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ORIGINAL ARTICLE

EXOGENOUS APPLICATION OF GROWTH REGULATORS IN SNAP BEAN UNDER WATER AND SALINITY STRESS

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Salinity and moisture limiting crop productivity due an imbalance between concentrations of auxins, gibberellins, cytokinin, ABA and ethylene. applications of growth regulators to alleviate salinity stress can be an economic and safe alternative to environment. The objective of this study was to determine the effect of salinity (0, 1000 and 2000 ppm of NaCl), soil moisture (30 and 60%) and exogenous application of growth regulators (control, 5 mL L^{-1} at the Beginning of flowering and 5 mL L^{-1} in vegetative stage + Beginning of flowering) and the combinations of three factors in snap bean (*Phaseolus vulgaris* L.). Concentrations of 2000 ppm reduced biomass and pod production in 35.5 and 45%, respectively. The humidity had no effects in biomass production and pod number. With the application of growth regulators in the vegetative stage + beginning of flowering, the weight and number of pods were increased. The greater biomass and yield was observed with 0 ppm, 60% humidity and application of growth regulators in the beginning of flowering. However, with: 1000 ppm, 30% of humidity and application in B. flowering, the biomass production was statistically similar. Our conclusion suggests that the application of growth regulators can be economical and easily applicable for farmers.

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Salinity and moisture are the major factors limiting crop productivity in the world (Levitt, 1980; Christiansen and Lewis, 1982). The causes of soil salinity are often associated with the origin of the soil and agricultural practices, such as using water with high concentrations of dissolved salts (Pessarakli and Szabolcs, 1999; Jacoby, 1999). Most saline soils are found in areas where moisture is limited, bringing two sources of stress.

The effects of salinity on crops are an imbalance between hormone levels. Concentrations of auxins, gibberellins and cytokinin are reduced; in contrast, ABA and ethylene are increased. When salinity conditions are prolonged reductions in growth, the death of organs (leaves, flowers and fruits) and ultimately death can be observed (Mussel and Staples, 1977; Hale and Ocurtt, 1987; Jones *et al.*, 1989).

Among the possible solutions to grow in saline soils has been tested with the introduction of species resistant to salinity like *Atriplex sp., Medicago sp., Trifolium sp., Puceinellia sp., Chenopodium sp. y Kochia.* The creation of genetically modified species to tolerate salinity is a trend that has increased recently (Herdt, 2007). However, there is much controversy over the loss of local varieties and the high cost of inputs and research.

Another possible solution to reduce the effects of stress on plants is the application of exogenous growth regulators, especially those that delay leaf senescence (cytokinins), to prevent the abortion of fruits (auxins and gibberellins) and increase the area leaf (gibberellins) (Belakbir *et a.,* 1998; Bultynck and Lambers, 2004; Vlcˇkova´ *et al.*, 2006).

Exogenous applications of growth regulators to alleviate salinity stress can be an economic and safe alternative to environment. However, the growth regulators have a threshold limited action; under severe stress conditions can accentuate the damage. Actually in a few studies has been studied all the cycle life, generally only germination and seedling are studied (Nieman and Bernstein, 1959; Stuart and Cathey, 1961; Younis *et al.,* 1994; Sibole *et al.,* 1998; Montero *et al.*, 1998; Reddy and Iyengar, 1999; Suleiman *et al.*, 2002; Bayuelo-Jiménez and Ochoa, 2005, 2005b).

The objetive of this study was to determine the effect of salinity, soil moisture and application of exogenous growth regulators as well as the interactions between these factors in snap bean cv. “Strike”.

Materials and Methods

The study was conducted in a greenhouse of the Colegio de Postgraduados, Montecillo campus (19 ° 29 'N and 98 º 53' W and 2250 meters above sea level) during the spring of 2007. Plastic pots 24 cm high and 28 cm in diameter were used.

The snap bean variety used was the "Strike" with growth type I, legumes generally do not tolerate salinity or water deficit, so it may be a good model for studying these types of stress (Nieman and Bernstein, 1959; Levitt, 1980; Montero *et al*., 1998; Pessarakli, 1999; Bayuelo-Jiménez and Ochoa, 2005, 2005b).

We evaluated three concentrations of NaCl in the irrigation water (0, 1000 and 2000 ppm), two levels of soil moisture (30 and 60%) and three stages of application of growth regulators (control, beginning of flowering and Vegetative + B.F.). We used nine replicates for each treatment, the experimental design was completely random with a factorial arrangement.

Concentrations of NaCl in water

The application of salinity treatments was conducted by the irrigation water. For the control group (0 ppm) using distilled water. Two groups were adjusted to concentrations of 1000 and 2000 ppm. The initial conditions of electrical conductivity and soil pH were evaluated on the basis of the saturation paste extract (E.C. = 1.7 dS m^{-1}, pH = 7.3).

Determination of moisture levels

The determination of moisture made on 100% of field capacity and 0% by weight of dry soil (48 hours, 105 º C). From these data we used the following equation:

DSW=WSW*% desired100+1

DSW=dry soil weight (48 hours, 105° C)

WSW=wet soil weight (field capacity)

Initially the pots were irrigated to field capacity, when the weight of pots was reduced to desired weight (30 or 60%) due evapotranspiration, the pots were irrigated newly to field capacity.

Application of growth regulators

The growth regulators used were a commercial mixture (Vitarise ®), which has as its principal components gibberellins, auxins and cytokinins

(Table 1). The application of growth regulators was foliar, concentrations used were 0 mL L^{-1} (control) and 5 mL L^{-1} at the beginning of flowering (BF), treatment with a double application (vegetative + beginning of flowering) was added. The determination of concentrations and stages of implementation was based on previous studies (Torres-García *et al.,* 2007).

At the end of the experiment (85 days), the number and weight of pods and total biomass was evaluated. Plants were separated into their different organs (stems, leaves and pods) and placed in paper bags and introduced in a forced air oven at 80 º C for 72 hours.

The data were analyzed using the PROC GLM procedure in SAS, under the design completely randomized with factorial arrangement, when the results had a significative difference, the mean comparison test Tukey was realized. An analysis of variance of all the treatments was made to determine which combination increased the biomass production and yield.

Table 1. Percentage composition of commercial growth regulators (Vitarise®). According to the product label.

Active compounds	% weight
Gibberellins	500 ppm
Auxins	500 ppm
Cytokinin	200 ppm
Cysteine	500 ppm
Thiamin	1100 ppm
Inositol	200 ppm
Total nitrogen	9.0%
Calcium	0.8%
Zinc	2.0%
Sulfur	0.8%
Citric acid	0.4%

Table 2. Final terms of soil salinity in the pots (E.C. and pH) in relation to the application of different concentrations of NaCl in irrigation water.

NaCl (ppm)	E. C. (dS m^{-1})	pH
0	2.3	7.21
1000	15.22	7.26
2000	21.74	7.21

Table 3. Effect of the factors: salinity, humidity and growth regulators on the pods number, biomass and pods production (g plant^{-1}) in snap bean (*Phaseolus vulgaris* L.) cv. "Strike".

Factors		Pods number	Pods g plant^{-1}	Biomass
	ppm			
	0	6.17a	1.45a	3.83a
Salinity	1000	6.54a	1.38a	3.57a
	2000	5.87a	0.80b	2.47b
HSD		1.80	0.37	0.94
	%			
Humidity	30	5.74a	1.07b	2.98a
	60	6.64a	1.35a	3.60a
HSD		1.22	0.25	0.65
	Stage			
Growth Regulators	Control	6.80a	1.29ab	2.93a
	Beginning flowering	4.81b	0.99b	3.16a
	Vegetative + BF	6.96a	1.36a	3.78a
HSD		1.80	0.37	0.95
	Salinity * Humidity	NS	*	NS
Interactions	Salinity*GR	NS	NS	NS
	Humidity*GR	NS	NS	NS
	Salinity*Humidity*GR	***	***	***

*, *** P≥0.05 y 0.001, respectively; NS=No significative difference (Tukey 0.05)

Table 4. Number of pods, biomass and pods production (g plant^{-1}) in function of salinity, humidity and application of growth regulators (GR) in snap bean (*Phaseolus vulgaris* L.) cv. "Strike".

Salinity (ppm)	Humidity %	GR Stage	Pods Number	Pods g plant^{-1}	Biomass
		C	6.22abc	1.60abc	**3.24abc**
	30	B.F.	1.78bc	0.30cd	**1.69bc**
0		Veg + BF	6.67abc	1.34abcd	**4.46ab**
		C	7.00abc	1.66ab	**2.90abc**
	60	B.F.	8.00ab	2.02a	**6.18a**
		Veg + BF	7.33abc	1.77ab	**4.48ab**
		C	4.00abc	0.83abcd	**1.73bc**
	30	B.F.	7.22abc	1.68ab	**4.31ab**
1000		Veg + BF	8.78a	1.68ab	**3.99abc**
		C	7.56abc	1.49abc	**3.67abc**
	60	B.F.	5.00abc	1.21abcd	**3.66abc**
		Veg + BF	6.67abc	1.4abcd	**4.08abc**
		C	6.67abc	1.02abcd	**2.93abc**
	30	B.F.	5.78abc	0.64bcd	**2.44bc**
2000		Veg + BF	4.56abc	0.53bcd	**2.08bc**
		C	9.33a	1.13abcd	**3.12abc**
	60	B.F.	1.11c	0.08d	**0.68c**
		Veg + BF	7.78ab	1.42abc	**3.61abc**
	HSD		6.6	1.34	3.48

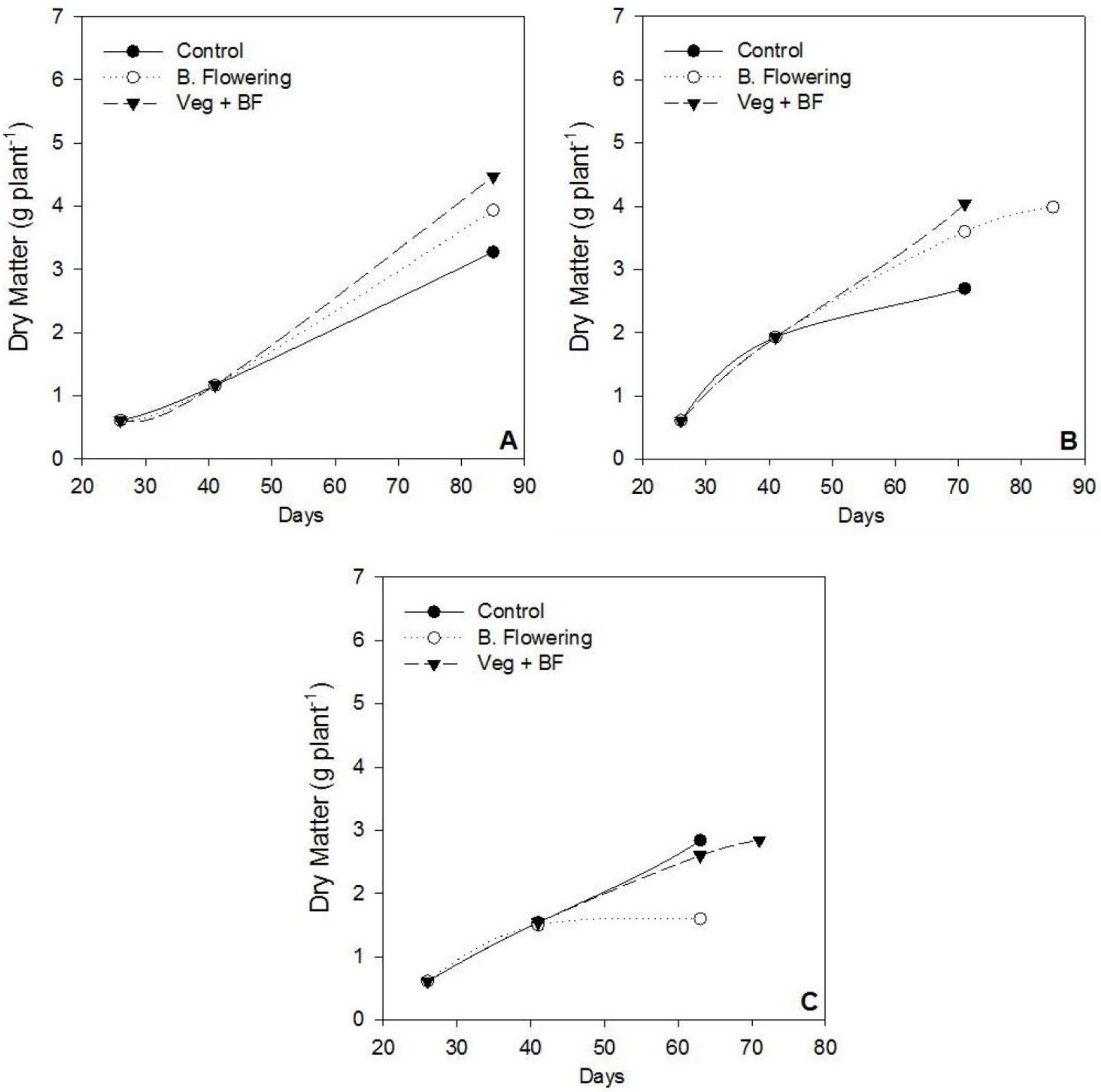

Figure 1. Dynamic of biomass production (g plant^{-1}): A) control (0 ppm), B) 1000 ppm and C) 2000 ppm.

Results

At the end of the experiment, the E.C. of soil of the treatments under salinity increased significantly (Table 2). In the case of pH were not found wide variations.

Salinity

The salinity factor showed significant differences ($P \geq 0.05$) in biomass production and pods depending on the concentration of NaCl. The effect of salinity was not significant at concentrations of 0 and 1000 ppm, the differences were found by increasing the concentration to 2000 ppm, where the death of nearly 50% of the plants was observed, this reduced the production of biomass and yield up to 35.5 and 45% respectively. Regarding the number of pods produced no changes were found, which suggests that the size was reduced.

Humidity

The humidity factor had no significant differences in biomass production or the number of pods. However, the differences were found in the production of pods where treatment with 60% humidity reached the highest performance.

Growth regulators

The exogenous applications of growth regulators not have significant effect on biomass production. On the other hand differences were found in weight and number of pods where the application of growth regulators in the vegetative + beginning of flowering increase its production significantly (Table 3).

Interactions

The interactions between the factors studied showed that only salinity and moisture interact in the production of pods, for greater interaction, differences were highly significant for all variables.

Finally, when analyzing all treatments studied, we found that the greater accumulation of biomass and yield were observed by the application of 0 ppm, 60% humidity and application of growth regulators at the beginning of flowering. This means that the snap bean show sensitivity to salinity and water deficit. But even in stressful conditions: 1000 ppm and 30% humidity, with the application of growth regulators in the beginning of flowering the biomass production was statistically similar (Table 4).

A part that is important to mention is that in the treatments subjected to 1000 and 2000 ppm and low humidity (30%) showed a reduction of life cycle, characterized by falling leaves and fruits. With the application of plant growth regulators in vegetative stages + the beginning of flowering was delayed by a week until senescence (Figure 1).

Discussion

The Snap bean cv. "Strike" was found to be sensitive to the factors studied of salinity and humidity, as is reported for most legumes cultivated, the pod production was the most sensitive parameter (Khafagi *et al.,* 1986; Sibole *et al.,* 1998; Pessarakli, 1999).

The values of E.C. at the end of study (21.7 dS m^{-1}) were above those reported for tolerance of bean, resulting in some treatments with 1000 and 2000 ppm the death of some individuals or anticipated senescence was presented. Subbarao and Johansen (1999) mentioned that in an interval of 1.3 to 3.2 dS m^{-1} production of beans is reduced from a 10 to 50%. The pH was not limiting for plant development because the source of saline was NaCl, which causes more problems of water deficit and toxicity by the presence of Cl^- (Levitt, 1980).

The increase in soil salinity by irrigation with saline water resulted in reductions in growth, yield and life cycle probably caused by increased concentrations of ABA and ethylene as inhibiting growth and promoting the abortion of the fruits and leaves (Mussel and Staples, 1977; Levitt, 1980; Hale and Ocurtt, 1987), although there were no differences in the number of fruits produced, differences in weight were observed.

Soil moisture and application of growth regulators did not affect biomass production but if the number and weight of pods. Low levels of humidity to which the plants were subjected during the reproductive stage modified the allocation of resources, decreasing the translocation of reserves into the pods and in some plant growth regulators, pod production was increased when the plants received applications in the vegetative + beginning flowering stages, caused mainly by increased production of biomass during the vegetative stage, which served as a source for allocating resources to the formation of pods (Yang *et al.,* 2003).

Of all the interactions tested, only the interaction between salinity and moisture for the production of pods was statistically significant as reported for Aldesuquy and Ibrahim (2001), Medhi and Borbora (2002). They mentioned that under conditions of salinity stress on reproductive structures are the most affected. Often the stress increase or decrease according to available soil water to keep out the salts of the radical zone and prevent absorption.

The analysis of all combinations studied showed that the salinity tolerance of some treatments could be caused by the addition of exogenous growth regulators such as gibberellins, auxins and cytokinins that inhibited the synthesis of ABA and ethylene, increased fruit size and delayed leaf senescence (Katz *et al.,* 1978; Khafagi *et al.,* 1986; Bayuelo-Jiménez, 2005, 2005b; Boutra and Sanders, 2001). Although production of pods under stress and application of growth regulators did not have a dramatic increase, if it was possible to increase the life cycle of plants and match production under limiting conditions, which opens a large window possibilities for future studies with other crops that show increased resistance to limiting conditions.

The application of growth regulators in agriculture has been left to one side by the use of molecular techniques, but here it was shown that this technology can be economical and easily applicable for farmers.

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