The physiological and biochemical responses of directly seeded and transplanted maize (*Zea mays* L.) supplied with plant growth-promoting rhizobacteria (PGPR) under water stress

Saeed Rezazadeh[1], Mohammadnabi ilkaee[1*], Fayaz Aghayari[1], Farzad Paknejad[1], and Mehdi Rezaee[2]

1. Department of Agronomy and Plant Breeding, Karaj Branch, Islamic Azad University, Karaj, Iran
2. Department of Horticulture, Shahrood University of Technology, Shahrood, Iran

Abstract

The purpose of the present study was to investigate the effect of plant growth-promoting rhizobacteria (PGPR*)* on physiological and biochemical properties of maize (*Zea mays* L.) in different cultivation methods under water stress. The experiment was carried out as split-plot design including water stress (well-watered, mild stress, and severe stress) as main plot and also cultivation (transplanting and direct seeding) and PGPR application (uninoculated and inoculated with *Pseudomonas putida)* as subplots. Water stress decreased biological and kernel yield. In direct seeding and no *Pseudomonas* application, severe stress decreased the kernel yield by 37% compared to the well-watered condition. Water stress resulted in significant reduction of chlorophyll content. However, it was increased by *Pseudomonas* application. In transplanting plants, well-watered and *Pseudomonas* application increased the chl. a+b by 41% in comparison with severe stress and no *Pseudomonas* application. Catalase (CAT) and superoxide dismutase (SOD) activities were gradually raised by increasing the water stress. In transplanted plants inoculated with *Pseudomonas*, severe stress decreased relative water content (RWC) by 23% compared to well-watered plants. In transplanted plants inoculated with *Pseudomonas*, severe stress increased Malondialdehyde (MAD) by 46% compared to well-watered plants. Total phenolic content (TPC) and total flavonoid content (TFC) increased by mild water stress and decreased by severe water stress. In directly seeded plants inoculated with *Pseudomonas,* mild water stress increased TPC by 11% compared to well-watered condition. Compared to well-watered treatment, 54% increases were observed in TFC by mild stress in transplanted plants treated with *Pseudomonas*. Transplanting and *Pseudomonas* was concluded to alleviate the adverse effects of water stress on physiological and biochemical traits of maize.

Keywords: cultivation method*; Pseudomonas* application*;* relative water content; malondialdehyde; phenol

Rezazadeh, S., M. ilkaee, F. Aghayari, F. Paknejad and **M. Rezaee.** 2019. 'The physiological and biochemical responses of directly seeded and transplanted maize (*Zea mays* L.) supplied with plant growth-promoting rhizobacteria (PGPR) under water stress'. *Iranian Journal of Plant Physiology,* 10 (1), 3009- 3021.

Introduction

*Corresponding author
E-mail address: ilkaeemohammadnabi@gmail.com
Received: October, 2019
Accepted: December, 2019

In natural environments, plants are exposed to both biotic and abiotic environmental stresses of pathogens, nutrient imbalance, extreme temperatures, salinity, and drought, which have adverse effects on plant growth and

productivity (Ruiz-Lozano et al., 2016). Recently, adverse impacts of abiotic stresses like salinity and drought have been hazardously increased (Golldack et al., 2014). Also, this is exacerbated by global climate change worldwide (Trenberth et al., 2014; Srivastava, 2017). Drought due to its significant limiting role on plant growth and yield is considered as the most important abiotic stress (Trenberth et al., 2014). Drought stress can alter plant characteristics physiologically and biochemically (Ruiz-Lozano et al., 2016). Hence, most plant processes are directly or indirectly influenced by the water shortage (Barzana et al., 2015). Plants use various mechanisms to cope with drought stress such as morphological acclimatization, osmotic adaptation, optimization of water resources, advancement of antioxidant system, improvement of root system, diminishment of growth and photosynthesis rate, all aimed to optimize water application (Farooq et al., 2009; Osakabe et al., 2014; Ruiz-Lozano et al., 2016). Plants use intrinsic and extrinsic protective systems against environmental stresses. Extrinsically, they attempt to make a corporation with favorable microorganisms presented in the rhizosphere to quench the stress effects (Liu et al., 2011). Plant growth-promoting rhizobacteria (PGPR) are important microorganisms for increasing the growth and yield of plants (Naiman et al., 2009). Pseudomonas strains have been specified as phosphorus solubilizers producing organic acids and phosphatases by facilitating the solubilization of phosphorus and other nutrients (Sharma et al., 2013).

In agricultural systems, the common uses of plant material are based on seed or seedling. Transplanting or replanting is applied as an effective technique by moving a plant from one location to another. Transplanted plants are raised under greenhouse or nursery condition before they are replanted in another, usually outdoor, growing location (Lampayan et al., 2015). Transplanting conserved the plants from environmental concerns like pests and diseases in the susceptible stage of plant life, i.e., the initial stage of plant growth specially germination. The advantages and disadvantages of transplanting are not similar for all plant species. However, the transplant shock - the damage induced in the process - is the main issue for most cases. In this technique, plants need an acclimatization period, known as hardening off. The growth stage of transplanting and the environmental conditions such as the temperature, moisture, and rhizosphere conditions are the main factors in transplanting (Johnkutty et al., 2006; Lampayan et al., 2015).

Recently, the climate changes and excessive use of chemical fertilizers have dramatically imposed the environmental issues and concerns in agricultural lands. However, the global perspective in producing the agricultural plants is based on the sustainable agricultural operations and proper management strategies such as bio-fertilizer application to alleviate environmental threats and increase the plant productivity. Previous studies have shown PGPR symbiosis alleviates the adverse effects of water stress, making the host plant more tolerant to stress (Gontia-Mishra et al., 2016; Calvo-Polanco et al., 2016; Danish and Zafar-ul-Hye, 2019). On the other hand, transplanting is the cultivation method to improve the yield of crops. However, a few studies have shown the advantages of transplanting relative to direct seeding in different plants (Dong et al., 2005; Birnbaum et al., 2011) and there is no published work on transplanting and direct seeding of maize inoculated with *Pseudomonas* under water stress condition. *Pseudomonas* has positive effects on quality and quantity of the plants. Therefore, the purposes of the study were (I) to assess the simultaneous effects of water stress, *Pseudomonas* inoculation, and cultivation method (transplanting and direct seeding) on plant yield and photosynthesis, (II) to evaluate these effects on antioxidant activities, (III) to measure these effects on water content and lipid peroxidation, and (IV) to assess the effects on phenol and flavonoid content of maize.

Material and methods

Soil and climate of experimental site

The present study was carried out in the research farm of University of Tehran (1312 m asl, 35°48´45” N, 51°01´30” E), Karaj, Iran. At the beginning of the experiment, a compound of the surface (0-30 cm) soil samples was collected, air-

Table 1
Physical and chemical properties of experimental field soil

Depth	texture	EC (ds m^{-1})	pH	TNV (%)	O.C (%)	N (%)	P (mg kg^{-1})	K (mg kg^{-1})	Fe (mg kg^{-1})	Cu (mg kg^{-1})
0-30	Loam		7.8	33	0.91	0.08		196		

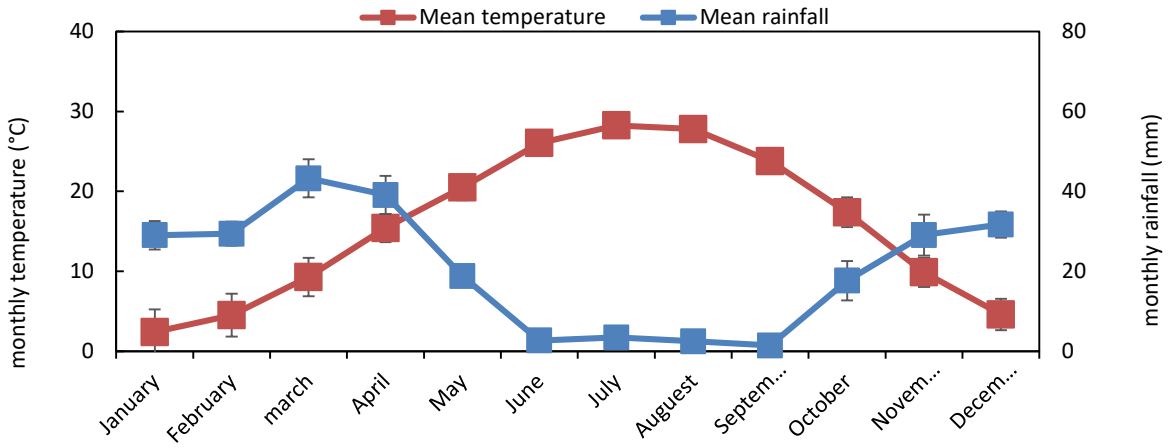

Fig. I. The climate conditions of Karaj during 2018 (National Meteorological organization, Iran)

dried, passed through a 2-mm sieve and the soil's physical and chemical properties were measured (Table 1). During the study period mean maximum temperature fluctuated from 17.3 °C to 28.2 °C whereas mean minimum temperature varied from 2.6 °C to 15.4 °C. The highest precipitation occurred in March (43.2 mm) and April (39.1 mm) (Fig. I).

Experimental design and treatments

The experiment was designed as split plot based on a randomized complete block design (RCBD) with three replications. Water stress was applied as the main plot at three levels as well-watered (25% soil moisture depletion), moderate water stress (50% soil moisture depletion), and severe water stress (75% soil moisture depletion). *Pseudomonas* inoculation was used at two levels as inoculated with *Pseudomonas putida* and uninoculated. The cultivation method was selected at two levels namely, direct seeding and transplanting.

Maize seeds (*SC704)* were purchased from Pakan-Bazar Company, Isfahan, Iran. The seeds were surface-sterilized in 10% (v/v) H_2O_2 for 15 min, and they were subsequently washed with deionized water. Uniform seeds were cultured after germination on moist filter paper at 25 °C for 36-48 h. Two different cultivation methods were used, namely (direct seeding and transplanting). For transplanting, the seeds were cultured in the greenhouse of University of Tehran, Karaj, using a culture media including coco peat and perlite (1:1) for 14 days. After that, the seedlings were transplanted in the open field (research farm of University of Tehran) on 25 may of 2018.

Pseudomonas inoculum

Luria Broth medium was used to culture *Pseudomonas putida*, and they were kept in a shaking incubator (150 rpm) for 24 h under 27 ± 1 °C.

Moisture treatments

All plots were uniformly irrigated after transplanting. To adapt the plants to the new environment, the second and third irrigations were applied according to 25% moisture depletion. The water stress treatments were exerted after the third irrigation according to the depletion of soil water. Irrigation regimes were used by the weighing method, through repeated soil sampling from the depth of root development

in the middle of experimental plots. Electric pump and tape were applied in the drip irrigation method. The crop water demand (Ig) was measured according to the following equation:

$$Ig = \frac{(\theta fc - \theta pwp) \times t \times \rho \times D \times A100}{Ea}$$

where ϑfc is the soil moisture content at field capacity, ϑpwp is the soil moisture content at wilting point, t is the soil moisture depletion content, ρ is the soil bulk density, D is the depth of root development, A is the plot area, and Ea is irrigation water efficiency, which was examined as an average of 90% (Tafteh and Sepaskhah, 2012).

Pigment content assay

Arnon (1949) method was used to measure chlorophyll a, chlorophyll b, and total chlorophyll contents. Two hundred (200) mg of fresh leaf sample was homogenized in 8 ml acetone (80%). Then, homogenates were centrifuged at 4 °C for 15 min (3000 rpm). Supernatants were used for analyzing pigments at 645, 663, and 470 nm.

Determination of leaf relative water content (RWC)

Leaf discs were obtained from the expanded leaves of each plant in the morning. The leaf samples were immediately weighed to find the fresh weight (FW), and submerged in distilled water for 4 h at 4 °C in dark condition and then weighed to obtain turgor weight (TW). After that, the leaves were dried in a forced-air oven at 70 °C for 24 h and the dry weight (DW) was measured. The RWC of samples was calculated using the following equation (Khoyerdi et al., 2016):

$$RWC = [(FW–DW)/(TW–DW)] \times 100$$

Malondialdehyde (MAD) concentration

To perform MDA assay, 0.25 g of fresh leaves were ground separately in 5 ml of 1% trichloroacetic acid (TCA) and centrifuged for 15 min (5000 rpm). A mixture was prepared by 1 ml of the supernatant and 4 ml of 20% TCA (containing 0.5% thiobarbituric acid). It was heated at 90 °C for 40 min, then quickly cooled in an ice bath and the absorbance was read at 450, 532, and 600 nm by a spectrophotometer. The MAD content was calculated using the following equation (Zhao et al., 1993):

$$\text{MDA}(\mu\text{mol g}^{-1}\text{FW}) = 6.45\,(\text{OD532} - \text{OD600}) - 0.56\,\text{OD450}$$

Superoxide dismutase (SOD, EC 1.15.1.1) activity

The activity of SOD was calculated as described by Beauchamp and Fridovich (1971). The reaction mixture consisted of 1.17×10^{-6} M riboflavin, 0.1 M methionine, 2×10^{-5} M KCN, and 5.6×10^{-5} M nitroblue tetrazolium salt (NBT) dissolved in 3 ml of 0.05 M sodium phosphate buffer (pH 7.8). Three (3) ml of the reaction medium was added to 1 ml of enzyme extract. Illumination was started to initiate the reaction at 30 °C for 1 h. Identical solutions that were kept under dark served as blanks and 560 nm was used to read the absorbance in the spectrophotometer against the blank. The activity of SOD was revealed in units (U mg^{-1} protein). One unit (U) is described as the change in the absorbance by 0.1 h^{-1} mg^{-1} protein.

Catalase (CAT, EC 1.11.1.6) activity

The activity of CAT was assayed based on the method described by Chandlee and Scandalios (1948). Briefly, 0.5 g of leaf samples was homogenized in a pre-chilled pestle and mortar with 5 ml of ice-cold 50 mM sodium phosphate buffer (pH 7.5) containing 1 mM phenylmethylsulfonyl fluoride (PMSF). The solution was centrifuged at 4 °C for 20 min. at 12,500 rpm. The supernatant was selected for enzyme assay. The mixture consisted of 2.5 ml of 50 mM potassium phosphate buffer (pH 7.0), 400 µl of 15 mM H_2O_2, and 40 µl of enzyme extract. The H_2O_2 decomposition was followed by the reduction in the absorbance at 240 nm and showed in U mg^{-1} protein.

Table 2
The effect of *Pseudomonas* application on biological yield and chlorophyll characteristics of direct seeding and transplanting maize under water stress condition

Irrigation regime	Cultivation method	*Pseudomonas*	Biological yield (ton/ha)	Kernel yield (ton/ha)	Chl.a+b (mg/g FW)	Chl. a:b ratio
Well-watered	Direct seeding	-	12.37 ±0.35de	5.67±0.08b	1.08±0.03ab	2.37±0.11de
		+	12.67±0.26cd	5.60±0.31b	1.10±0.02a	2.34±0.07de
	Transplanting	-	13.57±0.30ab	6.40±0.26a	1.03±0.01c	2.61±0.07b-d
		+	14.10±0.32a	6.57±0.08a	1.11±0.02a	2.35±0.07de
Mild stress	Direct seeding	-	11.30±0.36f	5.57±0.17b	1.07±0.01ab	2.30±0.08e
		+	11.83±0.15ef	5.53±0.14b	1.08±0.01ab	2.34±0.08de
	Transplanting	-	13.1±0.20ab	6.33±0.05a	1.05±0.01bc	2.51±0.10c-e
		+	13.87±0.30a	6.70±0.12a	1.08±0.13ab	2.40±0.09de
Severe stress	Direct seeding	-	8.40±0.32h	3.57±0.09e	0.79±0.03f	3.04±0.20a
		+	8.730±0.70h	3.70±0.05de	0.84±0.04e	2.70±0.03bc
	Transplanting	-	9.73±0.56g	4.03±0.09cd	0.78±0.03f	2.87±0.17ab
		+	10.23±0.50g	4.17±0.16c	0.88±0.01d	2.63±0.12b-d
Significance						
Ir			**	**	**	**
CM			**	**	ns	ns
Ps			**	ns	**	**
Ir*CM			ns	*	ns	ns
Ir*Ps			ns	ns	**	ns
CM*Ps			ns	ns	**	ns
Ir*CM*Ps			ns	ns	ns	ns

**, *, and ns show significant difference at 1%, 5%, and no significant difference, respectively.

Measurement of total phenolic content (TPC)

TPC was spectrophotometrically determined using Folin–Ciocalteu reagent. One hundred (100) µl of the MeOH solution of the precisely measured samples of the investigated plant 1–10 (2.54, 2.58, 2.25, 4.03, 4.80, 2.13, 4.62, 1.47, 1.58, 15.05 mg/mL respectively) were mixed with 0.75 mL of Folin–Ciocalteu reagent and allowed to stay at 22° C for 5 min. The mixture was enriched by 0.75 ml of $NaHCO_3$ solution and kept at room temperature for 90 min. The absorbance was measured at 725 nm by a UV–vis spectrophotometer (Varian Cary 50), and its standard curve was calibrated by Gallic acid (0–100 mg/L). The calibration curve revealed the linear regression at r>0.99, and the outcomes were showed as mg Gallic acid (GAE)/g DW.

Measurement of total flavonoid content (TFC)

Aluminum chloride method was applied to measure the TFC (Zhishenm, Mengcheng, and Jianming 1999). Briefly, 300 µL of $NaNO_2$ (1:20 w/v) were added to 0.5 mL of sample. The mixture was vortexed for 10 s and kept at 24 º C for 5 min. Subsequently, 2 mL of NaOH (1 M), 300 µL of AlCl3 (1:10 w/v), and 2 mL of distilled water. The absorbance was determined at 510 nm by UV–vis spectrophotometer (Varian Cary 50). Quercetin concentrations ranging from 0 to 1200 µg/mL were prepared and linear fit was used for calibration of the standard curve.

Statistical Analysis

All data were analyzed statistically by analysis of variance using SAS version 9.3 (Cary, NC: SAS Institute Inc., 2011). The means were separated using the Duncan test at P< 0.05 level.

Results

Biological and kernel yield

Biological yield was significantly influenced by water stress, cultivation method, and *Pseudomonas* application (P≤0.05, Table 2).

Table 3
The effect of *Pseudomonas* application on antioxidant enzymes and phenol and flavonoid content of direct seeding and transplanting maize under water stress condition

Irrigation	Cultivation method	*Pseudomonas*	CAT (U/mg protein)	SOD (U/ mg protein)	TPC (mg GA /g DW)	TFC (mg quercetin /g DW)
Well-watered	Direct seeding	-	0.47±0.017e	25.0±0.47bc	35.7±1.3bc	9.7±1.2c
		+	0.49±0.008e	25.3±1.67bc	37.0±0.9b	10.0±0.95c
	Transplanting	-	0.47±0.016e	22.7±1.63c	33.3±1.6c	9.3±1.6c
		+	0.48±0.020e	22.3±0.95c	26.0±1.7d	9.7±0.82c
Mild stress	Direct seeding	-	0.55±0.009d	31.0±1.24a-c	41.0±1.2a	14.0±1.7b
		+	0.53±0.026d	28.3±0.85a-c	41.3±0.8a	14.7±1.6ab
	Transplanting	-	0.54±0.012d	30.7±0.81a-c	41.0±1.3a	15.0±1.3ab
		+	0.52±0.016d	29.0±1.21a-c	43.3±0.9a	16.3±.81a
Severe stress	Direct seeding	-	0.78±0.012a	28.7±0.84a-c	35.7±1.4bc	10.7±0.47c
		+	0.74±0.014bc	36.0±1.24ab	35.0±1.2bc	10.0±1.1c
	Transplanting	-	0.77±0.014ab	37.0±0.82a	35.3±0.8bc	10.7±1.2c
		+	0.72±0.017c	36.3±0.92ab	36.0±1.4bc	11.3±0.78c
Significance						
Ir			**	**	**	**
CM			ns	ns	**	*
Ps			*	ns	ns	ns
Ir*CM			ns	ns	**	*
Ir*Ps			**	ns	**	ns
CM*Ps			ns	ns	ns	ns
Ir*CM*Ps			ns	ns	**	ns

**, *, and ns show significant difference at 1%, 5%, and no significant difference, respectively.

Biological yield decreased by increasing the rate of water stress. Under severe stress condition and *Pseudomonas* application, a 17% increase of biological yield was observed in transplanted plants compared to directly seeded plants (Table 2). Kernel yield was affected by water stress, cultivation method, and their interaction (P≤0.05, Table 2). In direct seeding and no *Pseudomonas* application, severe stress decreased the kernel yield by 37% in comparison with well-watered condition (Table 2).

Chlorophyll contents

The chl. a + b was significantly influenced by water stress, *Pseudomonas* application, and the interactions of water stress and *Pseudomonas* application, cultivation method and *Pseudomonas* application (P≤0.05, Table II). Chlorophyll content decreased over the increasing of water stress. However, it was increased by *Pseudomonas* application. In transplanted plants, well-watered and *Pseudomonas* application increased the chl. a + b by 41% in comparison with severe stress and no *Pseudomonas* application (P≤0.05, Table 2). The ratio of chl. a: b under severe stress was higher than other irrigation regimes. Under severe stress and transplanting method, *Pseudomonas* application decreased ratio of chl. a: b by 9% compared to no *Pseudomonas* application (Table 2).

Catalase (CAT) and superoxide dismutase (SOD) activity

The CAT activity was affected by water stress, *Pseudomonas* application, and the interaction of water stress and *Pseudomonas* application (P≤0.05, Table 3). CAT activity was enhanced gradually by increasing water stress. In transplanted plants inoculated with *Pseudomonas*, severe water stress increased the CAT activity by 50% in comparison with well-watered plants (Table 3).

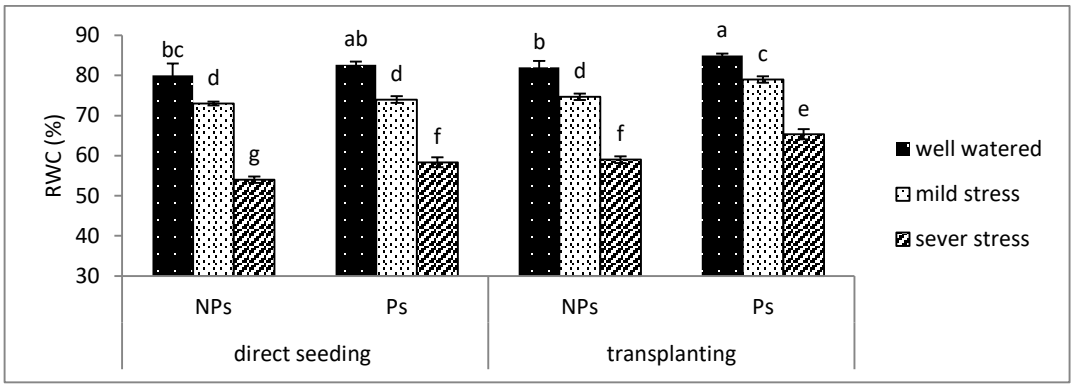

Fig. II. The effect of *Pseudomonas* application on relative water content (RWC) of direct seeding and transplanting maize under water stress condition

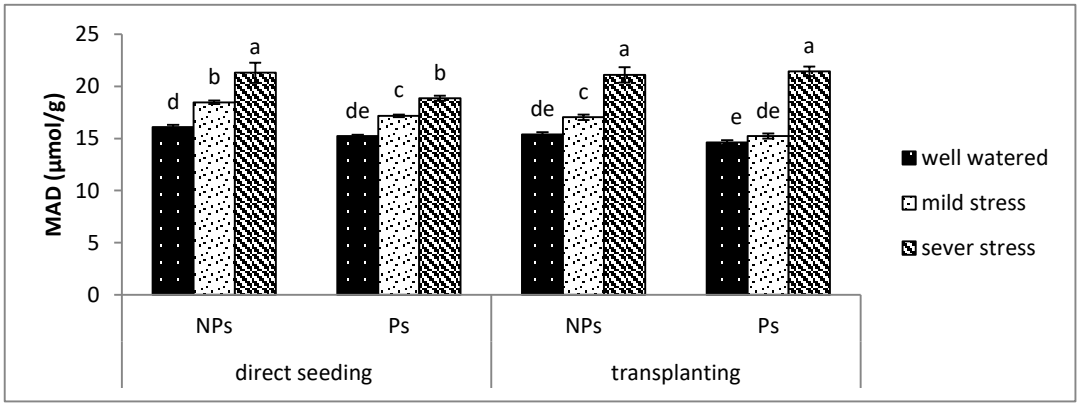

Fig. III. The effect of *Pseudomonas* application on Malondialdehyde (MAD) content of direct seeding and transplanting maize under water stress condition

Relative water content (RWC) and Malondialdehyde (MAD) concentration

RWC was significantly decreased by water stress, but increased by *Pseudomonas* application. This was higher in transplanted plants compared to direct seeded ones. In transplanted plants inoculated with *Pseudomonas*, severe stress decreased RWC by 23% compared to well-watered plants (Fig. II). Water stress significantly decreased MAD, and *Pseudomonas* reduce the MAD accumulation. In transplanted plants inoculated with *Pseudomonas*, severe stress increased MAD by 46% compared to well-watered plants (Fig. III).

Total phenolic content (TPC) and total flavonoid content (TFC)

The effect of water stress, cultivation method, and mutual interactions of water stress and cultivation method, water stress and *Pseudomonas* application as well as the triple interaction of these factors were significant on TPC (P≤0.05, Table 3). TPC was gradually increased by mild water stress and decreased by severe water stress. In directly seeded plants supplied with *Pseudomonas,* mild water stress increased the TPC by 11% compared to well-watered treatment (Table 3). TFC was affected by water stress, cultivation method, and the interaction of water stress and cultivation method (P≤0.05, Table 3). Compared to well-watered treatment, mild stress improved the TFC by 54% in transplanted plants with no *Pseudomonas* application (Table 3).

Discussion

In arid and semiarid areas, plants are exposed to drought, the most serious problems influencing plant growth and development. Favorable effects of PGPR have been reported on plant yield under water stress (Sandhya et al., 2010). Therefore, an understanding of symbiosis

between plants and microorganisms is required to reach the optimum plant productivity. In our experiment, the role of *P. putida* under stress condition was more pronounced in comparison with well-watered condition for maize biomass. Under dry conditions, the rhizobia cterial population probably improved root system and soil microbial respiration, which affected the soil porosity. *P. putida* stimulates plant growth through improvement in nutrient uptake and water holding in root zone (Ghosh et al., 2018). Previous studies have shown that PGPR can adjust the adverse effect of water stress on growth and development of different plant species (Calvo-Polanco et al., 2016; de Oliveira et al., 2018; Kaushal and Wani, 2018; Zaheer, 2019). Transplanting induced more plant yield relative to direct seeding. The date of maize cultivation in Iran, especially in semiarid areas, is late spring, enduring the environmental problems induced by increased temperature and lack of rainfall. Therefore, in this situation, the seedlings are more capable in connection with *Pseudomonas* and tolerate the adverse effects of harsh conditions (Sudahir et al., 2011).

Water stress significantly limits photosynthesis rate through stomatal closure, which restricts CO_2 uptake by leaves and prevents the water loss through transpiration resulting in the reduction in leaf turgor and/or water potential (Osakabe et al., 2014). Water stress restrains photochemical efficiency of photosystem PS II by reduction in electron transportation, omission of external proteins, and release of calcium and magnesium ions from their binding (Sourour et al., 2017). Severe water stress decreases photosynthesis rate because of reduction in Rubisco activity (Osakabe et al., 2014). Previous studies documented that chlorophyll content is significantly reduced under water stress conditions (Zai et al., 2012; Lakra et al., 2015; Shivakrishna et al., 2018; Zhang et al., 2018). Water stress can alter the ratio of chlorophyll 'a' and 'b' and carotenoids (Farooq et al., 2009). Chlorophyll 'a' and 'b' are the main photosynthesis pigments in high plants and their ratio is important under stress and fertilizer treatment. We found that the reduction in chlorophyll a is more than that in chlorophyll b. in our study where *Pseudomonas* increased chlorophyll content under different irrigation regimes.

Under stress conditions, bacteria in rhizosphere may enhance the plant resistance via various mechanisms such as improving the supply of nutrients, optimizing the synthesis of phytohormones IAA and ACC, and improving the antioxidant capacity (Timmusk et al., 2014). Typically, the oxidative damage is controlled by several enzymatic and non-enzymatic mechanisms. SOD as an essential component of the antioxidative defense system, converts superoxide radicals to H_2O_2 at a very quick rate and plays an important role in the protection of the cells against the toxic effect of oxygen species (Verma and Dubey, 2003). In transplanted plants which were also inoculated with *Pseudomonas,* severe water stress increased the SOD activity by 63% compared to well-watered plants (Table 3). Increased SOD activity contributed to the enhancement in superoxide radical concentrations, which is because of the de-novo synthesis of enzymatic proteins (Verma and Dubey, 2003, Fatima and Ahmad, 2004). The SOD activity in plants inoculated with *Pseudomonas* revealed that *Pseudomonas* increased the plant capacity to produce more SOD. The next step in controlling the ROS antioxidative process is the CAT activity through catalyzing H_2O_2 and converting it into the water (Noctor and Foyer, 1998). The enhancement of CAT activity is mainly related to the overproduction of hydrogen peroxide in stress conditions. In our study *Pseudomonas* application decreased antioxidant activity. There are different responses of antioxidant enzyme activity in plants inoculated with *Pseudomonas* under water stress. For example, Sandhya et al. (2010) found the lower antioxidant activity in plants under *Pseudomonas* application and water stress. In contrast, Rahimzadeh and Pirzad (2017) observed an increased activity of antioxidant activity.

High RWC is a resistant strategy to water stress and is related to higher osmotic regulation or lower elasticity of tissue cell wall. In osmotic adjustment, we can observe the accumulation of solutes in response to water stress through maintaining turgor in tissues. Osmotic adjustment strongly depends on photosynthesis. When dehydration is towards severe stress,

photosynthesis decreases, resulting in a smaller solute supply for osmotic adjustment. With continued water limitation, osmotic adjustment delays, but cannot completely prevent dehydration (Chen et al., 2019). Osmotic adjustment is not permanent and typically, plants rapidly respond to reduce water content. Osmoregulation and turgor maintenance allow the root to optimally absorb the soil moisture by plant root (Djemel et al., 2018t). The reduction of RWC in maize was found in different studies (Ye et al., 2016; Anjum et al., 2016; Chen et al., 2016; Djemel et al., 2018), but the transplanting and direct seeding in the presence of *Pseudomonas* used in our study helped us to get a better picture of the effect of PGPR and the cultivation method on maize. Transplanting due to its better condition in maize plants reached the higher RWC compared to direct seeding.

Water stress may damage membranes and macromolecules by producing more ROS such as O_2, H_2O_2, and OH^{-1} (Mittler 2002). In this study, there was no significant change in MAD content in plants inoculated with *Pseudomonas*. Gontia-Mishra et al. (2016) and Tiwari et al. (2016) observed a significant reduction of MAD content in PGPR-inoculated seedlings relative to control seedlings under water stress. MDA content, a product of lipid peroxidation, indicates the degree of oxidative membrane damage due to the stress (Gontia-Mishra et al. 2016). Oxidation of unsaturated fatty acids by singlet oxygen produces distinctly different products, such as MAD.

Phenolic compounds are reported to promote lignification indirectly in plants, which acts as a barrier to water loss and pathogen attack and improves plant growth (Gharbi et al., 2017; Grabber et al., 2019). In our study, mild stress got the highest TPC, which resulted in preventing the growth reduction of maize under drought after dual inoculation, which in turn lignified the cell wall and prevented water loss, being higher RWC in respect to severe stress. Similarly, two fold increases in TPC of *E. prostrata* was obtained after PGPR application under water stress compared to well-watered treatment (Sinha and Raghuwanshi, 2016). Flavonoids as a main secondary product in plant have an eminent role in plant physiology (Ma et al., 2014). Flavonoids pathway was reported to respond to biotic and abiotic stress situations in *Ligustrum vulgare* (Guidi et al., 2008), *S. baicalensis Georgi* (Yuan et al., 2012), maize (Chiristie et al., 1994), and rice (Ithal and Reddy, 2004). The increased TFC was reported in our study under drought stress, particularly in mild stress, which is similar to the results of Liu et al. (2013) and Yuan et al. (2012). This shows the protective role of flavonoids when plants are exposed to water stress. The protective role of flavonoids is because of their particular structure, e.g. hydroxyl group, double carbon bonds and modifications like glycosylation, prenylation, and methylation (Guidi et al., 2008). Flavonoids may fulfill their antioxidant role by preventing the generation of reactive oxygen species (ROS) and scavenging ROS once formed (Ohyama et al., 2018).

Conclusion

The present study tried to show the advantages of transplanting in comparison to direct seeding of the maize plants inoculated with *Pseudomonas* under irrigation regimes. According to the findings, transplanting was better in increasing the growth and antioxidant capacity of maize relative to direct seeding strategy. In terms of irrigation regimes, severe stress induced a remarkable reduction in biological yield and kernel compared to mild and wild stress and well-watered condition. Phenol and flavonoid contents as a main secondary product in plants were increased by mild stress and decease by severe stress. Finally, we can conclude that the simultaneous application of transplanting and *Pseudomonas* can alleviate the adverse effects of water stress on physiological and biochemical characteristics of maize.

References

Ahmad, I., T. Hamid, M. Fatima, H. S. Chand, S. K. Jain, M. Athar and **S. Raisuddin,** 2000. 'Induction of hepatic antioxidants in freshwater catfish (*Channa punctatus* Bloch) is a biomarker of paper mill effluent exposure'. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 1523(1): 37-48.

Anjum, S. A., M. Tanveer, U. Ashraf, S. Hussain, B. Shahzad, I. Khan and **L. Wang.** 2016.

'Effect of progressive drought stress on growth, leaf gas exchange, and antioxidant production in two maize cultivars'. *Environmental Science and Pollution Research*, 23(17): 17132-17141.

Arnon, D. 1949. 'Copper enzyme in isolated chloroplast and chlorophyll expressed in terms of mg per gram'. *Plant Physiology*, 24(1): 15-23.

Bárzana, G., R. Aroca and **J. M. Ruiz-Lozano.** 2015. 'Localized and non-localized effects of arbuscular mycorrhizal symbiosis on accumulation of osmolytes and aquaporins and on antioxidant systems in maize plants subjected to total or partial root drying'. *Plant, cell & environment*, 38(8): 1613-1627.

Birnbaum S J, J. M. Poole and **P. S. Williamson.** 2011. 'Reintroduction of star cactus *Astrophytum asterias* by seed sowing and seedling transplanting, Las Estrellas Preserve, Texas, USA'. *Conservation Evidence*, 8: 43-52.

Brand-Williams, W., M. E. Cuvelier and **C. L. W. T. Berset,** 1995. 'Use of a free radical method to evaluate antioxidant activity'. *LWT-Food science and Technology*, *28*(1): 25-30.

Beauchamp, C. and **I. Fridovich.** 1971. 'Superoxide dismutase: improve assays and an essay applicable to acrylamide gels'. *Annals of Biochemistry*, 44: 276-287

Calvo-Polanco, M., B. Sánchez-Romera, R. Aroca, M. J. Asins, S. Declerck, I. C. Dodd and **J. M. Ruiz-Lozano.** 2016. 'Exploring the use of recombinant inbred lines in combination with beneficial microbial inoculants (AM fungus and PGPR) to improve drought stress tolerance in tomato'. *Environmental and Experimental Botany*, 131: 47-57.

Chandlee, J. M. and **J. G. Scandalios.** 1984. 'Analysis of variants affecting the catalase developmental program in maize scutellum'. *Theoretical and applied genetics*, 69(1): 71-77.

Chen, D., S. Wang, B. Cao, D. Cao, G. Leng, H. Li, and **X. Deng**. 2016. 'Genotypic variation in growth and physiological response to drought stress and re-watering reveals the critical role of recovery in drought adaptation in maize seedlings'. *Frontiers in Plant Science*, 6: 1241-1249.

Chen, G. F. and **D. W. Schemske.** 2019. 'Adaptation to seasonal drought in two closely related species of *Neotropical Costus* (Costaceae)'. *Biotropica*, 51(3): 311-315.

Christie, P. J., M. R. Alfenito, and V. Walbot. 1994. 'Impact of low-temperature stress on general phenylpropanoid and anthocyanin pathways: enhancement of transcript abundance and anthocyanin pigmentation in maize seedlings'. *Planta*, 194(4): 541-549.

Danish, S., and **M. Zafar-ul-Hye.** 2019. 'Co-application of ACC-deaminase producing PGPR and timber-waste biochar improves pigments formation, growth and yield of wheat under drought stress'. *Scientific reports*, 9(1): 5999.

Dat, J. F., H. Lopez-Delgado, H. Foyer and **I. M. Scott.** 1998. 'Parallel changes in H_2O_2 and catalase during thermos tolerance induced by salicylic acid or heat acclimation in mustard seedlings'. *Plant Physiology*, 116(4): 1351-1357.

de Oliveira, D. M., A. L. A. de Lima, N. B. Diniz, C. E. Santos, S. L. F. da Silva and **A. D. N. Simões.** 2018. 'Inoculation of plant-growth-promoting rhizobacteria in *Myracrodruon urundeuva* Allemão supports in tolerance to drought stress'. *Journal of plant interactions*, 13(1):91-99.

Djemel, A., F. Z. Cherchali, M. Benchikh-Le-Hocine, R. A. Malvar and **P. Revilla.** 2018**.** 'Assessment of drought tolerance among Algerian maize populations from oases of the Saharan'. *Euphytica*, 214(8): 149.

Dong H Z, Li W J, W. Tang, Z. H. Li, and **D. M. Zhang.** 2005.' Increased yield and revenue with a seedling transplanting system for hybrid seed production in Bt cotton'. *Journal of agronomy and crop science*, 191(2): 116-124.

Farooq, M., A. Wahid, N. Kobayashi, D. Fujita and **S. M. A. Basra.** 2009. 'Plant drought stress: effects, mechanisms and management'. In *Sustainable agriculture* (pp. 153-188). Springer, Dordrecht.

Gharbi, Y., M. Barkallah, E. Bouazizi, K. Hibar, R. Gdoura and **M. A. Triki.** 2017. 'Lignification, phenols accumulation, induction of PR proteins and antioxidant-related enzymes are key factors in the resistance of *Olea*

europaea to Verticillium wilt of olive'. *Acta physiologiae plantarum*, 39(2): 43-49.

Ghosh, D., S. Sen and **S. Mohapatra**. 2018. 'Drought-mitigating *Pseudomonas putida* GAP-P45 modulates proline turnover and oxidative status in *Arabidopsis thaliana* under water stress'. *Annals of microbiology*, 68(9): 579-594.

Golldack, D., C. Li, H. Mohan and **N. Probst.** 2014. 'Tolerance to drought and salt stress in plants: unraveling the signaling networks'. *Frontiers in plant science*, 5: 151.

Gontia-Mishra, I., S. Sapre, S., A. Sharma and **S. Tiwari, S**. 2016. 'Amelioration of drought tolerance in wheat by the interaction of plant growth-promoting rhizobacteria'. *Plant Biology*, 18(6): 992-1000.

Grabber, J. H., C. Davidson, Y. Tobimatsu, H. Kim, F. Lu, Y.Zhu, M. Opietnik ,N. Santoro,CE Foster, F. Yue ,**D. Ress ,X. Pan** and **J. Ralph** 2019. 'Structural features of alternative lignin monomers associated with improved digestibility of artificially lignified maize cell walls'. Plant Science. 287, 110070. https://doi.org/10.1016/j.plantsci.2019.02.004.

Guidi, L., E. Degl'Innocenti, D. Remorini, R. Massai and **M. Tattini.** 2008. 'Interactions of water stress and solar irradiance on the physiology and biochemistry of *Ligustrum vulgare*'. *Tree Physiology*, 28(6): 873-883.

Ithal, N. and **A. R. Reddy**. 2004. 'Rice flavonoid pathway genes, OsDfr and OsAns, are induced by dehydration, high salt and ABA, and contain stress responsive promoter elements that interact with the transcription activator, OsC1-MYB'. *Plant Science*, 166(6): 1505-1513.

Johnkutty I, G. Mathew and **J. Mathew.** 2006. 'Comparison between transplanting and direct-seeding methods for crop establishment in rice'. *Journal of Tropical Agriculture*, 40: 65-66.

Kaushal, M., and **S. P. Wani.** 2016. 'Plant-growth-promoting rhizobacteria: drought stress alleviators to ameliorate crop production in drylands'. *Annals of Microbiology*, *66*(1), 35-42.

Khoyerdi, F. F., M. H. Shamshiri and **A. Estaji.** 2016. 'Changes in some physiological and osmotic parameters of several pistachio genotypes under drought stress'. *Scientia horticulturae*, 198: 44-51.

Lakra N, N. K. Nutan, P. Das, K. Anwar, S. L. Singla-Pareek and **A. Pareek.** 2015. 'A nuclear-localized histone-gene binding protein from rice (OsHBP1b) functions in salinity and drought stress tolerance by maintaining chlorophyll content and improving the antioxidant machinery'. *Journal of plant physiology*, 176: 36-46.

Lampayan R, M. K. Samoy-Pascual, E. B. Sibayan, V. B. Ella, O. P. Jayag, R. J. Cabangon and **B. A. M. Bouman.** 2015. 'Effects of alternate wetting and drying (AWD) threshold level and plant seedling age on crop performance, water input, and water productivity of transplanted rice in Central Luzon, Philippines'. *Paddy and Water Environment*, 13(3): 215-227.

Liu, H., X. Wang, D. Wang, Z. Zou and **Z. Liang.** 2011. 'Effect of drought stress on growth and accumulation of active constituents in *Salvia miltiorrhiza* Bunge'. *Industrial Crops and Products*, 33(1): 84-88.

Liu, M., X. Li, Y. Liu and **B. Cao.** 2013. 'Regulation of flavanone 3-hydroxylase gene involved in the flavonoid biosynthesis pathway in response to UV-B radiation and drought stress in the desert plant, *Reaumuria soongorica*'. *Plant physiology and biochemistry*, 73: 161-167.

Ma, D., D. Sun, C. Wang, Y. Li and **T. Guo.** 2014. 'Expression of flavonoid biosynthesis genes and accumulation of flavonoid in wheat leaves in response to drought stress'. *Plant physiology and biochemistry*, 80: 60-66.

Naiman, A. D., A. Latrónico and **I. E. G**. **de Salamone.** 2009. 'Inoculation of wheat with *Azospirillum brasilense* and *Pseudomonas fluorescens*: impact on the production and culturable rhizosphere microflora'. *European Journal of Soil Biology*, 45(1): 44-51.

Ohyama, Y., J. Ito, V. J. Kitano, J. Shimada and **Y. Hakeda.** 2018. 'The polymethoxy flavonoid sudachitin suppresses inflammatory bone destruction by directly inhibiting osteoclastogenesis due to reduced ROS production and MAPK activation in osteoclast precursors'. *PloS one*, 13(1): e0191192.

Osakabe, Y., K. Osakabe, K. Shinozaki and **L. S. P. Tran.** 2014. 'Response of plants to water stress'. *Frontiers in plant science*, 5: 86.

Rahimzadeh, S. and **A. Pirzad.** 2017. *'Arbuscular mycorrhizal* fungi and *Pseudomonas* in reduce drought stress damage in flax (*Linum usitatissimum* L.): A field study'. *Mycorrhiza*, 27(6): 537-552.

Ruiz-Lozano, J. M., R. Aroca, A. M. Zamarreño, S. Molina, B. Andreo-Jiménez, R. Porcel and **J. A. López-Ráez.** 2016. '*Arbuscular mycorrhizal* symbiosis induces strigolactone biosynthesis under drought and improves drought tolerance in lettuce and tomato'. *Plant, Cell & Environment*, 39(2): 441-452.

Sánchez, F. J., M. Manzanares, E. F. de Andres, J.L. Tenorio and **L. Ayerbe.** 1998. 'Turgor maintenance, osmotic adjustment and soluble sugar and proline accumulation in 49 pea cultivars in response to water stress'. *Field crops research*, 59(3): 225-235.

Sandhya, V. S. K. Z., S. Z. Ali, M. Grover, G. Reddy and **B. Venkateswarlu.** 2010. 'Effect of plant growth promoting Pseudomonas spp. on compatible solutes, antioxidant status and plant growth of maize under drought stress'. *Plant Growth Regulation*, 62(1): 21-30.

Sarker, U. and **S. Oba.** 2018. 'Response of nutrients, minerals, antioxidant leaf pigments, vitamins, polyphenol, flavonoid and antioxidant activity in selected vegetable amaranth under four soil water content'. *Food chemistry*, 252: 72-83.

Sharma, S. B., R. Z. Sayyed, M. H. Trivedi and **T. A. Gobi.** 2013. 'Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils'. *Springer Plus*, 2(1): 587-292.

Shivakrishna P, K. A. Reddy and **D. M. Rao.** 2018. 'Effect of PEG-6000 imposed drought stress on RNA content, relative water content (RWC), and chlorophyll content in peanut leaves and roots'. *Saudi journal of biological sciences*, 25(2): 285-289.

Sinha, S. and **R. Raghuwanshi.** 2016. 'Synergistic effects of *Arbuscular mycorrhizal* fungi and mycorrhizal helper bacteria on physiological mechanism to tolerate drought in *Eclipta prostrata* (L.).' *J Pure Appl Microbiol*, 10(2): 1117-1129.

Sourour, A., O. Afef, R. Mounir and **B. Y. Mongi.** 2017.' A review: morphological, physiological, biochemical and molecular plant responses to water deficit stress'. *Int J Eng Sci*, 6: 1-4.

Srivastava, S., S. Bhugra, B. Lall and **S. Chaudhury.** 2017. 'Drought stress classification using 3D plant models'. In *Proceedings of the IEEE International Conference on Computer Vision* (pp. 2046-2054).

Sudahir, Y., G. Gill. E. Humphreys, S. S. Kukal and **U. S. Walia**. 2011. 'Effect of water management on dry seeded and puddled transplanted rice'. Part 1: Crop performance. Field Crops Research, 120(1) :112-122.

Tafteh, A. and **A. R. Sepaskhah** 2012. 'Yield and nitrogen leaching in maize field under different nitrogen rates and partial root drying irrigation'. *International Journal of plant production*, 6(1): 93-114.

Timmusk, S., I. A. A. El-Daim, L. Copolovici, T. Tanilas, A. Känn aste, L. Behers and **U. Niinemets.** 2014. 'Drought-tolerance of wheat improved by rhizosphere bacteria from harsh environments: enhanced biomass production and reduced emissions of stress volatiles'. *PloS one*, 9(5): e96086.

Trenberth, K. E., A. Dai, A., G. Van Der Schrier, P. D. Jones, J. Barichivich, K. Briffa and **J. Sheffield, J.** (2014). 'Global warming and changes in drought'. *Nature Climate Change*, 4(1): 17-23.

Verma, S. and **R. S. Dubey.** 2003. 'Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants'. *Plant Science*, *164*(4), 645-655.

Ye, J., S. Wang, X. Deng, L. Yin, B. Xiong and **X Wang.** 2016. 'Melatonin increased maize (*Zea mays* L.) seedling drought tolerance by alleviating drought-induced photosynthetic inhibition and oxidative damage'. *Acta physiologiae plantarum*, 38(2): 48-53.

Yuan, Y., L. Shuai, S. Chen, L. Huang, S. Qin and **Z. Yang.** 2012. 'Flavonoids and antioxidative enzymes in temperature-challenged roots of *Scutellaria baicalensis* Georgi'. *Zeitschrift für Naturforschung C*, 67(1-2): 77-85.

Zaheer, M. S. 2019. 'Effect of Plant Growth Promoting Rhizobacteria (PGPR) and Cytokinins on the Growth and Yield of Wheat (*Triticum aestivum* L.) under drought'. (Doctoral dissertation, Islamia University, Bahawalpur.).

Zai X M, S. N. Zhu, P. Qin, X. Y. Wang, L. Che and **F. X. Luo. 2012**. 'Effect of *Glomus mosseae* on chlorophyll content, chlorophyll fluorescence parameters, and chloroplast ultrastructure of beach plum (*Prunus maritima*) under NaCl stress'. *Photosynthetica*, 50(3): 323-328.

Zhang T, Y. Hu Y, K. Zhang K, C. Tian and **J. Guo.** 2018. *'Arbuscular mycorrhizal* fungi improve plant growth of *Ricinus communis* by altering photosynthetic properties and increasing pigments under drought and salt stress'. *Industrial crops and products*, 117: 13-19.