b	0^a	24^b

of alfalfa at −9.1 bars. However, this combination was generally more effective than GA_3 in all the dicots except cotton.

In conclusion, GA_3 treatment stimulated the emergence of radicle and shoot in saline media more in the *Gramineae* investigated, but kinetin did in dicots. Although kinetin + GA_3 combination had more effect than kinetin and GA_3 in some of the *Gramineae*, it was not more successful than kinetin in dicots.

Discussion

In this work, exogenously applied GA_3 was found to overcome importantly inhibition of germination induced by salt stress in the seeds of

Gramineae tested. Exogenous kinetin treatments were not so successful as GA_3 in these seeds. In the other experiments with various dicot seeds, treatments with kinetin were observed to promote total percentage germination of these seeds under saline conditions much more than GA_3. According to the data obtained, seeds whose germination was normally inhibited by quite high levels of salts, may be grown in medium and rather high saline soils if they are pretreated with a suitable growth regulator. As a result, such soils then become convenient for agriculture.

In the works on this subject, mostly lettuce (e. g. KAHN & al. 1957, ODEGBARO & SMITH 1969, BRAUN & KHAN 1976), rarely wheat (KAUFMANN & ROSS 1970, MERT & YÜREKLI 1978), barley, tomato, and cotton (BOZCUK 1978) have been used. Most of workers mentioned above have studied the effect of only one, kinetin or GA_3 and they did not use another growth regulator in order to compare. The findings which we obtained both confirm their results and indicate that generally GA_3 application for monocot seeds, at least in the *Gramineae*, and kinetin treatment for dicots may be more useful to a great extent. BRAUN & KHAN 1976, working with Mesa 659, a not light-sensitive lettuce seed, and KABAR & BALTEPE 1990 with barley and lettuce, have obtained similar conclusions. These workers had used both kinetin and GA_3. In addition, our finding that kinetin $+GA_3$ combination was more effective on the germination and coleoptile emergence of some of the *Gramineae* tested in saline media than kinetin and GA_3 is in agreement with our previous work (KABAR & BALTEPE 1987). On the other hand, this combination was not more effective in the dicot seeds investigated than kinetin. BRAUN & KHAN 1976 reported a similar result too.

Furthermore, the results that shoot was more salt-sensitive than radicle and that the shoots (coleoptile) of the *Gramineae* better responded to GA_3 treatment, but dicot shoots (hypocotyl) to kinetin, accord with studies with lettuce and barley by KABAR 1985 and KABAR & BALTEPE 1987.

Probable roles of kinetin and GA_3 in the course of germination under saline conditions have been discussed in detail in our previous studies and in others (BRAUN & KHAN 1976, BOUCAUD & UNGAR 1976, KABAR & BALTEPE 1987, 1990). The results obtained agree with the germination model, a working hypothesis, proposed by KHAN 1971. As known, gibberellins play a primary role in germination. Cytokinins are secondary agents and are necessary to remove the inhibitor(s) effect presumably formed within seed in the case of a stress (KHAN & DOWNING 1968, ITAI 1978, LERNER 1985). In addition, it is shown that cytokinin-inhibitor antagonism is effective on both germination and seedling growth (SANKHLA & SANKHLA 1968, KHAN 1969). However, in overcoming probable inhibitor effect appeared at the rather high levels of salt, GA_3 may be more successful in the *Gramineae,* or endogenous amount of cytokinins in seed may increase after stimulation of germination by exogenous GA_3, but not by cytokinins (BOUCAUD & UNGAR 1976), and so GA_3 may not to feel the need for exogenous help of kinetin. On the other hand, GA_3 application may be more insufficient to inhibitors in the case of dicots and may need exogenous kinetin treatment. GA_3 effect on the coleoptile percentages of the *Gramineae* and influence of kinetin on the hypocotyl percentages of dicots in saline media may be similarly interpreted as well. Moreover, it was reported that exogenous kinetin application increased amylase activity in bean hypocotyl, but not GA_3 (CLUM 1967).

The present research indicates that probably more than one mechanism control salt tolerances of dicot and monocot seeds, at least *Gramineae*. GA_3 may be directly involved in the *Gramineae,* but kinetin in dicots, in alleviating osmotically induced inhibition of seed germination. This preliminary study will to pave the way for further and more detailed studies to test the hypothesis proposed here.

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