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Research Article

## PHYSIOLOGICAL PERFORMANCE OF PINTO BEAN CULTIVARS UNDER SALINITY

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**ABSTRACT :** Salinity is one of the major abiotic stresses that adversely affect crop productivity and quality in many arid and semi-arid parts of the world. Effects of four levels of salinity (0, 4, 8 and 12 dS m<sup>-1</sup> NaCl) on performance of three pinto bean cultivars (COS<sub>16</sub>, Talash and Khomain) were investigated in 2010. A factorial experiment based on randomized complete block design with three replications was carried out at the Greenhouse of University of Tabriz, Iran. Ten seeds were sown in each pot filled with 900 g perlite. After emergence, seedlings were thinned and 4 plants were kept in each pot. Results indicated that At the most stages of development, leaf chlorophyll content index (CCI) under severe salinity (12 dS m<sup>-1</sup>) was lower than that under other saline and non-saline conditions. Proline accumulation in leaf tissues increased gradually with the increase of salinity up to 8 dS m<sup>-1</sup> and thereafter slightly decreased. The highest CCI and proline content were found in Talash compared with other cultivars. Root/shoot ratio of COS<sub>16</sub> was higher than that of Talash and Khomain. Flowering and podding were delayed under moderate and severe salinities, but grain yield decreased as salinity increased. The highest and the lowest grain yield per plant were obtained from Khomain and COS<sub>16</sub>, respectively. However, differences in grain yield between Khomain and Talash and between Talash and COS<sub>16</sub> were not statistically significant. It was concluded that pinto bean is a sensitive crop to salinity, but the extent of sensitivity differs among cultivars.

**Keywords:** Chlorophyll content, grain yield, pinto bean, proline.

## INTRODUCTION

Salinity is one of the major abiotic stresses that adversely affect crop productivity and quality. About 20% of irrigated agricultural land is adversely affected by salinity [1]. Salinity influences almost every aspect of the physiology and biochemistry of plants and significantly reduces yield. High exogenous salt concentrations cause ion imbalance, leading to ion toxicity and osmotic stress [2, 3].

The most important process that is affected by salinity in plants, is photosynthesis. Reduced photosynthesis under salinity is not only attributed to stomata closure and reduction of intercellular CO<sub>2</sub> concentration, but also to non-stomata factors. There is strong evidence that salt affects photosynthetic enzymes, chlorophylls and carotenoids [4]. According to yeo and flowers [5], chlorophyll content of salt stressed rice can be described as a function of the leaf laminae reduces net photosynthesis and growth. This decline in photosynthetic activity has been directly resulted in reduction of yield.

Salinity reduces the ability of plants to utilize water and causes a reduction in growth rate, as well as changes in plant metabolic processes [6]. Kaymakanova and Stoeva [7] indicated that reduction of the biomass in beans under saline condition was indicative of several growth limitations. Salinity had adverse effects not only on the biomass, but also on other morphological parameters such as plant height, number of leaves, root length and shoot/root ratio. Proline accumulation is an important physiological index for plant response to salt stress [8]. A large number of plant species accumulate proline in response to salinity stress and that accumulation may play a role in combating salinity stress. Measurement of proline accumulation is also an important criterion for determination of plant tolerance to salt stress [9]. In salt tolerant and relatively salt tolerant plants like , *Beta vulgaris* [10], *Brassica juncea* [11] and alfalafa [12] sharp increases in proline levels were reported under the effect of salinity. All plants tolerate salinity up to a certain threshold level without any yield reduction. Thereafter, an increasing in salinity rate significantly reduces yield [13]. Crop yields decline dramatically when PH of the soil solution exceeds 8.5 or EC value goes over 4 dS m<sup>-1</sup>. If soil EC values were too high, crop yields would be seriously affected [14]. Reduction in crop yield as a result of salt stress has also been reported for sunflower [15], cotton and wheat [16], canola [17].

Common bean is the most important food legume grown worldwide. Although, this crop is sensitive to salinity like many other leguminous crops [18], the physiological responses of that crop to different levels of salinity are not well understood. Thus, this research was aimed to investigate the association of specific physiological traits with resistance to salinity and also to determine the relationship between these traits and grain yield of common bean.

## MATERIAL AND METHODS

A Factorial experiment based on randomized complete block design with three replications was carried out to evaluate yield and yield components of three pinto bean cultivars (COS<sub>16</sub>, Talash and Khomain) under a non-saline (control) and three saline (4, 8 and 12 dSm<sup>-1</sup>NaCl) conditions. Ten seeds were sown 3 cm deep in each pot filled with 900 g perlite, using 40 pots in general. Pots were then placed in the Greenhouse of the Faculty of Agriculture, University of Tabriz, Iran. Tap water and saline solutions were added to the pots in accordance with the treatments to achieve 100% FC. After emergence, seedlings were thinned to keep 4 plants in each pot. During the growth period, the pots were weighed and the losses were made up with Hoagland solution (EC = 1.3 dSm<sup>-1</sup>).

Perlites within the pots were washed every 20 days and non-saline and salinity treatments were reapplied in order to prevent further increase in electrical conductivity (EC) due to adding the Hoagland solution. Days to flowering and days to podding were recorded.

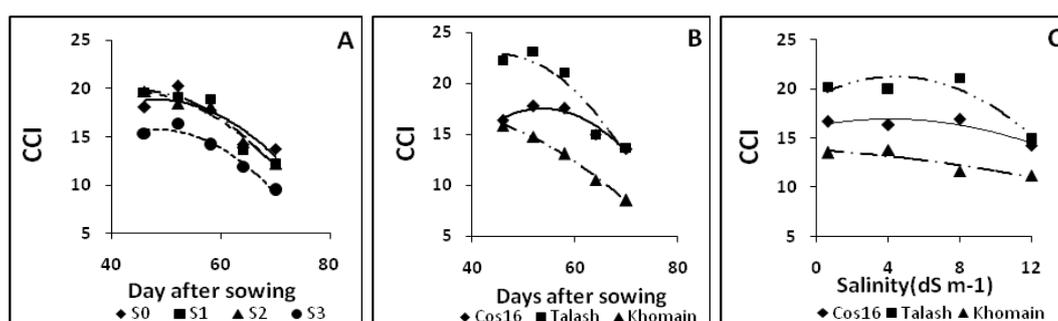
Chlorophyll content index (CCI) of leaves was measured by a portable chlorophyll-meter (CCM-200) in six days intervals, started 46 days after sowing. To determine free proline level, 0.5 g of leaf samples from each group were homogenized in 3% sulphosalicylic acid and then homogenate filtered through filter paper [19]. Mixture was heated at 100 °C for an hour in water bath after addition of acid ninhydrin and glacial acetic acid. Reaction was then stopped by ice bath. The mixture was extracted with toluene and the absorbance of fraction with toluene aspired from liquid phase was read at 520 nm. Proline concentration was determined using calibration curve and expressed as μmol proline g<sup>-1</sup> FW.

At maturity, all plants from each pot were harvested. Then grains were detached from the pods and grain yield per plant was determined. Shoots and roots of plants from each treatment were separately oven dried at 80 °C for 72 h and then were weighted.

Analysis of variance appropriate to the experimental design was conducted, using SPSS software. Means of each trait were compared according to Duncan multiple range test at p≤0.05.

## RESULTS

Chlorophyll content index (CCI) of pinto bean leaves diminished with progressing plant development at reproductive stages. At the most stages of development, leaf chlorophyll content index under severe salinity (12 dS m<sup>-1</sup>) was lower than that under other saline and non-saline conditions (Figure 1-A). CCI of all cultivars decreased with increasing plant senescence during reproductive stages. Reduction in CCI of Khomain was started earlier than that of other cultivars, but the rate of reduction was much higher for Talash than for Khomain and COS<sub>16</sub> (Fig. 1-B). Under all salinity treatments, Talash had the highest CCI, followed by COS<sub>16</sub> and Khomain, respectively (Figure 1-C).



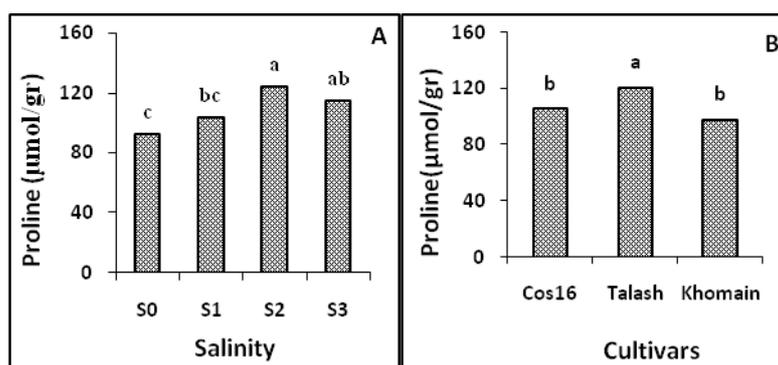
**Figure 1: Changes in leaf chlorophyll content index (CCI) of pinto bean during reproductive stages for different levels of salinity (A) and cultivars (B) and for cultivars under different salinity treatments (C) S<sub>0</sub>, S<sub>1</sub>, S<sub>2</sub> and S<sub>3</sub>: 0, 4, 8 and 12 dS m<sup>-1</sup> NaCl, respectively.**

Proline content of leaves was significantly affected by salinity and cultivars. The interaction of salinity  $\times$  cultivar for this trait was not significant (Table 1). Proline accumulation in leaf tissues increased gradually with the increase of salinity up to 8 dS m<sup>-1</sup> and thereafter slightly, but not significantly, decreased (Figure 2-A). The highest leaf proline content was recorded for Talash, but there was no significant difference in proline content of COS<sub>16</sub> and khomain (Figure 2-B).

**Table 1. Analysis of variance of the effects of salinity and on various traits of pinto bean cultivars**

Source of Changes	df	Mean of squares				
		proline	Days to flowering	Days to podding	Root/shoot ratio	Grain yield
Block	2	347.16 <sup>n.s</sup>	0.694 <sup>n.s</sup>	3.44 <sup>n.s</sup>	0.07 <sup>n.s</sup>	0.21 <sup>n.s</sup>
(Cultivar (C	3	*1624.35	**29.77	*95.528	**0.29	**7.49
(Salinity (S	2	*1661.83	*18.18	*40.54	01 <sup>n.s</sup>	**0.96
C $\times$ S	6	316.51 <sup>n.s</sup>	2.51 <sup>n.s</sup>	5.04 <sup>n.s</sup>	038 <sup>n.s</sup>	**0.82
Error	22	315.39	1.93	2.50	026.	0.17
(%) CV		16.38	3.53	3.39	40.31	18.32

\*\*, \*: significant at  $p \leq 0.01$  and  $p \leq 0.05$ , respectively.



**Figure 2. Mean proline content of pinto bean for salinity treatments (A) and cultivars (B) S<sub>0</sub>, S<sub>1</sub>, S<sub>2</sub> and S<sub>3</sub>: 0, 4, 8 and 12 dS m<sup>-1</sup> NaCl, respectively.**

Days to flowering and days to podding were also significantly affected by salinity and cultivars, but the interaction of salinity  $\times$  cultivar for these traits was not significant (Table 1).

Mean number of days to flowering and podding under moderate (S<sub>2</sub>) and severe (S<sub>3</sub>) salinities were statistically similar, but were longer than those under non-saline (S<sub>0</sub>) and low salinity (S<sub>1</sub>) conditions. Days to flowering and podding were longer for COS<sub>16</sub> than for Talash and Khomain. Difference between Talash and Khomain was not significant (Table 2).

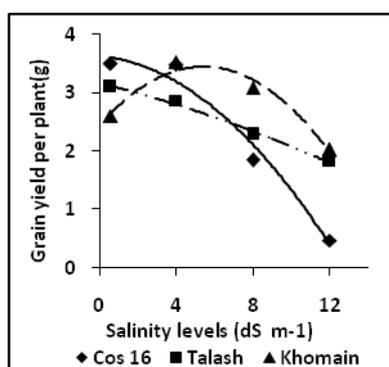
Root/shoot ratio affected only by cultivars. However, grain yield per plant was significantly influenced by salinity and cultivar. The interaction of salinity  $\times$  cultivar for grain yield per plant was also significant (Table 1). COS<sub>16</sub> had the highest root/shoot ratio, while the difference between Talash and Khomain was not significant. Grain yield per plant under non-saline condition (S<sub>0</sub>) and low salinity (S<sub>1</sub>) were statistically similar, but were considerably higher than that under moderate (S<sub>2</sub>) and severe (S<sub>3</sub>) salinities. The highest and the lowest grain yield per plant were obtained from Khomain and COS<sub>16</sub>, respectively. However, differences in grain yield between Khomain and Talash and also between Talash and COS<sub>16</sub> were statistically similar (Table 2).

**Table 2. Means of days to flowering and podding, root/shoot ratio and grain yield of three pinto bean cultivars under saline and non-saline conditions**

Treatment		Days to flowering	Days to podding	Root/shoot ratio	Grain yield (per plant(g)
Cultivar	Cos <sub>16</sub>	41 <sup>a</sup>	49.75 <sup>a</sup>	0.57 <sup>a</sup>	2.24 <sup>b</sup>
	Talash	38.5 <sup>b</sup>	45.33 <sup>b</sup>	0.32 <sup>b</sup>	2.52 <sup>ab</sup>
	Khomain	38.33 <sup>b</sup>	44.5 <sup>b</sup>	0.29 <sup>b</sup>	2.80 <sup>a</sup>
Salinity (dS m <sup>-1</sup> )	0	38.11 <sup>b</sup>	44.00 <sup>c</sup>	0.39 <sup>a</sup>	3.20 <sup>a</sup>
	4	38.11 <sup>b</sup>	45.55 <sup>b</sup>	0.35 <sup>a</sup>	3.33 <sup>a</sup>
	8	39.88 <sup>a</sup>	48.11 <sup>a</sup>	0.41 <sup>a</sup>	2.10 <sup>b</sup>
	12	41.00 <sup>a</sup>	48.44 <sup>a</sup>	0.43 <sup>a</sup>	1.41 <sup>c</sup>

Different letters in each column for each treatment indicate significant difference at  $p \leq 0.05$ .

The highest grain yield per plant under non-saline condition was obtained for COS<sub>16</sub>, but under salinity treatments, it was recorded for Khomain. Grain yield of all cultivars decreased with increasing salinity from 4 to 12 dS m<sup>-1</sup>. The extent of this reduction was higher for COS<sub>16</sub> than for other cultivars (Figure 3).

**Figure 3. Changes in grain yield per plant of pinto bean cultivars under saline and non-saline conditions.**

## DISCUSSION

Chlorophyll is the main pigment of photosynthesis in plants. To some extent, the Chlorophyll content can reflect the photosynthesis rate of plant. It is strongly influenced by environmental factors [20]. Reduction in CCI under severe salinity (Figure 1) can be attributed to a salt-induced weakening of protein-pigment-lipid complex [21] or increased chlorophyllase enzyme activity [22]. The decrease in chlorophyll content under salt stress is commonly reported phenomenon and in various studies, because of its adverse effect on membrane stability [23, 24]. Reduction in leaf chlorophyll content index due to severe salinity stress may limit photosynthesis and yield.

Stress mediated changes in free proline levels in relation to salinity have been studied in different plants and possible roles conferring stress resistance have been proposed. Among these roles balancing capacity as an osmolyte, stabilizing proteins, regulating cytosolic pH and scavenging of hydroxyl radicals could be given [25]. Salinity increased markedly the proline content in different salt sensitive and salt tolerant species/cultivars: with greater proline accumulation in salt tolerant ones, which is supposed to correlate with the adaptation to salinity [26, 27]. Our results (Fig. 2) suggest that salinity stress increases proline accumulation in leaves of pinto bean, differing among cultivars. The increased level of proline under salt stress has also been reported for barely [28], wheat [29], broad bean [30] and common bean [7].

Flowering is a life history trait determined by plant genotype, the environment, and the interaction between them [31]. Because salinity exerts multiple stresses on plants, including osmotic imbalance, nutritional deficits, and cellular toxicity [32, 33], it may be difficult to identify the specific mechanisms that alter life history shifts in important traits such as flowering. One potentially important factor facilitating delayed flowering and podding (Table 2) could be reduced biomass as a result of growth reduction under salt stress [6].

A reduction in energetic reserves of this magnitude could impede the development of floral structures and delay anthesis. Delay in flowering due to salinity stress was also reported for broad beans [17] and rice [34, 35]. Variation in root/shoot ratio, flowering and podding times and grain yield among pinto bean cultivars (Table 2) could be attributed to the differences in genetic constitution of these cultivars.

Decreasing grain yield per plant with increasing salinity could be mainly related to reduction in chlorophyll content under saline conditions (fig. 1), similar to that reported for soybean (36). Disturbed water and nutritional balance of plants may cause reduced crop yield in saline conditions [37]. Reduction in grain yield due to salinity stress was also reported for soybean [36], broad bean [38], and rice [39]. It can be concluded that pinto bean is a sensitive crop to salinity, but the extent of sensitivity differs among cultivars.

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