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CHITOSAN INFLUENCE ON THE AMINO ACIDS AND PROLINE CONTENT IN THE PLANTS UNDER DROUGHT STRESS

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ABSTRACT

Some of the most important results we came to in this study are, the clarity of the impact of drought stress on the amino acids content in the plant stem , and this will be increased by rising drought stress. It was also found that there is a role for amino acids in osmotic adjustment, and in the end it was concluded the role of the Chitosan and its obvious impact in the increase of the total amino acids. As well as the impact of Proline on the plants leaves where its concentration raised in the plants groups treated with Chitosan compared to the untreated plants groups, and the reason for this is that Chitosan is working to raise the concentration of the amino acid in some of those plants.

INTRODUCTION

The amino acids are simple nitrogen organic compounds which are water dissolvable to different degrees and its origin came from metabolize sugars and nitrogen. Amino acids play important roles within the plant as they are included in proteins composition, they also modify the osmotic inside cells when plant is exposed to environmental stress, as it was found, the amino acids has an important role in organizing the osmotic process in tomato plant that is under salt stress (Franco and Garcia, 2010). Also when plants are exposed to drought stress its initial response is by maintaining the cells full and this was done by assembling what is known by the osmotic organizers such as Proline , sucrose , sugars dissolved , glycine and other solutions in the cytoplasm in order to maintain the continued water absorption from the soil, a good example is wheat plants when exposed to drought stress, It collects osmotic organizers such as Proline which belongs to the Amino acids family for resisting drought stress (Nayyar and Walia , 2003) . Also found the production and accumulation of free amino acids in the maize tissues during the drought, especially proline(Caballero *et al.* , 2005). Both Ali-Ahmad and Basha (1998) also noted that an increase in the free amino acids content in the peanut plant leaves when exposed to drought stress, not only that, but the drought stress increases the amino acids content or some of the root nodes. It was also found that the Asparagine amino acid is an essential to the root nodules of soybean plants well irrigated, however, an increase in this acid content up to six times higher was found in the root nodules exposed to draught stress (Ramos *et al.* , 2005). It was also noted an increase in the free amino acids content in two plants pulses of all plant parts exposed to draught stress, especially in the leaves and the root nodules (Ashraf and Iram , 2005).On the other side, we found that drought stress practised a change in amino acids metabolism (Barnett and Naylor , 1966). As we said previously the drought stress increases the amino acids content ,however, it was found that drought stress does the exact opposite in other cases, this is

confirmed by Chen *et al.* (1964) as they have noticed that a decrease in some of the amino acids concentration in the plant under draught stress.

As for the Chitosan effect (Chibu and Shibayama , 2001) have found that soil treated with Chitosan has a positive effect in terms of adding amino compounds to the soil, when Chitosan is decomposed in the soil, thus benefiting plants.

Proline is considered as a free amino acids, which increases its concentration in the plant when exposed to stress. Many studies has shown that Proline can increase its content in the plant actually under ice stress (Charest *et al.*, 1990 and Come , 1992), or under the influence of salinity (Diaz *et al.*, 1999 and Lin-Chuanchi *et al.* , 2002), and also when exposed to draught stress conditions. It was found that Proline increases its level in the potatoes leaves exposed to draught stress (Knipp and Honermeier , 2005), as well as in the tomato leaves (Nahar and Gretzmacher , 2002) as a result of being put under draught stress.Proline content also increased in the okra plant as when is under draught stress (Amin *et al.*, 2009), A high Proline content was also found in the Protoplast of the *Nicotiana rustica* plant exposed to draught stress (Pahlich *et al.*, 1983) and another study conduced by (Aziz and Khan , 2003) proved that the high content of Proline is linked to the increase of draught stress on plants. Also drought stress is considered as a catalyzer for the accumulation of proline in plant tissues (Unyayar *et al.*, 2004), and the Proline plays a major role in the osmotic modification in the potatoes plant Büssis, and Heineke (1998), while proline forms a small part of the total concentration in the solutions that Organize the osmotic process in the tomatoes plants (Perez-Alfocea *et al.*, 1993). One of the tasks proline performs is the protection of membranes as well as proteins from the impact of the high concentrations of the non organic ions and the extreme temperatures (Santoro *et al.* , 1992).) About the impact of the Chitosan on proline concentration in plants, an increase in the proline concentration was found in the cucumber leaves exposed to low temperature stress and treated with the Chitosan.

The present experiment was conducted to study the effect of chitosan application on quality of amino acids and proline content of *Phaseolus vulgaris* under drought conditions

MATERIALS AND METHODS

Plant substance used:

In this study bean seeds classified Super strike (*Phaseolus vulgaris* Super stryke) was used, it was obtained from a company called Asgrow vegetable seeds imported by the agricultural machinery and materials company.

Chemical used:

In this study (chitosan) substance was used, it was obtained from the company (Paris, kentuky 40361) where irrigation water was added at the concentration of 0.5% (i.e. 0.5 gm / 100ml of water) and plants was treated in the beginning of the experiment .

Agriculture method:

Seeds were sterilized by sodium hypochlorite at (5%) of concentration for 3 minutes, then washed with distilled water several times and soaked in distilled water for one hour. At the end of the soaking period seeds were put in Petri dishes on Watman No. 1 (Whatman No1) wet filter paper of 9 cm diameter, and transferred directly to incubator at a temperature of 25 C for twenty-four hours. Then planted in plastic pots of eight groups, (26 cm of diameter, and 23 cm of height) ,each one filled with equal amounts of soil, so that it contains all the potted 15 seeds. Mainly we are divided the pots to two groups . First and second group without and with chitosan respectively . Irrigation was carried out according to usual practice by adding equal amounts of water to remain the water holding capacity at 100 %(control) , 45% , 25% and 15% . Plants were exposed to normal day length with natural illumination in the greenhouse to allow plants exposure to environmental conditions of natural lighting heat intensity and moisture. After 7 days we have reduced the plants density so that only 10 seedlings were left in all pots and each group contained 10 pots, and then plants underwent to the irrigation system specified when soil group were 100%dry.

Sampling:

Samples were taken at the vegetative stage (21-day old) and the content of amino acids and prolin were estimated.

Extraction of amino acids:

The Rosein (1957) method was used to extract amino acids, and 0.1 g of dry tissue was weight and added to 20 ml of the ethanol at the concentration of 70% and shaken for one hour and left to rest overnight then shaken again for half an hour and filtered. The amino acids were measured in the filtrate.

Estimation of amino acids:

Amino acids were estimated by taking 1 ml of the sample and plus 0.5 ml of Ninhydrin solution and 0.5 ml of balancing solution. Then placed in a water bath for 15 minutes and cooled down and add to it 5 ml and transferred directly to incubator at a temperature and measured using a spectrophotometer) device in a control sample (replaces the sample with distilled water) at the wave length of 570 nm making a standard curve together with the aspartic acid.

Preparation of Ninhydrin solution:

50 mg of Cadmium Acetate was added to 5 ml of acetic acid solution (1 ml acetic acid + 4 ml distilled water), then added to the previous solution 1 gm of Ninhydrin, then 45 ml acetone 50% was added.

Preparation of the balancing solution:27 grams of Sodium Acetate was added to 20 ml of distilled water and 5 ml of vinegar ice acid, and the volume size was completed by adding 75 ml of distilled water to reach the PH (3.5-4.5).

Proline Extraction and estimation: The method of Bates *et al.* (1973) was followed and all first steps were done in the cold (snowy bath) and all solutions used must be cool. 0.5 g of fresh leaves were taken and crushed by the crushing tool in 10 ml of Sulphosalicylic acid. The result was filtered

through Whatman paper No. 2, then 2 ml of the filtrate solution was taken and add to it 2 ml of Ninhydrin acid, as well as 2 ml of Glacial acetic **acid,** then the tubes were placed in a hot bath for an hour at 100°C. In order to stop any further reaction, tubes were taken after incubation to the snowy bath, and 4 ml of toluene was added, and using absorbing tool the rack color scheme separated from the fluid and the reading was recorded at 520 nm using the UV /VIS spectrophotometer.

Preparation of Acid Ninhydrin solution:1.25 g of Ninhydrin was dissolved in 30 ml of Glacial acetic acid with heating and stirring and added to it 20 ml of phosphoric acid and the solution was kept at low temperature of 4°C and used within 24 hours.

All data were analyzed statistically using one-way ANOVA, followed by Duncan's Multiple Range Test using COSTAT software. The values presented are all mean for three samples in each group.

RESULTS AND DISCUSSION

As shown in the results in Table 1 and Figure 1 , we see clearly the impact of drought stress on the amino acids content of the shoot, as we have found that the amino acids content in the plants roots under water stress is significantly less compared to amino acids content in the plants legs in the controlling group (100%), and when we focused on the amino acids content in the plants roots under water stress, we found that , the content increases when drought stress increases and this increase in amino acids have a role in modifying osmotic process inside the plant cells. An increase was found in the free amino acids content in the pine root as a result of the change in water relations within the plant cells when exposure to drought stress (Cyr *et al.* , 1989), again the free amino acids level increased in corn from 32 mM to 39 mM when exposed to an average stress, while the level of amino acids increased from 29 mM to 45mM when a plant exposed to severe drought stress (Jones *et al.* ,1980), yet again (Jones *et al.*, 1980) have found an increase in the following amino acids: Aspartate , Alanine and Glutamate in the corn leaves exposed to drought stress.The following amino acids were also found: glycin betain , glutamin and aspargin and others with high concentrations in the stressed tissues (Drossopoulos *et al.* ,1985). When we see groups treated with chitosan, we note a significant decrease in the amino acids content in the plant roots under drought stress, this is when compared to the of the amino acids content in the plants shoots of the controlling group. However, when we see the of amino acids content in plants under stress we have found that it content rises as drought stress increased, and this is because of the role of amino acids in modifying the osmotic process, as it was found that there are a number of processes that occur in the plant as a result of drought stress, including the production of plant compounds that work to modify the osmotic process such as Proline and glycine betaine (Wang *et al.* , 2003) and (Hasegawa *et al.* , 2000) which are amino acids, but it must be noted the positive role of chitosan in raising the amino acids content, both in the control group or groups under drought stress . Looking back at Table 1 and Figure 2 , we found that , a decrease in the amino acids

content in the plants roots exposed to drought stress and this decrease is significant when compared to the amino acids content in the plants roots in the controlling group, we have also found that groups treated with Chitosan behave in a similar way, and this is due to the impact of acids content in the roots, as (Xu and Zhou , 2004) drought stress on the amino have found a decrease in the amino acids content in the plants tissues exposed to drought stress. But when comparing groups treated with Chitosan in terms of roots content of the amino acids to the corresponding ones untreated with Chitosan, we found a superiority of amino acids content in the groups treated with Chitosan, and this is due to the role of Chitosan in raising of amino acid content in the roots. The study conducted by scientists (Abdel-Mawgoud *et al.* , 2010) has indicated that the application of Chitosan on strawberry plants has increased the total acids in the fruit. When we see the results of Table 2 and Figure 3 , we find a significant increase in the Proline concentration in the beans leaf under drought stress, when you compared with the Proline concentration in the plants leaves in the controlling group, and this was noticed in the plant groups untreated with the Chitosan and exposed to drought stress as well as the groups treated with Chitosan. This result is similar to what (Johari-Pireivatlou ,2010) has reach in his research of the increase of Proline content in wheat plant under drought stress, also all of (Naylor ,1972) and (Hsiao , 1973) have reached in their research an increase in the free amino acids content of the plant, specially the Proline, which reaches its concentration in some cases up to 10 and 25 times and to 1% of the dry weight of the leaves, this was seen in plants under drought stress.

But when comparing plants groups treated with Chitosan with the ones untreated in terms of Proline concentration, we found an increase in Proline concentration in plants groups untreated with Chitosan against the ones treated with Chitosan, and this is due to the fact that Chitosan may work to raise the concentration of amino acid other than the Proline, such as glycine betain for example.

CONCLUSION

It can be concluded that chitosan may play an important role in the growth and productivity of *Phaseolus vulgaris* plants grown under water stress conditions, perhaps because they can produce various metabolites as amino acids with special regrds proline and thus more water become available to plants for better growth and production.

Level of irrigation	Shoot amino acid		Root amino acid	
	Without chitosan	With chitosan	Without chitosan	With chitosan
100%	81.49±.014	85.99±.014	56.00±.000	61.89±.014
45%	72.29±.014	74.50±.014	46.49±.014	57.99±.014
25%	75.99±.014	78.99±.014	36.99±.014	40.49±.014
15%	78.99±.014	81.51±.014	36.99±.000	37.49±.014

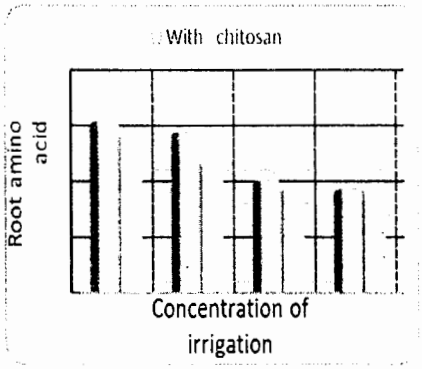

Figure 1

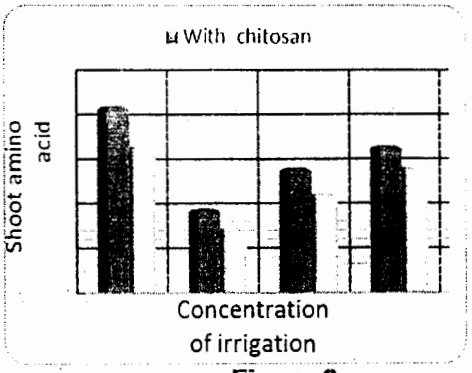

Figure 2

Table (2): Effect of different drought stress concentration in the culture field either alone or in combination with (0.5gm chitosan/100ml water) on the Proline concentration of 21-day-old *Phaseolus vulgaris* plants. Each value is the mean of 3 samples calculated as µg/gm dry weight .

Level of irrigation	Proline concentration	
	Without chitosan	With chitosan
100%	0.03±.014	0.02±.000
45%	0.06±.014*	0.05±.000
25%	0.09±.014*	0.05±.014
15%	0.012±.014*	0.06±.014

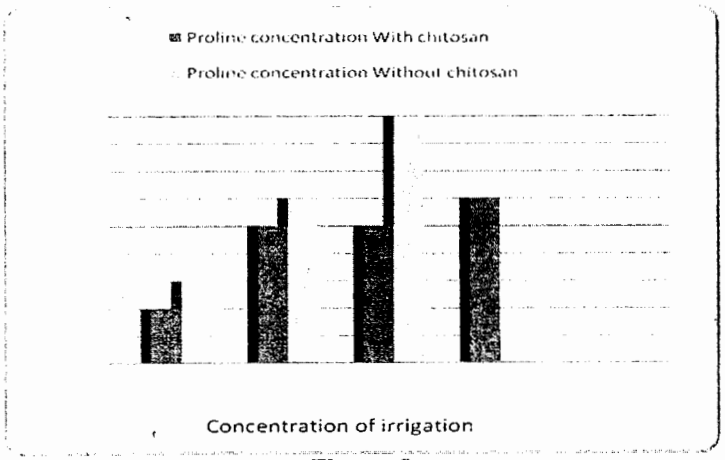

Figure 3

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تأثيرالكيتوزان على الأحماض الأمينية و البرولين في النباتات المعاملة تحت إجهاد الجفاف

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استخدمت في هذه الدراسة بذور الفاصوليا صنف سوبر ستراياك (*Phaseolus vulgaris* Super Stryke) حيث تم الحصول عليها من شركة Asgrow vegetable seeds المستوردة بواسطة شركة الآلات والمواد الزراعية. و ذلك بهدف دراسة تأثير الكيتوزان على الأحماض الأمينية و البرولين في النباتات المعاملة تحت إجهاد الجفاف . تم تعقيم البذور بواسطة هيبوكلوريت الصوديوم تركيزه (٥%) لمدة ٣ دقائق.جرى غسيل البذور بالماء المقطر عدة مرات ثم تم نقعها في ماء مقطر لمدة ساعة. وبعد انتهاء فترة النقع وضعت البذور في أطباق بتري على ورق ترشيح واتمان رقم ١ (Whatman No1) قطر ٩سم مبلل، ونقلت مباشرة إلى حضان عند درجة حرارة ٢٥ م لمدة أربعة وعشرين ساعة.ثم زرعت في ثمان مجموعات في أصص بلاستيكية (قطر كل منها ٢٦سم و ارتفاعه ٢٣سم) مملوءة بكميات متساوية من التربة،بحيث احتوى كل أصيص ١٥ بذرة،ووضعت الأصص داخل صوبة تسمح بتعريض النباتات لظروف البيئية الطبيعية من شدة إضاءة وحرارة ورطوبة. .ومن ثم تم ري كل مجموعة أصص بواحد من نظم الري المختارة (١٠٠%، ٤٥%، ٢٥% ، ١٥%) منفردة مرة و مضافا اليها الكيتوزان مرة أخرى حيث أضيفت لماء الري وكان تركيزها ٠.٥% (أي ٠.٥جم كيتوزان/١٠٠مل ماء) وتم معاملة النباتات في بداية التجربة. بعد ٧ أيام خفضت كثافة النباتات بحيث ترك في كل أصيص ١٠ بادرات وبحيث كل مجموعة احتوت على ١٠ أصيص،ومن ثم خضعت النباتات لنظام الري المحدد عند جفاف تربة المجموعة ١٠٠%. و تم تحليل البيانات احصائيا .

و قد كانت النتائج المتحصل عليها وضوح تأثير إجهاد الجفاف على محتوى الأحماض الأمينية في الساق النبات، وهذه الزيادة بسبب ارتفاع اجهاد الجفاف. ووجد أيضا أن هناك دورا للأحماض الأمينية في تعديل الاسموزية، وفي النهاية تم التوصل إلى دور الكيتوزان وأثره الواضح في زيادة من المجموع الكلي للأحماض الامينية . فضلا عن تأثير البرولين على اوراق النباتات حيث يترك تركيزه أثير في مجموعات النباتات المعاملة مع الكيتوزان بالمقارنة مع مجموعات النباتات غير المعاملة ، والسبب في ذلك هو أن الكيتوزان يعمل على رفع تركيز الأحماض الأمينية في بعض تلك النباتات.

و توصى هذه الدراسة بإمكانية استخدام الكيتوزان كأحد المواد التى تساعد نباتات الفاصوليا لمقاومة الاجهاد الناتج عن الجفاف .