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Effects of Kinetin and Gibberellic acid in Overcoming High Temperature and Salinity (NaCl) Stresses on the Germination of Barley and Lettuce Seeds

By

Kudred KABAR* and Şener BALTEPE**

With 2 Figures

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Summary

KABAR K. & BALTEPE Ş. 1990. Effects of kinetin and gibberellic acid in overcoming high temperature and salinity (NaCl) stresses on the germination of barley and lettuce seeds. – Phyton (Horn, Austria) 30 (1): 65–74, 2 figures. – English with German summary.

Gibberellic acid (GA_3), kinetin, and a combination of these two hormones applied axternally to dry seeds of barley (*Hordeum distichon* L.) and lettuce (*Lactuca sativa* L.) removed high temperature and/or salinity (NaCl) stresses on the germination of these seeds to varying extents. Kinetin alone had more effect on lettuce, while GA_3 was more effective on barley. However, as the germination temperature and salt levels were increased, the synergistic effect of GA_3 and kinetin combination was required. The application of high temperature stress (35° C) was found as reversible in lettuce, and as irreversible in barley. Nevertheless, the salinity stress was osmotic and reversible in both seeds.

Zusammenfassung

KABAR K. & BALTEPE Ş. 1990. Aufhebung des Temperatur- und Salzstreß (NaCl) bei der Keimung von Gersten- und Lattichsamen durch Kinetin und Gibberellinsäure. – Phyton (Horn, Austria) 30 (1): 65–74, 2 Abbildungen. – Englisch mit deutscher Zusammenfassung.

*) K. KABAR, Atatürk Üniversitesi, K. Karabekir Eğitim Fakültesi, Biyoloji Bölümü, TR-25240 Erzurum, Turkey.

**) Ş. BALTEPE, Fırat Üniversitesi, Fen-Edebiyat Fakültesi, Biyoloji Bölümü, TR-23169 Elâzığ, Turkey.

Nach äußerlicher Einwirkung von Kinetin, GA_3 und einer Kombination beider Wirkstoffe werden die Wirkungen von Temperatur und/oder Salz-(NaCl-) Streß bei der Keimung von Samen von Gerste (*Hordeum distichon* L.) und Lattich (*Lactuca sativa* L.) in verschiedenem Ausmaß aufgehoben. Kinetin ist bei Lattich wirksamer, GA_3 hingegen bei Gerste. Mit steigender Temperatur und Salzkonzentration wird das Zusammenwirken beider Wirkstoffe erforderlich. Temperaturstreß (35° C) ist bei Lactuca reversibel, irreversibel bei Gerste, Salzstreß als osmotischer Streß ist bei beiden Samen reversibel.

Introduction

Seeds of various plants have been known to enter into a period of thermodormancy when placed in supraoptimal temperatures (KEYS & al. 1975, SAINI & al 1986).

Changes in moisture level in the germinating medium profoundly affect seed performance. Water stress has been shown to increase the levels of abscisic acid (ABA) and decrease the levels of cytokinins in plant tissues. These adverse hormonal changes occur when plant tissues are subjected to water stress by drought (ITAI & VAADIA 1965, ZEEVAART 1971, LIN & al. 1986), salinity (ITAI 1978, BOZCUK & TOPÇUOĞLU 1984, LERNER 1985), or supraoptimal temperatures (DAIE & CAMPBELL 1981, BOZCUK & TOPÇUOĞLU 1982).

Treating seeds with various combinations of growth regulators has been shown to be highly effective in relieving inhibition of germination induced by salt stress (BRAUN & KHAN 1976, BOZCUK 1978, KABAR 1987), overcoming thermodormancy induced by high temperature (RAO & al. 1975, SAINI & al. 1986). However, there are different reports on this subject: Some researchers have reported that treatment by GA_3, and cytokinins singly or in combination removed the inhibitory effect of high temperatures on germination (KHAN 1975, DUNLAP & MORGAN 1977, BEWLEY 1980). Others reported that GA_3 and kinetin applied singly failed to overcome thermodormancy but that the combination of GA_3 and kinetin proved to be effective (SMITH & al. 1968, KEYS & al. 1975, SAINI & al. 1986). Also, several workers have reported that GA_3 had little effect, whereas kinetin promoted germination at supraoptimal temperatures (SHARPLES 1973, RAO & al. 1975, BEWLEY & BLACK 1982). In addition, there are conflicting reports about whether thermodormancy is reversible or not. Most of the above reports are those of studies with lettuce.

In this research work, the effect of GA_3 and kinetin pretreatments singly and/or in combination in removing inhibition of the germination of lettuce, a dicot, and barley seeds, a monocot, under high temperature and salinity stresses was studied. Lettuce seed was selected to serve a comparison with barley in the response to hormone treatments. The salt tolerances of these seeds and the permanence of injury caused by osmotic and high temperature stresses were investigated.

Materials and Methods

Germination experiments were carried out at various constant temperatures (20°, 25°, 30° C), in continuous dark. Barley (*Hordeum distichum* cv. Nutans Schubl) and lettuce (*Lactuca sativa* cv Salad Bowl) seeds were imbibed by soaking for 1 h at room temperature, in distilled water (control), or in aqueous solutions of kinetin (50 ppm), GA_3 (100 ppm), and a combination of GA_3 and kinetin (100 ppm GA_3 + 50 ppm kinetin). At the end of this pre-treatment, the solutions were decanted off and then seeds vacuum dried for 1 h. 25 lettuce or 20 barley seeds from each treatment were placed in 9 cm Petri dishes on 2 sheets of Whatman No. 1 filter paper moistened with distilled water or NaCl solutions at various concentrations. 5 ml of 0.75, 1.0, 1.25, 1.5 and 1.75% NaCl solutions for barley, and 4 ml of 0.5, 0.75, 1.0, 1.1, and 1.25% NaCl solutions for lettuce were used. After placement of the seeds, the Petri dishes were transferred to one of 3 incubators at 20°, 25°, or 30° C for temperature treatment. Barley and lettuce seeds were considered germinated when the radicles reached to 10 mm, and 1 mm in length, respectively (SHARPLES 1973, UNGAR 1974). The germination percentages were recorded after incubation for 120 h. For lettuce, the percent shares of typical (radicle protrusion and subsequent radical elongation) and atypical (cotyledon protrusion and/or stunted radicle) germination were determined. An arcsin transformation was made on percent data for analysis of variance (STEEL & TORRIE 1960).

Seeds kept for 120 h at 20° C, in germination media of 2.0, 3.0, 4.0, and 5.0% NaCl did not germinate. When these seeds were removed from NaCl solutions and transferred into Petri dishes with distilled water, they germinated as previously described. In addition, seeds kept at 35° C and in germination medium of distilled water were not able to germinate for 120 h. These seeds were transferred to 20° C so that they could germinate. These two experiments were done in order to test the permanence of injury of the salt and high temperature to the seeds.

Each treatment was repeated 3 times. Hormone and salt solutions were kept in a refrigerator at 4° C until used.

Results

1. Interaction of salt and temperature

The increasing levels of salinity and of temperature inhibited the germination of the control seeds of both species (Fig. 1, 2). As temperature increased, osmotic inhibition appeared to be more drastic. At 30° C, 1.25 and 0.75% NaCl completely inhibited the germination of barley and lettuce seeds, respectively. However, inhibition was not complete even in 1.5 and 1.75% NaCl in barley, and in 1.0 and 1.1% NaCl in lettuce at 25° and 20° C, respectively.

2. Effects of kinetin and GA_3 on salt and high temperature inhibition

In overcoming salt stress at all the temperatures, GA_3 alone had more effect in barley, whereas kinetin was more effective in lettuce. However, it was seen that the synergistic effect of GA_3 and kinetin combination was much more marked (Fig. 1, 2). At all the temperatures, as the level of salt

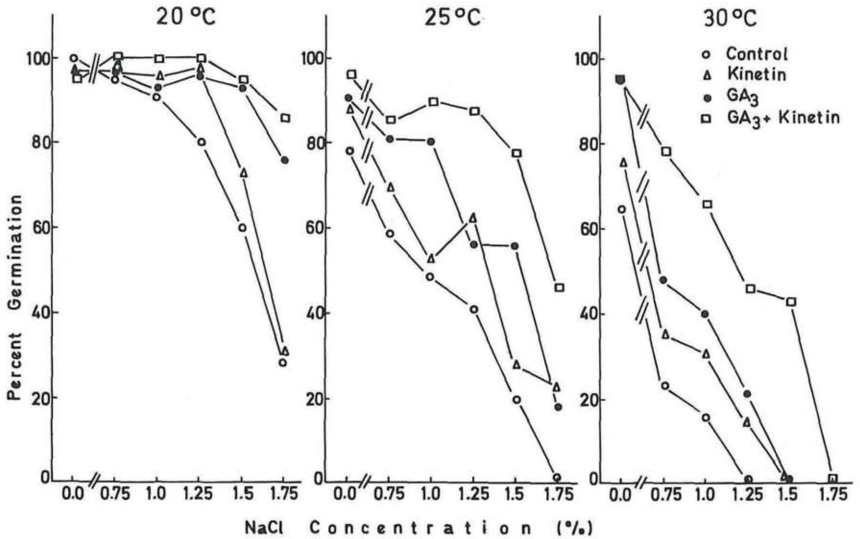

Fig. 1. Interactions among kinetin, GA_3, salt concentration, and temperature on the germination of barley seeds. LSD (0.05) = 3.95.

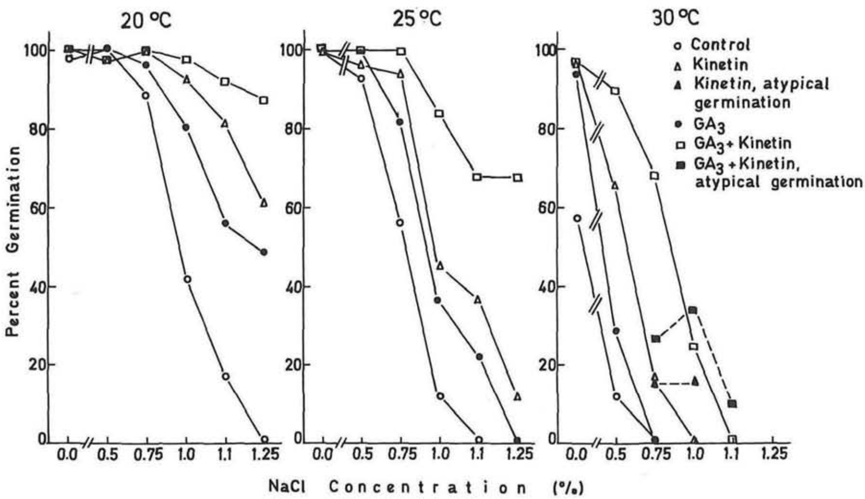

Fig. 2. Interactions among kinetin, GA_3, salt concentration, and temperature on the germination of lettuce seeds. LSD (0.05) = 3.20.

increased, the positive effects of hormone pre-treatments were more prominent. Hormone pre-treatments almost completely overcame the inhibitive effect of high temperature stress (30° C) on germination in distilled water.

As the result of this treatment, the seeds behaved as if they were returned to normal conditions (20° C). However, in overcoming thermodormancy in barley, GA_3 had more effect than kinetin. Untreated (control) seeds, even at 25° C, were not able to reach to the germination percentages of barley seeds in 1.0% NaCl, and of lettuce seeds in 0.75% NaCl by GA_3 and kinetin combination at 30° C.

At low levels of salt, kinetin or GA_3 alone was sufficient. But, as salt and/or temperature levels increased, a combination of GA_3 and kinetin was required.

3. Recovery from salt and high temperature inhibition

Barley and lettuce seeds failed to germinate in distilled water at 35° C even for 120 h. These seeds were transferred to an optimal temperature (20° C). Although lettuce seeds germinated 96.0% in 120 h after transfer, the germination of barley seeds was never observed (Table 1 a).

Table 1a

Percentages of barley and lettuce seeds germinating after transfer from 35° C to 20° C

% germination at 35° C after 120 h.		Barley	Lettuce
		0.0	0.0
Hours after transfer to 20° C	24	0.0	32.0
	48	0.0	64.0
	72	0.0	80.0
	96	0.0	90.0
	120	0.0	96.0

On the other hand, seeds of both species failed to germinate in the media of 2.0, 3.0, 4.0, and 5.0% NaCl at 20° C for 120 h. When these seeds were transferred to distilled water at the same temperature, they showed maximum germination in 120 h (Table 1 b).

Discussion

In this work, inhibition of the germination of barley and lettuce seeds by moderately high temperatures and/or salt levels was removed by GA_3, kinetin, and a combination of these two hormones to varying extents. Kinetin alone had more effect on lettuce, but GA_3 was more effective on barley seeds. However, as the germination temperature and/or salt concentration was increased, GA_3 and kinetin combination was required for the promotion of germination of both the seeds. Some researchers obtained

Table 1b

Percentages of barley and lettuce seeds germinating after transfer from NaCl solutions of high concentrations to distilled water

% NaCl		Barley 2.0	Barley 3.0	Barley 4.0	Barley 5.0	Lettuce 2.0	Lettuce 3.0	Lettuce 4.0	Lettuce 5.0
Hours after transfer to distilled water	24	91.7	75.0	18.3	0.0	92.0	64.0	9.3	4.0
	48	93.3	85.0	91.7	70.0	100.0	97.3	66.7	66.7
	72	95.0	95.0	91.7	80.0	100.0	98.7	92.0	85.3
	96	96.7	95.0	91.7	85.0	100.0	100.0	98.7	96.7
	120	96.7	95.0	93.3	90.0	100.0	100.0	98.7	97.3

similar results based on the effect of kinetin treatment on the seeds of lettuce (ODEGBARO & SMITH 1969, BRAUN & KHAN 1976, BRAUN & al. 1976), cotton and tomato (BOZCUK 1978). Our finding that there was a synergistic effect of GA_3 and kinetin combination on alleviating thermodormancy and/or salt stress in the germination of lettuce seed is in accord with those obtained by other workers (KEYS & al. 1975, BRAUN & KHAN 1976, SAINI & al 1986). However, we have scarcely come across report concerning that GA_3 may be more effective than kinetin in the alleviation of salt and temperature stresses on the germination of barley or other monocot seeds in literature (MERT & YÜREKLI 1978, KABAR 1987). These data may indicate that probably more than one mechanism is involved in overcoming inhibition of seed germination induced by salinity and temperature stresses. In the investigated monocot (barley) GA_3 may more effectively alleviate the stresses, whereas the dicot (lettuce) may better respond to kinetin treatments.

Removal of inhibition of germination by growth regulators depends on the conditions under which this inhibition has been induced. It is evident that more than one growth regulator is required as the seeds enter into a deeper state of dormancy by a rise in temperature (20° C vs. 30° C), or by an increase in salt level (e. g. in barley 0.75% vs. 1.75%, or in lettuce 0.5% vs. 1.25% NaCl). The requirement of more than one growth regulator is indicative of an increase in the complexity of biochemical and biophysical alteration responsible for the induction of dormancy induced by salinity and/or temperature stresses. Much research has indicated that the regulation of both dormancy and dormancy breaking is through a balance between endogenous hormonal promoters and inhibitors (KHAN 1971, 1977, EL ANTABLY 1976). An increase in the level of ABA (ÇETINGÜL & BALTEPE 1983, LACHNO & BAKER 1986) and a decrease in that of GA_3 (BOUCAUD & UNGAR 1976) and of cytokinins (BROWNING 1973, KHAN 1978, ÇETINGÜL & BALTEPE 1983) have been reported to occur in tissues under various conditions of stress such as salinity and high temperature. The improved germination may result from a direct restoration of the depleted level or the active ratio of hormones or by membrane-related changes that could prevent or slow down the degradative processes leading to changes in the level of hormones.

The increased response by GA_3 in the presence of kinetin could result from the removal of ABA block to GA_3 action by kinetin. It may be supposed that the lack of GA_3 is a factor limiting the germination in the seeds which cytokinins cannot overcome the inhibitive effect of ABA. Actually, in our study, kinetin alone failed the germination of barley seeds under stress, but GA_3 added to this hormone, due to synergism, had to a great extent a positive effect. These observations are consistent with the model of seed germination proposed by KHAN 1971. The fact that the lowering of the effect of GA_3 depending on the rising levels of the salt and temperature can be due to its inefficiency to overcome the inhibitors depending on the stress after a definite level in the absence of kinetin in the medium.

Cytokinin action is suggested to be related to production of higher osmolarity or an increased resistance of root system to stress (TAL & IMBER 1971). Also, we saw this case in both the seeds, particularly in lettuce. Kinetin effect also appears to be related to the growth (expansion) of cotyledons (LETHAM 1969). Atypical germination of lettuce seeds indicates this.

Several workers succeeded overcoming thermodormancy by kinetin, GA_3, ethylene, CO_2, and by various combinations of these (RAO & al. 1975, BRAUN & al. 1976, SAINI & al. 1986). Also, salt stress is reported to be overcome by kinetin, GA_3, ethylene and by combinations of these (BRAUN & KHAN 1976, BOZCUK 1978, KABAR 1987).

The multiplicity of the factors that can overcome high temperature and/or salt stresses suggests that the germination under conditions of such stress may be regulated by a number of control points (KETRING 1977, KHAN 1980, SAINI & al. 1986). It will be interesting to investigate these aspects in future with more elaborate techniques.

On the other hand, we saw that when ungerminated barley and lettuce seeds held for 120 h in an imbibed state at 35° C were transferred to 20° C, lettuce seeds germinated but not barley seeds. Whereas seeds held in an imbibed state at a supraoptimal temperature have been suggested to become thermodormant and to lose their ability to germinate when subsequently incubated at an optimal temperature (KHAN 1980, BEWLEY & BLACK 1982). However, return of the seeds to optimal temperatures is reported to alleviate thermodormancy (KEYS & al. 1975). Referring to our data, it should be more appropriate to say that high temperature stress, when returned to optimal conditions, may be reversible in some seeds and irreversible in others.

In addition, the fact that the seeds of both the species recovered from salt stress germinated indicates that the effect of the salt on these seeds is osmotic and reversible. KURTH & al. 1986 and UNGAR 1974 have also found the same results.

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