Iranian Medicinal Plants Society

Ethno-Pharmaceutical products

Journal homepage: Http://js.kgut.ac.ir

Ministry of Science Research and Technology
Graduate University of Advanced Technology

Response of fenugreek to short-term salinity stress in relation to lipid peroxidation, antioxidant activity and protein content

Amin Pasandi Pour [1], Hassan Farahbakhsh [2•], Mehri Saffari [2]

1- Ph.D Student of Agronomy, Member of Young Researcher Society, Shahid Bahonar University of Kerman
2- Department of Agronomy and Plant Breeding, Faculty of Agriculture, Shahid Bahonar University of Kerman

Article information

Article history:
Received: 14 Sep. 2013
Accepted: 1 Jan. 2014
Available online: 15 Mar. 2014
EPP 2014; 1 (1): 45 -52

Keywords:
Catalase
Fenugreek
MDA
Salinity stress
Protein

*Corresponding author:
Department of Agronomy and Plant Breeding, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran
E-mail:
hfarahbakhsh@yahoo.com

Abstract

To investigate the effect of salinity stress on membrane stability index, membrane lipid peroxidation, catalase activity and protein content of fenugreek (*Trigonella foenum*), an experiment with five levels of short-term salinity stress (0, 50, 100, 150 and 200 mM) was carried out in laboratory of Agriculture Faculty of Shahid Bahonar University of Kerman, Iran. The treatments were arranged based on a completely randomized design with three replicates. Salinity stress caused a significant reduction in protein content and membrane stability index compared to control. The mentioned parameters decreased with increasing in salinity concentration, so that the lowest and highest values of these traits belonged to 200 and 0 mM salinity. malondialdehyde and other aldehydes content of shoot and root as the measure of membrane lipid peroxidation and catalase activity were increased in response to short-term salinity stress. Increased catalase in this plant indicates the tolerance capacity of the plant to protect itself from oxidative damage induced by NaCl.

Introduction

Fenugreek (*Trigonella foenum-graceum* L.) is an annual dicot belonging to the subfamily Papilionaceae of the Fabaceae family. Fenugreek has an indeterminate growth habit, and plant growth will continue until heavy frost or dessication (Acharya et al., 2006). Fenugreek plants in North America grow typically 40 – 60 cm high (Acharya et al., 2006; Slinkard et al., 2006). This plant has been described as 'malodorous' (Lust., 1974) due to its distinct smell. It is trifoliate with branched stems, and has white or yellow flowers (Acharya et al., 2008). Slinkard et al., (2006) has described the chemical composition of fenugreek seeds as 32% insoluble dietary fibre, 13% soluble dietary fibre, 36% protein, 6% oil, 3% ash, 1.6% starch, and 0.4% sugar. Fenugreek is known to have several pharmacological effects such as hypoglycemia (Zia et al., 2001), hypocholesterolemia (Srinivasan., 2006), gastroprotective (Suja et al.,2002), chemopreventive (Amin et al., 2005), antioxidant (Kavirasan et al., 2007), anti-inflammatory, antipyretic (Ahmadiani et al., 2001), laxative (Riad & El-Baradie., 1959) and appetite stimulation (Petit et al., 1993) attributes. This plant is known to contain alkaloids (Jain & Madhu., 1988), flavonoids (Kamal &Yadav.,1991), salicylate (Swain et al.,1985), and nicotinic acid (Rajalakshmi et al., 1964).

Salt stress constitutes a major problem in arid and semi-arid regions. Approximately 7% of the world land area, 20% of the world cultivated land, and nearly 50% of the irrigated lands are affected with the high salt contents (Szabolcs., 1994). Salinity stress induces numerous biochemical and physiological responses in plants. The generation of Reactive oxygen species (ROS) and subsequent oxidative damage during stress, is well documented in plants. The production of ROS increases under salinity stress conditions, causing oxidative damage and impairment of normal metabolism (Bartoli et al., 2004).

The activated oxygen species can seriously disrupt normal metabolism through oxidative damage to

lipids, proteins and nucleic acids (Fridovich., 1986). The enzyme superoxide dismutase (SOD) converts O_2^- into H_2O_2. Catalase (CAT) and a variety of peroxidases (POD) catalyze the breakdown of H_2O_2 (Asada., 1994). The balance between the production of reactive oxygen species and the quenching activity of antioxidants becomes upset when plants are subjected to environmental salt stresses, often resulting in oxidative damage. Increased CAT activity is closely related to salt tolerance of many plants as reported in various researches (Rahnama & Ebrahimzadeh., 2005; Azevedo Neto et al., 2006; Koca et al., 2007).

Lipid peroxidation disrupts the membrane integrity of the plant cell. As a result, essential solutes leak out of organelles and from the cell, causing disruption in membrane function and metabolic imbalances (Mundree et al., 2002). The level of Malondialdehyde (MDA) as the product of lipid peroxidation in plant cells, increases under stress conditions. Lipid peroxidation is a major parameter to identify the degree of stress to which a plant has been exposed, by determining cell membrane stability (Parvanova et al., 2004).

Total soluble protein content was not affected in *Lupinus angustifolius* plant exposed to both drought and salt stress, but the decrease in protein content was shown in root, young and old leaves of *Helianthus annuus* and *Coleus blumei* plants (Dos Santos et al., 1999; Gilbert et al., 1998; Yu & Rengel, 1999). Similarly the decline in total soluble protein content was shown in *Lycopersicon esculentum*, *Oryza sativa*, *Vicia faba*, *Amaranthus tricolor* and *Brugiera parviflora* plants under NaCl stress (Alaghabary et al., 2004; Alamgir et al., 1999; Gadallah., 1999; Parida & Das., 2005; Parvaiz & Satyavati., 2008; Wang & Nil., 2000). Increase in total soluble protein content was reported at high NaCl concentration, and decrease at low concentration in *Pancratium maritimum* plants (Khedr et al., 2003). In contrast to this, increase in plants protein contents were reported in *Arabidopsis thaliana* and *Fragaria ananassa* cv. *Camarosa* (El-Baz et al., 2003).

Little information is known about the changes of CAT activity and lipid peroxidation with respect to salt stress in Fenugreek seedlings. Therefore, an investigation on protein contents, MDA, other aldehydes, membrane stability index and catalase activity in Fenugreek plants under salinity condition, not only provides a background for further research but also is of great importance to cultivation of this medicinal plant.

Materials and Methods

- **Plant material and stress treatments**

An experiment based on a completely randomized design with three replicates was carried out at the laboratory of agriculture faculty of Shahid Bahonar University of Kerman, Iran. Fenugreek (*Trigonella foenum*) seeds used in the experiment were provided from seed and plant researches center. Seeds were surface-sterilized with a 3% sodium hypochlorite solution, rinsed in distilled water for 3 times and dried before the experiment. Afterwards, the seeds were sown in pots containing peat and placed in growth chamber (Conviron PGR-15) with a 14 h photoperiod (300 µmol m^{-2} s^{-1} PAR) at 25/22˚C (day/night). The seedlings were irrigated with distilled water for the first 5 days and then after with a half strength Hoagland solution, pH 6.2-6.5 (Arnon & Hoagland., 1940). When the plants had 5-6 leaves on shoot, were transferred to hydroponic culture for 3 days. After this time, the plants was treated with different concentrations of NaCl (0, 50, 100, 150 and 200 mM) for 48 hr, then plant materials were harvested and stored in -80˚C for measuring some physiological parameters.

- **Membrane stability index**

The leaf samples (0.2 g) were placed in test tubes containing 10 ml of double distilled water. The leaves were cut into discs of uniform size (5 mm length). The tubes were incubated in a water bath at 32˚C for 2 h and the initial electrical conductivity of the medium (EC1) was measured. The samples were autoclaved at 121˚C for 20 min to release all the electrolytes, cooled to 25˚C and the final electrical conductivity (EC2) was measured (Dionisio-Sese & Tobita., 1998). The membrane stability index (MSI) was calculated using the fallowing formula:

$MSI = 1- (EC1 \div EC2) \times 100$.

- **Determination of lipid peroxidation**

Membrane Lipid peroxidation was measured in terms of malondialdehyde (MDA), and other aldehydes content (Dhindsa et al., 1981). Approximately 0.2 g of leaf and root tissues from control and treated plants were cut into small pieces and homogenized by the addition of 1ml of 5% trichloroacetic acid (TCA) solution. The homogenized leaf and root tissues were ground using cold mortar and pestle in ice bath. The homogenates were transferred to fresh tubes and centrifuged at 12000 rpm for 15 minutes at room temperature. One ml of supernatant was mixed with 5 ml of 20% (v/v) trichloroacetic acid containing 0.5% thiobarbituric acid (TBA). The mixture was heated at 100˚C for 30 minutes, quickly cooled and centrifuged at 10000g for 10 min. The absorbance of the supernatant was recorded at 532 and 450 nm for MDA and other aldehydes respectively. The concentration of MDA and other aldehydes were calculated by means of an extinction coefficient of 155 $mM^{-1}cm^{-1}$ (Heath & Packer., 1968) and 0.475×10^5 $mM^{-1}cm^{-1}$ (Meir et al., 1992) respectively. The results were expressed as µmol MDA g^{-1} FW.

- **Preparation of enzyme extract**

Coomassie Brilliant Blue G-250 (100 mg) was dissolved in 50 ml 95% ethanol. To this solution 100 ml 85% (w/v) phosphoric acid was added. The resulting solution was diluted to a final volume of 1 liter. Final concentrations in the reagent were 0.01% (w/v) Coomassie Brilliant Blue G-250, 4.7% (w/v) ethanol, and 8.5% (w/v) phosphoric acid.

- **Determination of protein content**

Total soluble protein content was measured according to Bradford (1976). 100 µl of enzyme extract were added to 5 ml of protein reagent and after 25 min transferred to spectrophotometer cuvettes and the absorbance was measured at 595 nm.

- **Assay of catalase activity**

Catalase activity was determined by consumption of H_2O_2 using the method of Dhindsa et al.,(1981). The reaction mixture contained 50 mM potassium phosphate buffer with pH 7.0, 15 mM H_2O_2 and enzyme extract. The consumption of H_2O_2 was spectrophotometrically monitored at 240 nm (e = 0.28 $mM^{-1}cm^{-1}$). The enzyme activity was expressed in terms of unit mg^{-1} protein. One unit of CAT is the mount of enzyme that decomposes 1 mM H_2O_2 in 1 minute.

- **Statistical analysis**

Analysis of variance (ANOVA) was performed on the data using SAS (SAS, 2001). Statistical significance was evaluated with the LSD test, and differences were considered significant if *P* values were ≤0.05.

Results

The same response was observed in the measured traits of fenugreek when exposed to short-term salinity stress. Analysis of variance revealed that salinity treatment was significant for all the traits (Table 1).

Table 1. Results of variance analysis of some physiological characteristics in fenugreek under short-term salinity stress

Source of Variation	df	MDA in Shoot	Other aldehydes in Shoot	MDA in Root	Other aldehydes in Root	MSI	Protein content	Catalase activity
Salinity	4	0.2818**	4.54**	0.00866**	0.4863**	8375**	4736.8**	2.978**
Error	10	0.00032	0.0095	0.0000152	0.00027	7.252	9.878	0.0963

Superscript ** denotes significant difference at P < 0.01.

Salinity-induced increases in lipid peroxidation, as estimated through malondialdehyde (MDA) and other aldehydes production, were observed in the shoot of Fenugreek. MDA value was increased with all concentrations of NaCl, but the effects of high concentrations were greater. Statistically significant increases in shoot MDA content was observed at 50 mM NaCl. Peroxidation rates increased by 113% and 373%, compared to control plants at 100 and 200 mM NaCl respectively (Figure 1).

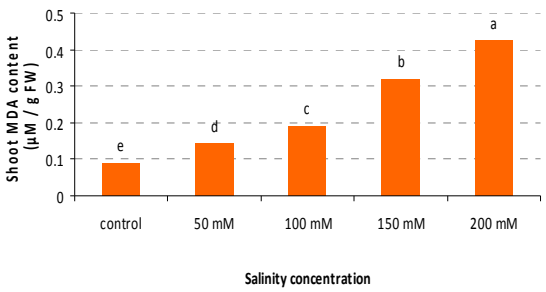

Figure 1. Effect of different salinity concentrations on MDA content in shoot of fenugreek

Under high NaCl concentrations, membrane lipids are damaged by ROS. Oxidative damage to tissue lipids was estimated by MDA and other aldehydes content. Shoot other aldehydes content were 0.538 µmol g^{-1} FW in the control group and were increased to 0.806 µmol g^{-1} FW in plants grown in the presence of 50 mM NaCl. This value increased with increasing in salinity concentration so that, for the highest NaCl concentration (200 mM), it was 1.943 µmol g^{-1} FW (Figure 2).

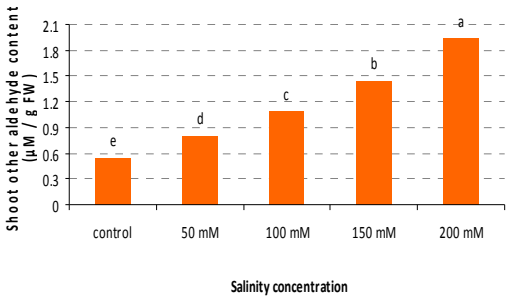

Figure 2. Effect of different salinity concentrations on other aldehyde content in shoot of fenugreek

The response of root MDA content was the same as that of shoot. Root MDA content was significantly affected by salinity treatment (Table 1). The mean of

root MDA content was 0.0492 µmol g^{-1} FW in all treated plants that ranged from 0.0197 to 0.0764 µmol g^{-1} FW belonged to control and 200 mM NaCl treatments respectively (Figure 3).

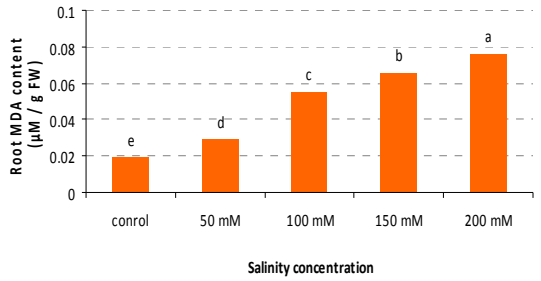

Figure 3. Effect of different salinity concentrations on MDA content in root of fenugreek

Mean comparisons showed that different salinity concentrations affected the other aldehydes content of roots significantly. As shown in figure 4, the minimum of this trait belonged to control plants (0.1256 µmol g^{-1} FW) while short-term salinity stress of 200 mM NaCl increased the root other aldehydes content to the maximum value of 0.5456 µmol g^{-1} FW (Figure 4).

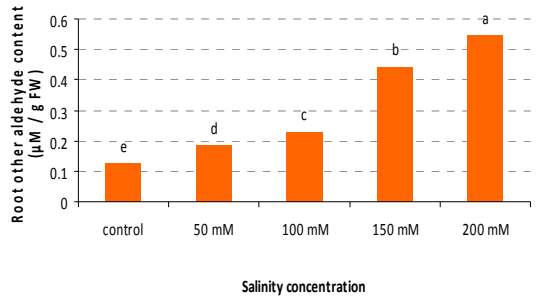

Figure 4. Effect of different salinity concentrations on the other aldehyde content in root of fenugreek

Response of membrane stability index (MSI) to salinity levels was different. The highest and lowest of MSI belonged to control and 200 mM treatments with value of 58.3% and 5% respectively. Application of the first level of short-term salinity stress (50 mM) caused a reduction by 13.99% in MSI, while this reduction for 100, 150 and 200 mM concentrations were 30.5%, 64.9% and 91.4% respectively (Figure 5).

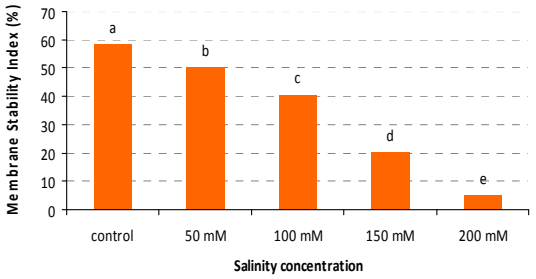

Figure 5. Effect of different salinity concentrations on membrane stability index of fenugreek

As figure 6 shows, the protein content was affected by different levels of short-term salinity stress. The highest protein content was recorded for control plants (129.5 mg g^{-1} FW). It was decreased by 2.77% when plants treated with 50 mM NaCl. Maximum decrease in total protein content was recorded for 200 mM NaCl concentration (33.2%) and this followed by 150 and 100 mM NaCl application (Figure 6).

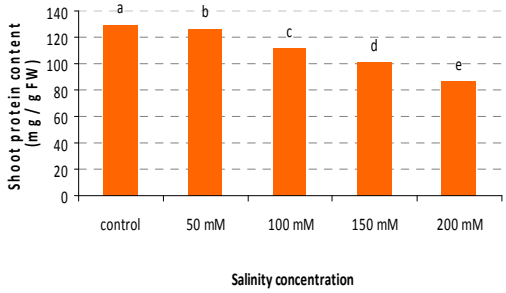

Figure 6. Effect of different salinity concentrations on protein content in shoot of fenugreek

Effects of different NaCl concentrations on CAT activity are presented in figure 7. CAT activity increased gradually with increasing NaCl concentrations up to 100 mM. The highest increase in activity was 184.9% compared to the control treatment (Figure7). Increasing salinity to more than 150 again reduced CAT activity but still was significantly higher than control.

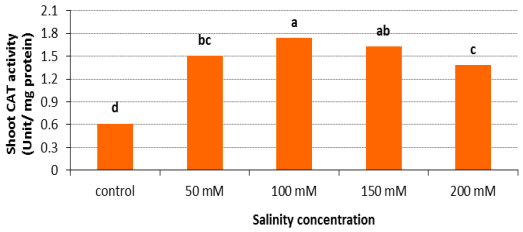

Figure 7. Effect of different salinity concentrations on catalase activity in shoot of fenugreek

Discussion

Salinity plays a major role in limiting cellular processes; thus, dealing with this stress is very important for plant growth. Mechanisms used by plants to overcome the detrimental effects of abiotic stresses, are very complicated because the growth and development mechanisms of the plants and the stress effectors mechanisms need to be balanced.

Our findings showed that under salinity stress, caused by different concentrations of NaCl, MDA levels in Fenugreek increased significantly compared to control plants, and this could be an indication for damaging cell membrane and reduction in its stability. Malondialdehyde (MDA) content, a product of lipid peroxidation, has been considered as an indicator of oxidative damage (Lima et al., 2002). Variations in MDA content have been reported for different plant species under different conditions. Since Catalaes activity increases in plants exposed to salinity, it implies that plant mechanism has been activated to quench ROS and alleviate fallowing oxidative damage resulted from salinity stress. An increase in H_2O_2 and MDA concentration upon salt stress has been reported in different plant species (Lee et al., 2001, Sudhakar et al., 2001; Sairam & Srivastava., 2002; Bandeoglu et al., 2004) and it is shown that this is related to stress levels and well correlated with lipid membrane damage. Lipid peroxidation under salt stress has also been reported in rice leaves (Dionisio-Sese & Tobita., 1998), tomato (Shalata et al., 2001), sugar beet (Bor et al., 2003) and cotton (Meloni et al., 2003).

One of the mechanisms affected by salt stress in plants is protein synthesis. It is known that soluble protein content is an important indicator of physiological status of plants. In this study, 48 h after applying salinity treatment, total protein content was decreased in plants grown under 50, 100, 150 and 200 mM NaCl concentrations. In response to increasing salinity, harmful effects increase, many metabolic pathways are inhibited, and many proteins are damaged and/or degraded either due to oxidative damage or proteolytic activities. The final result is decreased total protein content under high stress. Increased levels of protein synthesis are therefore important in order to restore damaged proteins for full restoration of the plant cell's metabolic activities and general growth. Demiral & Turkan (2006) detected that total protein content of salt tolerant rice cultivar (Pokkali) increased with salinity. However, Sibole et al., (2003) reported that salt stress could decrease or increase soluble protein content in legumes, and their response to salt stress was related to the tolerance of different species to salt stress. Our results were in agreement with those of Demir & Kocacliskan., (2001) and Shibli et al., (2007). In line with our findings, Yurekli et al., (2004) reported that short-term salinity stress severely reduced leaf protein content in *Phaseolus vulgaris* plants. Similarly, Porgali & Yurekli (2005) reported that compared with control plants, protein content in salt sensitive tomato (*Lycopersicon esculentum)* plants decreased with the salt application.

CAT, which is involved in the degradation of hydrogen peroxide into water and oxygen, is the most effective antioxidant enzymes in preventing oxidative damage (Willekens et al., 1995;Mittler., 2002). According to our results, the peak CAT activity in the shoot was observed at concentration of 100 mM NaCl. Similar to our findings, increased CAT activity was reported in *Cassia angustifolia* L. (Agarwal & Pandey., 2004), maize (Azevedo Neto et al., 2006), *Sesamum indicum* (Koca et al., 2007) and *Jatropha curcas* L. (Gao et al., 2008) differing in salt tolerance. The changes in CAT activity may depends on the species, the development and metabolic state of the plant, as well as on the duration and intensity of the stress (Chaparzadeh et al., 2004). Therefore, our findings showed that increased CAT activity plays an important protective role in the ROS-scavenging process and the active involvement of this enzyme is related, at least in part, to salt-induced oxidative stress tolerance in *Trigonella foenum* plants. This study showed that MDA and other aldehydes content, in both shoot and root, increased when exposed to increasing NaCl concentrations, while MSI and protein content decreased. Increased CAT in fenugreek (*Trigonella foenum*) indicates the tolerance capacity of this plant to protect itself from oxidative damage induced by NaCl. Although it can be said that through this enzyme fenugreek is able to tolerate the salinity of 150 mM but further experiments are necessary to understand the induction and regulation of this enzyme as well as the regulation mechanism of metabolism in this plant *Trigonella foenum* against salt stress.

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