Scientia Horticulturae 233 (2018) 61–69

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Integrative application of cyanobacteria and antioxidants improves common bean performance under saline conditions

Mostafa M. Rady[a,*], Sahar S. Taha[b], Sebnem Kusvuran[c]

[a] Botany Department, Faculty of Agriculture, Fayoum University, 63514, Fayoum, Egypt
[b] Vegetable Crops Department, Faculty of Agriculture, Cairo University, Giza, Egypt
[c] Cankiri Karatekin University, Kizilirmak Vocational High School, 18100, Cankiri, Turkey

ARTICLE INFO

Keywords:
Antioxidative activity
Blue-green algae
Phaseolus vulgaris
Photosynthetic efficiency
Salt stress

ABSTRACT

Cyanobacteria (CB), known as blue-green algae, are useful microscopic organisms that can lead to improve the nutrient uptake, plant growth, and plant tolerance to abiotic stress such as salinity. The impacts of CB, with or without glutathione (GSH) and ascorbic acid (AsA) and their combinations on morphological, physiological, metabolic, and enzymatic activity status in common bean plant grown on salt stressed soil were investigated. All single (CB, AsA or GSH), or combined (CB + AsA, CB + GSH, CB + AsA + GSH or CB + GSH + AsA) applications significantly increased plant length, number and area of leaves, plant fresh and dry weights, yield parameters (green pods weight per plant, dry seed weight per plant and 100-seed weight) and leaf photosynthetic pigments and their photochemical efficiency (*Fv/Fm* and PI) of common bean plants compared to the control in 2015 and 2016 growing seasons. In addition, relative water content, membrane stability index, contents of soluble sugar, proline, AsA, GSH, N, P and K^+ ion contents, and activities of superoxide dismutase, catalase and guaiacol peroxidase were significantly increased with all of the mentioned applications. In contrast, electrolyte leakage and Na^+ ion content was significantly decreased. The best response was obtained with the integrative CB + AsA + GSH and CB + GSH + AsA treatments, with distinguish of the former. Overall, these results suggest that supporting the seed CB application with foliar application of AsA and GSH helped to increase the defense systems of the common bean plant to tolerate the adverse effects of soil salinity.

1. Introduction

Salinity is one of the most important abiotic stresses that cause reduction in plant growth, development, and productivity worldwide, particularly in arid and semi-arid regions, where soil salt content is naturally high and precipitation can be insufficient for leaching (Kusvuran et al., 2016). Growth mediums with high salinity cause many adverse effects on plant growth, which can possibly be due to a low osmotic potential in soil solutions, effects of specific ions (salt stress), imbalance in nutrition, or a combination of such factors. All the factors mentioned have negative effects on plant development at physiological and biochemical levels. During the onset and development of salt stress within a plant, all the major processes such as photosynthesis, protein synthesis, energy and lipid metabolism are affected. Photosynthetic capacity is reduced, due to the osmotic stress and partial closure of stomata (Paul and Lade, 2014). –

Salt stress changes the morphological, physiological, and biochemical responses of plants. There is evidence that high salt concentrations cause an imbalance in cellular ions, resulting in ion toxicity and osmotic stress, leading to the generation of reactive oxygen species (ROS), which cause damage to DNA, lipids, and proteins. Concurrently, ROS cause chlorophyll degradation and membrane lipid peroxidation, decreasing membrane fluidity and selectivity. To prevent the negative effects of ROS, plants have developed various antioxidant enzyme systems including non-enzymatic antioxidants (e.g. ascorbic acid, glutathione and carotenoids) and antioxidative enzymes (e.g. glutathione reductase; GR, superoxide dismutase; SOD and ascorbate peroxidase; APX). The enzyme SOD belongs to a group of enzymes that accelerate the conversion of $O_2^{-}\cdot$ to H_2O_2 (Hodges et al., 1997). While, catalase (CAT) peroxidases detoxify toxic H_2O_2, superoxide is broken down into water and oxygen by catalysis by SOD. The H_2O_2 is then further scavenged by CAT and APX into H_2O and O_2 (Anjum et al., 2012). The APX reduces H_2O_2 using ascorbate as an electron donor in the ascorbate-glutathione cycle. Oxidized ascorbate is then reduced by GSH generated from GSSG catalyzed by GR at the expense of NADPH. Previous studies showed that the level of antioxidative enzymes increases when plants are exposed to oxidative stress including salinity (Zhu et al., 2004; Sevengör et al., 2011; Kusvuran et al., 2016). Plants with high levels of

* Corresponding author.
E-mail address: mmr02@fayoum.edu.eg (M.M. Rady).

https://doi.org/10.1016/j.scienta.2018.01.047
Received 29 April 2017; Received in revised form 9 January 2018; Accepted 16 January 2018

antioxidants, either constitutive or induced, have been reported to have greater resistance to this oxidative damage (Siringam et al., 2011). The reports suggested that the extent of oxidative cellular damage in plants exposed to abiotic stress is controlled by the capacity of their antioxidant systems.

Ascorbic acid (AsA) is a small, water-soluble molecule, which acts as a primary substrate in the cyclic pathway for detoxification and neutralization of superoxide radicals and singlet oxygen. Ascorbate functions co-ordinately with glutathione and several enzymatic antioxidants to inactivate superoxide, which is produced by the Mehler reaction and photorespiration (Noctor and Foyer, 1998). Ascorbate is also believed to detoxify singlet oxygen and hydroxyl radicals and AsA is also involved in regulating photosynthetic capacity by controlling stomatal movement (Athar et al., 2008; Dolatabadian et al., 2009). The AsA has also been shown to play multiple roles in plant growth, such as in cell division and wall expansion, and other developmental processes (Smironoff, 1996; Conklin, 2001; Pignocchi and Foyer, 2003).

Glutathione (GSH) is widely used as a marker of oxidative stress in plants, although its part in plant metabolism is a multi-faceted one (Tausz et al., 2004). GSH is the most important non-protein thiol present in plant cells, and the physiological effects of GSH can be divided into two categories: effects on sulphur metabolism and effects on the defense system. In addition, Mullineaux and Rausch (2005) reported that GSH plays an important role in the protection of the cell against oxidative stress. It is involved in the ascorbate-glutathione cycle and the regulation of protein thiol-disulphide redox status of plants in response to abiotic and biotic stress.

Cyanobacteria (CB) are an important component of soil. Some CB can grow successfully on saline soil, whereas most plants cannot. The fertility of soil can be improved by adding CB. They are a group of gram negative photo autotrophic bacteria (Nayak et al., 2001), and are also the most important nitrogen-fixing agents in many agricultural soils (Vargas and Novelo, 2007). They have the ability to secrete growth promoting substances such as hormones, vitamins and amino acids. They can also increase both the water holding capacity through their jelly-like structure and soil biomass after their death and decomposition (Alam et al., 2014). The CB inoculation is known to reduce the content of oxidized matter in soil, provide oxygen to submerged rhizospheres, ameliorate salinity and buffer the pH, solubilize phosphates, increase the efficiency of fertilizer usage of plants, and enhance plant growth (Mandal et al., 1999; Kaushik and Krishna Murti, 1981; Nain et al., 2010). The CB respond to high salinity by restricting the entry of Na ions and preventing cell injury by keeping a low internal Na concentration (Roychoudhury et al., 1985; Jha et al., 1987).

Classifying as a salt-sensitive plant (Maas and Hoffman, 1977), the common bean (*Phaseolus vulgaris* L.) is one of the most important vegetable crops belonging to *Fabaceae*. Food legumes, including beans, are an important component of the agricultural sector in developing countries due to their capacity to produce significant quantities of protein-rich seed for human nutrition.

This work was designed to investigate the impacts of seed inoculation with CB in addition to foliar application of AsA or GSH, and their integrations on the salt tolerance of *Phaseolus vulgaris*. To examine this purpose, the antioxidantive enzymatic (SOD, GPOX, and CAT) and non-enzymatic (AsA, GSH, and proline) activities, which are among the main antioxidative defences in plants, were assessed.

2. Material and methods

2.1. Experimental conditions and treatments

The seeds of the common bean (*Phaseolus vulgaris* L., cv. Bronco) were obtained from the Agricultural Research Center, Giza, Egypt. Two pot experiments were conducted in two successive summer seasons (2015 and 2016) at the Experimental Farm of the Faculty of Agriculture in Fayoum, Egypt. Seeds were inoculated with cyanobacteria (CB) before being sowed on 25 February of each season in plastic pots (50 cm in diameter, 50 cm in depth) containing saline soil. The soil analyses were carried out according to Black et al. (1965) and Jackson (1967). The soil characteristics are shown in Table 1. Based on the determined EC values (7.35–7.42 dS m^{-1}), the soil is classed as being saline according to Dahnke and Whitney (1988).

Table 1
Physical and chemical properties of the experimental saline soil.

Parameter	2015	2016
Clay	51.25	50.50
Silt	31.50	32.50
Sand	17.25	17.0
Soil texture	Clay	
pH	7.82	7.86
EC (dS m^{-1})	7.35	7.42
Organic matter%	0.81	0.79
CEC* ($cmol_c\ kg^{-1}$)	5.68	5.56
Field capacity (%)	32.9	32.2
Available water (%)	30.1	29.7
Available N (mg kg^{-1} soil)	12.1	11.8
Available P (mg kg^{-1} soil)	147.4	140.9
Available K (mg kg^{-1} soil)	12.5	11.9
Available Fe (mg kg^{-1} soil)	119.8	116.7
Available Mn (mg kg^{-1} soil)	30.5	28.9
Available Zn (mg kg^{-1} soil)	9.7	10.2

* CEC; cation exchange capacity.

The recommended mineral fertilization program for common bean in newly-reclaimed saline soils is a total of 450, 450 and 225 kg ha^{-1}, calcium superphosphate (15.5% P_2O_5), ammonium nitrate (33.5% N), and potassium sulphate (48% K_2O), respectively. This means that each pot (12 kg soil) was received 0.2, 0.2 and 0.1 g of these fertilizers, respectively. Soil in each pot was saturated with water and left until reached a soil water holding capacity. Seeds were then sown at a rate of 5 seeds per pot, and after full emergence seedlings were thinned to 3 per pot. Pots were irrigated with an equal volume of tap water day after day or once every 3 days according to the climate to maintain optimum soil moisture for plants. Using of pesticides was avoided so as not to interfere with the treatments effects, and weeds were collected manually in case of their emergence.

A preliminary study was performed using small plastic pots (0.75 kg soil) to select the best concentration of ascorbic acid (AsA) and glutathione (GSH) to use in the main study. The concentration of 1.0 mM from AsA and 0.75 mM from GSH was generated the best vegetative growth under the tested saline soil (data not shown). Each experiment was consisted of 7 treatments: control, 1.0 mM AsA foliar spray, 0.75 mM GSH foliar spray, inoculation of seeds with CB + AsA foliar spray, CB + GSH foliar spray, CB + AsA foliar spray first time + GSH foliar spray second time, and CB + GSH foliar spray first time + AsA foliar spray second time.

The AsA and GSH, at the amounts mentioned before, were sprayed on the foliage of plants to run off at 25 and 35 days after sowing for each or as a sequenced application of AsA then GSH or GSH then AsA. To ensure optimum penetration into leaf tissues, 0.1% (v/v) Tween-20 solution was added to the spraying solution as a wetting agent. The experiments were arranged in a randomized block design with one level of each of AsA (1.0 mM) and GSH (0.75 mM), applied singly or in combination with CB, with 20 replications/pots (3 plants pot^{-1}) per treatment.

2.2. Measurements of growth and yield characteristics

Forty five days after sowing, 9 plants were randomly chosen from each treatment and their growth characteristics: plant length, number of leaves per plant, leaf area per plant, plant fresh weight and plant dry weight were measured. Green pod characteristics were assessed from