RESEARCH ARTICLE

Biotic priming with *Pseudomonas fluorescens* induce drought stress tolerance in *Abelmoschus esculentus* (L.) Moench (Okra)

P. Pravisya¹ · K. M. Jayaram¹ · A. Yusuf¹

Received: 21 June 2018 / Revised: 17 August 2018 / Accepted: 31 October 2018 / Published online: 29 November 2018
© Prof. H.S. Srivastava Foundation for Science and Society 2018

Abstract Drought is a major abiotic stress which negatively affects plant growth and yield. The decrease in rainfall and ensuing drought affect crop production to a larger extent. The present investigation was aimed to evaluate the drought stress mitigation potential of *Abelmoschus esculentus* (L.) Moench (okra) plants primed with *Pseudomonas fluorescens* (PF). Okra seeds were primed with 10^{-7} CFU of PF, germinated and the plants were exposed to drought stress for 7 days, and the recovery potential was assessed after re-watering the plants. Physiological and biochemical parameters were evaluated during stress and recovery. PF treated plants mitigated the effect of drought stress by increasing relative water content (RWC), accumulated metabolites such as sugar, free amino acids and enhanced the activity of non enzymatic antioxidants; phenolics, ascorbate (AsA) and glutathione (GSH) and reactive oxygen species scavenging enzyme like superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX). Drought stress related membrane damage and protein denaturation were also alleviated by PF treatment. During re-watering, PF treated plants regained RWC, total sugar, total amino acid, protein, AsA, GSH, phenolics, SOD, CAT, APX and GPX to appreciable levels. Thus, this study suggests that PF can be used as an agent to effectively mitigate drought stress in okra plants.

Keywords Biotic priming · Drought · Antioxidant enzyme · Recovery

Introduction

Water availability is a primary factor that controls normal growth, development and yield in plants. Drought is a major factor affecting global agriculture production as total global water utilization tripled over the past 50 years, of which two-thirds is used for agriculture according to United Nations water development report (WWAPU 2015). Climate change induced precipitation pattern and related drought stress in plants depends on age, genotype and duration of stress (Lata et al. 2015). Water scarcity induces an array of morphological, physiological and biochemical reactions such as, movement of stomata, reduction in cell enlargement, changes in net photosynthesis, relative water content (RWC), change in photosynthetic pigments, increased respiration, increase in root:shoot ratio, overproduction of reactive oxygen species (ROS) and subsequent membrane damage (Pyngrope et al. 2013), leading to yield loss in plants. Drought-induced membrane damage and ROS production are alleviated by the upregulation of antioxidant enzymes, osmolytes, other stress-related proteins etc., that assist plants to increase stress tolerance (Kusvuran 2012).

To mitigate the effects of water stress on growth and yield, different approaches were used (Eisenstein 2013). Plant breeding methods are used for developing drought-resistant crop varieties, however, varieties raised through breeding does not guarantee the transfer of required traits to the succeeding generation and is time-consuming (Philippot et al. 2013). Genetic engineering assures drought tolerance at a higher level, yet its success is limited due to

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s12298-018-0621-5>) contains supplementary material, which is available to authorized users.

✉ A. Yusuf
akkara69@yahoo.co.in

¹ Department of Botany, University of Calicut, Calicut, Kerala 673635, India

the inherent problems and very costly (Fedoroff et al. 2010). Priming or pre-treatment of the seeds/seedlings, with bioinoculants, is another method used to ameliorate stress. Depending on the type of pre-treatment, priming is classified as biotic priming (using plant growth promoting bacteria, mycorrhiza etc.) and abiotic priming (using various chemicals). Plant growth promoting bacteria (PGPB) facilitate the production of phytohormones (indole acetic acid, gibberellic acid and cytokinins), increase the solubility of mineral phosphate by siderophore production and reduce the production of ABA during stress (Rai et al. 2014; Nautiyal et al. 2013).

PF is a plant growth promoting bacteria, that produces a wide range of enzymes and metabolites that help plants to mitigate the harmful effects of various biotic and abiotic stresses (Saravanakumar et al. 2011). According to Matthijs et al. (2007), PF has phosphate solubilizing capacity, assisting in plant growth improvement. PF inoculation increases the root-soil adhesion, increased soil moisture and enhances the soil/root tissue ratio favouring plant growth under drought stress (Niu et al. 2018). PF regulate plant growth by inducing the production of auxins, gibberellins and cytokinins (Marschner and Timonen 2006) and the balanced synthesis of these hormones under stress improves plant growth. *Zea mays* primed with *Pseudomonas spp.* improved osmotic balance by increased production of osmolytes under drought stress (Sandhya et al. 2010). *P. fluorescens* treated rice enhanced the expression of drought tolerant genes and improved drought stress tolerance potential (Saakre et al. 2017).

Global food security is under threat due to various environmental stresses, led to malnutrition worldwide (WHO 2016). The demand for increased nutrition warrants the production of quality vegetables which serves as a reservoir of micronutrients needed for healthier diets (Schreinemachers et al. 2017). *Abelmoschus esculentus* (Okra) is an economically important nutrient-rich vegetable crop grown in tropical and sub-tropical parts of the world (Gemedet et al. 2015). Fresh leaves, buds, flowers, pods, stems and seeds are used for various purposes. Immature fruits are consumed as vegetables and salads, soups and stews; mucilage of okra binds to cholesterol, reducing the risk of heart attack (Sengkhampan et al. 2009). Okra seeds are rich in oil (20–40%) with linoleic acid as the important constituent which is essential for human nutrition (Andras et al. 2005). The okra fruits have abundant antioxidant enzymes, contributing to beneficial health effects on a number of human diseases like cardiovascular diseases, type 2 diabetes, digestive diseases and some cancers (Sabitha et al. 2011; Atawodi et al. 2009; Kahlon et al. 2007). Drought stress during the reproductive stages of okra severely affects the flower formation, fruit development and the quality of fruits (Anyaocha et al.

2017). Though the role of PF in alleviating drought stress has been described in various plant species, detailed information on the antioxidative system mediated stress response is not reported in horticultural plants. The present study analysed the drought tolerance potential of PF treated okra, with emphasis on RWC, membrane damage, different metabolites and enzymatic and non-enzymatic antioxidant system.

Materials and methods

Plant material and PF treatment

Healthy okra seeds were surface sterilized with 1% (v/v) sodium hypochlorite for 5 min, washed 6 or 7 times with sterile double distilled water. Cultures of PF was procured from Institute of Microbial Technology, Chandigarh, India, reconstituted and maintained in King's B medium. The seeds were incubated with PF cultures 10^{-7} CFU ml $^{-1}$ [10^{-7} CFU ml $^{-1}$ was selected as optimal concentration, by using concentrations ranging from 10^{-5} to 10^{-9} CFU ml $^{-1}$ for PF infection in okra (supplementary data Fig. 1)] for 6 h at 28 °C on a rotary shaker at 250 rpm.

Field experiment

PF inoculated and un-inoculated okra seeds were germinated in polythene grow bags filled with sterilized soil (5 kg/bag) and one healthy plant was retained in each bag with 30 replicates. After 17 days of germination, one half (15 plants) from the PF inoculated (WW + PF) and un-inoculated plants (WW) were watered using sterile distilled water and the other half retained un-watered to induce drought stress, divided into two; one half treated with PF (WS + PF) and the other half without PF treatment (WS). Leaf samples for biochemical and physiological analysis were collected from all the treatments on the 0, 1st, 3rd, 5th and 7th day. Beyond the 7th day of stress (i.e., 9th and 11th day) the plants showed a deprivation of metabolites and pigments and dried (supplementary data Fig. 1). After 7 days of drought exposure, the plants were re-watered for 3 days to initiate recovery. The relative water content, total protein, total amino acid, MDA content, non-enzymatic and enzymatic antioxidant activity were analyzed during the 0, 1st, 3rd, 5th and 7th day of stress and 1st, 2nd and 3rd day of recovery. The field experiments were conducted in a greenhouse, established at the Department of Botany, University of Calicut at a temperature of 28 ± 2 °C, in a light intensity of 100 ± 5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and relative humidity of $60 \pm 5\%$.

Relative water content (RWC)

The relative water content was determined according to Bars and Weatherly (1950). Leaf samples were cut into uniform size, fresh weight (FW) was measured and the leaf samples were floated on distilled water in dark at room temperature. After 6 h, the turgid weight (TW) of the samples was measured. Further, the samples were kept in a hot air oven at 60 °C, until the dry weight becomes constant.

$$\text{Relative water content \%} = \frac{(\text{Fresh weight} - \text{Dry weight})}{(\text{Turgid weight} - \text{Dry weight})} \times 100$$

Quantification of metabolites

The total sugar from the in leaf tissues was quantified using the phenol-sulphuric acid method (Dubois et al. 1951). The quantity of sugar was calculated from the standard curve prepared using a gradient concentration of D-glucose at 490 nm. Protein content was determined following Lowry et al. (1951). The quantity of protein was calculated with reference to a standard curve prepared using gradient concentrations of bovine serum albumin (BSA, 1 mg/ml). Free amino acids from the leaf samples were quantified according to Moore and Stein (1948), using ninhydrin reagent and the quantity of accumulated free amino acids was calculated from the standard curve prepared using gradient concentrations of leucine (1 mg/ml).

Lipid peroxidation

Lipid peroxidation was determined as the quantity of malondialdehyde (MDA; $\text{MDA} \epsilon = 155 \text{ mM}^{-1} \text{ cm}^{-1}$), the product of lipid peroxidation according to Heath and Packer (1968).

Assay of non-enzymatic antioxidants

Total phenolic contents were quantified according to Folin and Denis (1915). Fresh leaf tissue (1 mg) was homogenized in 80% (v/v) ethanol and centrifuged at 10,000 rpm for 20 min. One ml. of the supernatant was pipetted out and made up to 2 ml using distilled water and equal volume of 20% (w/v) sodium carbonate was mixed with 0.5 ml of 1 N Folin–Ciocalteu reagent. This mixture was kept for 15 min. in a boiling water bath, cooled to room temperature and the absorbance was read at 700 nm. A gradient concentration of tannic acid (1 mg/ml) was used as the standard.

AsA and GSH were quantified according to Chen and Wang (2002). Leaf tissue (200 mg) was homogenized in 5 ml 5% (w/v) TCA and centrifuged at 12,000 rpm for 15 min at 4 °C. 1 ml of the supernatant was mixed with 0.3 ml of 200 mM sodium phosphate buffer, 0.5 ml of 10% (v/v) TCA, 0.4 ml of 42% (v/v) H_3PO_4 , 0.4 ml of 4% (w/v) bipyridyl and 0.2 ml of 3% FeCl_3 (w/v). The mixture was incubated at 36 °C for 15 min, and the absorbance was measured at 524 nm and AsA content was calculated from a standard curve prepared using gradient concentration AsA (1 mg/ml). For GSH quantification leaf tissue (200 mg) was homogenized in 5 ml TCA (5% w/v) and centrifuged at 12,000 rpm for 15 min at 4 °C. 0.5 ml of the supernatant was added to the reaction mixture containing 2.6 ml sodium phosphate buffer (pH 6.8), 0.18 ml of 3 mM 5 dithio-bis-2-nitrobenzoic acid (DTNB). This mixture was kept in a boiling water bath for 20 min. The absorbance was recorded at 412 nm and GSH content was calculated from a standard curve using gradient concentrations of reduced glutathione (1 mg/ml).

Assay of enzymatic antioxidants

Superoxide dismutase (SOD, EC 1.15.1.1) activity was quantified using the modified protocol of Giannopolitis and Ries (1977). One unit SOD activity is defined as the enzyme required for 50% photoreduction of nitroblue tetrazolium to blue formazan. The CAT (EC 1.11.1.6) activity was determined following the method of Kar and Mishra (1976), by measuring the decrease in absorbance at 240 nm for 1 min following the decomposition of one unit of the enzyme and expressed as $\mu\text{mole H}_2\text{O}_2$ decomposed $\text{min}^{-1} \text{ mg protein}^{-1}$. Cytosolic APX (EC 1.11.1.11) activity was assayed according to Nakano and Asada (1981), one unit enzyme activity is defined as the quantity of the enzyme required to oxidize one μmol of AsA min^{-1} at room temperature. Guaiacol peroxidase activity (EC 1.11.1.7) was measured according to Gaspar et al. (1975), one unit activity GPX was defined as the quantity of enzyme required for the formation of 1 μM of tetraguaiacol per minute.

Statistical analysis

The experiments were conducted in a randomized block design with 15 replicates and each experiment was repeated thrice. Statistical analysis of the physiological and biochemical parameters was carried out according to Duncan's test at 5% probability level and Pearson's correlation was calculated using the SPSS software (Version 16.0, SPSS Inc., Chicago, USA).

Results

Relative water content

Relative water content decreased in WS + PF plants and WS plants under drought stress. A drastic decrease in RWC ($30.08 \pm 3.88\%$) was observed in the WS plants, but in WS + PF plants the RWC was maintained at $60.54 \pm 2.55\%$ on the 7th day of stress. During re-watering, the WS + PF plants recovered faster compared to control plants, with the increase in RWC content ($80.33 \pm 1.39\%$) on the first day and further increased to $91.12 \pm 2.08\%$ on the second day. In the WS plants, the recovery of RWC content was $67.02 \pm 1.33\%$ on the first day and ($90.49 \pm 2.17\%$) on the third day of re-watering (Table 1).

Quantification of metabolites

The total protein content in the WW + PF and WW plants did not show appreciable difference, however, the total protein content in the WS + PF plants showed a progressive increase in protein content till the third day of stress and from the 5th day to the 7th day the protein content reduced drastically in the WS + PF plants (Fig. 1b). WS + PF plants showed faster recovery in total protein content ($12.40 \pm 0.16 \text{ mg g}^{-1}$) on the first day of re-watering compared to WS plants ($6.69 \pm 0.08 \text{ mg g}^{-1}$). The WS + PF plants showed rapid recovery of total protein ($15.9 \pm 0.34 \text{ mg g}^{-1}$) on the second day of re-watering whereas, in WS plants, the protein content ($12.26 \pm 0.56 \text{ mg g}^{-1}$) recovered on the third day of re-watering. The correlation between the protein content and RWC was significant in WS + PF under stress and recovery ($R = 0.977$, $P \leq 0.001$ and $R = 0.679$, $P \leq 0.001$) and in WS plants ($R = 0.951$, $P \leq 0.001$) during stress and recovery ($R = 0.750$, $P \leq 0.001$) (Table 3). The correlation between the protein content and MDA was significant in WS + PF plants under stress and recovery ($R = 0.992$, $P \leq 0.001$ and $R = 0.674$, $P \leq 0.001$) and in WS plants ($R = 0.847$, $P \leq 0.001$) during stress and recovery ($R = 0.755$, $P \leq 0.001$) (Table 2).

Free amino acid accumulation was more prominent in WS + PF plants, with a progressive increase in amino acid content from the third day to the 7th day. The higher quantity of amino acids ($27.69 \pm 0.69 \text{ mg g}^{-1}$) was observed on the seventh day of stress in WS + PF plants and in WS plants ($11.79 \pm 0.47 \text{ mg g}^{-1}$). On the first day of rewatering, the amino acid content ($6.60 \pm 0.25 \text{ mg g}^{-1}$) in the WS + PF plants reduced and attained the amino acid content equal to the control on the second day of rewatering, whereas in WS plants the

recovery comparable to the control plants was observed on the third day of rewatering (Fig. 1c). The correlation between free amino acid and RWC was significant in WS + PF plants under in stress and recovery ($R = 0.759$, $P \leq 0.001$ and $R = 0.939$, $P \leq 0.001$) and in WS plants $R = 0.841$, $P \leq 0.001$ during stress and $R = 0.227$, $P \leq 0.05$ in recovery (Table 3). The correlation between free amino acid and MDA was significant in WS + PF plants under stress and recovery ($R = 0.755$, $P \leq 0.001$ and $R = 0.939$, $P \leq 0.001$) and in PF un-inoculated plants ($R = 0.847$, $P \leq 0.001$) during stress and recovery ($R = 0.223$, $P \leq 0.05$) (Table 2).

Total sugar content increased during stress in WS + PF and WS plants. The increase in total sugar was more prominent in WS + PF plants ($29.55 \pm 0.66 \text{ mg g}^{-1}$) whereas in WS plants the sugar content was $21.16 \pm 0.66 \text{ mg/g}^{-1}$ on the 7th day of stress (Fig. 1a). WS + PF plants showed faster recovery of total sugar content ($5.61 \pm 0.32 \text{ mg g}^{-1}$) on the second day of watering, but WS plants acquired the normal level ($4.93 \pm 0.44 \text{ mg g}^{-1}$) on the third day of re-watering. The correlation between sugar content and RWC was significant in WS + PF plants during stress and recovery ($R = 0.758$, $P \leq 0.001$ and $R = 0.889$, $P \leq 0.001$) and in WS plants during stress ($R = -0.990$, $P \leq 0.001$) and recovery ($R = 0.961$, $P \leq 0.001$) (Table 3). The correlation between sugar content and MDA was significant in WS + PF plants during stress and recovery ($R = -0.754$, $P \leq 0.001$ and $R = 0.887$, $P \leq 0.001$) and in WS plants during stress ($R = -0.993$, $P \leq 0.001$) and recovery ($R = 0.966$, $P \leq 0.001$) (Table 2).

Malondialdehyde content

Membrane damage in water-stressed plants was assessed based on the MDA content. MDA accumulation was more prominent in WS plants compared to control plants. A higher quantity of MDA ($31.63 \pm 0.66 \text{ } \mu\text{mol g}^{-1}$) was observed in WS plants compared to WS + PF ($8.82 \pm 0.17 \text{ } \mu\text{mol g}^{-1}$) plants on the 7th day of stress (Fig. 2). During re-watering, the WS + PF plants showed fastest membrane damage recovery, with a lower MDA content on the first day of re-watering ($5.92 \pm 0.17 \text{ } \mu\text{mol g}^{-1}$) compared to WS plants ($23.7 \pm 0.34 \text{ } \mu\text{mol g}^{-1}$). In WS + PF plants progressive decrease in the MDA content ($3.42 \pm 0.22 \text{ } \mu\text{mol g}^{-1}$) on the second day of watering, whereas, in WS plants the recovery from the membrane damage was a slow process indicated by the MDA content ($5.07 \pm 0.17 \text{ } \mu\text{mol g}^{-1}$) and the MDA content comparable to control plants were attained on the third day of re-watering. The correlation between MDA and RWC was significant at $P \leq 0.001$ level in PF inoculated plants during stress ($R = -0.877$)

Table 1 Effect of PF treatment on RWC(%), phenolics (mg/g^{-1}), ascorbate (mg/g^{-1}), glutathione (mg/g^{-1}) in okra exposed to drought stress

Days	0	1	3	5	7	1R	2R	3R
RWC								
WW	90.41 $\pm$ 3.25 ^b	92.13 $\pm$ 3.33 ^a	94.85 $\pm$ 1.04 ^a	90.41 $\pm$ 3.45 ^a	92.13 $\pm$ 2.23 ^a	94.85 $\pm$ 1.06 ^a	90.41 $\pm$ 2.56 ^b	92.31 $\pm$ 2.31 ^a
WS	90.41 $\pm$ 2.10 ^b	84.64 $\pm$ 4.30 ^c	60.52 $\pm$ 5.01 ^c	54.28 $\pm$ 4.36 ^c	30.08 $\pm$ 3.88 ^c	67.02 $\pm$ 1.33 ^c	73.45 $\pm$ 2.11 ^c	90.49 $\pm$ 2.17 ^b
WW + PF	92.41 $\pm$ 2.16 ^a	92.13 $\pm$ 3.09 ^a	94.85 $\pm$ 2.10 ^a	90.41 $\pm$ 1.38 ^a	92.13 $\pm$ 2.78 ^a	94.85 $\pm$ 1.79 ^a	94.41 $\pm$ 1.47 ^a	92.36 $\pm$ 2.03 ^a
WS + PF	92.41 $\pm$ 2.16 ^a	91.33 $\pm$ 3.02 ^b	86.74 $\pm$ 3.04 ^b	77.74 $\pm$ 2.01 ^b	60.54 $\pm$ 2.55 ^b	80.33 $\pm$ 1.39 ^b	91.12 $\pm$ 2.08 ^b	91.99 $\pm$ 1.04 ^a
Phenolics								
WW	5.35 $\pm$ 0.24 ^a	5.21 $\pm$ 0.23 ^a	5.38 $\pm$ 0.24 ^a	5.35 $\pm$ 0.21 ^c	5.94 $\pm$ 0.23 ^c	5.93 $\pm$ 0.22 ^c	5.03 $\pm$ 0.24 ^b	5.11 $\pm$ 0.25 ^a
WS	5.35 $\pm$ 0.21 ^a	5.54 $\pm$ 0.33 ^b	8.19 $\pm$ 0.41 ^b	9.25 $\pm$ 0.20 ^b	10.43 $\pm$ 0.73 ^b	9.38 $\pm$ 0.48 ^a	6.15 $\pm$ 0.29 ^a	5.57 $\pm$ 0.30 ^a
WW + PF	5.59 $\pm$ 0.22 ^a	5.31 $\pm$ 0.27 ^c	5.66 $\pm$ 0.22 ^c	5.53 $\pm$ 0.25 ^c	5.44 $\pm$ 0.38 ^c	5.39 $\pm$ 0.22 ^c	5.69 $\pm$ 0.33 ^b	5.78 $\pm$ 0.43 ^a
WS + PF	5.59 $\pm$ 0.22 ^a	6.14 $\pm$ 0.42 ^a	9.36 $\pm$ 0.40 ^a	11.33 $\pm$ 0.84 ^a	15.47 $\pm$ 0.88 ^a	8.89 $\pm$ 0.54 ^b	5.53 $\pm$ 0.32 ^b	5.53 $\pm$ 0.49 ^a
Ascorbate								
WW	1.30 $\pm$ 0.12 ^a	1.18 $\pm$ 0.12 ^a	1.44 $\pm$ 0.13 ^c	1.43 $\pm$ 0.13 ^c	1.94 $\pm$ 0.02 ^c	1.83 $\pm$ 0.14 ^c	1.21 $\pm$ 0.13 ^b	1.76 $\pm$ 0.22 ^a
WS	1.30 $\pm$ 0.11 ^a	1.90 $\pm$ 0.11 ^a	2.08 $\pm$ 0.22 ^b	2.67 $\pm$ 0.22 ^b	3.04 $\pm$ 0.22 ^b	2.95 $\pm$ 0.24 ^a	1.94 $\pm$ 0.13 ^a	1.97 $\pm$ 0.23 ^a
WW + PF	1.55 $\pm$ 0.19 ^a	1.36 $\pm$ 0.19 ^a	1.68 $\pm$ 0.11 ^c	1.88 $\pm$ 0.10 ^c	1.95 $\pm$ 0.02 ^c	1.76 $\pm$ 0.28 ^b	1.15 $\pm$ 0.27 ^a	1.93 $\pm$ 0.27 ^a
WS + PF	1.56 $\pm$ 0.19 ^a	1.96 $\pm$ 0.10 ^a	2.90 $\pm$ 0.21 ^a	5.70 $\pm$ 0.27 ^a	9.50 $\pm$ 0.22 ^a	2.51 $\pm$ 0.21 ^a	1.11 $\pm$ 0.24 ^a	1.76 $\pm$ 0.24 ^a
Glutathione								
WW	1.12 $\pm$ 0.12 ^a	1.25 $\pm$ 0.15 ^c	1.16 $\pm$ 0.13 ^c	1.73 $\pm$ 0.12 ^c	1.77 $\pm$ 0.13 ^c	1.60 $\pm$ 0.12 ^c	1.01 $\pm$ 0.12 ^b	1.93 $\pm$ 0.12 ^a
WS	1.16 $\pm$ 0.12 ^a	1.09 $\pm$ 0.12 ^c	2.53 $\pm$ 0.22 ^b	3.67 $\pm$ 0.22 ^b	6.11 $\pm$ 0.22 ^b	4.20 $\pm$ 0.22 ^b	1.42 $\pm$ 0.22 ^a	1.89 $\pm$ 0.12 ^a
WW + PF	1.22 $\pm$ 0.13 ^a	1.83 $\pm$ 0.12 ^b	1.86 $\pm$ 0.02 ^c	1.88 $\pm$ 0.12 ^c	1.85 $\pm$ 0.12 ^c	1.76 $\pm$ 0.12 ^c	1.05 $\pm$ 0.13 ^b	1.93 $\pm$ 0.16 ^a
WS + PF	1.15 $\pm$ 0.13 ^a	2.30 $\pm$ 0.22 ^a	18.88 $\pm$ 0.40 ^a	29.91 $\pm$ 0.80 ^a	36.48 $\pm$ 0.78 ^a	8.35 $\pm$ 0.40 ^a	1.01 $\pm$ 0.12 ^b	1.86 $\pm$ 0.14 ^a

Different alphabetical letters indicate statistically different means at P 0.05. Values followed by the same letter in a column indicate no significant difference between treatment (P 0.05) following one-way ANOVA (Duncan's test)

WW, control plants without PF treatment; WS, water stressed plants without PF treatment; WW + PF, control plants with PF treatment; WS + PF, water stressed plants with PF treatment 1R, 2R and 3R recovery

Fig. 1 Effect of PF treatment on total sugar (mg g^{-1}), total protein (mg g^{-1}) and free amino acid (mg g^{-1}) content in okra exposed to drought stress [WW, control plants without PF treatment; WS, water stressed plants without PF treatment; WW + PF, control plants with PF treatment; WS + PF, water stressed plants with PF treatment; 1R, 2R and 3R denotes recovery days]. Each letter indicate statistical difference $P \leq 0.05$ value followed by the same letter in a bar indicate no significant difference between the treatment following one way ANOVA

Table 2 Pearson's correlation between various parameters MDA (malondialdehyde), RWC (relative water content), SU (sugar), PRO (protein), AA (amino acid), PHE (phenolics), AsA (ascorbate), GSH (glutathione), SOD (superoxide dismutase), CAT (catalase), APX (ascorbate peroxidase) and GPX (guaiacol peroxidase) in response to water stress and recovery

	MDA × RWC	MDA × SU	MDA × PRO	MDA × AA	MDA × PHE	MDA × ASA	MDA × GSH	MDA × SOD	MDA × CAT	MDA × APX	MDA × GPX
Control											
WW	– 0.883	– 0.673*	0.957**	0.519	0.336	0.983**	0.501	0.957**	0.685*	0.371	0.657
WW + PF	0.302	0.992**	0.950**	0.924**	0.512*	0.607	0.480	0.907**	0.839	0.990**	0.966**
Stressed											
WS	0.169	– 0.993**	0.847**	0.847**	0.696*	0.999**	– 0.966**	– 0.993**	– 0.849**	– 0.993**	0.848*
WS + PF	– 0.877	– 0.754**	0.992**	0.755**	0.979**	0.962**	0.974**	0.963**	0.835**	0.999**	0.959**
Recovery											
WS	0.414	0.966**	0.755**	0.223*	0.274	– 0.268	– 0.188	0.777*	0.826**	0.777*	0.695*
WS + PF	0.988**	0.887**	0.674**	0.939**	0.385	0.912**	0.890**	0.963**	0.996**	0.933**	0.635*

* $P \leq 0.05$; ** $P \leq 0.001$

and recovery ($R = 0.988$), and in WS + PF plants the values were significant at a $P \leq 0.05$ during stress ($R = 0.169$) and recovery ($R = 0.414$) (Table 2).

Antioxidant system

Nonenzymatic antioxidants

Drought stress led to an increase in the non-enzymatic antioxidants like phenolic substances, ascorbate and glutathione compared to control plants. Leaf phenolic content increased to $15.47 \pm 0.88 \text{ mg g}^{-1}$ in WS + PF plants, whereas, in WS plants, the quantity was $10.43 \pm 0.73 \text{ mg g}^{-1}$ on the 7th day of stress (Table 1). A significant increase in AsA content, with $9.50 \pm 0.22 \text{ mg g}^{-1}$ has observed on the 7th day in WS + PF plants and $3.04 \pm 0.22 \text{ mg g}^{-1}$ in WS plants (Table 1). GSH content of $36.48 \pm 0.78 \text{ mg g}^{-1}$ has observed on the 7th day in WS + PF plants and the activity was reduced to $6.11 \pm 0.22 \text{ mg g}^{-1}$ in WS plants (Table 1). On the first day of re-watering WS + PF plants recorded higher reduction in phenolics content ($8.89 \pm 0.54 \text{ mg g}^{-1}$) compared to WS plants ($9.38 \pm 0.48 \text{ mg g}^{-1}$), WS + PF plants exhibited a progressive recovery of phenolic content on the second day of re-watering ($5.53 \pm 0.49 \text{ mg g}^{-1}$). Similarly, AsA and GSH content (2.51 ± 0.21 and $8.35 \pm 0.40 \text{ mg g}^{-1}$) showed a progressive decrease in WS + PF plants and the values similar to control (1.11 ± 0.24 and $1.01 \pm 0.12 \text{ mg g}^{-1}$) were attained on the second day of re watering. In WS plants during the first day of rewatering, though the AsA and GSH content (2.95 ± 0.24 and $4.20 \pm 0.22 \text{ mg g}^{-1}$) reduced considerably, further reduced on the second day (1.94 ± 0.13 and $1.42 \pm 0.22 \text{ mg g}^{-1}$) and increased during the third day of rewatering. WS plants, developed phenolics, ascorbate and glutathione content (5.57 ± 0.30 , 1.97 ± 0.23 and $1.89 \pm 0.12 \text{ mg g}^{-1}$) little higher than the control on the third day of re watering attributing to their slow recovery. Correlation analysis showed the interaction between phenolic substances and MDA in WS + PF at $P \leq 0.001$ was significant with a $R = 0.979$ and recovery with $R = 0.385$ at $P \leq 0.05$ level. The correlation between AsA and MDA showed a significant R value 0.962 in WS + PF and a R value 0.912 during recovery at $P \leq 0.001$. The correlation between GSH and MDA was significant in WS + PF with $R = 0.974$, at $P \leq 0.001$ and recovery $R = 0.890$ at $P \leq 0.001$, in WS plants a correlation between AsA, GSH, phenolics and MDA during stress was significant ($R = 0.999$, $R = -0.966$, $P \leq 0.001$ and $R = 0.696$ $P \leq 0.05$) and in recovery it is non-significant ($R = -0.268$, $R = -0.188$ $R = 0.274$ $P \leq 0.05$) (Table 2).

Table 3 Pearson's correlation between various parameters RWC (relative water content), SU (sugar), PRO (protein), in response to water stress and recovery

	RWC × PRO	RWC × AA	RWC × SU
Control			
WW	0.873*	0.731*	0.907**
WW + PF	0.932**	0.992**	0.970**
Stressed			
WS	0.951**	0.841*	0.758*
WS + PF	0.977**	0.759**	0.990**
Recovery			
WS	0.750**	0.229*	0.889*
WS + PF	0.679**	0.939**	0.961**

* $P \leq 0.05$; ** $P \leq 0.001$ **Fig. 2** Effect of PF treatment on MDA content ($\mu\text{mol g}^{-1}$) in okra exposed to drought stress [WW, control plants without PF treatment; WS, water stressed plants without PF treatment; WW + PF, control plants with PF treatment; WS + PF, water stressed plants with PF treatment; 1R, 2R and 3R denotes recovery days]. Each letter indicate statistical difference $P \leq 0.05$ value followed by the same letter in a bar indicate no significant difference between the treatment following one way ANOVA

Enzymatic antioxidants

In the control plants, no significant difference in the enzymatic antioxidant activity was observed. However, on the 3rd day of drought stress, WS + PF plants showed a significant increase in the activity of ROS scavenging enzymes, SOD, CAT, APX and GPX. Higher activity of SOD (7.12 ± 0.22 Unit mg protein $^{-1}$) was recorded on the 7th day of stress in WS + PF plants, however, in the WS plants, the specific activity was 3.07 ± 0.18 unit mg protein $^{-1}$ (Fig. 3a). The CAT activity was significantly higher on the 7th day of drought exposure with an enzyme specific activity 5.74 ± 0.22 $\mu\text{mol mg protein}^{-1} \text{ min}^{-1}$ and the increase was more prominent in WS + PF plants, WS

plants showed CAT activity of 3.07 ± 0.18 $\mu\text{mol mg protein}^{-1} \text{ min}^{-1}$ (Fig. 3b). WS + PF plants significantly enhanced APX activity on the 7th day of stress with 16.26 $\mu\text{mol mg protein}^{-1} \text{ min}^{-1}$, but in WS plants the specific activity of APX decreased (5.16 ± 0.45 $\mu\text{mol mg protein}^{-1} \text{ min}^{-1}$) (Fig. 3c). PF inoculation significantly augmented the GPX specific activity (19.20 ± 0.45 $\mu\text{mol mg protein}^{-1} \text{ min}^{-1}$) on the 7th day of stress and in WS plants the GPX specific activity with 10.80 ± 0.42 $\mu\text{mol mg protein}^{-1} \text{ min}^{-1}$ compared to WW plants (Fig. 3d). During re-watering, the ROS scavenging enzyme activity was decreased faster in WS + PF plants than WS plants. WS + PF plants showed a higher reduction in SOD specific activity on the first day of re watering (2.86 ± 0.15 unit mg protein $^{-1}$) also the specific activity of CAT, APX and GPX (2.55 ± 0.09 , 4.47 ± 0.11 and 7.66 ± 0.14 $\mu\text{mol mg protein}^{-1} \text{ min}^{-1}$) was also reduced. WS + PF showed a decrease in specific activity of SOD (1.35 ± 0.11 unit mg protein $^{-1}$), CAT, APX and GPX (0.88 ± 0.27 , 1.61 ± 0.20 and 2.18 ± 0.31 $\mu\text{mol mg protein}^{-1} \text{ min}^{-1}$) on the second day of re watering. The specific activity of SOD (1.24 ± 0.12 unit mg protein $^{-1}$), CAT, APX and GPX (1.24 ± 0.12 , 1.45 ± 0.21 and 2.31 ± 0.34 $\mu\text{mol mg protein}^{-1} \text{ min}^{-1}$) in WS plants attained similar to the control on the third day of re-watering. The correlation between SOD, CAT, APX, GPX and MDA was significant in WS + PF ($R = 0.963$, $R = 0.835$, $R = 0.999$, $R = 0.995$, $P \leq 0.001$), during recovery, the correlation between SOD, CAT, APX, GPX and MDA was significant ($R = 0.963$, $R = 0.996$, $R = 0.933$, $P \leq 0.001$ and $R = 0.635$, $P \leq 0.05$) and in WS plants, the correlation between SOD, CAT, APX, GPX and MDA was significant in WS + PF plants during stress ($R = -0.993$, $R = -0.849$, $R = -0.993$, $P \leq 0.00$, $R = 0.848$, $P \leq 0.05$) also in recovery correlation between SOD, APX, GPX, CAT and MDA was significant ($R = 0.777$, $R = 0.777$, $R = 0.695$, $P \leq 0.05$ $R = 0.826$, $P \leq 0.001$) (Table 2).

Discussion

Colonization of the plant growth promoting bacteria, *P. fluorescens* (PF) in the roots of economically important plants, induces systemic stress tolerance in plants, by adjusting the metabolic processes (Saakre et al. 2017). Osmotic imbalance and relative water content act as indices of drought tolerance in plants (Ocampo and Robles 2000), however, such information is limited to economically important plant species. In spite of PF inoculation, drought-stressed okra plants initially showed a reduction in RWC, however, the water status improved during PF inoculation compared to un-inoculated okra plants, in

Fig. 3 Effect of PF treatment on ROS scavenging enzymes ($\mu\text{mol}/\text{protein}/\text{min.}$) in okra exposed to drought stress [WW, control plants without PF treatment; WS, water stressed plants without PF treatment; WW + PF, control plants with PF treatment; WS + PF, water stressed plants with PF treatment; 1R, 2R and 3R denotes recovery days]. **a** Specific activity of SOD, **b** specific activity of CAT, **c** specific activity of APX, **d** specific activity of GPX. Each letter indicate statistical difference $P \leq 0.05$ value followed by the same letter in a bar indicate no significant difference between the treatment following one way ANOVA

agreement with the results obtained in *Cicer arietinum* (Tiwari et al. 2016). The increase in osmolytes like proline and sugar improved RWC in PF treated drought stressed okra, is in concordance with the growth improvement in *Azotobacter chroococcum* and *Bacillus polymyxa* treated sunflower seedlings (Singh et al. 2015). The lesser RWC reduction in water-stressed okra, during drought is attributed to the faster recovery of RWC in PF treated plants during the recovery period. According to Zhang and Zhang (2001), PGPR improves the water status of plants by increasing root surface area, assisting to maintain water balance even under water stress. The RWC reduction in WS + PF plants significantly correlated with higher accumulation of osmolytes, as osmoregulation is an important mechanism involved in drought tolerance potential (Anjum et al. 2017). Higher accumulation of osmolytes like total protein, sugar and free amino acids resulted in an increase in the solute concentration in drought exposed plants assisting the absorption of water from dry soil (Fahad et al. 2017) and *Pseudomonas* sp. has the capacity to produce certain extracellular polymeric substances present in soil pores and increase water retention capacity of the soil under water deficient condition (Kroener et al. 2018).

Drought stress reduces the cellular water status which in turn disturbs the normal metabolic process in plants, hence mediated by the production of reactive oxygen species. Though MDA is not a direct measure for lipid peroxidation it can be used as an indicator of membrane lipid

peroxidation (Lata et al. 2015; Bhatt et al. 2011). The increased RWC in WS + PF plants, showed a decrease in MDA content proportionate to membrane protection, corroborating the results obtained in *Cicer arietinum* (Tiwari et al. 2016). The enhanced activity of antioxidant system in WS + PF plants significantly reduced the membrane damage, however, in WS plants the up-regulation of the antioxidant system was meager, similar observations were made in PGPR treated maize and in *Bacillus* treated maize plants (Gusain et al. 2015; Sandhya et al. 2010). Total protein content in plants increased during early days of stress, may be due to the enhanced synthesis of stress response proteins like dehydrin, HSPs, LEA etc. (Chutia and Borah, 2012). However, after the 3rd day of drought stress, reduction in protein content was observed in the WS + PF plants and WS plants, with lower levels in WS + PF plants. The reduction in protein content during stress is due to the hydrolysis of protein for metabolic functions (Vardharajula et al. 2011). The higher levels of free amino acids in WS + PF justify the role of amino acids in the osmotic adjustment under water stress. Studies revealed that the accumulation of amino acids like glycine betaine and proline during drought stress improves the plant's drought tolerance capacity (Sankar et al. 2007). Soluble sugars act as a nutrient molecule that regulates the plant growth and development (Rosa et al. 2009). During drought stress, soluble sugars maintain the turgidity of leaves and prevent dehydration of membranes and proteins (Sawhney and Singh 2002; Crowe et al. 1999). Soluble

sugar accumulation in WS + PF plants confirms the role of PF treatment to improve the efficiency of osmotic adjustment under water stress and the accumulation of sugar linearly correlated with the improvement in RWC and lowering of MDA content. However, it is observed that, the augmentation of sugar content in WS plants was not sufficient to alleviate stress-induced damages, supported by the results obtained in maize treated with different plant growth promoting bacteria (Sandhya et al. 2010) and in *Azospirillum* treated drought exposed maize plants (Bano et al. 2013).

Up-regulation of non-enzymatic antioxidants, phenolics substances, ascorbate and glutathione in WS + PF plants confirms the role of PF in improving the ROS scavenging mechanism during stress that mitigates the induced oxidative damage in okra under water stress. The synthesis of enzymatic and non-enzymatic antioxidants increase the stress tolerance potential in plants by acting as stress alleviator (Parsons and Fry 2010) and defense against oxidation (Horemans et al. 2000). The increase in AsA content in WS + PF plants helps to mitigate the negative effect of drought stress on photosynthesis by acting as an electron donor to photosystem II during photoinhibition and AsA facilitate the production of gibberellic acid which helps to maintain normal growth under stress condition (Smirnoff, 2011; Smirnoff and Wheeler 2000). The accumulation of GSH in WS + PF plants enhances the auxin content to maintain normal growth. GSH assist in cell division by activating the expression of genes related to cell cycle regulation (Diaz-Vivancos et al. 2010a, b), thus GSH accumulation promotes normal cell division during stress (Zechmann et al. 2011). The up-regulation of SOD activity in WS + PF plants confers reduced oxidative stress through active scavenging of superoxide radicals, compared to WS plants, as reported in *Cerasus humilis* seedlings (Ren et al. 2016). The significant increase in specific activity of APX, GPX and CAT in WS + PF plants underlines their active participation in the conversion of H₂O₂ to water. Water-stressed basil plants inoculated with *Pseudomonas* sp., *Bacillus* sp. and *Azospirillum brasilense*, significantly improved the CAT, GPX and APX specific activity (Heidari and Golpayegani 2012). PGPR stimulate plant growth and induce the up-regulation of stress alleviating enzymes SOD, CAT, POX and APX contributing the reduction in the ROS level in rice cultivars (Gusain et al. 2015), green gram (Saravanakumar et al. 2011) and potato under drought stress (Gururani et al. 2013).

Recovery from water stress in WS + PF plants is mediated by the synthesis of different metabolites and antioxidant enzymes during re-watering. The accumulation of amino acids and total sugars that act as osmolytes in WS + PF plants, assist to maintain osmotic balance in the

plant cell. Compatible solutes facilitate the osmotic potential decrease in cells and help to absorb water from the soil and maintain leaf cell turgor even under water stress (Chaves et al. 2003). Thus the accumulation of compatible solutes augments RWC recovery in water-stressed condition. Stress-induced ROS production causes denaturation and disintegration of the plasma membrane. Proteins induce the up-regulation of the activity of enzymatic and non-enzymatic antioxidants in WS + PF plants to reduce the ROS level assisting in the fast recovery of protein content and maintain membrane stability during re-watering.

Conclusion

The results of the present investigation confirm that, okra plants developed from PF treated seeds, develops improved drought stress tolerance capacity by maintaining the RWC, enhanced activity of enzymatic and non enzymatic antioxidants (AsA, GSH, SOD CAT APX and GPX) and increased accumulation of metabolites, suggesting that the biochemical and physiological changes in okra improve growth under drought stress. Therefore, PF can be used as an effective drought stress alleviator in okra.

Acknowledgments P.P. would like to acknowledge the funding in the form of e-grants received from Government of Kerala (India). Funding was provided by University of Calicut.

References

- Andras CD, Simandi B, Orsi F, Lambrou C, Tatla DM, Panayiotou C, Domokos J, Doleschall F (2005) Supercritical carbon dioxide extraction of “Okra” (*Hibiscus esculentus* L.) seeds. J Sci Food Agric 85:1415–1419. <https://doi.org/10.1002/jsfa.2130>
- Anjum SA, Ashraf U, Tanveer M, Khan I, Hussain S, Shahzad B, Zohaib A, Abbas F, Saleem MF, Ali I, Wang LC (2017) Drought induced changes in growth, osmolyte accumulation and antioxidant metabolism of three maize hybrids. Front Plant Sci 8:15. <https://doi.org/10.3389/fpls.2017.00069>
- Anyaocha CO, Orkpeh U, Fariyike TA (2017) The effects of drought stress on flowering and fruit formation of five okra genotypes in South-West Nigeria. Cont J Agric Sci 9:28–33. <https://doi.org/10.5707/cjagricsci.2015.9.1.28.33>
- Atawodi SE, Atawodi JC, Idakwo GA, Pfundstein B, Haubner R, Wurtele G, Spiegelhalter Owen RW (2009) Polyphenol composition and antioxidant potential of *Hibiscus esculentus* L. fruit cultivated in Nigeria. J Med Food 12:1316–1320. <https://doi.org/10.1089/jmf.2008.0211>
- Bano Q, Ilyas N, Bano A, Zafar N, Akram A, Hassan F (2013) Effect of *Azospirillum* inoculation on maize (*Zea mays* L.) under drought stress. Pak J Bot 45:13–20
- Bars P, Weatherly P (1950) Studies in the water relations of the cotton plant. New Phytol 49:81–97. <https://doi.org/10.1111/j.1469-8137.1950.tb05146.x>
- Bhatt D, Negi M, Sharma P, Saxena SC, Dobriyal AK, Arora S (2011) Responses to drought induced oxidative stress in five finger

- millet varieties differing in their geographical distribution. *Physiol Mol Biol Plants* 17:347
- Chaves MM, Maroco JP, Pereira JS (2003) Understanding plant responses to drought from genes to the whole plant. *Funct Plant Biol* 30:239–264. <https://doi.org/10.1071/FP02076>
- Chen JX, Wang XF (2002) Guide to plant physiological experiments. South China University of Technology Press, Guangzhou, pp 123–127
- Chutia J, Borah SP (2012) Water stress effects on leaf growth and chlorophyll content but not the grain yield in traditional rice (*Oryza sativa* Linn.) genotypes of Assam, India: II. Protein and proline status in seedlings under PEG induced water stress. *Am J Pl Sci* 3:971–980
- Crowe J, Hoekstra F, Crowe L (1999) Anhydrobiosis. *Ann Rev Plants Physiol* 54:579–599
- Diaz-Vivancos P, Dong YP, Ziegler K, Markovic J, Pallardó F, Pellny TK, Verrier P, Foyer CH (2010a) Recruitment of glutathione into the nucleus during cell proliferation adjusts whole cell redox homeostasis in *Arabidopsis thaliana* and lowers the oxidative defence shield. *Plant J* 64:825–838. <https://doi.org/10.1111/j.1365-313X.2010.04371.x>
- Diaz-Vivancos P, Wolff T, Markovic J, Pallard OFV, Foyer CH (2010b) A nuclear glutathione cycle within the cell cycle. *Biochem J* 431:169–178. <https://doi.org/10.1042/BJ20100409>
- Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1951) Colorimetric method for determination of sugars and related substances. *Anal Chem* 28:350–356. <https://doi.org/10.1021/ac60111a017>
- Eisenstein M (2013) Discovery in dry spell. *Nature* 501:S7–S9. <https://doi.org/10.1038/501S7a>
- Fahad S, Bajwa AA, Nazir U, Anjum SA, Farooq A, Zohaib A, Sadia S, Nasim W, Adkins S, Saud S, Ihsan MZ (2017) Crop production under drought and heat stress: plant responses and management options. *Front Plant Sci* 8:1147. <https://doi.org/10.3389/fpls.2017.01147>
- Fedoroff NV, Battisti DS, Beachy RN, Cooper PJM, Fishchhoff DA, Hodges CN, Zhu JK (2010) Radically rethinking agriculture for 21st century. *Science* 327:833–834. <https://doi.org/10.1126/science.1186834>
- Folin O, Denis W (1915) A colorimetric method for the determination of phenols (and phenols derivatives) in urine. *J Biol Chem* 22:305–308. <http://www.jbc.org/content/22/2/305.citation>
- Gaspar T, Penel C, Greppin H (1975) Peroxidase and isoperoxidation in relation to root and flower formation. *Plant Biochem J* 2:33–47. <https://doi.org/10.1034/j.1399-3054.2003.00223.x>
- Gemedé HF, Ratta N, Haki GD, Ashagrie Z, Woldegiorgis Beyene F (2015) Nutritional quality and health benefits of “Okra” (*Abelmoschus esculentus*): a review. *Int J Nutr Food Sci* 4:208–215. <https://doi.org/10.4172/2157-7110.1000458>
- Giannopolitis CN, Ries SK (1977) Superoxide dismutase-occurrence in higher plants. *Plant Physiol* 59:309–314. <https://doi.org/10.1104/pp.59.2.309>
- Gururani MA, Upadhyaya CP, Baskar V, Venkatesh J, Nookaraju A, Park SW (2013) Plant growth-promoting rhizobacteria enhance abiotic stress tolerance in *Solanum tuberosum* through inducing changes in the expression of ROS- Scavenging enzymes and improved photosynthetic performance. *J Plant Growth Regul* 32:245–258
- Gusain YS, Singh US, Sharma AK (2015) Bacterial mediated amelioration of drought stress in drought tolerant and susceptible cultivars of rice (*Oryza sativa* L.). *Afr J Biotech* 14:764–773. <https://doi.org/10.5897/AJB2015.14405>
- Heath RL, Packer L (1968) Phytoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch Biochem Biophys* 125:189–198. [https://doi.org/10.1016/0003-9861\(68\)90654-1](https://doi.org/10.1016/0003-9861(68)90654-1)
- Heidari M, Golpayegani A (2012) Effects of water stress and inoculation with plant growth promoting rhizobacteria (PGPR) on antioxidant status and photosynthetic pigments in basil (*Ocimum basilicum* L.). *J Saudi Soc Agric Sci* 11:57–61. <https://doi.org/10.1016/j.jssas.2011.09.001>
- Horemans N, Foyer CH, Potters G, Asard H (2000) Ascorbate function and associated transport systems in plants. *Plant Physiol Biochem* 38:531–540. [https://doi.org/10.1016/S0981-9428\(00\)00782-8](https://doi.org/10.1016/S0981-9428(00)00782-8)
- Kahlon TS, Chapman MH, Smith GE (2007) *In vitro* binding of bile acids by “Okra”, beets, asparagus, eggplant, turnips, green beans, carrot and cauliflower. *Food Chem* 103:676–680. <https://doi.org/10.1016/j.foodchem.2006.07.056>
- Kar M, Mishra D (1976) Catalase, peroxidase, and polyphenoloxidase activities during rice leaf senescence. *Plant Physiol* 57:315–319. <https://doi.org/10.1104/pp.57.2.315>
- Kroener E, Holz M, Zarebanadkouki M, Ahmed M, Carminati A (2018) Effects of mucilage on rhizosphere hydraulic functions depends on soil particle size. *Vadose Zone J* 17:2–11
- Kusvuran S (2012) Influence of drought stress on growth, ion accumulation and antioxidative enzymes in okra genotypes. *Int J Agric* 14:401–406
- Lata C, Muthamilarasan M, Prasad M (2015) Drought stress responses and signal transduction in plants. In: Pandey GK (ed) *Elucidation of abiotic stress signaling in plants*. Springer, New York. https://doi.org/10.1007/978-1-4939-2540-7_7
- Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951) Protein measurement with folin-phenol reagent. *J Biol Chem* 193:265–275
- Marschner P, Timonen S (2006) Bacterial community composition and activity in rhizosphere of roots colonized by arbuscular mycorrhizal fungi. In: Mukerji KS et al (eds) *Microbial activity in the Rhizosphere*. Springer, Berlin, pp 139–154
- Matthijs S, Tehrani KA, Laus G, Jackson RW, Cooper RW, Cornelis P (2007) Thioquinolobactin, a *Pseudomonas* siderophore with antifungal and anti-pythium activity. *Environ Microbiol* 9:425–434
- Moore S, Stein WH (1948) Photometric ninhydrin method for use in chromatography of aminoacids. *J Biol Chem* 176:367–388
- Nakano Y, Asada K (1981) Hydrogen peroxidase is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol* 22:867–880. <https://doi.org/10.1093/oxfordjournals.pcp.a076232>
- Nautiyal CS, Srivastava S, Chauhan PS, Seem K, Mishra A, Sopory SK (2013) Plant growth-promoting bacteria *Bacillus amyloliquefaciens* NBRISN13 modulates gene expression profile of leaf and rhizosphere community in rice during salt stress. *Plant Physiol Biochem* 66:1–9. <https://doi.org/10.1016/j.plaphy.2013.01.020>
- Niu X, Song L, Xiao Y, Ge W (2018) Drought-tolerant plant growth-promoting rhizobacteria associated with foxtail millet in a semi-arid agroecosystem and their potential in alleviating drought stress. *Front Microbiol* 8:2580
- Ocampo ETM, Robles RP (2000) Drought tolerance and osmotic adjustment in drought stressed mung bean. *Philipp J Crop Sci* 25:1–5
- Parsons HT, Fry SC (2010) Reactive oxygen species-induced release of intracellular ascorbate in plant cell-suspension cultures and evidence for pulsing of net release rate. *New Phytol* 187:332–342. <https://doi.org/10.1111/j.1469-8137.2010.03282.x>
- Philippot L, Raaijmakers JM, Lemanceau P, Van der Putten WH (2013) Going back to the roots: the microbial ecology of the rhizosphere. *Nat Rev Microbiol* 11:789–799. <https://doi.org/10.1038/nrmicro3109>
- Pyngrope S, Bhoomika K, Dubey RS (2013) Oxidative stress, protein carbonylation, proteolysis and antioxidative defense system as a

- model for depicting water deficit tolerance in Indica rice seedlings. *Plant Growth Regul* 69:149–165. <https://doi.org/10.1007/s10725-012-9758-3>
- Rai M, Rathod D, Agarkar G, Dar M, Brestic M, Pastore GM, Marostica MR Jr (2014) Fungal growth promotor endophytes: a pragmatic approach towards sustainable food and agriculture. *Symbiosis* 62:63–79. <https://doi.org/10.1007/s13199-014-0273-3>
- Ren J, Sun LN, Zhang QY, Song XS (2016) Drought tolerance is correlated with the activity of antioxidant enzymes in *Cerasus humilis* seedlings. *Biomed Res Int*. <https://doi.org/10.1155/2016/9851095>
- Rosa M, Prado C, Podazza G, Interdonato R, Gonzalez JA, Hilal M, Prado FE (2009) Soluble sugars- metabolism, sensing and abiotic stress. *Plant Signal Behav* 4:388–393
- Saakre M, Baburao TM, Salim AP, Ffancies RM, Achuthan VP, Thomas G, Sivarajan SR (2017) Identification and characterization of genes responsible for drought tolerance in rice mediated by *Pseudomonas fluorescens*. *Rice Sci* 24:291–298. <https://doi.org/10.1016/j.rsci.2017.04.005>
- Sabitha V, Ramachandran S, Naveen KR, Panneerselvam K (2011) Antidiabetic and antihyperlipidemic potential of *Abelmoschus esculentus* (L.) Moench. in streptozotocin-induced diabetic rats. *J Pharm Bio Allied Sci* 3:397–402. <https://doi.org/10.4103/0975-7406.84447>
- Sandhya V, Ali SKZ, Grover M, Reddy G, Venkateswaralu B (2010) Effect of plant growth promoting *Pseudomonas* spp. on compatible solutes antioxidant status and plant growth of maize under drought stress. *Plant Growth Regul* 62:21–30
- Sankar B, Jaleel CA, Manivannan P, Kishorekumar A, Somasundaram R, Panneerselvam R (2007) Drought-induced biochemical modifications and proline metabolism in *Abelmoschus esculentus* (L.) Moench. *Acta Bot Croat* 61:43–56
- Saravanakumar D, Kavino M, Raguchander T, Subbian P, Samiyappan R (2011) Plant growth promoting bacteria enhance water stress resistance in green gram plants. *Acta Physiol Plant* 33:203–209. <https://doi.org/10.1007/s11738-010-0539>
- Sawhney V, Singh DP (2002) Effect of chemical desiccation at the post-anthesis stage on some physiological and biochemical changes in the flag leaf of contrasting wheat genotypes. *Field Crops Res* 77(1):1–6
- Schreinemachers P, Simmons EB, Wopereis MC (2017) Tapping the economic and nutritional power of vegetables. *Glob food Secur* 16:36–45
- Sengkhampan N, Verhoef R, Schols HA, Sajjaanantakul T, Voragen AG (2009) Characterisation of cell wall polysaccharides from “Okra” (*Abelmoschus esculentus* (L.) Moench). *Carbohydr Res* 344:1824–1832. <https://doi.org/10.1016/j.carres.2008.10.012>
- Singh NB, Deepmala Singh D, Singh A (2015) Biological seed priming mitigates the effects of water stress in sunflower seedlings. *Physiol Mol Biol* 21:207–214. <https://doi.org/10.1007/s12298-015-0291-5>
- Smirnoff N (2011) Vitamin C: the metabolism and functions of ascorbic acid in plants. *Adv Bot Res* 59:107–177. <https://doi.org/10.1016/B978-0-12-385853-5.00003-9>
- Smirnoff N, Wheeler GL (2000) Ascorbic acid in plants: biosynthesis and function. *Crit Rev Plant Sci* 19:267–290. <https://doi.org/10.1080/10409230008984166>
- Tiwari S, Lata C, Chauhan PS, Nautiyal CS (2016) *Pseudomonas putida* attunes morphophysiological, biochemical and molecular responses in *Cicer arietinum* L. during drought stress and recovery. *Plant Physiol Biochem* 99:108–117. <https://doi.org/10.1016/j.plaphy.2015.11.001>
- Vardharajula S, Ali SZ, Grover M, Reddy G, Bandi V (2011) Drought-tolerant plant growth promoting *Bacillus* spp.: effect on growth, osmolytes, and antioxidant status of maize under drought stress. *J Plant Inter* 6:1–14. <https://doi.org/10.1080/17429145.2010.535178>
- WHO (2016) Database on Anaemia, Vitamin and Mineral Nutrition Information System (VMNIS). World Health Organization, Geneva
- WWAPU (2015) The United Nations world water development report 2015: water for a sustainable world. United Nations World Water Assessment Programme
- Zechmann B, Koffler BE, Russell SD (2011) Glutathione synthesis is essential for pollen germination *in vitro*. *BMC Plant Biol* 11:1–11. <https://doi.org/10.1186/1471-2229-11-54>
- Zhang GS, Zhang RZ (2001) The influence of nitrogen, phosphorus nutrition on the root characteristic matters of spring wheat under water stress. *J Gansu Agric Univ* 36:163–167. <https://doi.org/10.1071/A96159>