



Physiological and biochemical responses of Mung bean (*Vigna radiata*

L. Wilczek) to salt stress conditions

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Abstract:

This study was carried out to investigate the effect of salinity on responses of Mung bean plant which were measured by a range of physiological parameters: chlorophyll, proline and Nitrate reductase activity.

Salt concentrations (0, 50, 100, and 150 mEq.L⁻¹) were applied to Mung bean plant that grown in plastic pots filled with a mixture of sand and peat (2:1 v/v).

As a result of analyzing the cultures under salinity stress, it was determined that the chlorophyll decreased significantly. At low salinity regimes, a slight decrease was noted in chlorophyll a and b, and total chlorophyll content, but under high salinity conditions a significant reduction in the content of these pigments was observed. The chlorophyll a: chlorophyll b ratio also varied significantly under salinity stress, while the proline concentration increased considerably under salinity restraint. Therefore, the stress induced by salt caused an accumulation of proline. Nitrate reductase activity (NRA) decreased progressively with increasing salinity. This activity was inhibited in both leaves and roots under salinity

Key words: Salinity, Mung bean, Chlorophyll, Proline, Nitrate Reductase

Introduction:

Abiotic stresses, such as salinity, chemical toxicity, drought, external temperatures and oxidative stress are serious threats to agriculture and result in the deterioration of environment (Marshner, 2002 and Nasir Khan *et al.*, 2007).

Salinity affects almost every aspect of the physiology and biochemistry of plants and significantly reduces yield. It is well known that salt stress induces various biochemical and physiological responses in plants and affects almost all plant functions, including photosynthesis, growth and development (Abbas *et al.*, 2010). To cope with these stresses, plants adapt various changes in physiomorphological and enzymatic processes (Vinocur and Altman, 2005). Salt stress leads to a decreased efficiency of photosynthesis and is known to influence the chlorophyll content of plant leaves (Meloni *et al.*, 2003). The decrease of chlorophyll synthesis may be due to a decrease of δ -aminolevulinic acid dehydratase (ALAD) activity under environmental stress (Vajpayee *et al.*, 2000), but there is no clear information on the behavior of this enzyme in leaves under salt stress conditions (Yildiz and Terzi, 2013).

Proline accumulation is one of the adaptations of plants to salinity. It has also been widely advocated that proline accumulation uses as parameter of selection for salt stress tolerance (Ramanjulu & Sudhakar, 2001).

Nitrate reductase mediates the reduction of nitrate to nitrite, which is regarded as a rate limiting step in plant growth and development (Solomonson and Barber, 1990). Nitrate reductase activity (NRA) provides a good estimate of the nitrogen status of plants and is correlated with growth and plant yield (Srivastava, 1980 ; Garg and Singla, 2005).

Several studies have reported NO₃⁻ uptake and NR activity decrease in plants under salt stress. On the other hand, numerous



studies on the effect of exogenous NaCl on NR activity have given contradictory results. Both inhibition (Gouia *et al.*, 1994) and stimulation (Sagi *et al.*, 1997) have been reported.

Mung bean is an important legume species and provides an important source of human diet. This species is a summer crop with short duration (70 – 90 days) and high nutritive value (Vijayalakshmi *et al.*, 2003). Mung bean like many other crops is sensitive to water availability though it is sensitive to water stress at all growth stages, it is more sensitive to drought at flowering and grain development stage (Zubair *et al.*, 2002; Thaloot *et al.*, 2006). The objective of this work is to evaluate the physiological and biochemical responses of Mung bean (*Vigna radiata* L. Wilczek) to salinity stress.

Material and Methods:

Plant materials and growth conditions

Seeds of Mung bean (*Vigna radiata* L. Wilczek) were obtained from Agriculture Research Center of Cairo. Pot trial was conducted on at the experimental garden of Biology Department, Sciences Faculty, Mostaganem University. They were surface sterilized with 5% (w/v) commercial bleach sodium hypochlorite solution (NaOCl) three times for 30 min, washed in deionizer water. Seeds were planted in pots (25 cm upper diameter, 17 cm lower diameter and 25 cm in height with a bottom drainage hole), each pot contained 3 kg soil [sand and peat (2:1 v/v)]. In this experiment a Hoagland's nutrient solution was used as a main culture solution, this solution is consisting of 1.91 mM KNO₃, 1.29 mM Ca(NO₃)₂ · 4H₂O, 2.10 mM NH₄NO₃, 0.61 mM Mg SO₄ · 7H₂O, 0.54 mM K H₂PO₄, 0.34 mM K₂HPO₄ · 3H₂O, 0.02 mM MnCl₂ · 4H₂O, 0.002 mM CuSO₄ · 5H₂O, 0.03 mM H₃BO₃, 0.002 mM MO₇O₂₄(NH₄) · 7H₂O, 0.001 mM EDTA and added to distilled water. Saline solution was prepared by adding a mixture

of NaCl and CaCl₂ in a 1:1 molar ratio. The irrigation is for one time each three days. Application of saline concentrations was practiced one week before the collecting the samples for each treatment. In this case the Hoagland's solution was stopped. Salt treatments were set up 60 days after sowing on the plants treated by salt solutions (50, 100 and 150 mEq.L⁻¹). Plants grown in nutrient solution only served as control.

Determination of physiological and biochemical parameters

Chlorophyll content

The chlorophyll from the fresh leaves was extracted in 80% acetone and the absorbance was read spectrophotometrically at 663 and 645 nm. Chlorophyll content was estimated by the method of Arnon (1949).

Proline content

Proline was quantified by using ninhydrin reagent and measured according to Bates *et al.* (1973). Proline was extracted from 0.5 g of leaf in 10 ml of 3% sulfosalicylic acid. Two ml of extract was reacted with 2 ml acid- ninhydrin and 2 ml of glacial acetic acid for 75 min at 100 °C. The reaction was terminated in an ice bath. The reaction mixture was extracted with 4 ml of toluene and vortexes. The absorbance of toluene layer was spectrophotometrically determined at a wavelength of 520 nm. Concentration was determined from a standard curve and calculated on a fresh weight basis (μmol proline.g⁻¹ fw).

Nitrate Reductase Activity (NRA)

NR activity was determined by the method of Silveira *et al.* (1998). Leaf disc from the second youngest fully expanded leaves (200 mg fresh mass) were infiltrated twice for two minutes with 5 ml of reaction mixture containing 100 mM/l Potassium Phosphate buffer (pH 7.5) ; 25 mM/l KNO₃ ; and 1% isopropanol. The reaction mixture was incubated at 35°C for 30 minutes in the dark. NR activity was estimated from the amount of NO₂⁻ formed during the incubation period and released from the leaf discs to the medium after boiling for 5



minutes. Aliquots were mixed with 2 ml of (1:1) 1% sulfanilamide in 2.4 mol /l HCl ; 0.02% N-1-naphtyl-ethylenediamine and the absorbance was taken at 540 nm.

Statistical analysis

All data were subjected to ANOVA test and means were compared by the Newman-Keuls test. Comparisons with P values <0.05 were considered significantly different.

Results and Discussion

Effect of Salinity on Leaf Chlorophyll Concentration

Table 2 show that salt stress caused significant reduction ($P < 0.05$) of the leaves contents in chlorophyll a (25%) and in chlorophyll b (30%) as compared with control treatment, leading to an increase in the Chl a/b ratio (tab.1). Our results are in agreement with those reported by Iqbal *et al.* (2006) and Molazem *et al.* (2010), who reported that chlorophyll content was decreased under saline conditions. Moussa (2006) showed that salt stress significantly decreased both chlorophyll a and b in an experiment done by Ashrafuzzaman *et al.* (2000) showed with increasing salinity Chlorophyll a, b, total Chlorophyll (a + b) and Chlorophyll a/b were reduced.

Table 1. Mean chlorophyll content ($\text{mg}\cdot\text{g}^{-1}$ leaf tissue) of Mung bean (*Vigna radiata*) grown at different salt concentrations. Significance level = * $P < 0.05$

Chlorophyll content	NaCl + CaCl ₂ ($\text{mEq}\cdot\text{L}^{-1}$)	
	0 (control)	50
Chlo total	1,89±0,52	1,70±0,43
Chlo a	1,34±0,24	
Chlo b	0,74±0,41	
Chlo a/b ratio	1,78±0,29	1,61±0,19
	1,24±0,12	
	0,45±0,09	
	1,22±0,18	1,15±0,11
	1,05±0,13	
	0,37±0,11	
	1,45±0,34	1,40±0,22
	1,18±0,25	
	1,21±0,14	

The effect of salinity on chlorophyll a/b ratio was significantly varied by the different levels of salinity. The chlorophyll a declined more than that of chlorophyll b with increasing of salinity level. These results corroborate with those of Islam (2001) who stated that salinity decreased chlorophyll b content more than chlorophyll a and thus increased the chlorophyll a/b ratio. Pushpam and Rangasamy (2002) found that chlorophyll a, chlorophyll b and total chlorophyll contents and chlorophyll a/b ratio decreased with increasing of salinity level.

NaCl stress led to a significant reduction in the contents of chlorophylls a and b. This effect agrees with earlier work and may results from an inhibition of chlorophyll biosynthesis or from an activation of the chlorophyllase (Lycoskoufis *et al.*, 2005).

Decrease in chlorophylls level under salt stress may be due to reduction in pigment biosynthesis or enzymatic chlorophyll degradation (Yang *et al.*, 2009).

The elevation of chl b/chl a ratio under saline condition suggested salinity had most adverse effect on Chlorophyll b content. Similar results obtained by Stoeva and Kaymakanova (2008) on *Phaseolus vulgaris* under salt stress.

The chlorophyll level is an index of the photosynthesis (Xu *et al.*, 2008) and decrease in Chlorophyll level lead to reduction in growth parameters.

Effect of Salinity on proline content

Results presented in Table 3 clear that proline content of leaves, stems and roots significantly increased gradually by increasing the salinity levels as compared with control treatment.

Proline content of salt stressed plants was higher than of the control although salt stressed lands having no significant increase in proline content.

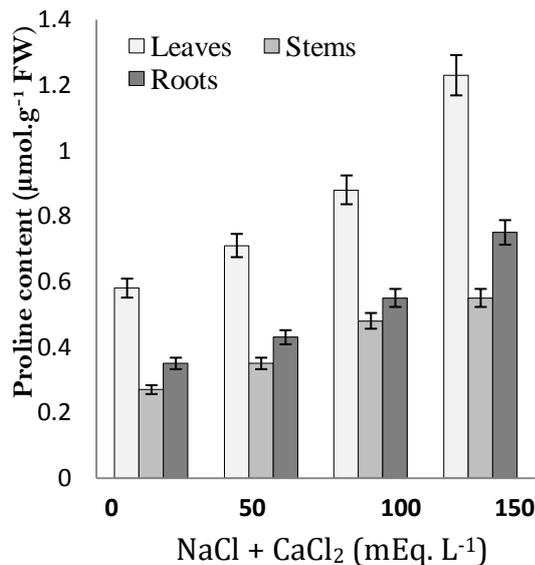


Figure 1: Effect of salinity (NaCl + CaCl₂) on proline content of 70 days old plants of Mung bean. Results are means of three replicates. Error bars (-) show LSD at 5% level.

From the results we can conclude that the chlorophyll pigment may be involved with salt tolerance of Mung bean plant that accumulates proline to a high level to prevent damage from osmotic stress. Wanichananan *et al.* (2003) and Al-Saad *et al.* (2012) were reported similar findings in salinity study of aromatic rice. One of the most important mechanisms by higher plants under salt stress is the accumulation of compatible solutes such proline. The accumulation of proline in salt stressed plants is a primary defense to maintain the osmotic pressure in a cell. Several reports show a significant role of proline in osmotic adjustment, protecting cell structure and its function in plants in salt-tolerant and salt-sensitive cultivars of many crops (Turan *et al.*, 2007) These results demonstrated that, the physiological function of proline which accumulated in mung bean under salinity stress may not only just behave as an osmolytes and protectants but also have other roles related to stress. Ashraf *et al.* (1998) reported that proline is an

important osmolyte to adjust the plant under drought/saline conditions. There is however reasons to believe that proline accumulation may play a role in the salinity tolerance.

High accumulation of proline in leaves is important adaptive mechanism of salt tolerance. Proline acts as an osmolyte and reduces the osmotic potential, thus reducing toxic ion uptake (Hare *et al.*, 1998).

A positive correlation between magnitude of free proline accumulation and salt tolerance has been suggested as an index for determining salt tolerance potentials between cultivars. The magnitude of increase in free proline accumulation was higher in the tolerant cultivars than in the sensitive ones (Misra & Gupta 2005; Kholová *et al.*, 2010). However, some researchers reported that proline accumulation cannot be used as a sole criterion for salt tolerance (Moradi & Ismail 2007). Firstly it is an osmolyte accumulated under stress in almost all the plant species. An increase in proline under the effect of salinity has been reported for various plants such as sugar beet (Ghoulam *et al.*, 2002), rice (Demiral and Türkan 2005), and maize (ChaUm and Kirdmanee 2009). Such an increase in the proline was also observed under the effect of other types of stress: water (Zhu *et al.*, 2005), thermal (Taulavuori *et al.*, 2005) and UV radiations (Saradhi *et al.*, 1995). Correlation between proline and chlorophyll content: NaCl+CaCl₂ affected both proline and chlorophyll content in Mung bean. The results indicated that high proline content positively correlates with the increase of chlorophyll pigment content (fig.3)

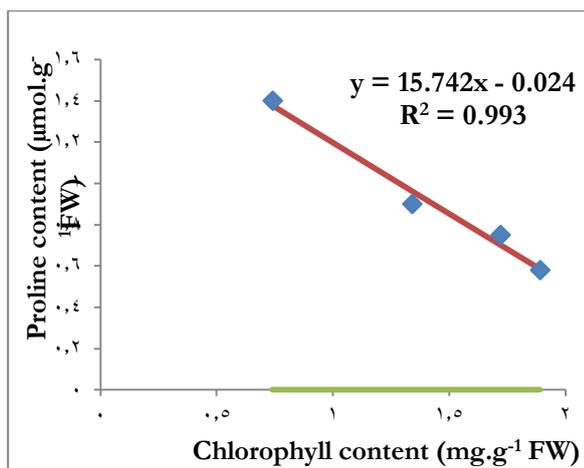
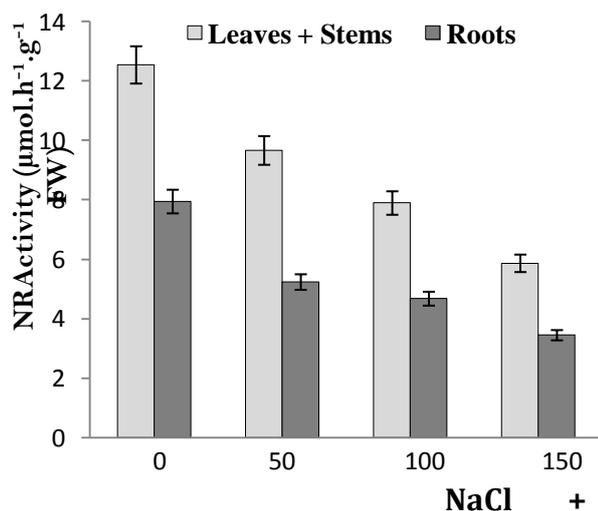


Figure 3: Correlation between proline and chlorophyll content of Mung bean influenced by NaCl + CaCl₂ for 8 days

Nitrate reductase (NR) activity

It appeared from the present results that increasing levels of salinity induced a substantial decline in NRA (Fig. 2). NR activity was significantly inhibited by salt treatments. The inhibition increased progressively with an increase in salt concentration from 50 to 150 mM. The NR activity was reduced from nearly 12,55 for control to 5,87 μmol.h⁻¹.g⁻¹ FW at 150 mM.



CaCl₂ (mEq.L⁻¹)

Figure 2: Effect of salinity (NaCl + CaCl₂) on NR activity of 70 days old plants of Mung bean. Results are means of three replicates. Error bars (-) show LSD at 5% level.

In vivo nitrate NRA was considerably greater in leaves than in roots. The addition of 150 mEq.L⁻¹ NaCl + CaCl₂ induced a substantial decline in NRA by about 43,5% in roots and 44,5% in leaves. On the other hand, a positive correlation was found between NRA and nitrate concentrations in leaves and roots (Meloni *et al.*, 2001). In this sense, cytosolic nitrate seems to protect the NR enzyme against the action of proteases and/or inhibitors besides triggering the novo synthesis of NR protein by induction of NR gene expression (Campbell, 1999). Even so, under conditions of restricted nitrate flux induced by salt stress or water deficit, NRA could be lowered initially due to enzyme degradation/inactivation and the reduction in gene expression and NR protein synthesis (Ferrario *et al.*, 1998). These changes in nitrate reduction were paralleled by those expressed by plant growth.

It leads to reduced concentration of nitrate in leaves, consequently reducing NR activity of leaves under salinity conditions. The reduction of NRA in leaves, under conditions of restricted nitrate flux induced by salt stress, could be due to the enzyme degradation/inactivation and the reduction in gene expression and NR protein synthesis. Ferrario *et al.* (1998) suggested that the reduction in NR mRNA levels is related to lower levels of NO₃- and glutamine in leaves. Salt reduced NO₃ fluxes from roots to leaves and impaired the NRA in leaves (Debouba *et al.*, 2007).

The reduction in NR and NiR under salt stress was reported in many plant genotypes (Surabhi *et al.*, 2008). Our results are in agreement with earlier reports.

Conclusion

It can be concluded that salt stress has affected the Mung bean development as well as its physiological process like



chlorophyll rate, proline content and nitrate reductase activity.

Based on the present results, it is concluded that Mung bean can be cultivated in Areas arid and semi-arid affected by a moderate salinity, which could increase its production of secondary metabolites at the plant level. Nonetheless, a detailed investigation regarding the suitability of this plant to varying salinity regimes, from very low to high, in addition to detailed field experiments are needed to confirm this conclusion.

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