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The Effect of Quality of Irrigation Water, Salinity, Moisture Stress and Kinetin on Germination of Three Cultivars of Alfalfa

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ABSTRACT. A comparison of the germination capacity of seeds of three cultivars of *Medicago sativa* L. of diverse geographic origin in water of different sources, solutions of sodium chloride and mannitol was investigated. The degree of germination retardation varies in the same species with the variation of the medium. The results indicate that salinity affects the rate of germination adversely and the presence of sodium chloride in the germination culture inhibited germination significantly more or less than that due to moisture stress [mannitol]. Kinetin applied simultaneously with NaCl or mannitol to seeds altered the stress response and the stressed seeds responded to kinetin more than did unstressed seeds. It is concluded that kinetin plays and important role in plant response to stress and that Hassawi and AS-49R appear to be very similar as salt tolerant cultivars and cv. Diabloverd is a little more tolerant at the highest salt levels.

Climatic conditions in Qatar are arid with scanty rainfall. Agriculture for commercial production is recent in Qatar and is limited mainly by the non-availability of suitable soils and sweet irrigation water is scarce (Ismail 1983). In Qatar, sweet water (EC 1000 micro mhos/cm) present as fresh water lens aquifer in the central north of the country – is limited, and most of the irrigation waters have EC range 1000-3000 micro mhos/cm in the north and EC more than 6000 micro mhos/cm in the south. According to Strongov and Kabanov (1973) and Yaron *et al.* (1973) irrigation water having EC 2250-5000 micro mhos/cm is classified as very highly saline and is not suitable for irrigation under ordinary conditions.

The chemical composition of water may affect germination in a number of ways. The water may have a salt content which may osmotically retard or completely prevent germination, or it may incur an ionic balance unfavourable to

germination. Little information is available on the role of the quality of water on germination, however, Hussein (1981) reported that salts are added to the root zone by saline irrigation water. Various criteria for appraising the tolerance of plants to saline water, particularly at the germination stage, received little attention.

The present study involved the effect of (a) various qualities of water in Qatar, (b) levels of osmotic stress and salinity using NaCl (Batanouny and Ziegler 1971, Waite and Hitchings 1978, Wainwright 1980), (c) moisture stress using mannitol (Jarvis and Jarvis 1963, Batanouny and Ziegler 1971, Wainwright 1980) and (d) the subsequent effect of kinetin (Benzioni *et al.* Katz *et al.* (1978) on these treatments on the germination capacity of three cultivars of alfalfa grown under Qatari conditions.

Material and Methods

Plant Material

Seeds of *Medicago sativa* L. cvv. AS-49R and Diabloverd were obtained from Ferry-Morse Seed Company (California, U.S.A.) and *Medicago sativa* cv. Hassawi (originally derived from Berseem Higazi but propagated in El-Ihssa, Saudi-Arabia) was obtained from Qatar, Ministry of Agriculture.

Experiments

Three germination experiments were carried out for each cultivar.

Experiment 1: Effect of Quality of Irrigation Water on Germination

The culture solutions used were distilled water, tap water, north water (water brought from wells in the northern part of Qatar), south water (water brought from wells in the southern part of Qatar) and half strength sea water (a 50% solution was made up from sea water). A litre of each solution was kept as a stock-solution (see Table 1a).

Experiment 2: Effect of Sodium Chloride Solutions on Germination

Culture solutions of NaCl having osmotic values of −2, −4, −8, −12, −16, −20 and −24 bar were used (Weast 1982), *i.e.*, a litre of each solution was prepared and kept as stock-solution for subsequent use (Table 2a).

Experiment 3: Effect of Mannitol on Germination

Mannitol culture solutions having moisture stress (osmotic) values of −2, −4, −8, −12, −16, −20 and −24 bar were used (Weast 1982), *i.e.* a litre of each solution was prepared and kept as stock-solution for subsequent use (see Table 3a).

Germination Conditions

Seeds were germinated in darkness at 25°C. For each cultivar, four replicates each of 20 seeds was sown in 9-cm diameter × 0.4 mm. Whatman Seed Test filter pads moistened by adding 5 ml of appropriate culture solution to each dish. In order to study the effect of kinetin on germination, two relevant stock solutions (100 ml) were prepared by adding kinetin in two different amounts to each of the culture solutions mentioned in Table 1a, 2a and 3a. The kinetin concentrations in the resulting relevant solutions were 20 and 30 ppm. 5 ml from each relevant solution (Culture solution including kinetin) were used to moisten the filter pads in the petridishes.

Seeds were examined daily and those which had germinated were removed and counted. A seed was considered to have germinated when the radicle had emerged from the testa. Recording was continued until no further increase in germination occurred.

The significance of main treatment effects and interactions were determined by analysis of variance using an arcsin transformation of percentage germination (Snedecor and Cochran 1969).

Results

Experiment 1

Both the rate of germination and the final percentage germination decline as salinity is increased (Fig. 1A; Table 1a, and b). The trend of decreasing germination with increasing salinity is significant ($P = 0.01$) throughout the treatments. The cultivars response to this treatment was the same. In all culture solutions, seeds began to germinate on the first day. No germination occurred in all treatments after the eighth day. At 0.25, 2.85, 6.98 (m mhos/cm) salinity level, the total germination percentage was reduced 5.7, 25.2 and 30.7 respectively, whereas at 23800 ppm germination was prevented.

In cultures having 20 ppm kinetin, the rate of germination increased significantly ($P = 0.01$) compared to the controls which had no kinetin. At 6.98 m mhos/cm salinity level, the percentage of germination was 59.3 for cv. AS-49R, 63.8 for cv. Diabloverd and 54.0 for cv. Hassawi but addition of 20 ppm kinetin to this saline culture increased the percentage of germination to 85.4, 85.4 and 71.6 respectively. In the cultures having 30 ppm kinetin the rate of germination at all salinity levels was about the same as in controls which were without kinetin. Kinetin promotion of germination was observed on the third day.

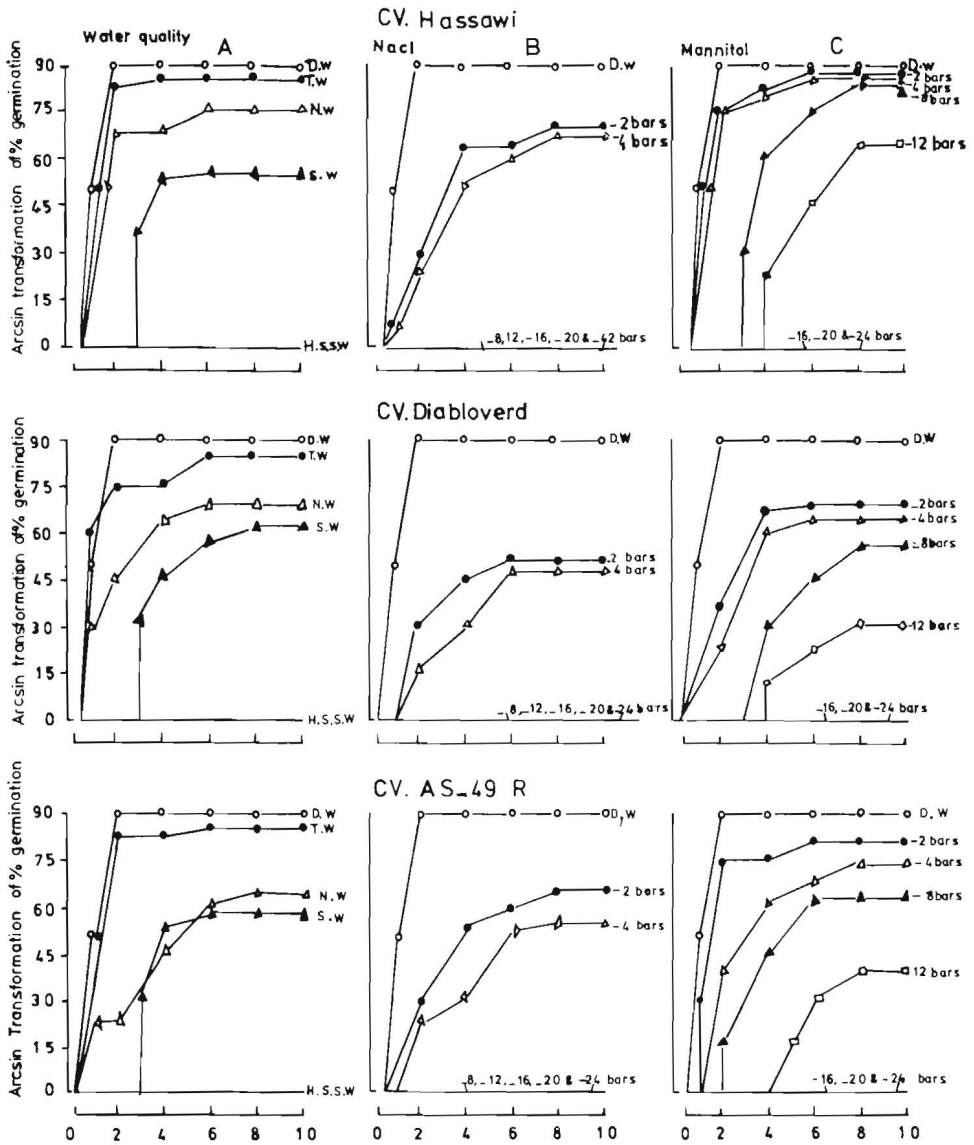

Fig. 1. Effect of different qualities of irrigation water and concentration of sodium chloride and mannitol on the germination of seeds of three cultivars of *Medicago sativa* L. [D.W = Distilled Water, T.W = Tap Water, N.W = North Water, S.W = South Water and H.S.S.W = Half-strength Sea Water].

Table 1a. Effects of quality of water and kinetin on the final percentage germination of three cultivars of *Medicago sativa* L. at 25°C after ten days. Each figure represents the germination percentage of four replicates mean using an arcsin transformation.

Kinetin concentration (ppm)	Quality of water used and its total dissolved salts in (E.C) and osmotic value in (bars)				
	Distilled water [0 m mhos/cm] 0 bars	**Tap water [0.25 m mhos/cm] > −1 bar**	**North water [2.98 m mhos/cm] > −2 bars**	**South water [6.98 m mhos/cm] > −6 bars**	**Half-strength Sea water [28.35 m mhos/cm] −20 bars**
cv. AS-49R					
0 [Control]	90.0	84.3	64.8	59.3	0.0
20	90.0	90.0	85.4	85.4	0.0
30	90.0	78.8	71.6	64.4	0.0
cv. Diabloverd					
0 [Control]	90.0	84.3	71.6	63.8	0.0
20	90.0	90.0	90.0	85.4	0.0
30	90.0	85.4	69.4	65.3	0.0
cv. Hassawi					
0 [Control]	90.0	84.3	75.6	54.0	0.0
20	90.0	90.0	85.4	71.6	0.0
30	90.0	83.9	73.4	57.0	0.0

Table 1b. Analysis of variance for the effects of different qualities of water and different kinetin concentrations on germination of three cultivars of *Medicago sativa* L.

[a] Cultivars		[b] Kinetin concentration		[c] Quality of Water	
AS-49R	74.943a*	20 ppm	81.690a	Distilled water	89.489a
Diabloverd	74.783a	30 ppm	79.620b	Tap water	81.106b
Hassawi	71.518a	0 ppm	59.935b	North water	75.567c
				South water	71.050d
				Half-strength sea water	51.531e

* In each column figures with the same letter are not significantly different [$P > 0.05$] but the figures with different letters are significantly different from each other [$P < 0.01$].

Experiment 2

The rate of germination under different concentrations of NaCl for a period of ten days is illustrated in Fig. 1B. The total germination percentages in the various solutions is shown in Table 2a. The analysis of variance for all the results

Table 2a. The effect of salinity [NaCl] and kinetin on the final percentage germination of three cultivars of *Medicago sativa* L. at 25° after ten days. Each figure represents the germination percentage of four replicates mean using an arcsin transformation.

Kinetin concentrations (ppm)	Distilled water	Sodium chloride osmotic stress values (in bars)						
		−2	−4	−8	−12	−16	−20	−24
cv. AS-49R								
0[Control]	90.0	63.4	52.3	0.0	0.0	0.0	0.0	0.0
20 ppm	90.0	85.4	70.4	58.6	0.0	0.0	0.0	0.0
30 ppm	90.0	51.0	48.2	18.4	0.0	0.0	0.0	0.0
cv. Diabloverd								
0[Control]	90.0	50.9	49.6	0.0	0.0	0.0	0.0	0.0
20 ppm	90.0	79.2	68.1	51.7	0.0	0.0	0.0	0.0
30 ppm	90.0	42.0	27.9	19.6	0.0	0.0	0.0	0.0
cv. Hassawi								
0[Control]	90.0	73.6	68.4	0.0	0.0	0.0	0.0	0.0
20 ppm	90.0	78.8	74.2	60.9	39.2	0.0	0.0	0.0
30 ppm	90.0	62.2	57.7	8.6	0.0	0.0	0.0	0.0

Table 2b. Analysis of variance for the effects of different osmotic stress values and different kinetin concentrations on germination of three cultivars of *Medicago sativa* L.

[a] Cultivars		[b] Kinetin concentrations		[c] Osmotic stress values	
				Distilled water	
Hassawi	52.703a*	20 ppm	59.433a	[0]	90.000a
AS-49R	47.850b	30 ppm	42.793b	−2	59.939b
Diabloverd	43.547c	0 ppm	41.873b	−4	58.344b
				−8	28.228c
				−12 (bars)	3.656d

* In each column figures with the same letter are not significantly different [$P > 0.05$] but the figures with different letters are significantly different from each other [$P < 0.01$].

is shown in Table 2b. In general, the final percentage germination declines as the level of salinity increases and the trends are significant for all the different concentrations and cultivars ($P = 0.01$). The maximal limit of osmotic values under which the seeds of the three cultivars germinated was −4 bar. Seeds failed to germinate in concentrations higher than this limit. For each cultivar, the final percentage germination at −2 or −4 bar was similar. Germination began by the second day and by the eighth day no germination occurred.

In cultures having 20 ppm kinetin, the rate of germination increased significantly ($P = 0.01$) compared to the controls which had no kinetin. At −8 bar osmotic value, the percentage of germination was 0.0 for all the cultivars but addition of 20 ppm kinetin to these saline media increased the percentage of germination 51.7, 58.6 and 60.9 for cvv. Diabloverd, AS-49R and Hassawi, respectively. At −12 bar osmotic value, germination was prevented for all cultivars, but addition of 20 ppm kinetin to this solution increased the percentage germination from 0.0 to 39.2 for cv. Hassawi only. In the solutions having 30 ppm kinetin, the rate of germination at all osmotic values was similar to the controls without kinetin. The reversal of the action of NaCl by kinetin began by the fourth day.

Experiment 3

Generally, germination is reduced by increasing the concentration of mannitol (Fig. 1C, Table 3a and b). In a solution of −2 to −4 bar osmotic value, seeds began to germinate on the first day, in a solution of −8 bar seeds germinated on the third day and in a solution of −12 bar seeds germinated by the fourth day for cvv. Hassawi and Diabloverd, and by the fifth day for cv. AS-49R. No germination occurred in all treatments after the eighth day. The maximal limit of osmotic values under which the seed of the cultivars germinated was −12 bar.

Table 3a. The effect of moisture stress [mannitol] and kinetin on the final percentage germination of three cultivars of *Medicago sativa* L. at 25°C after ten days. Each figure represents the germination percentage of four replicates mean using an arcsin transformation.

Kinetin concentrations (ppm)	Distilled water	Mannitol moisture stress values (in bars)						
		−2	−4	−8	−12	−16	−20	−24
cv. AS-49R								
0[Control]	90.0	80.8	74.2	64.2	40.4	0.0	0.0	0.0
20	90.0	90.0	90.0	71.6	67.5	54.0	40.6	39.2
30	90.0	90.0	90.0	78.8	72.4	69.0	63.8	54.0
cv. Diabloverd								
0[Control]	90.0	74.2	63.8	59.0	30.8	0.0	0.0	0.0
20	90.0	90.0	90.0	78.8	71.6	69.5	64.2	45.0
30	90.0	90.0	90.0	90.0	90.0	71.6	70.5	70.5
cv. Hassawi								
0[Control]	90.0	90.0	85.4	84.1	39.2	0.0	0.0	0.0
20	90.0	90.0	90.0	84.1	76.8	72.1	69.6	50.9
30	90.0	90.0	90.0	90.0	90.0	90.0	85.4	77.1

Table 3b. Analysis of variance for the effects of different moisture stress values and different kinetin concentrations on germination of three cultivars of *Medicago sativa* L.

[a] Cultivars		[b] Kinetin concentration		[c] Moisture stress values	
Hassawi	71.183a*	30 ppm	81.996a	Distilled water	90.000a
Diabloverd	67.946b	20 ppm	76.565b	−2 (bars)	87.217ab
AS-49R	64.281c	0 ppm	44.850c	−4	85.328b
				−8	89.989c
				−12	65.217d
				−16	46.328e
				−20	46.000e
				−24	41.350f

* In each column figures with the same letter are not significantly different [$P > 0.05$] but the figures with different letters are significantly different from each other [$P < 0.1$].

In cultures having either 20 or 30 ppm kinetin, the rate of germination increased significantly ($P = 0.01$) compared to the controls without kinetin. At osmotic values of −16, −20 and −24 bar, the percentage of germination was 0.0 for the three cultivars but addition of 20 or 30 ppm kinetin to these solutions altered and increased the percentage germination. The kinetin rate at 30 ppm increased the percentage germination more significantly than did the kinetin rate 20 ppm (Table 3a and b). The reversal of the action of mannitol commenced by the third day.

Discussion

Plant species differ greatly in their tolerance to salt and crop plants vary from very sensitive to fairly tolerant (Brenstien 1964, Strongov 1964). There is considerable vagueness about the level up to which NaCl can be tolerated in irrigation waters particularly for germination of seeds of crops. Alfalfa is amongst the most important fodder crop in Qatar (where sweet irrigation water is a limiting factor) because of its perennial character, high yield potential, palatability and therefore it occupies a prominent position in agricultural programme aimed at rehabilitation and improvement of pasture land in Qatar.

Laboratory germination tests shows that all the cultivars germinated under a wide range of conditions once their short-term dormancy – due to osmotic stress, sodium chloride or moisture stress – was lost. Numerous investigators indicated that germination was reduced by increasing the concentration of the medium (Ayers 1952, Batanouny and Ziegler 1971, Waite and Hutchings 1978, and Harradine 1982). The results show that the rate of decrease in germination percentage differed with the different solutions used. With the qualities of water used, the gradual increase of salinity produced no sharp rate of decrease, but with the introduction of half-strength sea water as a culture medium germination was completely prevented. Comparison of the effects of different solutions having the same osmotic value on germination shows that they vary widely. For example, the final germination percentage of the seed of cv. Hassawi in mannitol solution with an osmotic value of −8 bar is similar to that of a solution of NaCl with −4 bar osmotic value. The results show that mannitol solutions and NaCl solutions not only differed in their action on retardation of germination, but also on the rate of germination. Lang (1965) stated that the germination retarding effect of solutions was mainly an osmotic effect and that there were differences in the effectiveness of different solutes indicating the presence of specific effects of ions.

Benzioni *et al.* (1967) and Katz *et al.* (1978) reported that kinetin completely or partially reversed stress and inhibitory effects of NaCl and that the endogenous levels of cytokinins were altered in response to water stress whether imposed by salinity or drought. The results reported here clearly show that germination, salinity and moisture stress tolerance of the cultivars' seeds are enhanced when kinetin

was added. The results are in agreement with those of Benzioni *et al.* (1967) and Katz *et al.* (1978) that the addition of kinetin strongly induced germination and alleviated the effects of salinity and moisture stress. It is noteworthy that the germination percentage in mannitol solution with an osmotic value of −12 bar + Kinetin was three times that of NaCl solution with the same osmotic vaslue + Kinetin, and that kinetin was able to reverse the highest value of moisture stress exerted by mannitol but failed to remove the effect exerted by −16 bar osmotic value of NaCl (Fig. 2). In every case in, Table 1a, the 30 ppm kinetin reduced germination over the 20 ppm kinetin, while in non-ionic substances (mannitol-containing solutions, Table 3a) the 30 ppm kinetin increased germination over the 20 ppm kinetin. These results indicate that NaCl exerts an effect on seeds which is quite different from that exerted by mannitol and that kinetin is toxic at higher levels, *i.e.* 30 ppm in the presence of ionic substances. Although the mechanism by which kinetin partially reverses the action of salinity and completely reverses the action of mannitol is still unclear and needs further biochemical investigation,

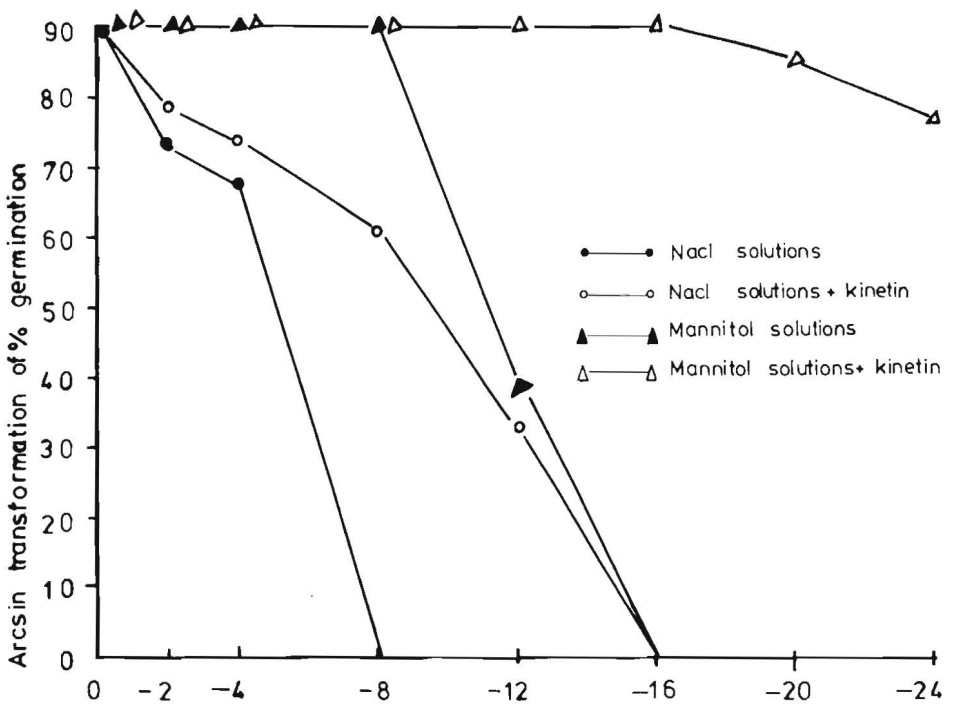

Fig. 2. The effect of osmotic values of solutions of sodium chloride and mannitol, sodium chloride + kinetin and mannitol + kinetin on the germination of *Medicago sativa* cv. Hassawi seed at 25°C in darkness.

yet there is evidence from the present study that kinetin plays a major role in plant stress responses.

A comparison of the ranking of cultivars with respect to their germination capacity in the series of experiments described shows many similarities. Thus, at different qualities of water and concentration of kinetin the cultivars seed capacity to germinate was similar at the different salinity levels. Cv. Hassawi and cv. AS-49 R appear to be very similar and cv. Diabloverd is a little more tolerant at the highest salt concentration. At different moisture stress values (mannitol) and kinetin the ranking corresponded less well. Cv. Hassawi had the highest percentage germination and cv. AS-49 R had the lowest percentage germination. These differences in ranking may simply reflect ecological adaptations to salinity and moisture stress for cv. Hassawi which has been derived and propagated from Saudi Arabian origin (Berseem Higazi) and is the cultivar presently used in Saudi Arabia and Qatar and it is concluded that cvv. AS-49 R and Diabloverd may as well be used under Qatari conditions.

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تأثير مياه الري والملوحة والجهد الرطوبي وهرمون الكاينتين على إنبات ثلاثة طرز مزروعة من البرسيم

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الدوحة ـ قطر

أجرى هذا البحث بهدف دراسة مقارنة لإنبات ثلاثة طرز من البرسيم من أماكن مختلفة من العالم تحت ظروف مختلفة من مياه الري والملوحة والجهد الرطوبي . دلت النتائج على أن الملوحة تؤثر تأثيراً عكسياً على نسبة الإنبات وأكثر من التأثير العكسي الذي يسببه الجهد الرطوبي . كما أوضح البحث فعالية هرمون الكاينتين في إزالة تأثير الملوحة والجهد الرطوبي على نسبة الإنبات النهائية .

وقد استنتج من البحث أن طرزي الحساوي (cv. Hassawi) وإس ـ ٤٩ ر (cv. AS-49 R) لهما صفات واحدة في تحمل الملوحة وأن طرز ديابلوفرد (cv. Diabloverd) أكثر تحملا للملوحة الشديدة من الطرزين السالفي الذكر .