INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES

IJPAES

VOLUME-2, ISSUE-3, JULY-SEPT-2012 ISSN 2231-4490

Coden : IJPAES www.ijpaes.com

Received: 02nd June-2012 *Revised: 06th June-2012* *Accepted: 10th June-2012*

Research article

LEAF CHARACTERISTICS AND GRAIN YIELD OF PINTO BEAN CULTIVARS UNDER SALT STRESS

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ABSTRACT: Effects of four levels of salinity (0, 4, 8 and 12 dS m^{-1}NaCl) on leaf characteristics and grain yield of three pinto bean cultivars (COS_{16}, 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 the 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. Leaf chlorophyll content index (CCI), leaf temperature, leaf water content, proline accumulation, leaf area index (LAI), specific leaf weight (SLW) and grain yield per plant were significantly affected by salinity and cultivar. SLW, leaf temperature and proline content of pinto bean leaves increased, but the leaf water percentage, LAI and CCI considerably decreased as a result of salt stress. COS_{16} had the highest leaf water percentage and leaf area index (LAI) and the lowest leaf specific weight (SLW) and leaf temperature. However the highest chlorophyll content index (CCI) and proline content were found in Talash, compared with other cultivars.

Keywords: Chlorophyll, leaf area index, pinto bean, proline content, yield

INTRODUCTION

Soil salinization is a serious problem in the entire world and which causing substantial loss in crop productivity [1] Currently, about 50% of irrigated land in the world, which has at least twice the productivity of rain-fed land is affected by salinization [2, 3]. Crop improvement for saline conditions requires an understanding of the mechanisms enabling salt tolerance. Response to elevated salt may differ considerably among plant species as a function of their inherent salt tolerance [4]. One important component is the evaluation of genetic variability of the cultivated species to identify a tolerant genotype that may sustain a reasonable yield on salt affected soils [5]. Excess soluble salts in root zone severely affect growth of higher plant species mainly glycophytes [6]. Salt stress causes nutrient imbalance in plants by influencing uptake, transport and utilization of different nutrients [7, 8] which may result in an excessive accumulation of Na^+ and Cl^- in tissues [9]. Furthermore, salt stress exposes the plant to secondary osmotic stress, which implies that all the physiological responses invoked by drought stress, can also be observed in salt stress [10].

Low soil osmotic potentials (due to dissolved salts) cause low water potentials in plants resulting in reduced leaf expansion rates, lower photosynthetic rates per unit leaf area and reduced growth [11]. As water availability limits, stomatal conductance and transpiration decrease and leaf temperature increases [12]. Plants have evolved complex mechanisms that contribute to the adaptation to osmotic stress caused by high salinity [13]. Osmotic adjustment has undoubtedly gained considerable recognition as a significant and effective mechanism of salinity tolerance in crop plants [14]. Proline is a key osmolyte which help plants to maintain cell turgor [15, 16]. A large number of plant species accumulate proline in response to salinity stress and that accumulation may play a role in defense against salinity stress. Several possible roles including osmoregulation under drought and salinity conditions, stabilization of proteins, prevention of heat denaturation of enzymes and conservation of nitrogen and energy for a post-stress period have been attributed to supra-optimal levels of proline [17].

The adverse effects of salinity on growth and yield may be caused by reduced cell expansion and leaf area [18] or by a reduced supply of photosynthates to the growing tissues. It may occur as a result of the shortening of the lifetime of individual leaves, thus reducing net productivity and crop yield [19]. Reduction in crop yield as a result of salt stress has also been reported for sunflower [20], cotton and wheat [21] and canola [22]. The objective of this work is to evaluate the effects of sodium chloride salinity on leaf characteristics and grain yield of three pinto bean cultivars.

MATERIALS AND METHODS

An experiment (using RCB design) with three replications was carried out in 2011 at the Greenhouse of the University of Tabriz to investigate changes in leaf characteristics and grain yield of pinto bean cultivars (COS_{16}, Talash and Khomain) under a non-saline (control) and three saline (4, 8 and 12 dS m^{-1}NaCl) conditions. Ten seeds after treating with 2 g/kg Benomyl were sown 3 cm deep in each pot filled with 900 g perlite, using 36 pots. The temperature in the greenhouse was about 20 ºC. Tap water (EC = 0.59 dSm^{-1}) and saline solutions were added to the pots in accordance with the treatments to achieve 100% FC.

After emergence, seedlings were thinned to keep four plants in each pot. During the growth period, the pots were weighed and the losses were made up with Hoagland solution (EC = 1.3 dS m^{-1}). Perlites within the pots were washed every 20 days and non-saline and salinity treatments were re-applied in order to prevent further increase in electrical conductivity (EC) due to adding Hoagland solution.

After seedling establishment, a plant was marked in each pot and Leaf temperature (ºC) and chlorophyll content index (CCI) of upper, middle and lower leaves were measured. Leaf CCI was measured by a chlorophyll meter (CCM-200, Opti-Science, USA) in weekly intervals for five weeks. Leaf temperature (ºC) was directly measured by an infra-red thermometer (TES-1327) at pod setting stage. Subsequently, mean Leaf temperature and CCI for each treatment and replicate were calculated.

To determine free proline level, 0.5 g of leaf samples from each group were homogenized in 3% (w/v) sulphosalycylic acid and then filtered through a filter paper [23]. After addition of acid ninhydrin and glacial acetic acid, the mixture was heated at 100 ºC for an hour in water bath. 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^{-1} FW.

At 60 days after sowing, one plant from each treatment was harvested. First, the fresh weight of leaves was determined. Then, leaf area of the pinto bean leaves were measured by using a portable Leaf area-meter (ADC-AM300). Thereafter, the leaves were oven dried at 80ºC for 24 hours and weighed. The leaf water content was calculated as (FW-DW)/FW*100, where FW is leaf fresh weight and DW is leaf dry weight. Specific leaf weight (SLW) was calculated by the following formula:

SLW= leaf dry weight in mg/ leaf area in mm^2

At maturity, plants of each pot were separately harvested and grain yield per plant for each treatment at each replicate was determined. Analysis of variance and comparison of means at $P \leq 0.05$ were performed, using SPSS soft-wares. Excel software was used to draw figures.

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^{-1}) was lower than that under other saline and non-saline conditions (Fig. 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_{16} (Fig. 1-b). Under all salinity treatments, Talash had the highest CCI, followed by COS_{16} and Khomain, respectively (Fig. 1-c).

Leaf temperature was increased during reproductive stages (Fig. 2-a). Increasing salinity led to linear enhancement in leaf temperature (Fig. 2-b). The highest and the lowest Leaf temperatures were recorded for Khomain and COS_{16}, respectively. However, differences in leaf temperature between Khomain and Talash and between Talash and COS_{16} were not statistically significant (Fig. 2-c).

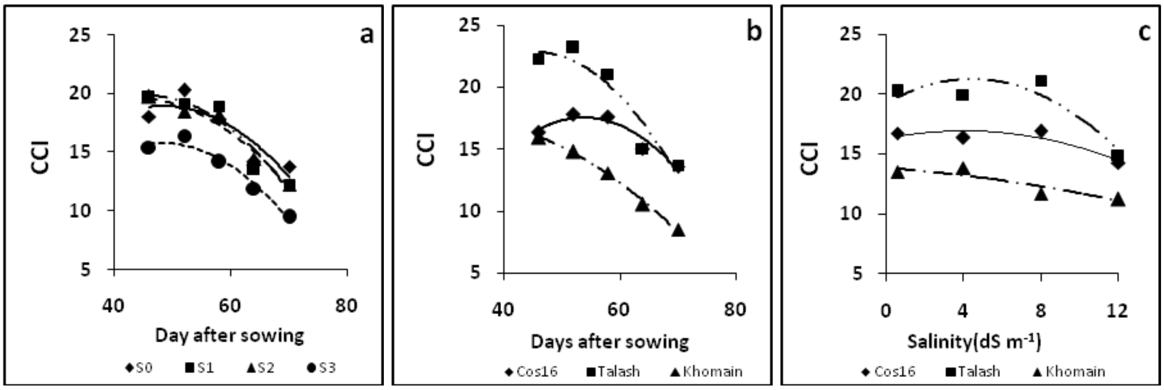

Fig. 1: Changes in leaf chlorophyll content index (CCI) of pinto bean during reproductive stages for different levels of salinity (a) and cultivars (b) and for cultivars under different salinity treatments (c) S0, S1, S2 and S3: 0, 4, 8 and 12 dS m^{-1} NaCl, respectively.

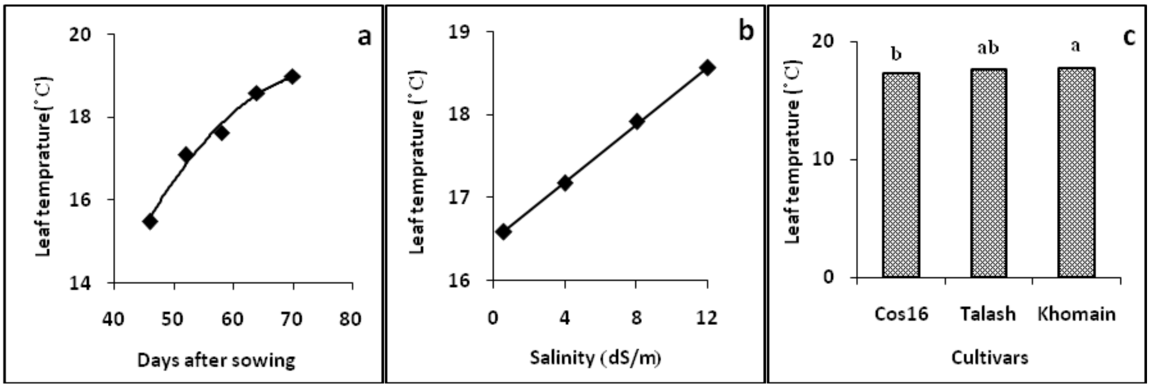

Fig. 2: Leaf temperature of pinto bean at different stages development (a) different levels of salinity (b) and for cultivars (c)

Analysis of variance of the data (Table 1) showed that leaf water content, proline content, leaf area index (LAI), specific leaf weight (SLW) and grain yield per plant were significantly affected by salinity and cultivar. The interaction of salinity × cultivar was only significant for grain yield per plant.

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

	Mean of squares					
Source of changes	df	Leaf water content	proline	LAI	SLW	Grain yield
Block	2	1.81^{ns}	347.16$^{n.s}$	0.40^{ns}	0.18**	0.21$^{n.s}$
Cultivar (C)	3	14.37**	1624.35*	0.52*	0.10**	7.49**
Salinity (S)	2	28.97**	1661.83*	1.67**	0.08*	0.96**
C × S	6	1.00^{ns}	316.51$^{n.s}$	0.15^{ns}	0.01^{ns}	0.82**
Error	22	1.60	315.39	0.13	0.01	0.17
CV (%)		1.44	16.38	19.07	15.95	18.32

**, *: significant at p≤0.01 and p≤0.05, respectively.

Leaf water percentage, LAI and yield per plant considerably decreased, but SLW increased as a result of salinity. Proline accumulation in leaf tissues increased gradually with increasing salinity up to 8 dS m^{-1} and thereafter slightly, but not significantly, decreased (Table 2). COS_{16} had the highest leaf water percentage and leaf area index and the lowest leaf specific weight. However the highest proline content was found in Talash, compared with other cultivars. The highest and the lowest grain yield per plant were obtained from Khomain and COS_{16}, respectively. However, differences in grain yield between Khomain and Talash and also between Talash and COS_{16} were not statistically significant (Table 2).

Table 2. Comparison of means of leaf water content, proline, LAI, SLW and grain yield of three pinto bean cultivars under saline and non-saline conditions.

Treatment		Leaf water percentage	Proline	LAI	SLW	Grain yield per (plant (g
Salinity (dS m^{-1})	0	89.26^{a}	92.44^{c}	2.05^{ab}	0.71^{b}	3.20^{a}
	4	88.07^{ab}	103.095^{bc}	2.40^{a}	0.70^{b}	3.33^{a}
	8	86.92^{b}	123.82^{a}	1.70^{bc}	0.81^{b}	2.10^{b}
	12	85.05^{c}	114.19^{ab}	1.40^{c}	0.93^{c}	1.41^{c}
Cultivar	Cos_{16}	88.75^{a}	105.79^{b}	2.12^{a}	0.70^{b}	2.24^{b}
	Talash	86.92^{b}	121.10^{a}	1.72^{b}	0.82^{a}	2.52^{ab}
	Khomain	86.51^{b}	98.27^{b}	1.81^{b}	0.85^{a}	2.80^{a}

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 from COS_{16}, but under salinity treatments, it was recorded for Khomain. Grain yield of all cultivars decreased with increasing salinity from 4 to 12 dS m^{-1}. The extent of this reduction was higher for COS_{16} than for other cultivars (Fig. 3).

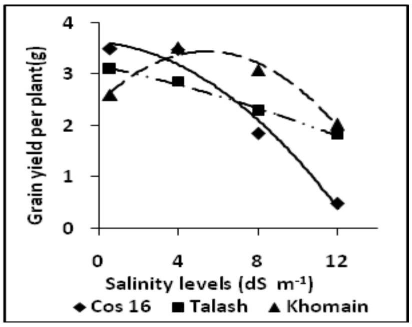

Fig. 3. Changes in grain yield per plant of pinto bean cultivars under saline and non-saline conditions.

DISCUSSION

Under adverse circumstances, the chlorophyll level is a good indicator of the photosynthesis function. Reduction in CCI under severe salinity (Fig. 1) can be attributed to increasing the activity of the cholorophyyllase [24], inducing the destruction of the chloroplast structure and the instability of pigment protein complexes [25]. It has been reported that chlorophyll content decreases in salt susceptible plants such as pea [26] and soybean [27].

Increasing leaf temperature with increasing salinity (Fig. 2) was the consequence of decreasing leaf water content (Table 2). High salt concentration in the soil solution is bound to create high osmotic pressure in the root zone and reduce availability of water to plants. Water deficit and salinity cause stomatal closure and reduce transpiration rate [28], leading to the enhancement of leaf temperature [29].

The proline content of pinto bean cultivars was increased by salinity, with greater proline accumulation in salt tolerant cultivar (Table. 2), which is supposed to correlate with the adaptation to salinity [30, 31]. High levels of free proline in cultivars may have had a protective effect on cells.

Leaf area index (LAI) was decreased, but specific leaf weight (SLW) was increased with increasing salinity (Table 2). Leaf area index is an indicator of the size of the assimilatory system of a crop [32]. When plants are grown under saline conditions, as soon as the new cell starts its elongation process, the excess of salts modifies the metabolic activities of the cell wall causing the deposition of various materials which limit the cell wall elasticity. Cell walls become rigid and consequently the turgor pressure efficiency in cell enlargement is decreased [33]. Salt stress reduced the leaf growth rate by shortening the length of the leaf elongating zone and decreasing the growth intensity in its central and distal portions [34]. Leaf growth inhibition by salinity must be expected to occur via an effect on this region [35]. Some plant species use accumulation of Na^+, Cl^-, Ca^+, K^+, and other compatible solutes to increase osmotic potential and in turn to reduce water potential, which leads to increase of SLW [36, 37].

Decreasing grain yield per plant with increasing salinity (Fig. 3) could be mainly attributed to reduction in chlorophyll content (Fig. 1), leaf water content and LAI (Table 2) and enhancement in leaf temperature (Fig. 2), proline content and SLW (Table 2) under saline conditions. Yield reduction under saline conditions was also due to reduced growth as a result of decreased water uptake, toxicity of sodium and chloride in the shoot cells as well as reduced photosynthesis [38]. Crop yield loss as a result of salt stress has also been reported for maize [39], broad bean [40], chickpea [41], rice [42] and soybean [43].

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