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Changes in Photo-Protective Energy Dissipation of Photosystem II in Response to Beneficial Bacteria Consortium in Durum Wheat under Drought and Salinity Stresses

Mohammad Yaghoubi Khanghahi, Sabrina Strafella and Carmine Crecchio *

Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy; mohammad.yaghoubikhanghahi@uniba.it (M.Y.K.); sabrina.strafella@uniba.it (S.S.)

* Correspondence: carmine.crecchio@uniba.it

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Abstract: The present research aimed at evaluating the harmless dissipation of excess excitation energy by durum wheat (*Triticum durum* Desf.) leaves in response to the application of a bacterial consortium consisting of four plant growth-promoting bacteria (PGPB). Three pot experiments were carried out under non-stress, drought (at 40% field capacity), and salinity (150 mM NaCl) conditions. The results showed that drought and salinity affected photo-protective energy dissipation of photosystem II (PSII) increasing the rate of non-photochemical chlorophyll fluorescence quenching (NPQ (non-photochemical quenching) and qCN (complete non-photochemical quenching)), as well as decreasing the total quenching of chlorophyll fluorescence (qTQ), total quenching of variable chlorophyll fluorescence (qTV) and the ratio of the quantum yield of actual PSII photochemistry, in light-adapted state to the quantum yield of the constitutive non-regulatory NPQ (PQ rate). Our results also indicated that the PGPB inoculants can mitigate the adverse impacts of stresses on leaves, especially the saline one, in comparison with the non-fertilized (control) treatment, by increasing the fraction of light absorbed by the PSII antenna, PQ ratio, qTQ, and qTV. In the light of findings, our beneficial bacterial strains showed the potential in reducing reliance on traditional chemical fertilizers, in particular in saline soil, by improving the grain yield and regulating the amount of excitation energy.

Keywords: chemical fertilization; non-photochemical chlorophyll fluorescence quenching; photosynthetic energy partitioning; plant growth-promoting bacteria; PSII photochemistry

1. Introduction

Durum wheat (*Triticum durum* Desf.) is a very important worldwide food crop widely cultivated all over the Mediterranean basin, where it often suffers from multiple and coincident environmental stresses such as drought and salinity [1–3]. The primary negative effects of such abiotic stresses appear on the photosynthesis process and photosystem II (PSII) activity [2], by over-reducing of reaction centers in PSII, especially in plants without the ability to dissipate the excess energy [4]. In fact, there are two photo-protection mechanisms for dissipating the excess of light energy absorbed by chlorophyll molecules including re-emitting as light-chlorophyll fluorescence (~2–5% of the absorbed energy) and dissipating as heat (~15–18%), which otherwise may result in the production of harmful molecular species like singlet oxygen [5,6]. These two mechanisms, along with photosynthesis pathway (as a consumer of ~80% of assimilated energy), occur in a competitive way, so that any increase in the efficiency of one will decrease the yield of the other two [7,8]. Basically, the non-photochemical chlorophyll fluorescence quenching is often used for estimation of non-radiative energy dissipation

within thylakoid membranes, when photosynthetic productivity is saturated by the electronic excitations of the pigment molecules [9].

On the other hand, there has been a growing interest in utilizing plant growth-promoting bacteria (PGPB) inoculants, as an eco-friendly and cost-effective strategy, not only to supply essential nutrients to the soil-plant system but also to decrease the adverse impacts of stresses, as well as to reduce the consumption of chemical fertilizers, while minimizing negative effects on the agro-ecosystem and food health [10,11]. It is already known that these beneficial bacteria can induce several mechanisms involving the increment of stress tolerance in the plant, such as reinforcing the primary and secondary metabolisms in crops, by the production of plant growth regulators e.g., auxins, cytokinins, and gibberellins [12,13], augmenting the crops antioxidant defense system by the increment of reactive oxygen species (ROS)-scavenging enzymes and the biosynthesis of antioxidants [13], quenching the hypersensitive response of ethylene-induced stress by the biosynthesis of 1-amino cyclopropane-1-carboxylate deaminase (ACC deaminase) and consequent formation of α-ketobutyrate and ammonia from ethylene precursor [13,14], and increasing nutrient solubilization and biological nitrogen fixation [10].

The present study aimed at estimating and comparing the ability of durum wheat leaves in the harmless dissipation of excess excitation energy in the form of heat (non-photochemical chlorophyll fluorescence quenching) under non-stress, salinity, and drought conditions. More relevantly, attempts were also extended to validate our hypothesis that the non-photochemical energy dissipation can be more affected by the application of native PGPB bacterial consortium, in comparison to traditional fertilization plans, under both non-stress and stress conditions.

2. Materials and Methods

2.1. Pot Experiment

Three pot experiments, including non-stress, drought, and salinity experiments, were conducted in a completely randomized design (CRD) with 4 replications. 24 pots were used in each experiment: 2 PGPB inoculation treatments (with and without PGPB bacterial consortium) × 3 doses of chemical fertilization treatments (full dose, half dose, and no chemical fertilization) × 4 replications; fertilization treatments are below described. Each pot contained 2.5 kg of soil that was collected from the same durum wheat field where the isolation of PGPB was conducted from. The main soil properties were: clay loam soil with 43% sand, 25% silt, 32% clay; 0.99% organic carbon; mass of carbon to mass of nitrogen (C:N) ratio 10.4; microbial biomass C 270 μg C g^{-1}; pH 7.5; water content at field capacity (−0.3 Mpa) 31%; wilting point (−15 Mpa) 17%; electrical conductivity of the soil saturation extract (EC) 0.44 dS m^{-1}. The temperature in greenhouse was keptat 20 °C under duration of a light/dark (14 h/10 h; light intensity: 200 μmolm^{-2} s^{-1} PAR) cycle. In order to avoid edge effects, pots were rotated weekly within the greenhouse.

2.2. Preparation of Plant Growth-Promoting Bacteria (PGPB) Inoculums and Seed Treatments

Four PGPB strains, *Acinetobacter pittii*, *Acinetobacter oleivorans*, *Acinetobacter calcoaceticus*, and *Comamonas testosterone*, previously isolated from a durum wheat field at Lavello (Basilicata region, Southern Italy), showed a great potential to solubilize the insoluble forms of phosphate, potassium, zinc, and to fix N_2 gas, respectively. Bacterial compatibility test, as fully described by Santiago et al. [15], was performed in order to use a mixture of 4 PGPB strains as inoculation treatment. Since colonies did not show any antagonistic symptom and there was no inhibition zone on the plates, a consortium of the four cultures for seed inoculation, by mixing equal volume of the grown bacterial cultures, was used. Briefly, the inoculums were prepared separatelyin flasks containing Nutrient Broth (NB, Sigma-Aldrich, Steinheim am Albuch, Germany) medium, starting from single colonies picked from Nutrient Agar (NA, Sigma-Aldrich, Steinheim am Albuch, Germany) plates, under agitation at 100 rpm for 24 h. For this reason, we used the optimizing growth conditions in NA medium for *A. pittii* (0.78% NaCl and 28

°C), *A. oleivorans* (0.67% NaCl and 25 °C), *A. calcoaceticus* (0.64% NaCl and 27 °C), and *C. testosterone* (0.92% NaCl and 23 °C) and then an equal proportion of each strain was used to obtain the mixture of 4 PGPB. The bacterial culture was concentrated by centrifugation at 5000 rpm for 15 min. The pellet was washed 3 times in a sterile potassium chloride (CAS Number: 7447-40-7, Sigma-Aldrich, Steinheim am Albuch, Germany) solution (0.9%, *w/v*) and then re-suspended in the same saline solution. The density of the bacterial suspension to be inoculated was brought to 10^6 CFU mL^{-1} corresponding to an optical density at 600 nm of 0.6–0.7.

Durum wheat seeds (var. Furio Camillo, an improved cultivar with large ears, black awn, 1000-grain weight 45–50 g, good resistance to lodging and cold, tolerance in diseases, high gluten index, and high protein content, provided from the Department of Agricultural and Environmental Science (DiSAAT), University of Bari Aldo Moro) were previously sterilized in 1% sodium hypochlorite solution for 10 min and washed several times with sterilized distilled water. The bacterial suspension was used for seeds inoculation before planting in pots (overnight at room temperature), as well as for pots inoculation every 3 weeks. Seeds were also treated with sterile potassium chloride solution (0.9%, *w/v*) for chemical fertilizers (full dose, half dose) and control (no fertilizer) treatments. Then, 10 seeds were randomly selected and planted in each pot. After the germination, 6 healthy seedlings were kept until sampling time.

2.3. Chemical Fertilization Treatment

Ammonium sulfate (21% N, AGRIFEED, Modena, Italy) was used as N fertilizer (290 Kg ha^{-1}), one-thirds of it was added to soil before planting, while the same amount was applied at tillering and flowering stages as topdressing fertilizer. Mono ammonium phosphate (52% P_2O_5; 11% N; AGRIFEED, Modena, Italy), Potassium sulfate (47% K_2O; AGRIFEED, Modena, Italy) and Zinc oxide (75% Zn, EverZinc, London, UK) were also applied equal to 115, 75 and 10 Kg ha^{-1}, respectively, as basal fertilizer before planting.

2.4. Drought/Saline Stress Experiment

Two stress experiments, drought at 40% field capacity and salinity (150 mM NaCl), were applied at the booting stage (63 days after sowing) when the head of the durum wheat plants developed and became visible beneath the sheath on the stalk. In order to avoid osmotic shock, salinity levels were applied by adding saline solutions to pots every 3 days (until 81 DAS (dark-adapted state)) and dividing each day amount into three parts added to the pots gradually every 2/3 h.

2.5. Chlorophyll Fluorescence Parameters Referring to the Non-Photochemical Quenching

The non-photochemical quenching parameters were estimated from the flag leaves at 92 days after sowing using a Pulse Amplitude Modulated fluorometer (PAM-2500, Walz, Effeltrich, Germany), as described by Genty et al. [16]. The DLC-8 leaves clips were used to acclimate the leaves to dark for 15 min before the measurements and consequently the basal fluorescence (F_0) and the maximum fluorescence (F_m) level were measured without interference of ambient light. Accordingly, the leaf was exposed to low intensity light (<0.1 µmol photons m^{-2} s^{-1}, red light). Afterwards, a saturating light pulse (>8000 µmol photons $m^{-2}s^{-1}$, white light) was turned on for 1 s (one pulse). Several theories and parameters have been defined for the quenching analysis and photosynthetic energy partitioning up to now, based on the different excitation and detection of fluorescence signal [17]; the parameters we used are presented in the Table 1.

Table 1. Summary of commonly used concepts of chlorophyll fluorescence parameters referring to the quenching.

Parameter	Equation	Reference
Non-photochemical quenching (NPQ)	$NPQ = \frac{F_m - F'_m}{F_m}$	Bilger and Björkman [18]
Quantum yield of thermal dissipation in the DAS (*L*)	$L = 1 - \frac{F_v}{F_m}$	Kato et al. [19]
Quantum yield of thermal dissipation in the LAS (*D*)	$D = \frac{F_v}{F_m} - \frac{F_v}{F_m}$	Kato et al. [19]
Fraction of light absorbed by PSII that is used in photochemistry (*P*)	$P = \frac{F_m - F_0}{F_m} \times \frac{F_m - F_s}{F_m - F_0}$	Demmig-Adams et al. [20]
Complete non-photochemical quenching of ChlF (qCN)	$qCN = \frac{F_v + F_0 - F_v - F_0}{F_m}$	Buffoni et al. [21]
Total quenching of variable ChlF (qTV)	$qTV = 1 - \frac{F_s - F_0}{F_m - F_0}$	Schreiber et al. [22]
Total quenching of ChlF (qTQ)	$qTQ = 1 - \frac{F_s}{F_m}$	Buffoni et al. [21]
Ratio of the quantum yield of actual PSII photochemistry in LAS to the quantum yield of the constitutive non-regulatory NPQ (PQ)	$PQ = \frac{F_m}{F_s} - \frac{F_m}{F_m}$	Lazár [17]

ChlF: chlorophyll fluorescence; F_0, F_m and F_v: Minimum, maximum and variable chlorophyll fluorescence yields in dark-adapted state; F_0', F_m' and F_v': Minimum, maximum and variable chlorophyll fluorescence yields in light-adapted state; F_S: Steady-state chlorophyll fluorescence yield in light-adapted state; DAS: Dark-adapted state; LAS: Light-adapted state; NPQ: Non-photochemical quenching.

2.6. Chlorophyll Content

Porra [23] method was used to estimate the chlorophyll *a* (Chl*a*) and *b* (Chl*b*) content in fresh samples of flag leaves (1.0 cm^2) at 84 days after sowing. Accordingly, the following formulas were used to compute the Chl*a* and Chl*b* content reading the absorption at visible wavelengths of 665.2 ($A_{665.2}$), 652.4 ($A_{652.4}$) and 470 (A_{470}) nm by using a spectrophotometer (Ultrospec 4000, Pharmacia Biotech Inc. Piscataway, NJ, USA):

$$\text{Chl}a\ (\mu g\ cm^{-2}) = 16.72\ A_{665.2} - 9.16\ A_{652.4} \quad (1)$$

$$\text{Chl}b\ (\mu g\ cm^{-2}) = 34.09\ A_{652.4} - 15.28\ A_{665.2} \quad (2)$$

All plants (six) in each pot were harvested at the maturity stage (124 days after sowing) to determine the fresh weight of roots (g/plant), plant height (cm) and grain yield (g/plant). For this purpose, the grains were oven-dried at 70 °C until constant weight. In order to calculate the rate of changes (%), the values of the chemical/microbiological treatments were divided by the control in each experiment, separately. Then the obtained values were minus one and multiplied by 100. Data presented as the means of four replicates have been statistically analyzed by two-way ANOVA, the least significant difference (LSD) test at 0.05% level of significance and the correlation analysis, by using the SigmaPlot software (SigmaPlot®v11.0, Systat Software Inc., London, UK).

3. Results

Given the results, the fraction of light absorbed by PSII that is used in photochemistry (*P*) increased by the fertilizer application in all non-stress, drought, and salinity conditions, while inversely, the fraction of absorbed light dissipated thermally, including the quantum yield of thermal dissipation in the dark-adapted state (*L*) and quantum yield of thermal dissipation in the light-adapted state (*D*), decreased or remained unchanged by the application of PGPB inoculation and chemical fertilization. The lowest and highest *P* values were determined in control (−I − CF) and PGPB inoculations combined with the half dose of chemical fertilizer (I + $\frac{1}{2}$CF treatment), respectively. This parameter ranged from 0.73, 0.66 and 0.66 in non-fertilized plants to 0.75, 0.71 and 0.73 in I + $\frac{1}{2}$CF treatment under non-stress, drought, and salinity conditions, respectively. In fact, PGPB inoculation, applied alone (I)

or in combination with chemicals (I + $\frac{1}{2}$CF and I + CF), showed the same statistical significance level in terms of the *P* values, but were significantly greater than control treatment, not only in non-stress condition but also under drought and salinity (Table 2). On the other hand, *D* values in all experiments significantly decreased when PGPB inoculants were applied alone (I) or combined with the chemical fertilizers (I + $\frac{1}{2}$CF and I + CF) as compared to the control. This reduction for *L* parameter was significant only in salinity stress by application of I + $\frac{1}{2}$CF and I + CF treatments (Table 2).

Table 2. Estimation of fraction of light absorbed by PSII that is used in photochemistry (*P*); quantum yield of thermal dissipation in the dark-adapted state (*L*) and quantum yield of thermal dissipation in the light-adapted state (*D*) in response to the chemical/microbiological fertilization management under non-stress, drought and salinity conditions, as well as their change rates as compared to the non-fertilized plants.

Experiment	PGPB Inoculation	Chemicals	*P* (±SD)	Rate of Change (%)	*L* (±SD)	Rate of Change (%)	*D* (±SD)	Rate of Change (%)
Non-stress	I	−CF	0.73 b (±0.02)	-	0.21 a (±0.03)	-	0.061 a (±0.00)	-
		$\frac{1}{2}$CF	0.74 ab (±0.03)	+1.84	0.21 a (±0.01)	−0.86	0.045 ab (±0.00)	−26.23
		CF	0.75 a (±0.04)	+2.54	0.20 a (±0.02)	−3.01	0.048 ab (±0.00)	−20.15
	+I	−CF	0.75 a (±0.03)	+3.25	0.20 a (±0.01)	−3.15	0.043 b (±0.00)	−28.24
		$\frac{1}{2}$CF	0.75 a (±0.02)	+3.57	0.20 a (±0.01)	−4.40	0.043 b (±0.00)	−27.78
		CF	0.75 a (±0.02)	+2.98	0.20 a (±0.02)	−3.32	0.044 b (±0.00)	−24.45
Drought stress	I	−CF	0.66 c (±0.03)	-	0.24 a (±0.02)	-	0.097 a (±0.01)	-
		$\frac{1}{2}$CF	0.68 bc (±0.04)	+2.02	0.24 a (±0.03)	−0.31	0.083 ab (±0.00)	−14.42
		CF	0.68 bc (±0.03)	+3.06	0.24 a (±0.03)	−0.45	0.073 ab (±0.00)	−24.49
	+I	−CF	0.69 ab (±0.03)	+4.57	0.24 a (±0.02)	−0.62	0.067 b (±0.00)	−30.93
		$\frac{1}{2}$CF	0.71 a (±0.05)	+7.20	0.24 a (±0.03)	−0.87	0.045 b (±0.00)	−53.61
		CF	0.69 ab (±0.04)	+3.93	0.24 a (±0.02)	−0.34	0.065 b (±0.00)	−32.99
Salinity stress	I	−CF	0.66 c (±0.04)	-	0.24 a (±0.02)	-	0.097 a (±0.00)	-
		$\frac{1}{2}$CF	0.69 bc (±0.05)	+3.38	0.23 ab (±0.03)	−2.55	0.081 ab (±0.00)	−16.83
		CF	0.70 bc (±0.04)	+4.98	0.23 ab (±0.03)	−2.94	0.071 b (±0.00)	−26.72
	+I	−CF	0.71 ab (±0.03)	+6.58	0.23 ab (±0.01)	−5.37	0.066 b (±0.00)	−31.96
		$\frac{1}{2}$CF	0.73 a (±0.05)	+10.59	0.22 bc (±0.02)	−8.32	0.047 c (±0.00)	−51.82
		CF	0.72 ab (±0.03)	+7.97	0.21 c (±0.02)	−10.09	0.068 b (±0.00)	−29.66

Means in each column and each experiment followed by the same letter(s) are not significantly different based on the least significant difference (LSD) test at 0.05 probability level. +I: Seed inoculation with the selected plant growth-promoting bacteria (PGPB) consortium (bacterial suspension was also added to pots every 3 weeks); −I: No PGPB inoculation (durum wheat seeds were treated with non-inoculated NB medium before planting); CF: Soil treated with full dose of chemical fertilizer; $\frac{1}{2}$CF: Half dose of chemical fertilizer; −CF: No chemical fertilizer; SD: Standard deviation.

However, non-photochemical quenching (NPQ) and complete non-photochemical quenching of ChlF (qCN) did not show a specific response to the chemical and inoculums treatments under drought stress, but both parameters intensely decreased by chemical and microbiological fertilizer

application as compared to the non-fertilized (control) plants in non-stress and saline conditions (Table 3). The reduction range was 24–56% for NPQ and 21–52% for qCN in comparison to the control. Nevertheless, an increasing trend was found in both NPQ and qCN for each chemical and inoculums level by imposing the drought and salinity stresses. In this context, NPQ ranged from 0.02 to 0.04 in non-stress, 0.12–0.18 in drought, and 0.08–0.17 in salinity experiments, while qCN ranged from 0.02 to 0.04, 0.11–0.15, and 0.08–0.14, respectively (Table 3).

Table 3. Photo-protective energy dissipation of photosystem II in response to the chemical/microbiological fertilization management under non-stress, drought, and salinity conditions.

Experiment	PGPB Inoculation	Chemicals	NPQ (±SD)	qCN (±SD)	qTV (±SD)	qTQ (±SD)	PQ (±SD)
Non-stress	I	−CF	0.04 a (±0.00)	0.04 a (±0.00)	0.93 c (±0.09)	0.74 b (±0.04)	2.81 b (±0.14)
		$\frac{1}{2}$CF	0.02 b (±0.00)	0.02 b (±0.00)	0.95 ab (±0.08)	0.75 ab (±0.06)	2.95 ab (±0.19)
		CF	0.02 b (±0.00)	0.02 b (±0.00)	0.94 bc (±0.07)	0.75 ab (±0.05)	3.03 ab (±0.17)
	+I	−CF	0.03 b (±0.00)	0.03 ab (±0.00)	0.95 ab (±0.010)	0.76 a (±0.04)	3.15 a (±0.23)
		$\frac{1}{2}$CF	0.02 b (±0.00)	0.02 b (±0.00)	0.95 ab (±0.05)	0.76 a (±0.08)	3.16 a (±0.24)
		CF	0.03 b (±0.00)	0.03 ab (±0.00)	0.96 a (±0.06)	0.76 a (±0.04)	3.11 ab (±0.14)
Drought stress	I	−CF	0.17 ab (±0.02)	0.15 a (±0.02)	0.91 c (±0.08)	0.71 c (±0.05)	2.30 b (±0.16)
		$\frac{1}{2}$CF	0.12 c (±0.00)	0.11 b (±0.01)	0.94 ab (±0.04)	0.71 c (±0.06)	2.34 b (±0.17)
		CF	0.12 c (±0.01)	0.11 b (±0.01)	0.92 bc (±0.07)	0.72 bc (±0.07)	2.44 b (±0.21)
	+I	−CF	0.15 a-c (±0.02)	0.13 ab (±0.01)	0.94 ab (±0.06)	0.73 b (±0.04)	2.62 ab (±0.12)
		$\frac{1}{2}$CF	0.18 a (±0.02)	0.15 a (±0.2)	0.95 a (±0.09)	0.75 a (±0.07)	2.90 a (±0.18)
		CF	0.13 bc (±0.01)	0.11 b (±0.01)	0.96 a (±0.06)	0.73 b (±0.05)	2.54 ab (±0.16)
Salinity stress	I	−CF	0.17 a (±0.03)	0.14 a (±0.02)	0.91 b (±0.09)	0.71 b (±0.07)	2.36 c (±0.16)
		$\frac{1}{2}$CF	0.12 bc (±0.02)	0.11 b (±0.01)	0.95 a (±0.06)	0.74 ab (±0.06)	2.62 bc (±0.12)
		CF	0.09 d (±0.01)	0.08 c (±0.01)	0.94 ab (±0.04)	0.74 ab (±0.07)	2.67 b (±0.19)
	+I	−CF	0.13 b (±0.02)	0.11 b (±0.01)	0.95 a (±0.09)	0.74 ab (±0.04)	2.71 b (±0.20)
		$\frac{1}{2}$CF	0.08 d (±0.00)	0.08 c (±0.01)	0.96 a (±0.06)	0.75 a (±0.08)	3.00 a (±0.17)
		CF	0.11 c (±0.01)	0.10 b (±0.01)	0.95 a (±0.07)	0.75 a (±0.06)	2.88 ab (±0.21)

Means in each column and each experiment followed by the same letter(s) are not significantly different based on the least significant difference (LSD) test at 0.05 probability level. +I: Seed inoculation with the selected PGPB (plant growth-promoting bacteria) consortium (bacterial suspension was also added to pots every 3 weeks); −I: No PGPB inoculation (durum wheat seeds were treated with non-inoculated NB medium before planting); CF: Soil treated with full dose of chemical fertilizer; $\frac{1}{2}$CF: Half dose of chemical fertilizer; −CF: No chemical fertilizer. NPQ: Non-photochemical quenching coefficient; qCN: Complete non-photochemical quenching of ChlF; qTV: Total quenching of variable ChlF; qTQ: Total quenching of ChlF; PQ: Ratio of the quantum yield of actual PSII photochemistry in LAS to the quantum yield of the constitutive non-regulatory NPQ; SD: Standard deviation.

All the treatments, especially the PGPB inoculations, improved the total quenching of variable ChlF (qTV) and total quenching of ChlF (qTQ) as compared to the control in all experiments. In this regard, the highest rate of qTV observed in I + CF (under non-stress and drought) and I + $\frac{1}{2}$CF (in saline condition) which was significantly higher than control treatment up to 3.5, 5.5, and 6%, respectively. However, there was no significant difference among all chemical and inoculums treatments in terms of qTV rate (Table 3). qTQ varied from 0.74 (control) to 0.76 (I, I + CF and I + $\frac{1}{2}$CF) in non-stress, 0.71 (control) to 0.75 (I + $\frac{1}{2}$CF) in drought, and 0.71 (control) to 0.75 (I + CF and I + $\frac{1}{2}$CF) in salinity conditions (Table 3).

According to the results in non-stress conditions, all chemical treatments and PGPB inoculums improved up to 13% the ratio of the quantum yield of actual PSII photochemistry in LAS to the quantum yield of the constitutive non-regulatory NPQ (PQ), as compared to the unfertilized (control) treatment. PGPB inoculations combined with half dose of the chemical fertilizer (I + $\frac{1}{2}$CF), significantly increased the PQ rate as compared to the control plants under drought and salinity stresses (26 and 27%, respectively), which was non-significantly higher than I + CF and I treatments in drought (14 and 11%, respectively) and I + CF in salinity (4%) conditions (Table 3).

Grain yield in all experiments was affected by PGPB inoculation and chemical fertilization. This parameter varied from 0.52 to 1.15 g/plant in non-stress, 0.26 to 0.46 g/plant in drought, and 0.35 to 0.69 g/plant in salinity condition. In this regard, the highest grain yield in non-stressed plants was achieved by I + CF treatment, which was 122, 53, 12, and 9% more than that in control, I, CF, and I + $\frac{1}{2}$CF treatments, respectively. Almost the same trend was observed in the saline condition, where the grain yield after treating by I + CF was significantly higher than control (+97%) and half dose of the chemicals (+41%), while it was non-significantly more than I (+13%), CF (+15%), and I + $\frac{1}{2}$CF (+25%) treatments. The responses of drought-stressed plants to the microbiological and chemical fertilization treatments were slightly different. Accordingly, the highest grain yield was found by the application of PGPB inoculants (I), which had no statistically significant difference with CF (+17%), I + $\frac{1}{2}$CF (+6%), and I + CF (+21%) treatments, while was significantly higher than $\frac{1}{2}$CF(+60%) and control (+82%) treatments (Figure 1).

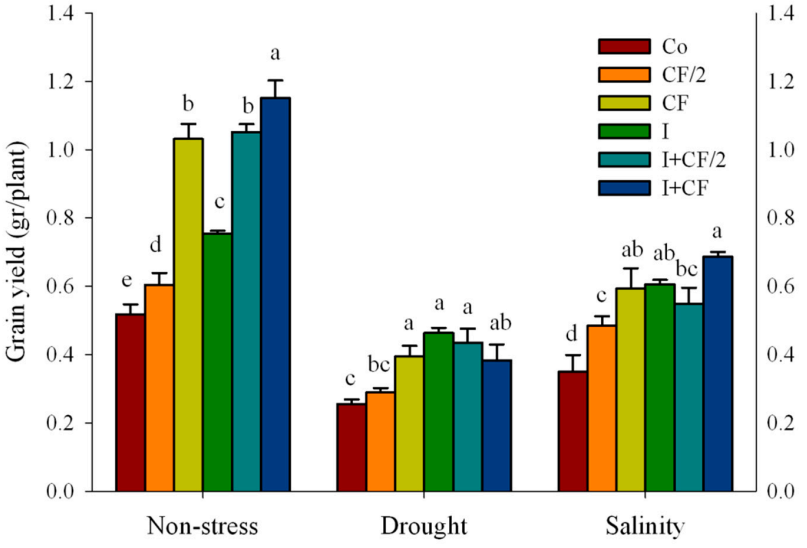

Figure 1. Grain yield in response to the chemical/microbiological fertilization managements under non-stress, salt, and drought conditions. Co: Control (no PGPB (plant growth-promoting bacteria) inoculation and no chemical fertilizer); I: Seed inoculation with the selected PGPB consortium (bacterial suspension was also added to pots every 3 weeks); CF: Soil treated with full dose of chemical fertilizer; CF/2: Half dose of chemical fertilizer; I + CF and I + CF/2: Inoculation and chemical fertilization. Means on the bars marked by the same letter(s) are not significantly different based on the least significant difference (LSD) test at 0.05 probability level.

Chlorophyll content (Chl*a* + *b*) was affected by microbiological/chemical fertilization in all experiments. Accordingly, this parameter reached the highest value equal to 14.72 µg cm^{-2} (I + $\frac{1}{2}$CF) in non-stress, 12.52 µg cm^{-2} (I + CF) in drought and 12.40 µg cm^{-2} (I + CF) in salinity conditions, which were significantly (26.2, 52.3, and 29.4%, respectively) higher than the control (−I − CF) in the related experiments (Table 4). The difference was not statistically significant among all the treatments for Chl*a*/*b* in all conditions (Table 4).

Based on the results, the maximum root weight in non-stress condition was obtained after PGPB inoculation (I), with 0.55 g/plant which was 34.1% higher than that in control. Indeed, plants treated with I + CF and I + $\frac{1}{2}$CF produced heavier roots under drought (0.67 g/plant) and salinity (0.44 g/plant) conditions, which were 17.5 and 29.5% greater than those treated with control treatment, respectively (Table 4). The tallest plants in the non-stress and drought conditions were detected after the PGPB inoculation (I), equal to 71 and 59 cm, which were 16.4 and 27.3% higher than non-fertilized control plants, respectively. Conversely, the highest plant height under salinity condition was observed in I + CF treatment, which was significantly taller than control (31.9%) (Table 4).

Table 4. Plant height, fresh weight of root, chlorophyll content (Chl*a* + *b*), and the ratio of Chl*a* to Chl*b* in response to the chemical/microbiological fertilization management under non-stress, drought, and salinity conditions.

Experiment	PGPB Inoculation	Chemicals	Plant Height (±SD)	Root Weight (±SD)	Chl*a* + *b* (±SD)	Chl*a*/*b* (±SD)
			cm	g/plant	µg cm^{-2}	
Non-stress	I	−CF	61.00 cd (±7.11)	0.41 c (±0.05)	11.66 b (±1.09)	3.68 a (±0.84)
		$\frac{1}{2}$CF	64.00 bc (±5.56)	0.52 ab (±0.07)	13.71 a (±0.88)	03.41 a (±0.46)
		CF	68.33 ab (±6.12)	0.48 b (±0.06)	14.24 a (±1.17)	3.59 a (±0.45)
	+I	−CF	71.00 a (±0.8.01)	0.55 a (±0.05)	13.49 a (±0.90)	3.84 a (±0.34)
		$\frac{1}{2}$CF	69.33 ab (±6.05)	0.50 b (±0.06)	14.72 a (±1.05)	4.33 a (±0.18)
		CF	68.33 ab (±7.17)	0.52 ab (±0.07)	14.71 a (±1.26)	4.16 a (±0.54)
Drought stress	I	−CF	46.33 c (±3.68)	0.57 bc (±0.05)	8.22 c (±1.18)	2.69 a (±0.45)
		$\frac{1}{2}$CF	54.00 b (±4.46)	0.55 bc (±0.06)	8.68 bc (±1.04)	3.07 a (±0.36)
		CF	54.00 b (±5.07)	0.64 ab (±0.08)	9.89 b (±1.47)	2.89 a (±0.67)
	+I	−CF	59.00 a (±6.58)	0.60 b (±0.06)	11.65 a (±0.56)	2.51 a (±0.24)
		$\frac{1}{2}$CF	55.33 b (±4.46)	0.60 b (±0.07)	11.30 a (±1.09)	2.71 a (±0.57)
		CF	52.00 b (±6.74)	0.67 a (±0.05)	12.52 a (±0.86)	2.41 a (±0.35)

Table 4. *Cont.*

Experiment	PGPB Inoculation	Chemicals	Plant Height (±SD)	Root Weight (±SD)	Chl*a* + *b* (±SD)	Chl*a*/*b* (±SD)
			cm	g/plant	μg cm^{-2}	
Salinity stress	I	−CF	51.67 d (±0.03)	0.34 c (±0.05)	9.58 c (±0.79)	2.37 a (±0.37)
		$\frac{1}{2}$CF	55.33 cd (±6.22)	0.39 b (±0.03)	10.27 b (±1.10)	2.84 a (±0.16)
		CF	59.33 c (±5.61)	0.36 bc (±0.05)	11.17 ab (±0.94)	2.47 a (±0.37)
	+I	−CF	64.67 ab (±7.31)	0.41 ab (±0.05)	10.32 b (±0.59)	2.59 a (±0.44)
		$\frac{1}{2}$CF	59.33 c (±5.50)	0.44 a (±0.06)	11.01 ab (±0.96)	3.06 a (±0.48)
		CF	68.67 a (±7.12)	0.40 ab (±0.05)	12.40 a (±1.07)	2.46 a (±0.26)

Means in each column and each experiment followed by the same letter(s) are not significantly different based on the least significant difference (LSD) test at 0.05 probability level. +I: Seed inoculation with the selected PGPB consortium (bacterial suspension was also added to pots every 3 weeks); −I: No PGPB inoculation (durum wheat seeds were treated with non-inoculated NB medium before planting); CF: Soil treated with full dose of chemical fertilizer; $\frac{1}{2}$CF: Half dose of chemical fertilizer; −CF: No chemical fertilizer; Chl*a* and *b*: Chlorophyll *a* and *b*; SD: Standard deviation.

The Pearson correlation coefficients indicated the grain yield in all experiments had a significant correlation with the fraction of light absorbed by PSII (*P*), qTQ, and PQ ($p < 0.05$). The significant relationship ($p < 0.05$) was also observed between grain yield and qTV under drought and salinity conditions. A significant negative correlation ($p < 0.05$) was found between grain yield and NPQ in salinity experiment (Table 5).

Table 5. Correlation coefficients (r) between grain yield and different fluorescence quenching parameters in response to the potentially beneficial bacterial ($N = 24$).

Variable	r		
	Non-Stress	Drought	Salinity
P	0.42 *	0.39 *	0.47 *
L	−0.37 NS	−0.20 NS	−0.33 NS
D	−0.09 NS	−0.18 NS	−0.34 NS
NPQ	−0.24 NS	−0.08 NS	−0.43 *
qCN	−0.20 NS	0.19 NS	−0.15 NS
qTV	0.09 NS	0.39 *	0.39 *
qTQ	0.41 *	0.47 *	0.40 *
PQ	0.40 *	0.44 *	0.39 *

*: Significant at $p < 0.05$ level; NS: Not Significant. *P*: the fraction of light absorbed by PSII that is used in photochemistry; *L*: Quantum yield of thermal dissipation in the dark-adapted state; *D*: Quantum yield of thermal dissipation in the light-adapted state; NPQ: non-photochemical quenching coefficient; qCN: Complete non-photochemical quenching of ChlF; qTV: Total quenching of variable ChlF; qTQ: Total quenching of ChlF; PQ: Ratio of the quantum yield of actual PSII photochemistry in LAS to the quantum yield of the constitutive non-regulatory NPQ.

4. Discussion

In the framework of this research, thermal dissipation associated with non-photochemical quenching, as an essential photo-protective mechanism of PSII [24], was investigated in durum wheat leaves under non-stress, drought, and salinity conditions, by comparing the application of a bacterial

consortium composed of four PGPB strains (as bio-inoculants) to a traditional chemical fertilization plan. Research on simulation of the energy dissipation are based on two principle theories defined by Demmig-Adams et al. [20] that were drawn up on the basis of puddle model of energy transfer, in which the light energy absorbed in antennae chlorophyll is always transferred to the same reaction centers, and by Kramer et al. [25] and Hendrickson et al. [26] that made on the basis of the lake model of energy transfer, in which the excitation energy of chlorophylls can be exchanged among reaction centers. In fact, what distinguishes these two models is how far excitation energy can travel before it is captured at a reaction center or is dissipated by other means [27]. From this perspective, in this study, we worked on some of the most important photo-protective energy dissipation parameters that have been introduced so far.

One of the early research on photosynthetic energy partitioning was carried out by Demmig-Adams et al. [20] which was further elaborated by Kato et al. [19] by introducing the fraction of light absorbed by PSII that is used in photochemistry (*P*) and quantum yield of thermal dissipation in the DAS (*L*) and LAS (*D*). According to the results of energy partitioning, *P*values increased by PGPB inoculation and chemical fertilization treatments while decreased under both stress conditions. The inverse results were obtained by application of these treatments for the fraction of absorbed light dissipated thermally, especially the *D* value. Nevertheless, there was no significant decrease in *L* rate in response to the PGPB inoculation and chemical fertilization treatments in non-stress and salinity conditions. In fact, *L* always exists in both healthy and stressed leaves due to its basic physics, as the constitutive non-regulatory (dark) non-photochemical quenching/dissipation [17]. On the other hand, our PGPB strains were able to affect light energy partitioning under both non-stress and stress conditions by increasing *P* and decreasing *D* values. *D* part is related to the non-photochemical quenching, which is formed only upon illumination to regulate the amount of absorbed light as a photo-protection mechanism of plants for energy quenching/dissipation of PSII [17]. It has been proved that the *P* rate increases by improving the status of leaf nutrients, including nitrogen, phosphorus, potassium, and zinc, while *D* rate decreases [28], which consequently lead to higher grain yield, as confirmed in our research by the significant positive relationship between *P* rate with grain yield. According to previous studies, these nutrients, released by microbial activity and chemical fertilizers, can improve the PSII photochemistry functions, electron transport systems, photosynthetic pigments biosynthesis and grain yield under both non-stress and stress conditions [29–31].

As a consequence of lower *P* and higher *D* and *L* values in plants under stress, as compared to those in non-stress, incoming light energy should be dissipated non-photochemically, as the most important photo-protection mechanism involved in the safe dissipation of excess excitation energy in PSII [5,32], which resulted in high NPQ and qCN values in stressed plants. On the other hand, owing to the higher *P* rate in fertilized plants, the increments in NPQ and qCN values were less than those in unfertilized (control) plants under salinity and non-stress conditions. In fact, the higher rate of *P* in the plants grown under microbiological/chemical fertilization, especially PGPB inoculation combined to the chemical fertilizers, indicated that the reaction centers in the thylakoid membrane were open, which led to less activity of NPQ process (lower rate of NPQ and qCN) [6], as compared to the close ones in control plants. Nevertheless, chemical and inoculums treatments did not have a specific effect on NPQ and qCN under drought condition. Another evidence for the discrepancy between plants grown under drought and salinity is the lack of a significant relationship between the NPQ and grain yield in drought stress, while their negative relationship was significant under salinity condition, which may be attributed to their different mechanisms to cope with the drought and salinity stresses, especially the regulation of the photochemical and non-photochemical quenching parameters, which depends on the stress and its severity [33] and a different response of pigments to the environmental stress [34]. Findings in drought experiment also brought out that the amount of grain yield, plant height, and PQ rate in plants under PGPB consortium inoculation, alone (I) or combined with the half dose of CF (I + $\frac{1}{2}$CF), were higher than the full dose of CF treatments (CF and I + CF). A possible

explanation could be given by salt accumulation in soil because of the intensive application of chemical fertilizers [35] which can contribute to an increasingly negative impact of drought stress.

Total quenching of ChlF (qTQ) and total quenching of variable ChlF (qTV) are two other quenching parameters, defined by Buffoni et al. [21] and Schreiber et al. [22], reflecting the simultaneous effects of both photochemical and non-photochemical processes during the light induction period. The difference between the two parameters is that the first one (qTQ) is calculated using the maximum ChlF yields in DAS (F_m) and steady-state ChlF yield in LAS (F_s) while the latter is based on the variable ChlF yields in DAS (F_v) and LAS (F_v') [9]. In our research, increments in the qTQ rate in fertilization treatmentsby the combined treatments of PGPB and chemicalsindicated an increase in the overall excitation energy consumption by the photochemical and non-photochemical processes in LAS. Similar results observed in qTV showed the higher activation of photochemical and non-photochemical processes in LAS under microbiological/chemical fertilization treatments, which resulted in higher quenching of F_V. Increment of the total quenching of ChlF (qTQ and qTV), despitethe reduction of non-photochemical quenching (NPQ and qCN), can be related to further increase of the photochemical quenching bychemical and inoculums treatments, especially PGPB inoculation (applied alone or combined with chemicals). This result was confirmed by PQ parameter that was influenced by fertilization (microbiological and chemical) treatments, especially undersalinity conditions. The PQ is defined as a ratio of the quantum yield of actual PSII photochemistry in LAS to the quantum yield of the constitutive non-regulatory non-photochemical quenching [17].

Based on the results, drought condition induced an improvement of root weight as compared to the non-stress, while the salinity condition reduced it. In addition, roots showed a positive response to the application of microbiological/chemical fertilizers. Furthermore, the effect of PGPB inoculums and chemical fertilizers on the increment of the plant height was almost significant as compared to the non-fertilized control plants. This difference between drought and salinity is a morphological adaptation mechanism and an indication of the photosynthetic source-sink balance in plants under various conditions, showing that the photosynthesis assimilation can contribute to growing plant organs by the elongation of the stem (plant height) under salinity and to growing roots, under drought, rather than sink organs such as grain [36–38]. Drought stress effects induced a biomass allocation into the root organ at the expense of the aerial parts, which can contribute to further absorb water from the soil. The increment of plant height and root weight in response to the PGPB inoculation and chemical fertilization has been already reported in several studies [12,36].

In the present research, chlorophyll pigments (Chl*a* + *b*) in plants treated by PGPB inoculations and chemical fertilizer were significantly higher than control plants. It has been previously reported that the optimal N, P, and K supply is considered essential for the biosynthesis of photosynthetic pigments, especially under stress conditions [36,37]. The tendency of the plants to maintain or increase the amount of chlorophyll content under stress conditions is a defense mechanism to conserve the photosynthesis system [29]. On the other hand, the ratio of Chl*a* to Chl*b* (Chl*a*/*b*) showed no significant changes under microbiological/chemical fertilization treatments. This may be caused by the different sensitivity of Chl*a* and *b* under external environment [31,34].

In view of these findings, the PGPB inoculants, applied alone or in combination with the half dose of chemicals, were able to regulate the amount of excitation energy reaching the reaction centers in PSII and avoid photo-damage in plants, not only in non-stress condition but also under drought and salinity. Moreover, the consortium of our native PGPB, isolated from a durum wheat field, significantly improved some quenching parameters, especially PQ, as compared to the half and full doses of chemical fertilizer. Similar results reported that the inoculation of beneficial bacteria can induce systemic tolerance against salinity and drought stresses [38] and improve the efficiency of chemical fertilizer [36,37], which consequently resulted in reducing the amount of the chemical fertilizer consumption [39], upon some subsequent direct and indirect mechanisms such as bio-availability of soil nutrients [40,41], production of IAA [13], regulation of the expression of specific genes [42],

and of carbohydrate metabolism [35], and synthesis of 1-aminocyclopropane-1-carboxylate (ACC) deaminase [43].

5. Conclusions

In conclusion, drought and salinity affected photo-protective energy dissipation of PSII in durum wheat leaves through increasing the rate of non-photochemical chlorophyll fluorescence quenching (NPQ and qCN) and decreasing the PQ ratio, qTQ and qTV. PGPB inoculants, as a consortium of four effective bacterial strains, can mitigate the adverse impacts of stresses on durum wheat leaves, especially the saline one, in comparison with the non-fertilized (control) treatment, by increasing the fraction of light absorbed by PSII antenna that can be used in photochemistry, PQ ratio and total quenching of chlorophyll fluorescence. However, the better performance of the PGPB in regulating the amount of excitation energy was observed when the half dose of chemical fertilizer was applied, suggesting an integrated approach of microbiological and chemical treatments. Considering also the grain yield results, we can conclude that our beneficial bacterial strains have the potential toreduce reliance on traditional chemical fertilizers, in particular in saline soil.

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