



Use of Thiamine, Pyridoxine and Bio stimulant for Better Yield of Wheat Plants Under Water Stress: Growth, Osmoregulations, Antioxidantive Defence and Protein Pattern

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Abstract

Drought stress is an inevitable challenges presents in various environments hindered plant biomass production and quality. Wheat crop productivity is hampered all over the world due to water stress. Therefore, exogenous treatments of plants with vitamins might be useful method to decrease drought growth and productivity losses. Thiamine and pyridoxine are organic nutritional agents needed for development of all living organisms. Cyanobacteria as biofertilizers are considered as environmentally friendly, cost-effective, and alternate to artificial and chemical fertilizers. Due to its physiological properties, cyanobacteria and vitamins are considered potential and environmentally friendly compound for agricultural plant protection and improvement productivity under diverse abiotic challenges such as drought. The aim of this investigation was to study the mechanism of thiamine (Th) or pyridoxine (Pyr) in the absence and presence of Cyanobacteria (Cya) in alleviating the reverse impacts of water deficit on wheat plant. A field experiment was conducted in the Farm of National Research Centre Nubaria region, Egypt. Plants were exposed to different leves of water irrigation requirements (100% or 75% WIR) and two concentrations of Th or Pyr (50, and 100 mg/l). Water stress decreased growth and grain yields and its components of wheat plant compared to 100% water irrigation requirement. Total solule sugars (TSS), proline (Pro) and total free amino acids (FAA) content increased under water-stressed and Th or Pyr treated plants, with and without of Cya. Meanwhile, foliar treatment of Th or Pyr in absence and presence of Cya increased markedly growth parameters, grain yield. Associating with an improvement of photosynthetic pigments, TSS, Pro and FAA. Furthermore, protein bands revealed in wheat treated with Th and Pyr without and with Cya at 100% and 75% WIR stimulates appearance of some polypeptides at 42 and 24 kDa. Also, one subunit at 18 kDa appeared in the presence of Cya at 75% and 100% WIR. Wheat plants treated with Th or Pyr increased density and intensity of protein bands at water deficit (75% WIR). Peroxidase and polyphenol oxidase isozymes were scored in wheat plant treated with Th and Pyr in without and with of Cya under water strees. In conclusion, exogenous treatment of thiamine and pyridoxine and cyanobacteria improved growth and productivity of wheat plants under normal water irrigation and could alleviate the reduced drought stress. The maximum growth and yield of wheat plant were gained at 100 mg⁻¹ Pyr or Th in the presence of Cya.

Keyword: Cyanobacteria, Electrophoretic protein patterns, Pyridoxine, Thiamine, Wheat, Water stress

1. Introduction

Water deficit is considered the major restrictive crieteria for plant growth and crop production globally. When plants confront water stress condition, they produce alterations in different physiochemical processes, molecular level, growth inhibition and

reduce photosynthetic rates [1]. Water stress produced in the creation of reactive oxygen species that led to leaving damage, and so, decrease the plant growth and productivity [2].

In recent years, large consideration has been focused on the potential of utilizing naturalist and

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healthy compounds to mitigate plant growth and yield of field crops reductions. In this respect, vitamins perform a significant function as plant bioregulators precursors which could improve plant growth and productivity under abiotic stress [3]. Vitamins are amongst the organic nutritional agents needed for the development of all living organisms. Thus, external treatment of vitamins induced promotive influences on plant development, carbon dioxide absorption, and protein composition. Several treatments of vitamins caused an efficient part through plant bioregulators which then impact various biochemical procedures and protects the plant from reverse influences of environmental stress [4].

Thiamine (vitamin B1) can be provided as a coenzyme in the decarboxylation of α -keto acids, like pyruvic acid and keto-glutamic acid that has its significance in the metabolism of fats and carbohydrates [5 & 6]. Kaya [7] found that, application of thiamine improved growth, development and chemical components of maize plants under environmental stresses. Thiamine induced an important function in plant reactions to water stress [8]. Exogenous application of thiamine can rise the antioxidant defense mechanism via involving the transketolase enzyme in the glycolytic cycle [9].

Pyridoxine (Vitamin B6) is a major coenzyme which incorporated in a broad extent of physiological processes, among them glycogen metabolism and biosynthesis of amino acids [10]. Pyridoxine plays a role as a co-enzyme for many metabolic enzymes and it is considering a potent antioxidant [11]. It improved the efficiency of photosynthetic carbon reactions and increased dry matter production. Meanwhile, various plants treated with pyridoxine led to increased cell division, enhanced growth and differentiation and increased nutrient uptake [12].

play a crucial role in carbon uptake, photosynthesis, the transport of photosynthates and transfer of organic and inorganic nutrients from soil to the plant [6]. Furthermore, potassium is important for: control of ionic balance, management of plant stomata and water use, activation of plant enzymes and, many other processes [4 & 7]. For the growth and development of plants, Boron (B) is a crucial micronutrient. Boron is taken by plants from soil solution in the form of boric acid [8]. It provides a variety of vital activities in plant, it is mainly engaged in cellular membrane and structural integration. B is cross-linked with pectin assembly, which maintains the tensile strength and porosity of the cell wall [9]. B play a crucial role in protein and enzymatic functions resulting in increased membrane integrity. Moreover, Adequate B levels improves the plasma membrane hyper polarization, whereas inadequate contents alter membrane potential and reduce H^+ -ATP ase activity

[10]. Boron impact of B on plasma membrane-bound proton-pumping ATPase affects ion flux: in *Vicia faba* under B-deficient conditions, alteration of H^+ , K^+ , PO_4^{3-} , Rb^+ , and Ca^{2+} ions across membrane was recorded [11]. The greater B demand in young growing tissues proves the critical effect particularly in cell division and extension [12]. B shortage severely inhibits root extension, with deformed flower and fruit formation because of decreased cell division in the meristematic area, while adequate B supply promotes advantageous root development [13]. Moreover, B is used in phenolic metabolism and plays a pivotal role in nitrogen (N) metabolism as it enhances nitrate levels and reduces nitrate reductase activity under decreased B levels [14]. Furthermore, B improves fruit setting and seed production, causing improved crop production [15]. B affects availability and uptake of other nutrients from the soil [16].

A huge ecological degradation throughout the world resulted from the increased use of traditional chemical fertilizers in agricultural land, causing soil infertility and loss of biodiversity [13]. Thus, it is of great importance to use various alternative fertilizers to reduce over using of chemical fertilizers. Using of different microorganisms such as Cyanobacteria as biofertilizers could be used to decrease the harmful effect of chemical fertilizers [14]. Biofertilizers are environmentally friendly, cost-effective, and alternate to artificial fertilizers. They promote agricultural production and reduce environmental pollution [15]. Cyanobacteria, formerly known as blue-green algae, are photosynthetic microscopic organisms, lead to the liberate of a various group of biologically active metabolites in the rhizosphere, and in stress status could evolve plants growth regulators thus improve plant growth and productivity [16]. Cyanobacteria are important of agricultural soils where they effectively participate in biological nitrogen fixation, assist in phosphate solubilization and mineral liberation to progress soil fertility and crop production [17]. Moreover, Cyanobacteria liberated different types of active substances such as proteins, amino acids, carbohydrates, vitamins, polysaccharides, and phytohormones, that role as signaling molecules to elevate plant growth and development [18].

Wheat (*Triticum aestivum* L) is the most important cereal crop grown in the world. At least one-third of the world's population relies on wheat as its main food staple [19]. It considers from the most important food grain due to the grains of wheat contain a large quantity of proteins, carbohydrates, several minerals, and vitamins. Thus, efforts must be made to enhance wheat yield, in order to reduce the gap between production and consumption. So, the cultivated lands could be increased by reclaimed new lands, supplying

them with water from wells and devoting them for wheat production [20].

So, the target of our work was to study the adverse influences of water stress on wheat plants (Gimeza 9 genotype) and the effect of soil addition with cyanobacteria and /or foliar treatment with thiamine and pyridoxine on growth, some biochemical aspects, protein electrophoretic patterns, isozymes, yield and yield components.

2. Materials and methods

2.1. Materials. Grains of the wheat cultivar (Gimeza 9) and Cyanobacteria extract were got from the Agricultural Research Centre in Egypt. Thiamine and pyridoxine were bought from Sigma – Aldrich Company.

2.2. Experiment location. Two field experiments were sowing in two successive winter seasons of 2018/2019 & 2019/2020. at the Farm of National Research Centre Nubaria region, Egypt, (30_86'67" N 31_16'67" E), and mean altitude 21 m above sea level).

. Experiment design

The object of this study was to examine the influence of Cyanobacteria supplemented to the soil with (120 litre/ha.) in the beginning of cultivation, and repeated with the same dose after 30 days and 55 days from cultivation. Foliar treatments with thiamine and pyridoxine (0.0, 50 and 100 mg⁻¹) on Gimeza 9 plants were carried out.

The experiment was designed split-split plot design with four replicates, which the water irrigation requirements (WIR) 100% (WW) & 75% (WS), occupy the main plots. while biofertilizer (Cyanobacteria extract Cya) in sub-plots.

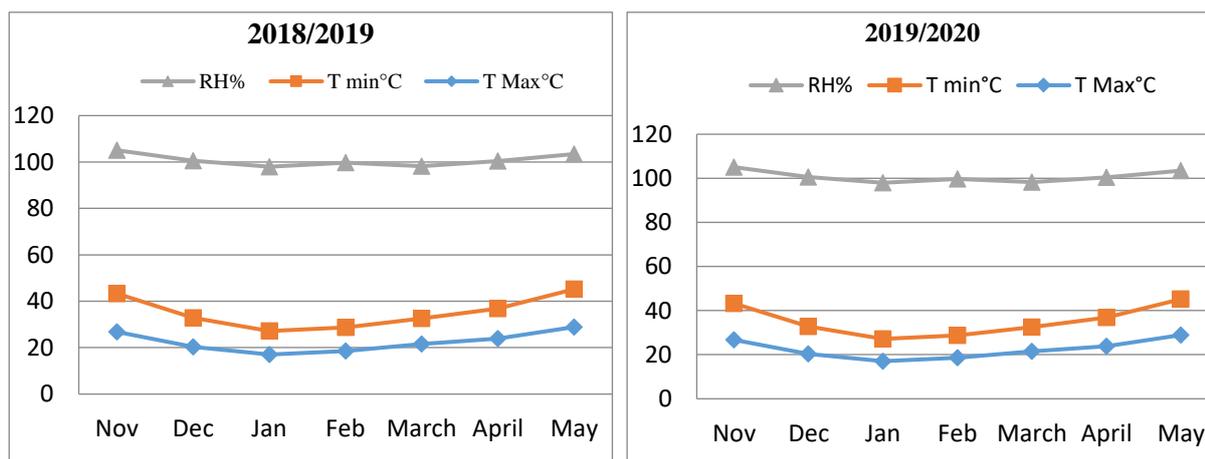


Figure 1 The data of maximum temperature (TMax °C), minimum temperature (TMin°C) & average relative humidity (RH%), got from weather station installed at the experimental station of National Research Centre, Nubaria shows the climatic data of the experimental site during the growing season.

Physical and chemical analysis of the sandy soil of the experimental site (Table 1) is determined according to [21].

Table 1: Mechanical, chemical and nutritional analysis of the experimental soil.

Sand		Silt 20-0µ%	Clay < 2µ%	Soil texture
Course 2000-200µ%	Fine 200-20µ %			
47.46	36.19	12.86	4.28	Sandy

Chemical analysis:

pH 1:2.5	EC dSm ⁻¹	CaCo ₃	OM%	Soluble Cations meq/l				Soluble anions meq/l			
				Na ⁺	K ⁺	Mg ⁺	Ca ⁺⁺	CO ₃ ⁻	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁻
7.60	0.13	5.3	0.06	0.57	0.13	0.92	1.0	0.0	1.25	0.48	0.89

Nutritional analysis:

Available nutrients						
Macro element ppm			Micro element ppm			
N	P	K	Zn	Fe	Mn	Cu
52	12.0	75	0.14	1.4	0.3	0.00

Meanwhile, the treatments of thiamine or pyridoxine concentrations were located randomly in sub-sub plots. In 26th November, the grains of wheat were cultivated in the two seasons in rows 3.5 m long, and the distance between rows was 20 cm apart, plot area was 10.5 m² (3.0 m in width and 3.5 m in length).

The agricultural practices were done as recommended of sowing wheat under sandy soil conditions, and the seeding rate was (140 Kg ha⁻¹). Pre-sowing, 360 kg ha⁻¹ of calcium super-phosphate (15.5 % P₂O₅) was added to the soil. Nitrogen fertilizer was added after plants emergence in the form of ammonium nitrate 33.5% at a rate of 180 Kg/ha⁻¹ was divided at five equal doses before the 1st, 2nd, 3rd, 4th, and 5th irrigation. Potassium sulfate (48.5 % K₂O) was divided at two equal doses of 120 kg/ha⁻¹, before the 1st and 3rd irrigation. Irrigation was carried out using the new sprinkler irrigation system where water was added every 5 days according to the amount of water irrigation used. Plants were sprayed with different concentrations of thiamine or pyridoxine after 45 and 60 days from sowing.

2.4. Plant sampling

Plant samples were gathered after 75 days from cultivation for measuring morphological parameters (plant height, tiller fresh and dry weights root fresh and dry weight). Biochemical aspects as photosynthetic pigments, total soluble sugars (TSS), proline, free amino acids, protein electrophoretic pattern and antioxidant isozymes were measured.

At harvest the following wheat characters were recorded on random patterns of ten girded plants in each treatment to measuring: plant height (cm), spike weight (g), grain weight/spike (g), 1000 grains weight (g), grain yield (ton/ hectare), straw yield (ton/ hectare), biological yield (ton/ hectare) and water productivity (WP) in kg m/m/ ha⁻¹ were measured at harvest (after 160 days from sowing).

2.5. Irrigation Water Requirements:

Two irrigation water requirements were calculated using Penman Monteith equation and crop coefficient according [22]. The average amount of irrigation water applied with sprinkler irrigation system were 5950 and 4760 m³ ha⁻¹ season⁻¹ as (100% and 75%) for two seasons after deducting the amount of rainwater that fell during the two growing seasons for wheat, (*Triticum aestivum* L.) variety Gimeza 9, where it was 680 m³ ha⁻¹ and was 616 m³ ha⁻¹ for the first and second season, respectively. The irrigation water requirements were calculated as the following:

$$IWR = \frac{[ET_0 \times K_c \times Kr \times I + LR]}{E_a} \times 4.2 \quad \text{Ea}$$

Where:

IWR = water irrigation quantities m³/ ha⁻¹

ET₀ = Evapotranspiration (mm/day)

Kc = Crop coefficient.

Kr = Reduction factor [23].

I = the period between two irrigations, day

Ea = Irrigation water efficiency, 90%.

LR = Leaching requirement = 10% of the total water requirement applied to the treatment.

2.6. Biochemical analysis:

Photosynthetic pigments content was determined according to the method explained by Lichtenthaler & Buschmann [24]. Using a spectrophotometer method (Shimadzu UV-1700, Tokyo, Japan). Total soluble sugars were extracted and analyzed according to [25] and [26] respectively. Proline was assayed depending on the method explained by Bates [27]. The free amino acid was determined depending on the method explained by Yemm & Willis [28]. Total carbohydrate was determined according to Dubois [29].

Protein electrophoretic analysis by Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was done according to the method described by Laemmli [30] as adapted by Studier [31]. For the assay of antioxidant enzymes peroxidase (POX EC 1.11.1.7) and polyphenol oxidase (PPO EC 1.10.3.1) were extracted based on the method of Stagemann [32]. PPO and POX isozymes were separated by Native-polyacrylamide gel electrophoresis (Native-PAGE). The activities of POX and PPO were defined depending on Brown [33] and Baaziz [34].

2.7. Water-productivity (WP):

WP was calculated by Howell [35]. water productivity (WP) is realized as the relation between the grain yield and the quantity of irrigation water. WP in kg /mm/ha was calculated by the following:

$$WP = E_y / E_t$$

Where WP is the Water productivity (kg/m³); E_y is the economical yield (kg ha⁻¹); E_t is the total utilized of irrigation water, m³ha⁻¹ /season.

2.8. Statistical analysis:

The data were statistically analyzed on complete randomized design at split plot system of MSTAT- [36] software. Means were compared by utilizing least significant difference (LSD) at 5% level of probability.

3. Results:

3.1. Growth criteria

Data showed that water deficit (75% WIR) significantly decreased $P \leq 0.05$ the growth criteria (plant height, leaves number/tiller, fresh and dry weight of tiller) contrast with the control (100% WIR) Table (2). Also, Cya addition to sandy soil increased significantly all these growth criteria contrast with the corresponding control (75% WIR) or (100% WIR). Foliar application of Th or Pyr with 50 & 100 mg l⁻¹ induced significant enhances in growth criteria in the presence and absence of Cya

compared with the corresponding control plants grown at (75% & 100% WIR). Application of Pyr and Th at 100mg l^{-1} recorded the maximum rises in all the growth character in the presence of Cya.

3.2. Photosynthetic pigments

Figure (2) illustrated that the impact of different concentrations of thiamine or pyridoxine with 50 and 100mg l^{-1} on photosynthetic pigments of Gimeza 9 variety grown under various water irrigation requirement (WIR). Data clearly shows that, water deficit (75% WIR) significantly decreased the contents of photosynthetic pigments contrasted to untreated plants. Meanwhile, foliar application of thiamine or pyridoxine with both concentrations significantly increased $P \leq 0.05$ in the photosynthetic pigments of the plants grown at 100% and 75% WIR. Meanwhile additive raises were detected for the plants cultivated in the presence of Cya contrasted to their corresponding treated plants cultivated without Cya. Application of pyridoxine at 100mg l^{-1} in the presence of Cya recorded the maximum increases of photosynthetic pigments.

3.3. Changes in compatible solutes:

Under water stress plants led to accumulate higher amounts of compatible solutes osmolytes [total soluble sugars (TSS), proline (Pro) and free amino acids (FAA)] Figure (3). Moreover, different Th and Pyr treatments caused more increases in the studied compatible osmolytes. Also, plant treated with Cya significantly increased $P \leq 0.05$ in TSS, Pro and FAA when contrast with plants cultivated in absence Cya under two levels of WIR.

3.4. Protein electrophoresis:

SDS-PAGE of total proteins extracted from wheat leaves treated with thiamine or pyridoxine in without and with of Cya was shown in (Fig.4 & Table 3). A total number of 12 bands were scored ranging from 8 to 165 kDa; three of these were monomorphic (25%), meanwhile nine were polymorphic (75% polymorphism). The maximum number of bands showed in the control plants without treatment with Cya at 75% WIR (11 bands) followed by the wheat plants treated with pyridoxine in the presence of Cya at 100% WIR and 75% WIR

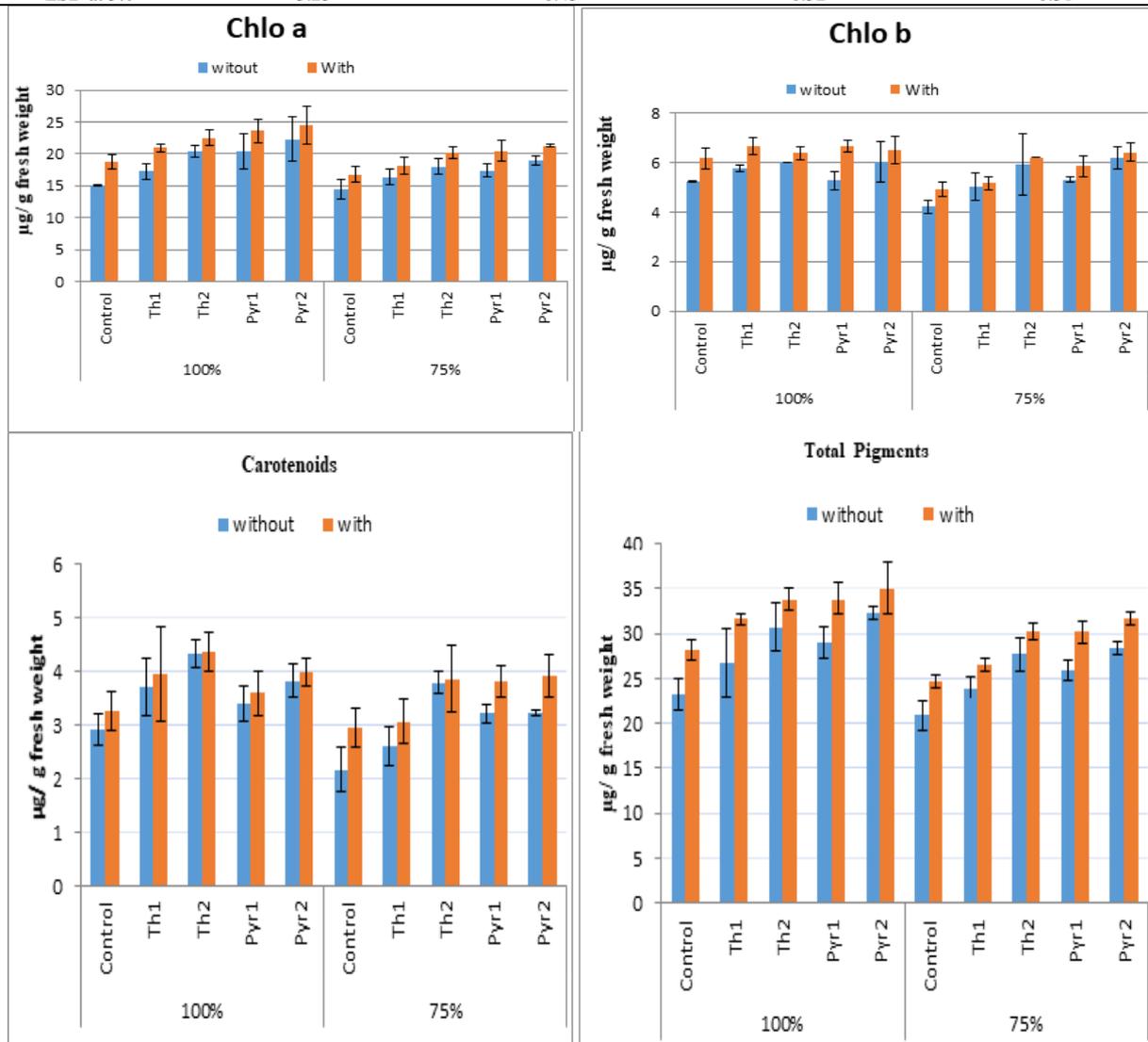
(9 bands). Moreover, the least number of polypeptides was observed in the untreated plants without Cya under 100% WIR (5 bands). Moreover, six polypeptides with Mwts 72, 65, 42, 24, 20 and 18 kDa revealed in the control wheat plants in absence of Cya under water deficit, while they disappeared in control plant (Fig. 4). The Cya at 100% WIR induced induced the protein bands at molecular weights 42, 24, 20, and 18 kDa contrast with the untreated plants in absence of Cya at 100% WIR. In addition, polypeptide at 42 and 24 kDa appeared in wheat plants treated with thiamine and pyridoxine without and with Cya under 100% and 75% WIR. Also, one subunit at 18 kDa appeared in wheat plants treated with thiamine and pyridoxine in the presence of Cya under 75% and 100% WIR except on 100% WIR in absence of Cya (Fig. 4 and Table 3). It was observed that, Gimeza 9 variety treated with thiamine and pyridoxine under water deficit increased the density of bands as compared to the same treatment at 100% WIR.

3.5. Peroxidase (POX) and Polyphenol oxidase (PPO) isozymes

POX profiles recorded eight bands with *RF* value ranging from 0.082 to 0.921 with changed in intensity and density. The maximum number of isozymes were shown in Gimeza variety treated with thiamine or pyridoxine in the absence of Cya under 75% WIR, Th or Pyr in the presence of Cya under 100% WIR and control plant treated with Cya at 75% WIR (7 bands). Meanwhile, the other treatments in absence and presence of Cya showed less number of isomers (six isoforms) (Fig. 5 & Table 4). One band with *RF* 0.120 was found in the wheat plants treated with thiamine and pyridoxine in absence of Cya under 75% WIR. Moreover, it appeared with Th treatment at 100% WIR and disappeared in the remaining treatments in the presence of Cya. In addition, one isoform (*Rf* 0.301) revealed in the wheat plants treated with Pyr in the presence of Cya under 100% WIR and the control plants in presence of Cya under 75% WIR. Meanwhile, it disappeared in the other treatments (Fig. 5 & Table 4).

Table 2: The influence of various levels of thiamine (Th1 50 & Th2 100mg^l⁻¹) and pyridoxine (Pyr1 50 & Pyr2 100mg^l⁻¹) treatments in without and with Cyanobacteria on vegetative growth parameters in various water irrigation requirement (WIR) of Gimeza 9 variety at 75 days from sowing.

WIR %	Treatment	Plant height (cm)		Leaves number/ tiller		Fresh weight of tiller (g)		Dry weight of tiller (g)	
		without	with	without	with	without	with	without	with
100 %	Control	52.67±0.67	56.33±1.86	6.00±0.00	6.33±0.00	6.20±0.40	7.45±0.15	1.81±0.10	2.31±0.04
	Th1	54.33±0.88	62.33±1.20	6.67±0.33	7.00±0.00	8.46±0.76	9.84±0.33	1.98±0.26	2.37±0.10
	Th2	57.50±1.33	65.50±1.45	6.33±0.33	7.50±0.33	9.89±0.42	10.09±1.17	2.16±0.15	2.42±0.39
	Pyr1	53.67±1.76	61.67±1.45	6.67±0.33	7.67±0.33	8.50±1.12	9.01±0.41	2.38±0.33	2.71±0.36
	Pyr2	58.50±0.33	66.00±1.15	6.67±0.33	7.67±0.33	10.11±0.89	11.22±0.49	2.46±0.33	2.87±0.14
75 %	Control	45.50±2.60	51.50±2.65	5.67±0.58	6.00±0.33	5.47±1.33	6.95±1.33	1.31±0.14	1.62±0.18
	Th1	49.00±2.40	60.00±1.73	6.00±0.33	6.67±0.33	7.34±0.27	8.02±0.27	1.81±0.39	2.10±0.22
	Th2	51.00±1.00	63.00±3.06	6.33±0.33	7.00±0.33	8.00±0.36	8.16±0.36	2.08±0.12	2.34±0.23
	Pyr1	49.67±1.53	59.33±0.88	5.67±0.33	6.67±0.33	8.70±0.60	8.99±0.60	2.05±0.10	2.58±0.10
	Pyr2	55.33±1.45	63.67±0.89	6.33±0.33	7.00±0.00	8.73±0.09	8.97±0.09	2.11±0.19	2.46±0.16
LSD at 5%		3.25		0.45		0.52		0.31	



LSD at 5% : Chlo a: LSD 5% :0.304, Chlo b: 0.057, Carotenoids: 0.304 and Total pigments: 0.057

Figure 2: The influence of various levels of thiamine (Th1 50 & Th2 100mg^l⁻¹) and pyridoxine (Pyr1 50 & Pyr2 100mg^l⁻¹) treatments in without and with Cyanobacteria on photosynthetic pigments (mg/g fresh wt) in various water irrigation requirements (WIR) of wheat leaves at 75 days from sowing.

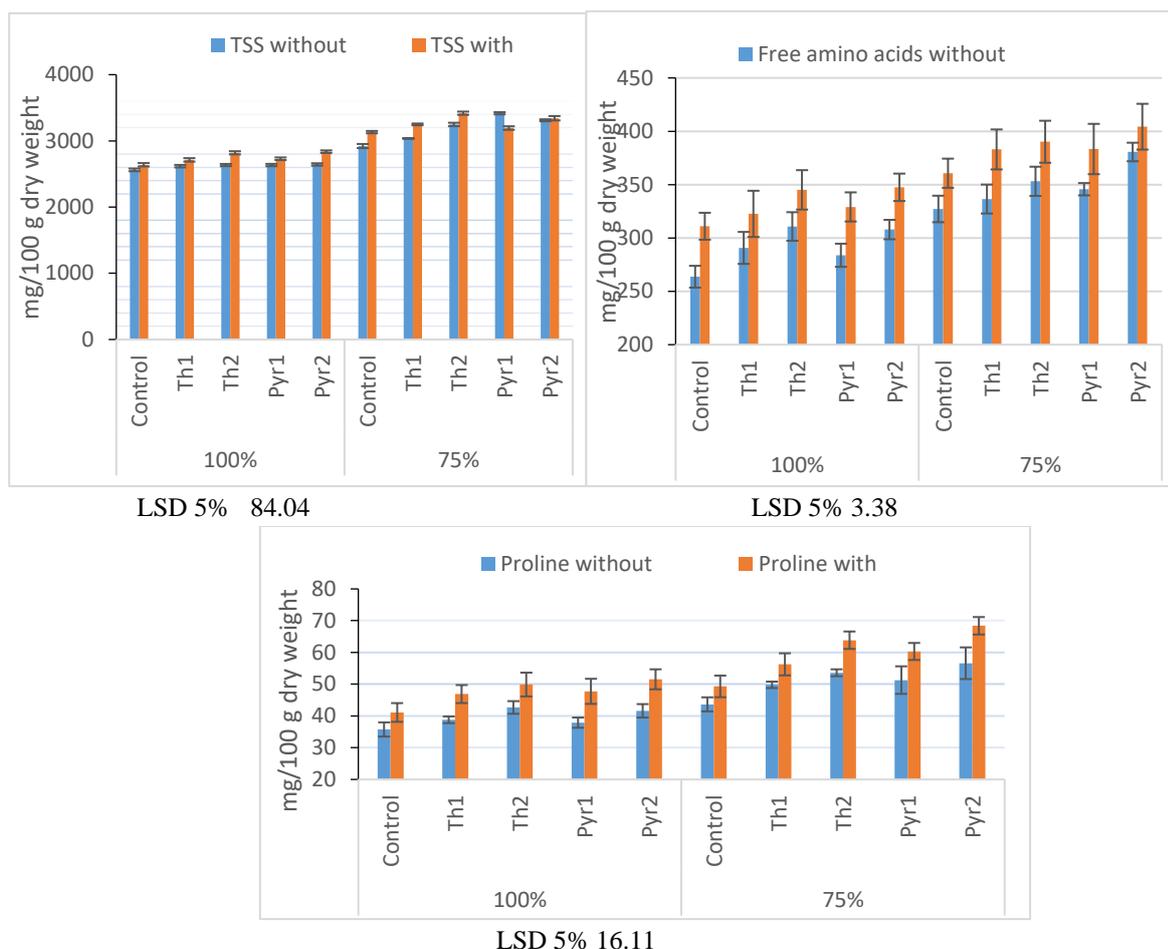


Figure 3: The influence of various levels of thiamine (Th1 50 & Th2 100mg^l⁻¹) and pyridoxine (Pyr1 50 & Pyr2 100mg^l⁻¹) treatments in without (-ve) and with (+ve) of Cyanobacteria on total soluble sugar, free amino acids and proline (mg/100g dry weight) in various water irrigation requirement (WIR) of wheat leaves at 75 days from sowing.

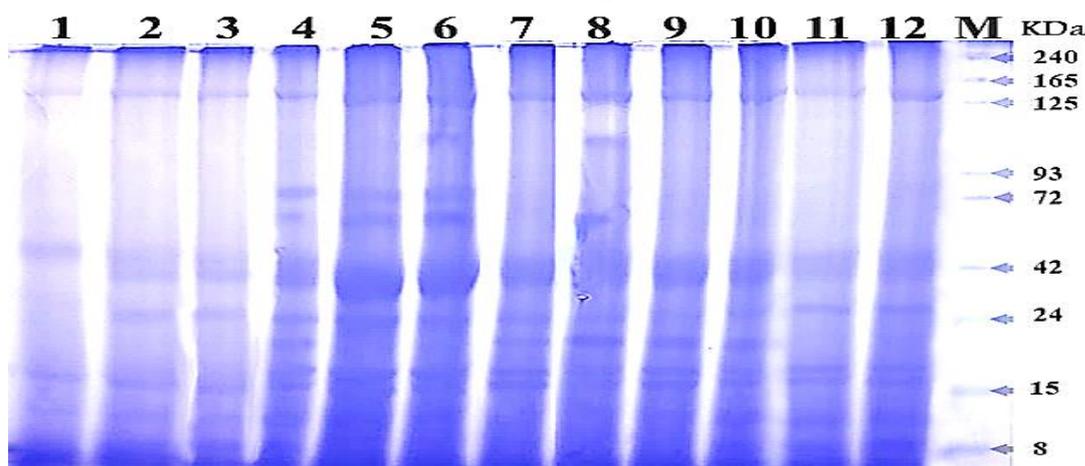


Fig. (4) The alteration of protein bands in response to various treatments of thiamine (100 mg^l⁻¹) and pyridoxine (100 mg^l⁻¹) without and with of Cyanobacteria on wheat leaves at various levels of water irrigation requirement (WIR). Each lane contains equal amounts of protein extracted from plant. Protein bands in the gel were visualized by Coomassie Blue Stain.

Lane M. Marker, In absence of Cyanobacteria (lane 1: 100% WIR, lane2: 100% WIR + 100mg/l thiamine, lane3: 100% WIR + 100mg/l pyridoxine, lane 4: 75% WIR, lane5: 75% WIR + 100mg/l thiamine, lane 6: 75% WIR + 100mg/l pyridoxine), in presence of Cyanobacteria (lane 7: 100% WIR, lane 8: 100% WIR + 100mg/l thiamine, lane 9: 100% WIR + 100mg/l pyridoxine, lane 10: 75% WIR, lane11: 75% WIR + 100mg/l thiamine, lane 12: 75% WIR + 100mg/l pyridoxine).

Table 3. The influence of thiamine (Th 100 mg l⁻¹) and pyridoxine (Pyr 100 mg l⁻¹) treatments without (-ve) and with (+ve) of Cyanobacteria on SDS-PAGE analysis protein electrophoresis of wheat leaves under different levels of water irrigation requirement (WIR) at 75 days from sowing

Band No	Mw(kDa)	Without Cya						With Cya					
		100 % WIR			75 % WIR			100 % WIR			75 % WIR		
		C	Th	Pyr	C	Th	Pyr	C	Th	Pyr	C	Th	Pyr
1	153	+	+	+	+	+	+	+	+	+	+	+	+
2	120	-	-	-	-	-	+	-	+	-	-	-	-
3	72	-	-	-	+	+	+	-	-	-	-	-	-
4	65	-	-	-	+	+	+	-	+	-	-	-	-
5	52	+	+	+	+	+	+	+	+	+	+	+	+
6	42	-	+	+	+	+	+	+	+	+	+	+	+
7	24	-	+	+	+	+	+	+	+	+	+	+	+
8	20	-	-	-	+	-	-	+	+	+	+	-	-
9	18	-	-	-	+	+	+	+	+	+	+	+	+
10	15	+	+	+	+	+	+	+	+	+	+	+	+
11	10	+	+	+	+	-	-	-	-	-	+	+	+
12	8	+	+	+	+	-	-	-	-	-	+	+	+
Total No. of bands		5	7	7	11	8	8	7	9	7	9	8	8

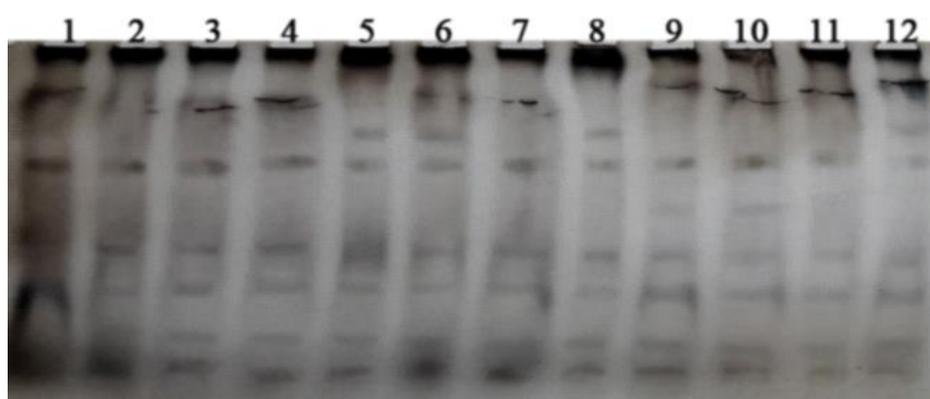


Fig. (5) The influence of thiamine (Th 100 mg l⁻¹) or pyridoxine (Pyr 100 mg l⁻¹) treatment in without and with of Cyanobacteria on isozyme peroxidase in response to different treatments on wheat leaves (Gimeza 9 cv.) at various levels of water irrigation requirement (WIR) at 75 days from sowing In absence of Cyanobacteria (lane 1: 100% WIR, lane 2: 100% WIR + 100mg/l thiamine, lane 3: 100% WIR + 100mg/l pyridoxine, lane 4: 75% WIR, lane 5: 75% WIR + 100mg/l thiamine, lane 6: 75% WIR + 100mg/l pyridoxine), in presence of Cyanobacteria (lane 7: 100% WIR, lane 8: 100% WIR + 100mg/l thiamine, lane 9: 100% WIR + 100mg/l pyridoxine, lane 10: 75% WIR, lane 11: 75% WIR + 100mg/l thiamine, lane 12: 75% WIR + 100mg/l pyridoxine)..

Table (4) The influence of thiamine or pyridoxine (100 mg l⁻¹) treatments in with or without of Cyanobacteria on isozyme peroxidase in wheat leaves at various levels of water irrigation requirement (WIR) at 75 days from sowing.

No	Rf	Without Cya						With Cya					
		100 % WIR			75 % WIR			100 % WIR			75 % WIR		
		C	Th	Pyr	C	Th	Pyr	C	Th	Pyr	C	Th	Pyr
1	0.082	+	+	+	+	+	+	+	+	+	+	+	+
2	0.120	-	-	-	-	+	+	-	+	-	-	-	-
3	0.202	+	+	+	+	+	+	+	+	+	+	+	+
4	0.301	-	-	-	-	-	-	-	-	+	+	-	-
5	0.565	+	+	+	+	+	+	+	+	+	+	+	+
6	0.620	+	+	+	+	+	+	+	+	+	+	+	+
7	0.692	+	+	+	+	+	+	+	+	+	+	+	+
8	0.921	+	+	+	+	+	+	+	+	+	+	+	+
Bands No= 8		6	6	6	6	7	7	6	7	7	7	6	6

(+) present (-) absent of band

PPO profiles showed five iso-forms with *Rf* values extending of (0.182 to 0.941) with changed in intensity and density of bands. The maximum number of isoenzymes was found in the wheat plants treated with thiamine in absence of Cya under 75 % WIR (five isozymes), followed by the plants treated with Pyr in the presence of Cya under water deficit (four bands). The least number of bands was scored in the remaining plants (three isoforms) (Fig. 6 & Table 5). Two isozyme markers with *Rf* 0.412 and 0.529 were scored in wheat plants treated with thiamine in absence of CYA under water deficit (Fig. 6 & Table 5).

3.6. Yield componets

Data in Table (6a & b) showed that the effects of different concentrations of Th or Pyr with 50 and 100 mg^l⁻¹ on yield and its components of wheat

plants grown under 100% & 75 % WIR. Data clearly showed that 75 % WIR decreased significantly ($P \leq 0.05$) the yield and yield components (shoot high (cm), Spike wt (g), grains weight / spike (g), 1000 grains weight (g), straw yield, biological yield and grain yields ton/ha) compared with control. Foliar application of Th or Pyr (50 and 100 mg^l⁻¹) increased significantly ($P \leq 0.05$) the entire above mentioned yield components for the plants grown under water deficit or normal condition. Application of Th or Pyr (100 mg^l⁻¹) recorded the maximum increments in grain yeilds ton /ha in the presence of Cya extract. Meanwhile additive increments were noticed for the plants cultivated with Cya as contrast to the corresponding treated plants cultivated in the absence Cya.

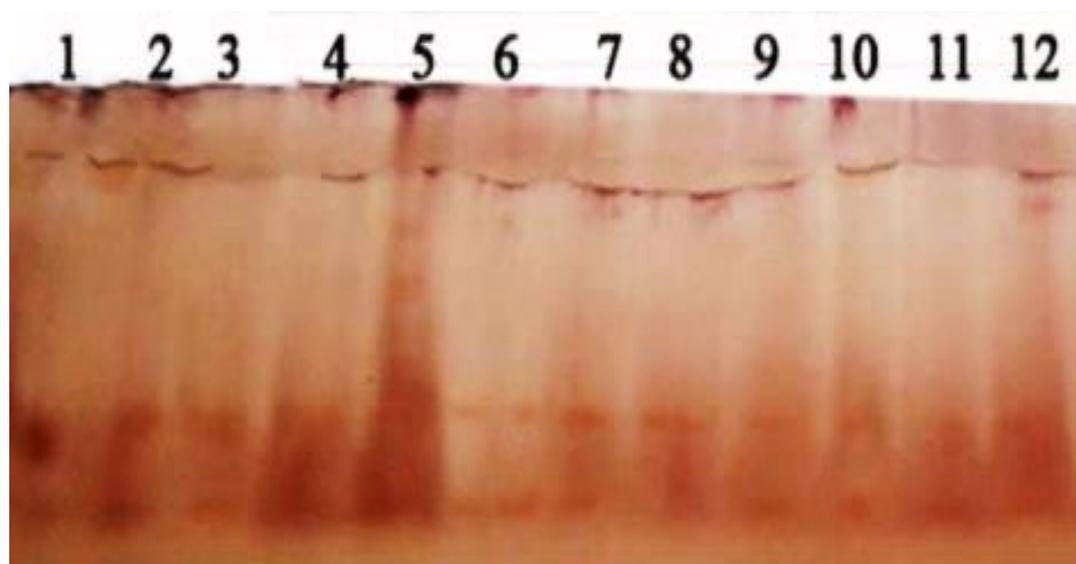


Fig. (6) The influence Of thiamine (Th 100 mg^l⁻¹) or pyridoxine (Pyr 100 mg^l⁻¹) treatment in without and with of Cyanobacteria on isoenzyme polyphenol oxidase on wheat leaves (Gimeza 9 cv.) at various levels of water irrigation requirement (WIR) at 75 days from sowing: In absence of CYANobacteria(lane 1: 100% WIR, lane2: 100% WIR + 100mg/l thiamine, lane3: 100% WIR + 100mg/l pyridoxine, lane 4: 75% WIR, lane5: 75% WIR + 100mg/l thiamine, lane 6: 75% WIR + 100mg/l pyridoxine), in presence of CYANobacteria(lane 7: 100% WIR, lane 8: 100% WIR + 100mg/l thiamine, lane 9: 100% WIR + 100mg/l pyridoxine, lane 10: 75% WIR, lane11: 75% WIR + 100mg/l thiamine, lane 12: 75% WIR + 100mg/l pyridoxine)..

Table 5. The influence of thiamine (Th 100 mg^l⁻¹) or pyridoxine (Pyr 100 mg^l⁻¹) treatment in without and with cyanobacteriaon isozyme polyphenol oxidase on wheat leaves at various levels of water irrigation requirement (WIR) at 75 days from sowing

No	N Rf	Without Cya						With Cya					
		100 % WIR			75 % WIR			100 % WIR			75 % WIR		
		C	Th	Pyr	C	Th	Pyr	C	Th	Pyr	C	Th	Pyr
1	0.182	+	+	+	+	+	+	+	+	+	+	+	+
2	0.412	-	-	-	-	+	-	-	-	-	-	-	+
3	0.529	-	-	-	-	+	-	-	-	-	-	-	-
4	0.722	+	+	+	+	+	+	+	+	+	+	+	+
5	0.941	+	+	+	+	+	+	+	+	+	+	++	+
Bands No= 5		3	3	3	3	5	3	3	3	3	3	3	4

Table (6 a): The influence of various levels of thiamine (Th1 50 & Th2 100mg^l⁻¹) and pyridoxine (Pyr1 50 & Pyr2 100mg^l⁻¹) treatments in without and with Cyanobacteria on yield components of Gimeza variety at various levels of water irrigation requirement (WIR).

WIR % Treatment	Plant height (cm)		Spike wt (g)		Grains wt /spike (g)		1000 grains wt (g)		
	without	with	without	with	without	with	without	with	
100 %	Control	73.50±1.76	76.93±2.40	2.42 ± 0.19	2.69 ± 0.39	2.03 ± 0.04	2.36 ± 0.02	43.50±1.08	44.98±1.08
	Th1	75.72±3.71	84.50*±0.33	2.79*±0.39	3.08*±0.18	2.32*±0.04	2.53*±0.04	44.87*±1.11	45.63*±0.120
	Th2	76.44±2.67	83.33*±1.86	2.96*±0.35	3.31*±0.22	2.52*±0.12	2.70*±0.02	44.91*±1.11	46.50*±1.35
	Py1	75.60±1.76	78.50 ±2.51	2.88*±0.15	3.12*±0.19	2.49*±0.02	2.69*±0.04	45.01*±1.41	46.62*±2.13
	Py2	76.68*±0.67	79.80*±2.52	2.93 ± 0.13	3.08*±0.19	2.18*±0.01	2.57*±0.03	45.43*±1.02	47.78*±2.30
75 %	Control	70.00*±3.18	73.50 ±0.00	2.13*±0.17	2.42±0.31	1.77*±0.01	1.94*±0.04	38.84*±1.59	40.83*±2.06
	Th1	77.67*±0.89	79.00*±2.40	2.41±0.12	2.58±0.11	1.94*±0.01	2.22*±0.08	40.02*±1.11	43.08*±1.39
	Th2	76.90*±1.20	80.40*±3.51	2.52±0.38	2.96*±0.36	1.92*±0.05	2.57*±0.09	42.00*±1.11	44.19*±2.06
	Py1	75.50±1.70	82.67*±1.45	2.44±0.20	2.88*±0.17	1.97*±0.06	2.55*±0.05	41.52*±2.16	44.63*±1.28
	Py2	78.00*±2.18	78.80*±1.45	2.39±0.45	2.77*±0.23	1.84*±0.03	2.25*±0.06	40.96*±1.42	44.92*±1.98
LSD at 5%		3.01		0.32		0.15		0.42	

Table (6b): The influence of various levels of thiamine (Th1 50 & Th2 100mg^l⁻¹) and pyridoxine (Pyr1 50 & Pyr2 100mg^l⁻¹) treatments in without and with Cyanobacteria on yield components of Gimeza variety at various levels of water irrigation requirement (WIR).

WI R (%)	Treatments (mg/l)	Grains yield ton /ha		Straw yield ton /ha		Biological yield ton /ha	
		without	with	without	with	without	with
100 %	Control	4.68 ±0.07	5.31±0.15	6.95±0.14	7.29±0.07	11.63±0.13	12.60±0.17
	Th1	5.21 ±0.07	5.65*±0.08	8.76*±0.05	8.88*±0.08	13.97*±0.12	14.53*±0.17
	Th2	5.35 ±0.05	6.05*±0.17	8.17*±0.05	9.49*±0.07	13.52*±0.19	15.54*±0.18
	Py1	5.30 ±0.13	5.74± 0.11	8.41*±0.07	8.85*±0.08	13.71*±0.24	14.59*±0.13
	Py2	5.42*±0.05	6.13*± 0.00	8.59*±0.08	9.17*±0.072	14.01*±0.16	15.30*±0.20
75 %	Control	4.12±0.09	4.63±0.11	5.41*±0.12	6.05*±0.07	9.53*±0.18	10.68*±0.13
	Th1	4.66 ± 0.15	5.06 ±0.08	6.02*±0.17	7.39*±0.09	10.69*±0.16	12.45*±0.21
	Th2	4.98*±0.07	5.37*±0.08	7.24*±0.09	7.90*±0.16	12.32*±0.19	13.23*±0.24
	Py1	5.02*±0.11	5.29*±0.09	6.76*±0.16	7.15*±0.06	11.86*±0.14	12.44*±0.19
	Py2	5.12*±0.09	5.38*±0.12	7.17*±0.08	7.68*±0.07	12.29*±0.24	13.06*±0.20
LSD at 5%		0.52		0.46		0.34	

3.7. Carbohydrates % in grains yield

Figure (7) showed that the influence of various concentrations of Th and Pyr (50 and 100 mg^l⁻¹) in absence and presence of Cya on carbohydrates percentage of wheat grains at various levels of WIR %. Data clearly showed that plant cultivated under water deficit 75% WIR decreased significantly ($P \leq 0.05$) on carbohydrates % when compared to plants grown under normal condition 100% WIR. Wheat plant was treated with various concentrations of Th or Pyr in absence and presence of Cya led to significant increase increases ($P \leq 0.05$) in carbohydrates % under different water levels. While, the plants were cultivated in the presence of Cya significant increases ($P \leq 0.05$) were observed as compared to the corresponding treated plants cultivated without Cya.

3.8. Water productivity (WP):

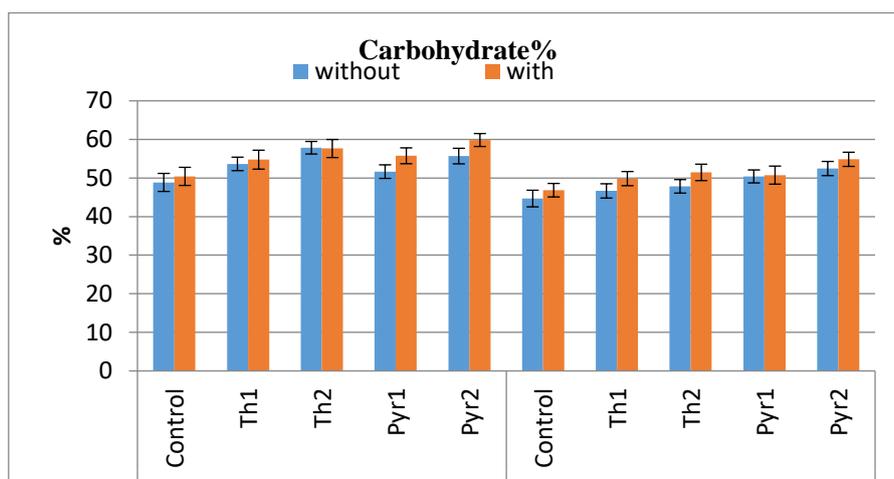
Figure (8) showed that the influences of Th or Pyr (50 and 100 mg^l⁻¹) in absence and presence of Cya on water productivity (WP) of Gimeza 9 variety at various levels of WIR. Exposure of plants to 75% increased significantly ($P \leq 0.05$) in WP when contrast to plants grown at normal condition 100% WIR. Wheat plants were treated with Th or Pyr in

absence and presence of Cya increased significantly WP at various water levels compared with the corresponding untreated plant at the same WIR %. While, the plants were cultivated in the presence of Cya significant increases ($P \leq 0.05$) were observed as compared to the corresponding treated plants cultivated without Cya.

4. Discussion

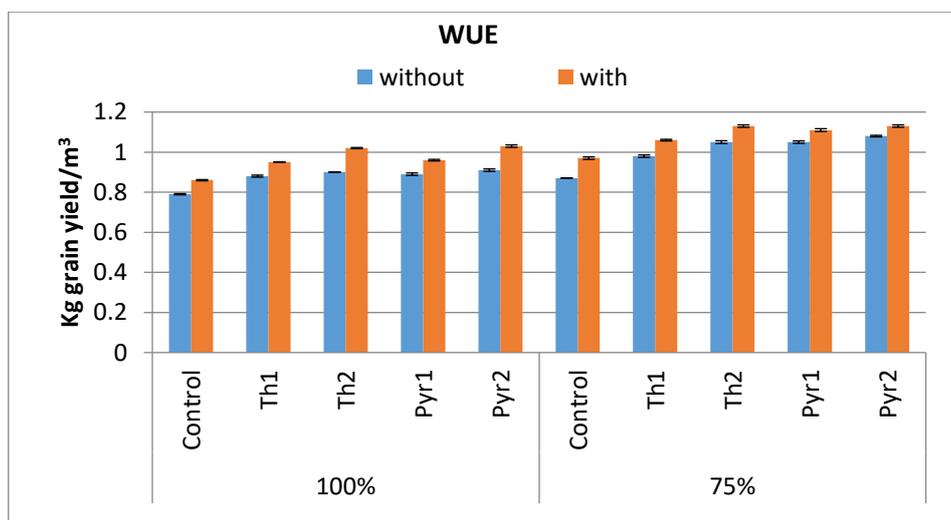
4.1. Growth parameters

Deficiency of water from the plant cells due to water stress decreases wheat growth came in line with *Khafagy et al.*, [37] on barley, *Bakhom et al.*, [38] on faba bean. These reduced effects might be due decline in cell enlargement as well as, decreased turgor pressure [39]. In addition, this decreased effect could be due to decreased water absorption, low water potential, increased different ions contents in cells and conductance of stomata of leaves [40]. *Jabeen et al.*, [41], *Ragaey et al.*, [42] and *Bkhom et al.*, [43] reported that, drought led to oxidative stress, nutritional imbalance, hormonal changes, repression/decay of proteins, depress enzymes activities, and disorder in secondary metabolism.



LSD 5% 2.89

Figure (7): The influence of various levels of thiamine (Th1 50 & Th2 100mg l^{-1}) and pyridoxine (Pyr1 50 & Pyr2 100mg l^{-1}) treatments in without and with Cyanobacteria on carbohydrates % of wheat plants under various levels of water irrigation requirement (WIR (combined analysis of two seasons).



LSD 5% 0.06

Figure (8): The influence of various levels of thiamine (Th1 50 & Th2 100mg l^{-1}) and pyridoxine (Pyr1 50 & Pyr2 100mg l^{-1}) treatments in without (-ve) and with (+ve) of Cyanobacteria on water productivity (Kg grain yield /m 3) of Gimeza 9 variety at various levels of water irrigation requirement (WIR).

In response to thiamine, Khafagy *et al.*, [37] observed that the treated barley plants with thiamine led to significantly enhanced in growth criteria. Thiamine is the main function of coenzyme thiamine pyrophosphate which plays a significant role in the balance of carbon metabolism in plants. Ghafar *et al.*, [44] found that foliar spray of thiamine (50 mM) enhanced plant growth under water deficit. They referred this growth improvement to thiamine-induced raise in the photosynthetic pigments and total phenolic contents of *Trifolium repens* L. plants under water stress.

Regarding to the results of pyridoxine, Barakat [12] found that, the treated of wheat plant with pyridoxine enhanced the cell division, increased the

growth and nutrient absorption that enhanced capacity of photosynthetic surface and increased dry matter production. Moreover, the efficiency of pyridoxine on the growth of *Lupinus termis* and sesame plants were investigated by Boghdady [45] and Nassar *et al.*[46] and Sadak [47].

The promotive influence of Cya on growth parameters of wheat plants are in harmony with those gained earlier by Ismail & Abo-Hamad [48] on *Hordium vulgare* and *Trigonella foenum-graecum* plants. These increases in growth criteria can be referred to fix atmospheric nitrogen. Furthermore, Cya recently in considered as a new bio fertilizer containing some of the important macroelements (N, P, K, Ca, Mg, and S) and microelements (Zn, Fe,

Mn, Cu, Mo, Co) as well as various bioactive compounds and growth regulators. These bioactive substances such as endogenous phytohormones, lipids and proteins were active on encouraging growth and development and increase plant tolerance to water deficit [49].

4.2. Photosynthetic pigments

Suppression in photosynthetic pigments in wheat plants cultivated under water deficit is in harmony with the results obtained by Bakhoum *et al.*, [50] on white lupine plant, Sadak [51] and Bakry *et al.*, [52] on flax. These decreases in photosynthetic pigments contents under the effect of drought stress might be because of the sensitivity of pigment-protein complex and devastation of pigments [53].

Meanwhile, foliar application of thiamine or pyridoxine with 50 and 100 mg l⁻¹ significantly improved in the photosynthetic pigments of the plants grown at 100% WIR or water deficit 75% WIR conditions. Whereas progress increments were observed for the plants cultivated with Cya in contrast to their corresponding treated plants cultivated in the absent of Cya (Fig 2).

The role of vitamins was enhancing chlorophyll biosynthesis or decreasing degradation. This enhancement was generally because of the defending role of vitamins which led to a decrease in oxidative stress. The positive role of thiamine on chlorophyll was stated in many plants because thiamine enhances or assist resynthesize chlorophyll [54]. El-Awadi *et al.*, [55] reported that thiamine treatment is mostly associated to suitable regulation of photosynthesis and energy-providing reactions in plants. Regarding to the impact of pyridoxine came on line with Hamada and Khulaef [54] in *Vicia faba* seedlings treated with pyridoxine. Moreover, Nassar *et al.*, [46] and Hendawy & Ezz-El-Din [56] confirmed the increased contents of photosynthetic pigments of *Foeniculum vulgare* var. *azoricum* and sesame plant by treatments of vitamin B as co-enzymes in the enzyme reactions in carbohydrates, protein and fats metabolism and used in respiration and photosynthesis.

The effect of Cya play an enhancing role on photosynthetic pigments which came in line with Abbas *et al.*, [57] on different plant species. These increased may be because of the large quantity of nitrogen assimilated through Cya from atmosphere and transferred to plant tissues [58].

4.3. Compatible solutes:

Under drought deficit plants accumulate higher amounts of compatible solutes, these compounds shield plants from stress by raising membrane stability and assistance in steadying proteins and enzymes composition [39] on wheat plants. They showed that the osmolytes (TSS, Pro, and FAA) shared a significant function in the adaptability of

cells to various unfavourable drought stress (Fig.3). They raised osmotic pressure in cytoplasm, stabilized proteins and membranes, and maintained the comparatively rising-water content which necessary for plant growth, development and cellular functions [59]. The accumulation of TSS in plants has been demonstrated as a reply to water deficit in spite of a significant reduction in CO₂ assimilation rate Elewa *et al.*, [60]. Moreover, an increment in Pro content can be referred to the reduction in proline oxidase activity at water stress conditions [61]. Furthermore, Pro is a source of nitrogen and carbon to rescue stress and perform as a stabilizer for membranes, several macromolecules, and a free radical scavenger and enzymes protection [62].

The obtained data proved that thiamine treatment has an important function in the regulating of carbon metabolism and protein synthesizes as an effective coenzyme in the metabolic passageways of these biochemicals. Cumulation of TSS by thiamine treatment in drought-stressed wheat plants could be a significant adaption reply to drought [63]. Similar alleviating response of wheat plant on TSS, FAA and Pro cumulation were noticed in *Zea maize* plants [7].

Pyridoxine is an important cofactor for many metabolic enzymes as well as amino acid metabolism and is needed for the growth and development of several plant species [64]. It may be dependent on its influence on the production of proline and another procedure in proline synthesis. Proline synthesise in this system depends on increment transcription of the D1-pyrroline-5-carboxylate synthesise and preventing of its degradation demands inactivation of the proline dehydrogenase enzyme [65].

Moreover, the wheat plant was cultivated in the presence of cyanobacteria encouraged a significant increase in TSS as compared with plants cultivated in absence of Cya. Also, the increase in TSS may be due to the Cya induced raising endogenous phytohormones or by acting as activators of carbohydrate synthesizes. In this connection, El-Bassiouny *et al.*, [66] and Sadak & Ahmed [67] confirmed these results on wheat plants. They found that, cyanobacteria supplemented to the soil increased free amino acids and proline contents.

4.4. Protein electrophoresis:

The alteration in protein electrophoresis patterns in leaves of wheat plant grown under water deficit in without and with Cya showed several kinds of alterations. Some polypeptides were improved and produce a new set of polypeptides. Mohammadkhani and Heidari [68] observed that drought stimulated several soluble proteins and inhibited others in *Zea maize* plants. New proteins appeared in wheat leaves exhibited as a result of

water deficit at molecular weights 18, 20, 24, 42,65 and 72 kDa (Kilo Dalton) in absence of Cya. This protein is specified as stress-associated protein. It was proposed that these proteins have an osmoprotectant role or kept cellular structures [62 & 69].

Wheat plants treated with Cya induced a slight increase in the protein contents in contrast with the untreated plants (Fig. 4 & Table 3). Mohiuddin *et al.*, [70] found that increases in the protein contents in wheat plants treated with biofertilizers. The polypeptides at Mwts 24 and 42 kDa in wheat plant could be deemed as affirmative markers for thiamine and pyridoxine and it was observed that these polypeptides disappear under the control. Barakat *et al.*, [12] observed that treated wheat cultivar with pyridoxine resulted in the creation of five polypeptides with M wts of 11, 18, 33, 52 & 68 KD and increment the density of almost polypeptides. The creation of new polypeptides and the significant rise in the density of several polypeptides suggest that vitamin B6 has a deep influence on the specific and quantitative alterations in the protein constituent of the plants. It is recognized that various vitamins B are recognized to be precursors of different coenzymes; working in various techniques [71].

4.5. Peroxidase (POX) and Polyphenol oxidase (PPO)

Wheat plants treated with Cya under water deficit led to an increase in activities of both POX and PPO contrast with the control in absence of Cya (Fig.5, 6). These results were agreement with El-Bassiouny *et al.*, [66] & Ashraf *et al.*, [72]. They recorded increment in the enzyme activities e.g., POX, CAT, and PPO activities in *Helianthus annuus*, *Sorghum durra*, and wheat plants which were treated with the CYAnobacterial filtrates. Jabeen *et al.*, [42] stated that, exogenous application of thiamine decreased the oxidative stress in white clover by enhancing the antioxidant enzyme activities.

Thiamine & pyridoxine treatments induced pronounced stimulation in the antioxidant enzymes activity of CAT, POD, SOD & APX compared to the reference controls [73]. Naresh *et al.*, [74] found enhancement in the antioxidant activities of flax that was treated with *Anabaena fertilissima*, and *Nostoc muscorum*, exudates. Likewise, the enzymes activity of phenylalanine ammonialyase and peroxidase in the *Oryza sativa* plants were determined as a result of inoculated with Cyanobacteria. Also, Cya contain different metabolites which may influence favorably on antioxidative defense system in the plant by improving SOD, POD, and CAT activities [75].

4.6. Yield and yield components

Table (6a & b) showed that water deficit decreased the yield components, Abd Elhamid *et al.*, [76] and Sadak & Ramadan [77] and Abdalla *et al.*, [78] confirmed these results on yield of moringa, lupine and moringa plants. Meanwhile, foliar application of thiamine or pyridoxine with 50 and 100 mg/l increased the entire above mentioned yield components for the plants grown under water deficit or normal condition. Meanwhile, the advanced enhances were showed in the plants cultivated in the presence of Cya. These results of Cya improving role came in accordance with the studies earlier [54]. These positive impacts might be due to the capability of Cya producing growth promoting hormones, these substances increased seed germination, growth, development and protein contents [79].

Nassar *et al.*, [46] reported that the pyridoxine improved the plant growth, yield, and seed quality of Sesame plant. The positive effect of Pyr was approved on the plant's productivity and tolerance to stress. In addition, Vitamin B could act as an antioxidant in improving plant tolerance. Lone *et al.*, [80] found that pyridoxine increases the growth of the root system and lead to the best nutrient absorbed and higher economic yield. In response to thiamine, El-Awadi *et al.*, [55] found that in Lupine plant treated with thiamine led to significant increments in seed yield, oil, and protein contents.

Sadak *et al.*, [39] instituted that, water stress induced a significant decrease in total carbohydrate contents in wheat cultivars Figure (7). The impact of thiamine or pyridoxine increased significantly the carbohydrate percentage of grains yield may be because of the rises in growth character and photosynthetic pigments. The photosynthetic production increased thus enhanced carbohydrates forming in leaves and so enhanced the transportation of carbohydrates to improved grains. El-Metwally & Sadak [9] and El-Karamany *et al.*, [81] found that, faba bean and Egyptian clover treated with thiamine and pyridoxine induced significant increases, yield constituents, carbohydrates and protein contents in seed yield.

4.7. Water productivity

Water productivity (WP) that is related to the proportion of grain yield to water utilized, in common, is inversely proportional to water deficit strength. The plants exposed to water deficit (75% WIR) induced a significant increase in WP when compared to plants grown under the level of 100% WIR (Fig. 8). Wheat plants treated with various concentrations of Th or Pyr in the absence or presence of Cya led to the increased WP under various water deficits as compared with the corresponding control of WIR %. The increases in WP indicates that the plant uses various techniques

under water stress as a mechanism this can decrease its water consumption while maintaining high biomass [39]. Abdelraouf *et al.*, [82] explained that wheat plants treated with biofertilizer led to increasing significantly in the WUE as compared to the control (non treated one).

So, utilizing agronomic rules like the irrigation management and application of Th or Pyr in presence of Cya can increase soil humidity preservation and WP would be the clef for decreasing water utilization and beneficent the possibility of low-input producing regulations.

5. Conclusion

The application of Cyanobacteria and/or thiamine and pyridoxine ameliorated the harmful effects of drought through enhancing the biosynthesis of the plant's bioactive compounds such as organic solutes (TSS, proline, FAA), antioxidant compounds (carotenoids) and isozyme (POX, PPO) and some soluble proteins. Cya, and/or thiamine and pyridoxine work on a cellular scale their influence the redox status of the cell. They enhanced growth, grains yield, and crop water productivity. It was observed that Thiamine and pyridoxine (100mg⁻¹) were, the most efficient in wheat exposed to water deficit in the presence of Cya. Thiamine and pyridoxine treatments in the presence of Cyanobacteria are a favorable strategy to progress water deficit tolerance and the production of the wheat plants. Moreover, it confirmed the probability and possibilities of utilizing Cya biofertilizers as renewable natural nitrogen resources for wheat. Cya is also nonpolluting, cheap, and uses renewable resources.

Data Availability

The authors state that all data generated or analyzed during this study are included in this article.

Author Contributions:

Hala Mohamed Safwat El-Bassiouny and Mervat Shamoon Sadak, designed and performed the experiment, responsible of all the physiological and biochemical analysis and also wrote and reviewed the manuscript. Sherin Amin Mahfouze and Magda Aly Mahmoud El-Enany, designed and performed the experiment, responsible of the molecular analysis, Tarek abd Elfattah Elewa, designed and performed the experiment, responsible of statistical analysis and also wrote and reviewed the manuscript All authors have read and agreed to the published version of the manuscript.

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