

EFFECT OF GIBBERELIC ACID APPLICATION ON BRASSICA SPECIES GROWN UNDER SALT STRESS

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ABSTRACT

*Three Brassica species of wild divergent origins, namely *B. napus*, (L.), *B. campestris* (L.) and *B. alba* (L.) Bioss were tested to study the effects of gibberellic acid (GA_3) on the productivity of these species under salt-stress. The obtained result indicate that the salt-stress imposed by 25 or 50 mM NaCl reduced substantially some plant characters; leaf area, leaf carbohydrate content, dry mass, leaf chlorophyll content, stomatal conductance and photosynthetic rate 50 days after emergence. Exceptional being, total seed protein showed a remarkable increase. The treatment with GA_3 application on plant species appears to mitigate the adverse effect of salinity stress and improved the productivity of the studied Brassica, Furthermore, the application of GA_3 was more pronounced in case of *B. alba* and *B. napus*, whereas, *B. campestris* took a special behavior than the other studied species .*

Key words: *Brassica species, gibberellic acid, growth, salt stress, chlorophyll, Photosynthesis, stomatal conductance and seed protein content.*

INTRODUCTION

Now a day, the production of *Brassica* is economically low due to the influence of number of biotic and abiotic stresses, among of which is the salt stress. This stress has an adversely affects on the germination, growth, physiology and productivity by causing ionic and osmotic stresses as well as oxidative damage (Iterbe-Ormaetex et al., 1998), change in C and N metabolism (Kim et al., 2004), decreased biosynthesis of chlorophyll (khan, 2003), responsible for an increased respiration rate, and ion toxicity (Sudhir and Murthy, 2004).

The role of the physiologist is, therefore, to get a solution to solve these problems. In this

accords, an attention has been become to be use of plant growth regulation such as gibberellic acid (GA_3) which control a number of stresses -induced genes, (Naqvi, 1999).

Gibberellic acid is one of the phytohormones which play an active role in plant growth substances. The important of the physiological effects of gibberellic acid on higher plants are: substitute for the stratification of dormant seeds, regulation of the cell division of the sub apical mersitem and promote short growth, flower formation of long day plants under non-induction condition, increasing the seedless grape berries and elongate the cluster pasts, delay the maturing, ripening and aging of fruits, creating new

product of RNA and protein in plants, (Reid et al., 1968, Pal and Bangarayer, 1968).

The effect of gibberellic acid application on the role of plant tolerant to saline stress still unsatisfactory and needed more studies, therefore, the present study was designed as an attempt to characterize the influence of GA₃ on the adverse effects of salt stress in three *Brassica* species with reference to basic growth, physiological and yield characters.

MATERIALS AND METHODS

MATERIALS :

Three *Brassica* species of wild divergent origins, namely *B.napus*, (L.), *B. campestris* and *B. alba* (L.) Bioss were experimentally tested to study the response of these species to foliar gibberellic acid (GA₃) application under salt stress. The experiment was conducted in pots (25 cm diameter) on a completely randomized block design at the Genetic Engineering Center (GEC), Cairo, Egypt.

A brief description of the studied B.species are outlined in the following, *Brassica napus* , *Brassica campestris* and *Brassica alba*. Description according to Tackholm (1974).

1- *Brassica napus* (L.)

Annual or biennial, when sown late and flowering the following spring, with slender or stout, hard, long, fusiform tuberous taproot; stems erect, much-branched, up to 1.5 m tall, often purple toward base; leaves glaucous, the lower ones lyrate-pinnatifid or lobed, with petioles 10-30 cm long, glabrous or with a few bristly hairs, upper stem leaves lanceolate, sessile, clasping, more or less entire; flowers pale yellow, 1.2-1.5 cm long, open flowers not overtopping buds of inflorescence; inflorescence much-branched, up to 1 m tall as an elongating raceme; silique 5-11 cm long, 2.5-4 mm wide, with slender beak 0.5-3 mm long. Underground part curved or crooked for 5-7.5 cm and then dividing into stout horizontal branches. Fl (Fig.1).

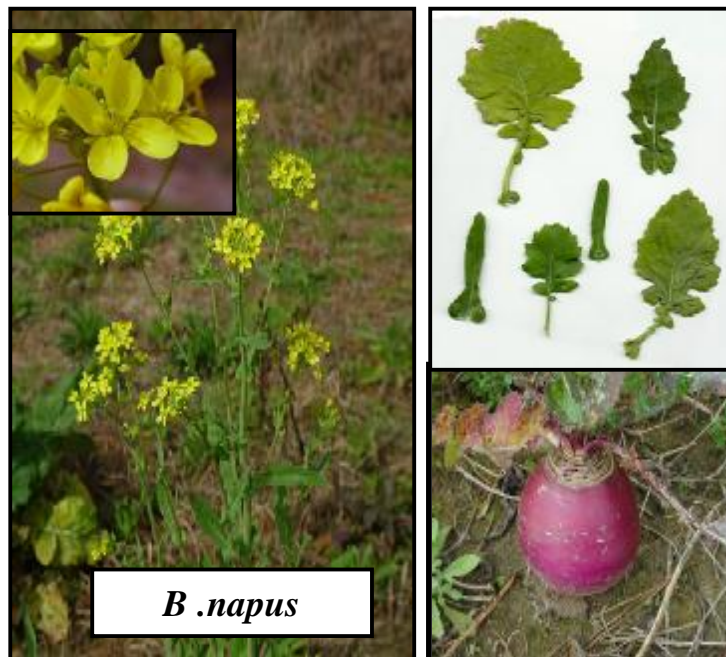


Fig. 1 : *Brassica napus*.

2- *Brassica campestris* (L.)

Plant is 75-100 cm in height. Stem is upright, branched, root is thin, unedible. Lower leaves are petiolate, lyre-pinnatifid, green, pubescent. Upper leaves are sessile, ovate with deep-cordate, amplexicaul base, dove-coloured, glabrous or slightly pubescent. Inflorescence is racemose, flowers are actinomorphic, divided into four parts, petals are golden-yellow, situated crosswise, and sepals are horizontally deflected by the end of flowering. Fruit is silicle, with long elongate-conical or subulate tip. Lower part of silicle is multi-seeded, dehiscent, upper part (tip) is in-

dehiscent and has one seed. Seeds are globular, slightly squeezed, from red-brown to red-black in color, lusterless, with fine reticular surface. Plant flowers in May-August, bears fruits in July-September. Minimum temperature for seed germination is 3-4°C. Seedlings appear in spring, in forest zone during all vegetation period. Seedlings that appear in autumn usually do not over-winter. Maximum productivity is up to 20,000 seeds/plant. Seeds have high germinability, germinate from depth of no more than 5-6 cm, and remain viable up to 6 years in soil. (Fig. 2).

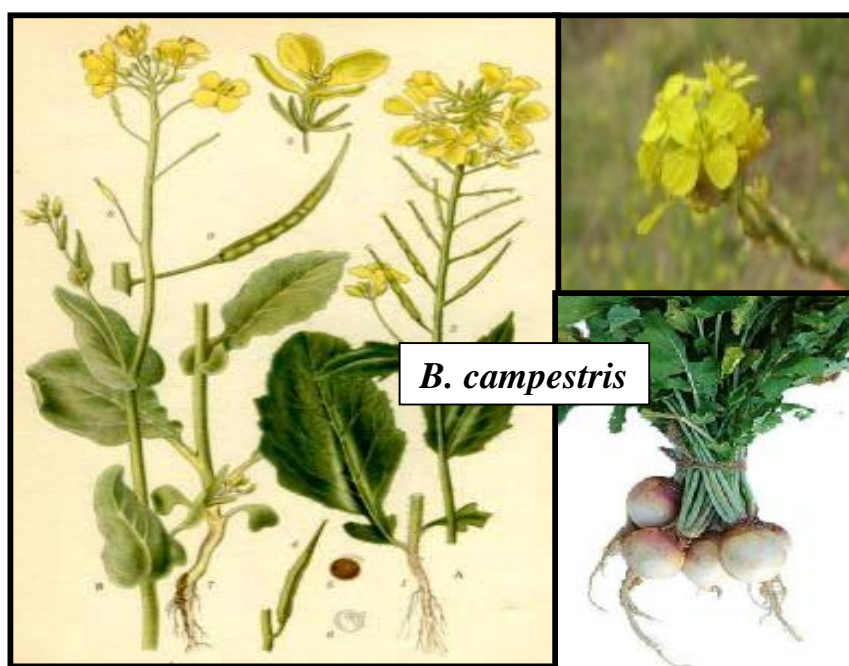


Fig. 2 : *Brassica Campestris*.

3- *Brassica alba* (L.) Bioess

The white mustard grows from 1 to 2 feet in height. It is more or less hairy, with stiff, spreading hairs. The lower leaves, which are 6 to 8 inches in length, are deeply lobed, but

the upper ones are lance shaped. The surface of the leaves is rough hairy. The light yellow flowers are borne in clusters at the ends of the stems from about June to September. The narrow, spreading seed pods which follow are

rough hairy, contracted between seeds, and are about an inch in length, containing

numerous roundish, pale-yellow seeds (Fig.3).

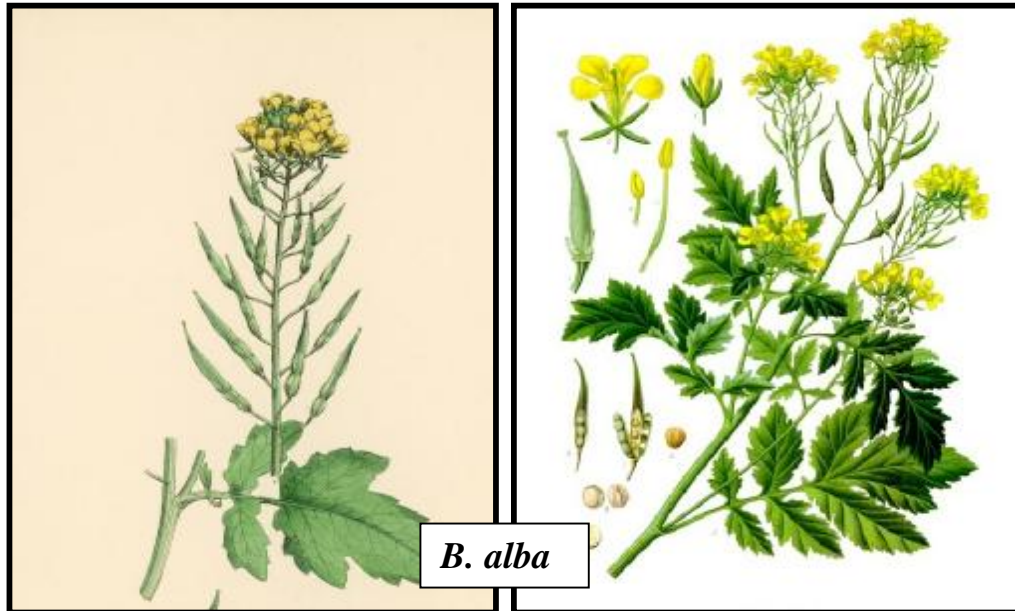


Fig. 3 : *Brassica alba*.

METHODS :

Seeds of the study plants were sown in the pots after sterilized with 0.01% HgCL₂ solution and rinsed using double distilled water. Each pot contains three plants from each species besides the controls were supplied every week with 200 cm³ of full strength Hoagland's solution containing 6 mM of NO³. After germination, salt treatments were applied where concentrations of 0.0, 0.25 and 0.50mM NaCL were added to Hoagland's solution and supplying daily for 20 days following germination. Then, daily supply of usual nutrient solution was provided till harvest and at 25 days after emergence (DAE), each plant was sprayed with 5 cm³ of 10⁻⁵ M GA₃, Shah et al., (2006). The control plants were sprayed with

distilled water and each treatment was done triplicate.

Leaf area (LA), dry mass (DM), chlorophyll content (Chl), stomatal conductance (g_s), photosynthetic rate (PN) and protein content were measured at 50 DAE and the yield together with the different characteristics were recorded at 90 DAE . (LA) was calculated using leaf area meter -Li- 300, (DM) per plant was manifested by drying the plants at 80°C for 24 h, (Chl) was measured as the method described by Mackinney (1941), and (g_s) and (PN) were analyzed using infrared gas analyzer on fully expanded uppermost leaves of 5 plants from each replicate. Protein content was measured colorimetrically using Lowry et al., (1951) method.

Treatment means were compared by analysis of variance using statistical package SPSS 90-5 (SPSS 7.5 for windows, standard version 1996). Least significant difference (LSD) was estimated at 0.05 level of probability.

RESULTS AND DISCUSSION

The results shown in Tables (1 and 2) and illustrated in figures (4 and 5) indicate that the concentrations of NaCl were induced a general degree in all studies parameters. The results tabulated in Table (1) and shown in figure (4) indicate that the parameters LA, DM, ChL, Gs and P N were decreased, in general, with increasing salt concentrations and the influence of decreasing was clearly noticed at the salt concentration of 50 mM. The treatments with GA₃ applications deal with the remarkable increase with the different concerned parameters, especially those of LA in *B.alba*. The former tendency was associated with the *B.napus* and *B. alba*, especially after Gibberellic acid (GA₃) application, whereas the *B. compestris* took another behavior than the other species. However, the GA₃ application of the studied parameters were decreased with increasing salt concentration.

The reduction observed in LA and DM of the salt-treated plants (Table 1) can be attributed to the changes in plant water relations under salt stress, which cause a reduction in meristem activity as well as cell elongation (Dorgham, 1991), thereby inhibiting leaf expansion (Bernstein et al. 1993). Furthermore, high salinity is known to induce ionic stress, which causes premature abscission and senescence of adult leaves, reducing the available photosynthetic area (Munns, 2002). Thus, the observed decrease in DM of the salt-

stressed plants can be traced to the scanty recovery of leaves following limited photosynthesis production. Moreover, these adverse effects of salinity were mitigated through treatment with foliar GA₃ (Table 1). Aldesuquy and Ibrahim (2001) proposed that hormones used during salt stress may reduce water loss rates and cause a concomitant increase in leaf water potential and carbon gain rates. In the present study, GA₃ application might have de-repressed the LA expansion and caused increased DM production in the salt-treated plants.

Leaf chlorophyll content, gs and PN were significantly reduced in the salt-treated plants (Table 1). Singh and Jain, (1981), suggesting that the observed low chlorophyll content could be a result of both decreased synthesis and increased degradation under salt stress. However, treatment of the salt-stressed plants with GA₃ was found to restore normal chlorophyll levels (Table 1). This may will be attributed to the GA₃-generated enhancement of ultra structural morphogenesis of plastids coupled with retention of chlorophyll and delay of senescence caused by the hormone treatment (Arteca, 1997).

The decrease in gs (Table 1) can be explained based on the fact that accumulation of salts triggers a transient water deficit which induces an increase in ABA accumulation and causes stomatal closure (Aldesuquy and Ibrahim, 2001). The obtained results showed that the application of GA₃ restored gs (Table 1) which can be accounted for by an inhibition of ABA through conjugation (Arteca, 1997) leading to a decline in ABA levels (Younis et al., 1991).

As indicated by the lowered levels of leaf chlorophyll and gs (Table 1), the PN of the salt-treated plants was clearly reduced as compared to the untreated control. This can be proposed to be a consequence of the oxidative damage to important photosynthetic cells (Iterbe-Ormaetxe et al., 1998). On the contrary, GA₃ is known to promote PN through enhancement of not only the carboxylase activity of (Yuan and Xu, 2001), but also the rates of cyclic and non-cyclic phosphorylations (Naidu and Swamy, 1995). This is probably because the GA₃-treated plants recovered efficiently from salt stress and exhibited greatly enhanced PN (Table 1).

Exposure to high salinity was found to induce a general reduction in all parameters studied, except seed protein content (Tables 2). In addition, the higher concentration of NaCl applied (50 mM) produced more deleterious effects. Meanwhile, hormone treatment with GA₃ clearly mitigated the adverse effects of salt stress based on the improved parameters studied as compared to the untreated plants, and there was a greater amelioration response in the 50 mM than the 25 mM salt treatment.

All yield parameters, except seed protein content, were substantially lowered due to salt treatment (Table 2). These results are consistent with those of Aldesuquy and Ibrahim (2001) and Afroz et al. (2005). It has been proposed that under salt stress conditions the thickness of the assimilate conducting pathway is reduced (Aldesuquy and Ibrahim, 2001), and leaves start behaving as sinks

rather than sources (Arbona et al., 2005). This causes inhibition of assimilate movement towards the developing reproductive organs, which might be the reason for the observed decrease in pod number, seeds per pod, 1000-seed weight and seed yield per plant (Table 2). On the other hand, these adverse effects of high salinity were alleviated by the hormone treatment, primarily by rejuvenation of the sink potential and enhancement of the duration or rate of dry mass accumulation in developing reproductive organs (Davies, 1995).

The obtained results showed that in contrast to the other yield parameters, seed protein content increased significantly under salinity stress, especially at 50 mM NaCl (Table 2). Similar results reported by Dorgaham (1991) have led to the suggestion that salinity promotes the fixation of inorganic nitrogen into protein, thus favouring protein synthesis. Application of GA₃ under such conditions was found to synergistically increase seed protein content, being in accordance with the results of Singh and Sharma (1996) and Aldesuquy and Ibrahim (2001). GA₃ is known to have a secondary enhancement effect on protein content through the intensification of nitrate reductase activity (Shah, et al. 2006). Stimulation of the enzyme protein synthesis by GA₃ stimulates the overall protein synthesis (Premabatidevi, 1998).

In conclusion, the results of the present study indicate that the ability of GA₃ to ameliorate all adverse effects of salt stress and rescue the productivity of *Brassica* species.

Table (1) : Effect of foliar spray with 10^{-5} M GA₃ on growth and physiological parameters of three *Brassica* species subjected to 25 and 50 mM NaCl treatments. Determinations were done at 50 DAE, - GA₃ without GA₃ spray; + GA₃ with GA₃ spray.

<i>B. napus</i> , Parameters	- GA ₃			+ GA ₃		
	0.0	0.25	0.50	0.0	0.25	0.50
LA(cm ² plant ⁻¹)	315.0	261.2	239.13	330.1	300.1	291.0
DM(g plant ⁻¹)	2.3	1.98	1.63	2.60	2.34	2.21
Chl (g kg ⁻¹ FM)	1.39	1.11	1.01	1.44	1.22	1.11
G _s (mol m ⁻² S ⁻¹)	2.5	1.88	1.01	2.60	2.49	2.21
P _N (μmol (CO ₂ Kg ⁻¹ S ⁻¹))	15.9	14.3	12.00	18.00	14.9	14.00
<i>B. campestris</i> Parameters	- GA ₃			+ GA ₃		
	0.0	0.25	0.50	0.0	0.25	0.50
LA(cm ² plant ⁻¹)	304.0	270.1	281.0	301.0	315.0	300.0
DM(g plant ⁻¹)	2.4	2.0	1.99	2.7	2.8	2.7
Chl (g kg ⁻¹ FM)	1.4	1.2	1.00	1.6	1.7	1.3
G _s (mol m ⁻² S ⁻¹)	2.90	1.99	1.00	2.89	2.99	2.4
P _N (μmol (CO ₂ Kg ⁻¹ S ⁻¹))	16.3	15.2	13.0	15.0	17.0	14.00
<i>B. alba</i> Parameters	- GA ₃			+ GA ₃		
	0.0	0.25	0.50	0.0	0.25	0.50
LA(cm ² plant ⁻¹)	319.0	275.0	249.0	330.0	301.1	280.0
DM(g plant ⁻¹)	2.51	2.16	1.94	2.56	2.35	2.23
Chl (g kg ⁻¹ FM)	1.27	1.21	1.17	1.53	1.42	1.26
G _s (mol m ⁻² S ⁻¹)	2.62	1.92	1.12	2.61	2.51	2.23
P _N (μmol (CO ₂ Kg ⁻¹ S ⁻¹))	16.83	13.32	12.13	18.33	15.20	14.90

L.S.D.at 5% . LA(cm² plant⁻¹) = 18.2
 DM(g plant⁻¹) = 0.19
 Chl (g kg⁻¹ FM) = 0.10
 G_s (mol m⁻² S⁻¹) = 0.11 and
 P_N (μmol (CO₂Kg⁻¹ S⁻¹)) = 1.02

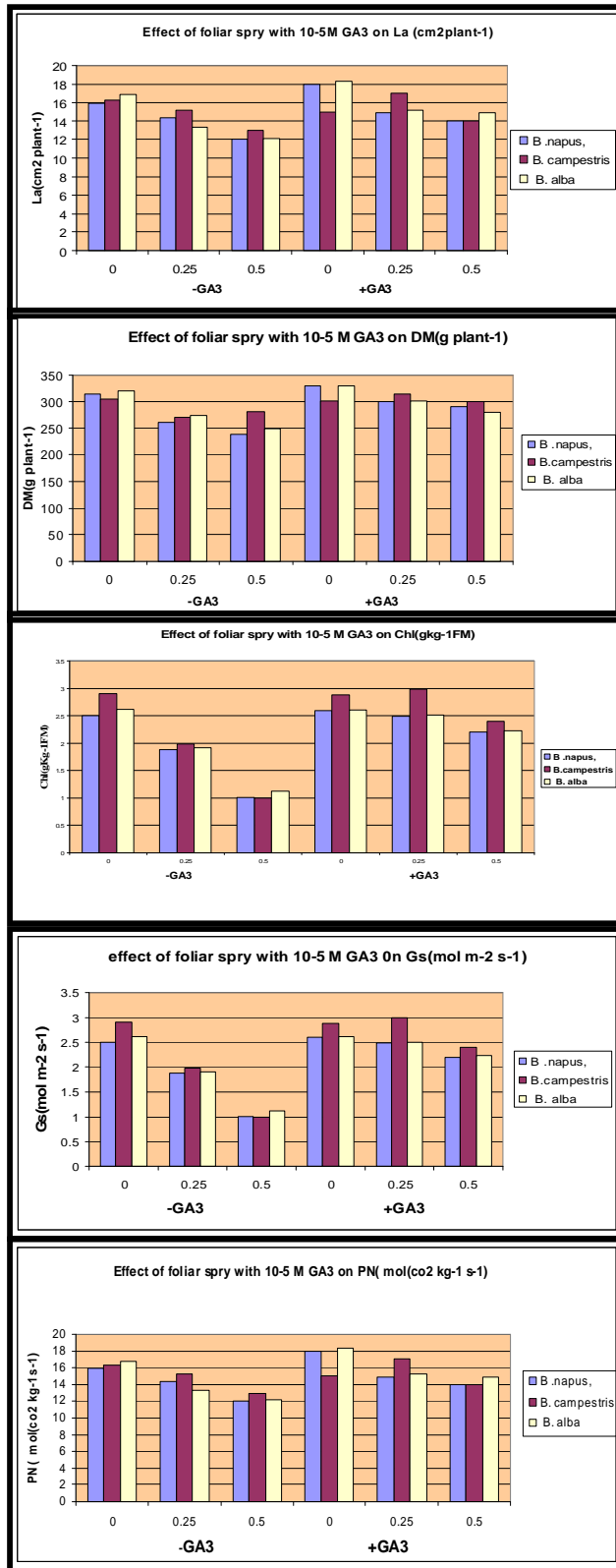


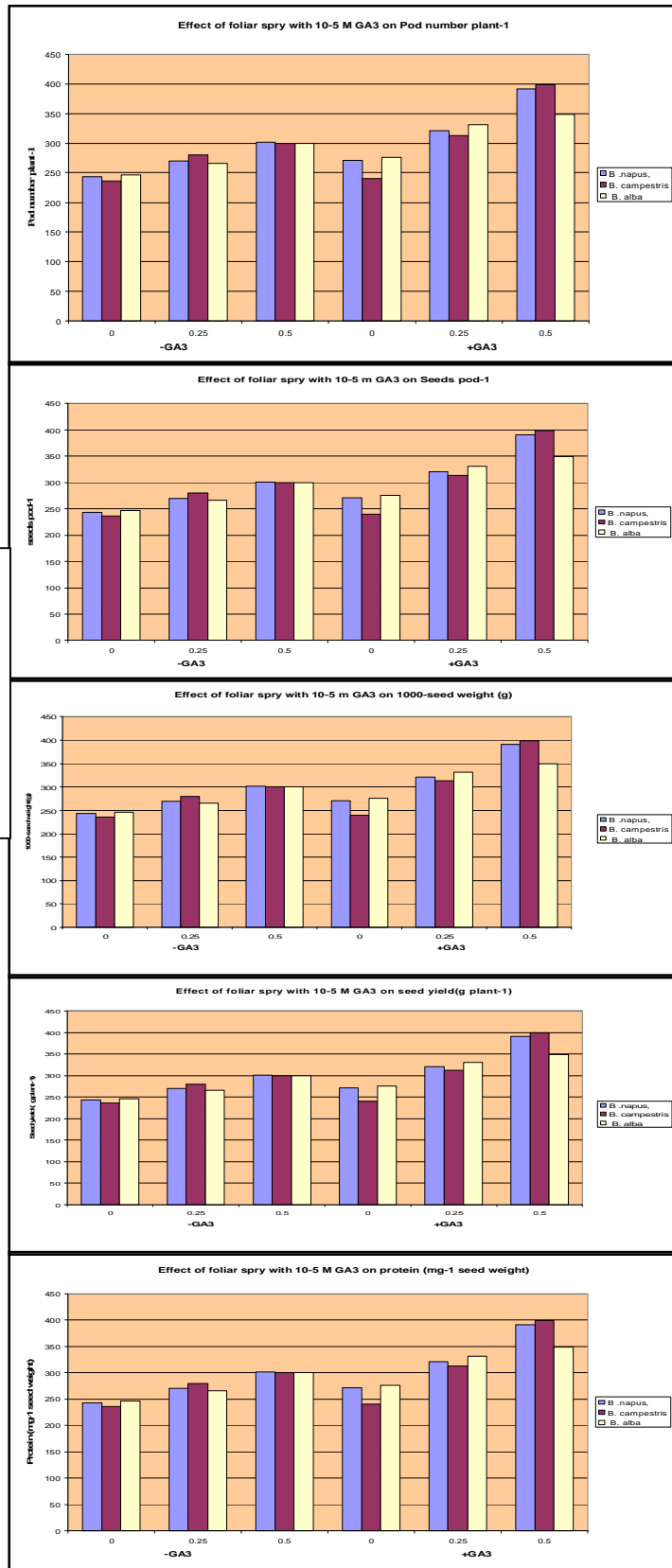
Fig. (4) Effect of foliar spray with 10^{-5} M GA_3 on growth and physiological parameters of three *Brassica* species subjected to 25 and 50 mM NaCl treatments. Determinations were done at 50 DAE, - GA_3 without GA_3 spray; + GA_3 with GA_3 spray.

Table (2) : Effect of foliar spray with 10^{-5} M GA₃ on growth and physiological parameters of three *Brassica* species subjected to 25 and 50 mM NaCl treatment. Determinations were done at 90 DAE, - GA₃ without GA₃ spray; + GA₃ with GA₃ spray.

<i>B. napus</i> , Parameters	- GA ₃			+ GA ₃		
	0.0	0.25	0.50	0.0	0.25	0.50
Pod number plant ⁻¹	176.1	170.0	160.0	182.1	180.1	174.0
Seeds pod ⁻¹	18.01	15.30	14.25	20.31	18.11	14.99
1000-seed weight (g)	4.00	3.80	3.00	4.51	4.00	3.12
Seed yield (g plant ⁻¹)	14.00	11.00	7.20	17.99	13.00	8.10
Protein(mg ⁻¹ seed weight)	243.13	270.0	301.11	271.0	321.0	391.2
<i>B. campestris</i> Parameters	- GA ₃			+ GA ₃		
	0.0	0.25	0.50	0.0	0.25	0.50
Pod number plant ⁻¹	187.0	183.0	173.0	194.0	186.0	183.0
Seeds pod ⁻¹	19.0	17.31	14.21	22.41	19.03	16.41
1000-seed weight (g)	5.00	4.81	3.99	4.99	4.20	3.98
Seed yield (g plant ⁻¹)	15.0	11.31	9.34	19.81	14.31	10.33
Protein(mg ⁻¹ seed weight)	236.0	280.0	300.0	240.0	313.0	399.0
<i>B. alba</i> Parameters	- GA ₃			+ GA ₃		
	0.0	0.25	0.50	0.0	0.25	0.50
Pod number plant ⁻¹	186.91	173.01	169.-0	192.00	184.0	181.0
Seeds pod ⁻¹	18.01	16.11	13.11	21.21	18.11	15.33
1000-seed weight (g)	4.50	3.85	3.06	4.65	4.10	3.17
Seed yield (g plant ⁻¹)	14.56	11.0	7.21	18.33	13.44	8.60
Protein(mg ⁻¹ seed weight)	246.12	266.0	300.10	276.0	331.0	349.0

L.S.D.at 5%. Pod number plant⁻¹ = 7.5
 Seeds pod⁻¹ = 1.12
 1000-seed weight (g) = 10.32
 Seed yield (g plant⁻¹) = 1.65 and
 Protein (mg⁻¹seed weight) = 15.21

Fig. (5) : Effect of foliar spray with 10-5 M GA3 on growth and physiological parameters of three Brassica species subjected to 25 and 50 mM NaCl treatment. Determinations were done at 90 DAE, -GA₃ without GA₃ spray; +GA₃ with GA₃ spray



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الملخص العربي

تأثير إضافة حمض الجبريليك على أصناف من نبات الخردل النامية تحت ظروف ملحية

فايقه منيب الجعلى

قسم النبات - كلية العلوم (فرع البنات) - جامعة الأزهر

أجرى البحث باستخدام ثلاث أصناف من نبات الخردل ذات أصول بريّة مختلفة وهي *B.napus* (L), *B.campestris* (L), *B. alba* (Bioss) وذلك لدراسة تأثير حمض الجبريليك على إنتاجية هذه الأصناف تحت الإجهاد الملحي.

أوضحت النتائج المتحصّل عليها أن تعرض النباتات للإجهاد الملحي بتركيزات ٢٥ ملليمتر و ٥٠ ملليمتر يقلل تلقائياً من بعض صفات النباتات مثل مساحة الورقة والثغور ومعدل التمثيل الضوئي بعد ٥٠ يوم من الزراعة ماعدا البروتين الكلي في البذور والذي أوضح زيادة ملحوظة. والمعاملة بحامض الجبريليك لأصناف نبات الخردل تحت الدراسة أوضحت علاج للتأثير العكسي للأملح وحسنت الإنتاج، علاوة على ذلك فإن المعاملة بحامض الجبريليك كانت أكثر وضوحاً في حالة *B. alba*, *B.napuu*s وكان العكس في حالة *B.campestris* والتي أخذت سلوك مختلف للأصناف الأخرى المدروسة.

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