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Enhancing *in vitro* Growth of Wheat Seedlings Under Water Stress using Biopriming

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ABSTRACT

Purpose: *Providing food for the world, growing population becomes more difficult with the increase of abiotic stresses due to climate change. The production of wheat, which is a major crop is under the threat of drought stress. The aim of this experiment was to find out to what extent biopriming reduced the effects of drought on wheat (Triticum aestivum L. cv. Slemani-2) seedling growth under in vitro conditions.*

Research Method: *In vitro planted seedlings were subjected to drought conditions using three different watering regimes (normal [control], ½ reduced, and ¾ reduced) after biopriming the seeds with Azospirillum lipoferum, Frateuria aurantia, and Chlorella saccharophila. Physiological traits and growth characteristics were measured. ANOVA was performed according to the factorial layout in completely randomized experimental design with three replications using Microsoft Windows based COSTAT software (version 6.3).*

Findings: *The experiment results showed that biopriming had reduced the effect of drought on chlorophyll concentration and root characteristics, especially under ½ reduced water application. A. lipoferum and C. saccharophila were more beneficial for the seedlings than F. aurantia. Physiological and growth characteristics such as chlorophyll concentration were enhanced using biopriming, hence photosynthesis, root characters, and nutrient uptake.*

Research Limitations: *The availability of previous studies and data on the microorganisms used were the main constrain in the study. Conducting a study under controlled greenhouse conditions until harvest might provide more information as more parameters will be measured and data collected, especially at the anthesis stage as it is a crucial stage.*

Originality/ Value: *This research provides a possible method to reduce drought impact on wheat crops by suggesting biopriming with new microorganisms.*

Keywords: *Azospirillum lipoferum, Biopriming, Chlorella saccharophila, drought, Frateuria aurantia, wheat*

INTRODUCTION

Drought has been one of the most damaging abiotic stress to agriculture in recent years and threatens world food security under the climate change scenario at present and in the future. It is a result of reduced precipitation and increased temperature. It dramatically decreases cereal production on a global scale by 9-10% via negative effects on growth, physiology, and grain development (Zhang *et al.,* 2018). The world's three major crops according to FAO are maize, wheat, and rice. Common wheat (*Triticum aestivum* L.) has an annual production of more than 758 million tons worldwide, and it has contributed more than 27% to global cereal production in 2020 (FAO,

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2020). The grain yield and quality of wheat have been affected by drought which is the most significant abiotic stress, reducing the quality and limiting the productivity of wheat worldwide. The loss is estimated at up to 86% irrespective of the genotype and the time of stress application (Tricker *et al.,* 2018; Qaseem *et al.,* 2019). It disrupts germination, vegetative growth, and reproductive stages especially the seed filling stage which is critical (Gooding *et al.,* 2003; Prasad *et al.,* 2011; Sehgal *et al.,* 2018).

Soil microorganisms have an important role in the improvement and maintenance of soil health as well as physical and chemical properties. These microorganisms have relationships with all multi-cellular organisms, and probably all eukaryotes. The association between plants and the microbial since the earliest evolution, which is essential for producing plant hormones, increases the availability of mineral nutrients, and increases resistance to pathogens (Kudoyarova *et al.,* 2015; Backer *et al.,* 2018). In the soil, various groups of bacteria are associated with the plant root efficiently and compete in the rhizosphere, which creates positive, neutral, or negative plant-microbe interactions. Plant growth-promoting bacteria include many species from the genera *Azospirillum, Bacillus, Enterobacter, Gluconacetobacter, Paenibacillus,* and *Pseudomonas* (Kudoyarova *et al.,* 2015). These bacteria are generally isolated from natural plant habitats which might substitute chemical fertilizers, hence reducing the environmental pollution.

Azospirillum spp. is a nitrogen-fixing bacteria that helps soil enrichment and allows plants to grow under abiotic stress. It produces hormone-like substances, auxins, gibberellins, and cytokinins. Plant tolerance to abiotic stresses is enhanced by *Azospirillum spp.* as it stimulates both rates of root elongation and the appearance of lateral and adventitious roots (Creus *et al.,* 2004; Enebe and Babalola, 2018). *Frateuria spp.* is a Potassium solubilizing bacterium that mobilizes a mixture of potassium from mica into a usable form of potassium for the plant (Debnath *et al.,* 2019).

Microalgae are polyphyletic microscopic size photosynthetic group organisms that enrich and enhance soil nutrient utilization. In order to promote plant growth, they generate growth hormones, polysaccharides, antimicrobial compounds, and other metabolites (Guo *et al.,* 2020). *Chlorella* genus is a microalga with the ability of nitrogen fixation, improving soil's physical and chemical properties, and producing substances that can promote plant development and infection control (Ortiz-Moreno *et al.,* 2019).

This experiment was conducted to explore priming potential with bacteria strains and microalga to improve drought stress tolerance of wheat. To understand the impact of bacterial priming on stress tolerance mechanisms of wheat, physiological and biochemical analyses were carried out.

MATERIALS AND METHODS

Experimental Design and Growing Media Preparation

Wheat (*Triticum aestivum* L. cv. Slemani-2) was studied for drought stress because it is a newly registered spring wheat cultivar in Iraq. The experiment was carried out at the Seed Science and Technology Laboratory, Department of Field Crops, Faculty of Agriculture, Van Yuzuncu Yil University, Turkey in 2020. The experimental units were arranged according to a factorial layout in completely randomized experimental design with three replications. Two plant growth-promoting bacteria (PGPB), (*Azospirillum lipoferum* ($1x10^6$ kob/ml) and *Frateuria aurantia* ($1x10^5$ kob/ml)), and one microalga (*Chlorella saccharophila* ($2x10^4$ kob/ml)) were used for seed priming. Seeds were treated for 10 minutes with sodium hypochlorite (5%), and then washed 3 times with de-ionized water (dl-H 2O). Sterilized seeds were soaked in suspension (10ml/1L for *Azospirillum lipoferum* and *Frateuria aurantia* and 5 % for *Chlorella saccharophila*) for two hours, and seeds of the control were left untreated (soaked in sterilized water without microorganisms). All treated seeds were rinsed and sown in 500 cm^3 dark pots containing a soil mixture of 1/3 soil: 1/3 turf: 1/3 perlite. The pots were arranged to grow in a controlled environment growth chamber at 25 °C and 65% humidity. After one week, plants were thinned and one seedling was left per pot. Seedlings were again replenished with 5 ml plant growth promoter suspension when seedlings reached 10 cm height in all pots. Basic fertilization was applied to all pots twice during the growing period. Plants were watered every 3 days with 50 ml distilled water for 14 days. Then, subsequently subjected to water stress, normal (control), ½ reduced and ¾ reduced distilled water per pot every 3 days for 20 days.

Observations Recorded

Nitrogen balanced index (NBI), leaf chlorophyll (Chl), flavonoid (Flav), and anthocyanin (Anth) were measured from the seedlings' leaves before harvesting, using a non-destructive optical leaf clip meter (DUALEX, FORCE-A, France). NBI, which is the Chl to Flav ratio in the leaf, is a useful indicator for nitrogen availability in the plant as it is less sensitive to phenology. The Leaf temperature (LT) data were recorded using a handheld temperature meter. Plants were harvested and separated into above-ground and root parts. Roots were cleaned from soil particles and washed with tap water and dried. Plant height- cm (PLH) and root length- cm (RL) were recorded using a measuring tape. Plant and root fresh weight- g (PFW, RFW) were weighed directly after harvest using a sensitive electronic balance (0.00 g), then dried for 48 h at 70 °C and weighed for the second time to measure plant and root dry weight- g (PDW, RDW).

Statistical Analysis

COSTAT software (version 6.3) for Windows was used to examine the data and evaluate variance and determine the statistical significance of treatment effects. The ANOVA analysis was performed according to the factorial layout in completely randomized experimental design. Treatment means with a probability threshold of $P<0.05$ were considered statistically significant. The significant differences were compared and grouped according to the Least Significant Difference (LSD) multiple comparison test. Differences in mean values indicated with different letters.

RESULTS

Plant Physiological Characters

The effect of biopriming varied for each measured parameter as presented in (Table 01). The mean value of seedling leaf temperature (LT) grown in a normal watering was 2.59 and 1.3 ^{0}C less than ½ and ¾ reduced water application respectively. Nitrogen balanced index (NBI) value showed a reduction with the increase in water stress, 39.96 at normal (control) water application and 20.03 at ¾ reduced water application. However, *C. saccharophila* biopriming at ½ reduced water application gave the highest value of nitrogen balanced index 53.4. The biopriming application had a positive effect on chlorophyll concentration (Chl) under ½ reduced water application, 23.8. The highest chlorophyll concentration was obtained from *C. saccharophila* biopriming at ½ reduced water application, 38.38, while the lowest value was for the control treatment under ¾ reduced water application, 9.7. Water stress significantly increases the flavonoid (Flav) and anthocyanin (Anth) concentration. Flavonoid concentration increased by 40% under water stress compared to the normal watering condition. The highest values were 0.91 and 0.88 obtained from *C. saccharophila* and *A. lipoferum* biopriming respectively. Anthocyanin (Anth) concentration increased by 50 and 66% under ½ and ¾ reduced water application compared to the normal watering condition.

Growth Parameters

The data analysis indicates that for most of the studied traits, normal and ½ reduced water application growing condition had the same effect on plant height (PLH), root length (RL), and root fresh (RFW) and dry weight (RDW) (Table 02).

Table 01: Effect of biopriming of wheat seed with plant growth-promoting bacteria and microalgae on plant physiological characteristics

Applications		LT - °C	NBI	Chl	Flav	Anth
Water stress	PGPR					
Normal irrigation (control)	Control (water)	27.93 def	52.20 b	16.00 g	0.46 f	0.05
	A. lipoferum	26.80 f	39.73 c	20.60 e	0.57 e	0.04
	F. aurantia	27.80 ef	34.53 d	21.87 d	0.60 de	0.07
	C. saccharophila	28.17 de	29.37 e	21.37 de	0.64 d	0.08
Mean		27.67 C	38.96 A	19.96 B	0.57 C	0.06 C
½ reduced irrigation	Control (water)	27.93 def	20.23 h	14.03 h	0.72 c	0.05
	A. lipoferum	32.90 a	27.67 f	19.60 f	0.71 c	0.17
	F. aurantia	30.17 b	35.21 d	23.20 c	0.64 d	0.08
	C. saccharophila	30.03 bc	53.40 a	38.38 a	0.78 b	0.04
Mean		30.26 A	34.13 B	23.8 A	0.71 B	0.09 B
¾ reduced irrigation	Control (water)	25.47 g	13.53 j	9.70 i	0.70 c	0.08
	A. lipoferum	29.40 cd	23.30 g	19.10 f	0.88 a	0.10
	F. aurantia	32.67 a	28.03 f	29.2 b	0.72 c	0.04
	C. saccharophila	28.57 de	16.33 i	14.1 h	0.91 a	0.17
Mean		29.03 B	20.3 C	18.02 C	0.80 A	0.10 A
CV (%)		2.53	1.75	2.28	5.57	13.80

*LT: leaf temperature; NBI: nitrogen balanced index; Chl: leaf chlorophyll; Flav: flavonoid; Anth: anthocyanin; CV (%): coefficient of variation; **Different letters indicate significant differences among treatments (P ≤ 0.05). Values with the same letter are in the same statistical group.*

Plant height (PLH) value decreased by 6.4 and 5.4 % at ¾ reduced water application compared to plants sown at normal watering and ½ reduced water application respectively. The differences in height of seedling grown under ½ reduced water application and *F. aurantia* biopriming and the control was 1.33 cm. Water stress had significantly reduced the values of plant fresh weight (PFW) and dry weight (PDW). At normal watering, the values were 2.80 and 0.43 g and these values decreased by 70 and 37% for of plant fresh and dry weight respectively. *A. lipoferum* (2.97 g) and *F. aurantia* (0.47 g) values were the highest for both plant fresh weight (PFW) and dry weight (PDW) respectively. The effect of biopriming on root length (RL), root fresh weight (RFW) and root dry weight (RDW) values for both normal watering condition and ½ reduced water application was significant. The root length value was lower by 14.8 and 17.7 %, root fresh weight 38.9 and 36.14%, root dry weight 6.9% for plants grown under ¾ reduced water application compared to normal watering and ½ reduced water application respectively. *C. saccharophila* treatment produced longer roots, 54 cm and *A. lipoferum* higher root fresh weight (RFW) value, 2.52 g. while no significant change was observed in root dry wight (RDW) for the different biopriming treatments.

Table 02: Effect of biopriming of wheat seed with plant growth promoting bacteria and microalgae on plant growth characteristics

Applications		PLH (cm)	PFW (g)	PDW (g)	RL (cm)	RFW (g)	RDW (g)
Water stress	PGPR						
Normal irrigation (control)	Control (water)	42 a	2.75 ab	0.41 abc	45.67 bcd	1.94 cd	0.35
	A. lipoferum	38 abc	2.97 a	0.46 ab	44 cd	2.12 bc	0.31
	F. aurantia	41 a	2.78 ab	0.47 a	41.67 de	1.74 de	0.21
	C. saccharophila	35.33 c	2.72 b	0.38 bc	50.67 ab	2.31 b	0.28
Mean		39.08 A	2.80 A	0.43 A	45.5 A	2.03 A	0.29 A
½ reduced irrigation	Control (water)	38 abc	1.36 e	0.27 de	50.67 ab	1.76 d	0.24
	A. lipoferum	38.67 ab	1.98 c	0.43 ab	49 bc	2.52 a	0.36
	F. aurantia	40.67 a	1.79 d	0.42 ab	34.67 f	1.56 ef	0.23
	C. saccharophila	37.33 bc	1.68 d	0.42 ab	54 a	1.95 cd	0.33
Mean		38.67 AB	1.70 B	0.38 B	47.08 B	1.95 A	0.29 A
¾ reduced irrigation	Control (water)	37.67 abc	0.77 g	0.24 e	37.67 def	1.03 h	0.26
	A. lipoferum	36.67 bc	0.95 f	0.24 e	42.33 d	1.38 g	0.28
	F. aurantia	35.67 c	0.69 g	0.26 e	38 def	1.12 h	0.24
	C. saccharophila	36.33 c	0.90 f	0.32 cd	37 ef	1.41f g	0.31
Mean		36.58 B	0.83 C	0.27 C	38.75 B	1.24 B	0.27 B
CV (%)		5.00	6.00	13.00	8.00	8.90	7.50

**PLH: plant height; PFW: plant fresh weight; PDW: plant dry weight; RL: root length; RFW; root fresh weight; RDW: root dry weight; CV (%): coefficient of variation; **Different letters indicate significant differences among treatments (P ≤ 0.05). Values with the same letter are in the same statistical group.*

DISCUSSION

Drought is the most aggressive abiotic stress and a major factor affecting agriculture across the world, particularly wheat (Shah and Paulsen, 2003; Fahad *et al.,* 2017; Wang *et al.,* 2018; Zhang *et al.,* 2018). Biopriming is an efficient technique that enhances seed vigor hence produce strong and healthy seedling with tolerant ability to abiotic stresses (Mahmood *et al.,* 2018; Dief *et al.,* 2020; Nawaz *et al.,* 2020).

In current project, seed biopriming treatments affected almost all plant physiological traits' growth parameters. Reduction in physiological like chlorophyll content was minimum under the influence of Plant Growth Promoting Bacteria (PGPB) and microalga treatment and the influence was greater at ½ reduced water application treatments (Table 1). Similarly, Shukla *et al.* (2014) and Rawat *et al.* (2016) demonstrate that biopriming reduces the effect of drought on bread wheat leaf greenness through measuring chlorophyll content especially under ¾ reduced water application. Stress significantly increases the flavonoid (Flav) and anthocyanin (Anth) concentration. Flavonoid concentration increased by 40% under water stress compared to the normal watering condition. In water stress condition, Anthocyanin (Anth) concentration increased by 66% compared to the normal watering condition. In plants exposed to drought, flavonoids and anthocyanin have a protective role; flavonoids special structure effectively counters stress-induced oxidative damage, and the anti-oxidative capacity of anthocyanin (Ma *et al.,* 2014). Nitrogen is an important element for a plant to grow and develop, its absence affects crop performance and its end product under. Nitrogen balanced index (NBI) is an indicator for measuring plant nitrogen status based on leaf chlorophyl to polyphenolics ratio (Cerovic, *et al.,* 2005). Biopriming increases the plant nitrogen content under stress as shown in our results and explained by previous scholars. (Mahmood, *et al.,* 2016; Meena, *et al.,* 2016). Different biopriming may also act through somewhat different mechanisms; Kasim *et al.* (2013) and Arzanesh *et al.* (2011) suggested that priming of drought stress tolerance is provided by somewhat different mechanisms by the different bacterial strains. In the current experiment, *A. lipoferum* increases shoot and root fresh weight; same results were observed by Arzanesh *et al.* (2009). The results show enhancing root growth characters that might be due to producing gibberellins, cytokinin and auxins by the microorganisms (Creus *et al.,* 2005; Kudoyarova *et al.,* 2015; Meena *et al.,* 2016).

CONCLUSION

Using biopriming is a promising technique for enhancing plant performance under drought stress especially, under ½ reduced water application drought condition. It enhances most of physiological and growth characters such as chlorophyll concentration hence photosynthesis, root characters and nutrient uptake. *A. lipoferum* and *C. saccharophila* were more beneficial for the seedlings than *F. aurantia.* Finally, based on the findings it can be suggested that Slemani-2 cultivar has a medium resistance to drought.

Statement of any Conflicts of Interest

The authors have no relevant financial or non-financial interests to disclose, have no conflicts of interest to declare that are relevant to the content of this article, no involvement or affiliations with any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript and no financial or proprietary interests in any material discussed in this article.

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