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Functional activity of biofertilizers for improving yield and its components of common bean plants under water stress.

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Abstract: Water stress is a world-wide problem that affects the functional and biochemical processes of plants resulting in varying growth, income and water associations of economic plants. The present work was evaluated to improve the yield of common bean plants (*Phaseolus vulgaris* L.) under different water stress levels by using microorganisms. The interaction effect between water stress (WW as recommended irrigation, WS_1 after 13 days and WS_2 after 18 days) holding water and inoculation with different microorganisms (AMF, EM, endophytic bacteria) used alone or in combination with each other were evaluated for their effect on yield production. Application of AMF and EM considerably increased the average values of all quality parameters and realized the highest mean values until WS_1. The inoculation with AMF and EM with WS_1 are the greater than other levels which common bean plants had a significantly higher number of pods, length of pod, pods weight, 100 seeds weight, yield (ton /Fed) and water-use efficiency (WUE, ton/ m^3). Concentrations of nutrients (N, K, P, Mg and Ca). In addition, vitamin B_1, folic acid, total carbohydrates, crude protein and crude fibers significantly increased at AMF+EM under second water stress (WS_1).

keywords: Arbuscular mycorrhizal fungi, (Phaseolus vulgaris L.)Yield components Water levels

Introduction

Ingestion of legumes, mostly dry beans, has favorable in some countries due to an improved understanding of consumers about the nutritional features [1]. Beans considered an important source of nutritional protein. Furthermore, the fiber content proscribes blood sugar levels from raising rapidly after a meal, creating these beans an especially good high-quality for individuals with diabetes [2]. Water is one of the most valued resources on earth, therefore, thirst for water will turn into one of the most insistent resource problems of the current century [3]. The main challenge fronting Egypt now is the robust need for better management and development of the available restricted resources of water, energy and land to bump into the needs of population progress [3]. Recently, there are exhausting new technologies to raise the production of this legume, such as mechanized harvesting and irrigation, to face to consume beans everyday [4]. Plants are wide-open to a diversity of biotic or abiotic stresses, such as salt loading, freezing and drought affect their growth, development and productivity. One of the major abiotic stresses that upset plant productivity is water stress resulting from salinity and drought [5]. Water stress affects the biochemical and biological processes of plants [6, 7], resulting in altering yield, growth, water relations [8 9, 10] and metabolic trails [11]. Reduction in plant growth under water stress is by reason of osmotic stress resulted from reducing the uptake of nutrients and soil water potential [12, 13]. AMF are pervasive soil microbes from the phylum Glomeromycota that ripen a mutualistic symbiosis with the excessive majority of land plants [14, 15]. Mycorrhizae are mutualistic symbiotic associations based on bidirectional nutrients between the roots and soil fungi of vascular plants, where AMF transfers water and inorganic nutrients to the plant and obtain carbohydrates un exchange [16, 17]. One of the microbiological preparations used to improve quantitative and qualitative yielding of plants is Effective Microorganisms (EM) [18]. EM can significantly improve soil fertility and flowering, promotes growth, fruit development and maturing in crops. It can raise crop yields and enhance crop quality other than rushing the breakdown of organic matter from crop remains [19]. Endophytes are microorganisms counting bacteria and fungi that expend the full of their life cycle colonizing in living tissue of different plants archetypally without causing any noticeable symptoms of disease [20, 21]. Therefore, application of endophytic bacteria resulting in the construction of phytohormones, biocontrol of phytopathogens in the root region (through creation of antibacterial agents or antifungal, nutrient competition, siderophore production and induction of systematically developed immunity, or host resistance [22, 23].

The purpose of the present work was to study the alleviation of the water stress by using microorganisms and improving the yield production of *Phaseolus vulgaris* L. plants under diverse water stress levels.

2. Materials and methods

Design of the experiment

This study was carried out from March to June of 2018 at the Station of Experimental Research, Science's Faculty, Mansoura University. The experimental layout was split - plot design in a randomized complete blocks design with three replicates, the main plots were the three water levels (WW, WS_1 and WS_2), whereas the sub plots were the microorganisms treatments. The plot was 1.68 m2 (2 lines× 120cm long×70 cm width).

2. Inoculum preparation of AMF

The inoculum of AMF species counting*: G. mosseae, G. clarum, G. monosporium and Gigaspora margarita* were initially insulated from rhizosphere soil of *Phaseolus vulgaris* L. plants using the wet sieving and decanting technique [24], and then identified by Dr. Abdel-Fattah G. M. The identified AMF spores were left to burgeon for 6 months on sudangrass plants (*Sorghum halepense* L.) under normal conditions. Plants were irrigated by tap water as required in addition to the nutrient solutions (Long- Ashton without phosphorus) were provided. A mixture of soil and infected sudangrass root, that confined spores were used as AMF inoculum. The AMF inoculums containing 5 g of rhizosphere soi1 (approx. 725 spores) and 0.5 g infected sudangrass root bits with an infection leve1 of 88.6% was inoculated to each plot.

3. Growth conditions and planting

Seeds of *Phaseolus vulgaris* L. (Nebraska), gaining from the Horti. Res. Center, Egypt and used during this study. Seeds were surface disinfected in 0.0l% mercuric chloride for 3 min, afterward washed with purified water. The disinfected seeds were permitted to germinate on wet disinfected cotton for 3 days. For AMF treatments, each block was infected with 5g of rhizosphere soil (approx.275 spores/g soi1) and 0.5 g of cut AMF fresh sudangrass roots (M = 88.6%). Below the soil surface of common bean plants, the inoculum was placed 3-5 cm to produce mycorrhizal plants. While, the effective of microorganisms (EM) and endophytic bacteria were added in a presoaking way for the seeds at planting time, and the second time in a foliar way after 37 days from sowing.

Measurements

1.Estimation of mycorrhizal root colonization

The stained parts were placed on slides and were detected with a microscope (Carl Zeiss, Italy) at 40× magnification. They were visually allocated to 6 classes of AM colonization (from 0: 5 depending on the existence of AMF erection in the root section) and to 4 levels of AMF richness (from A0: A3). AMF colonization levels of the stained roots were appraised by the method of Trouvelot *et al*. [25] using the *Mycocalcsoftware (http://www.dijon.inra.fr/mychintec/ mycocalcprg/download.html)*. This method calculates three parameters of mycorrhizal colonization as follows:-

F: Rate of root colonization.

M: Strength of cortical colonization.

A: Arbuscules rate in roots.

2. Yield components

No. of pods, length of one pod, pods weight/ plant, weight of l00 seeds, yield ton/Fed and WUE were determined proximately after harvest Grain premeditated from {yield / water applied at a sdifferent level of requirements }according to Stanhill [26]. [27]. Phosphorus was determined subsequent ammonium molybdate b1ue method [28]. K and Na were evaluated by a flame spectrophotometer (Corning 400, UK), while Ca and Mg were determined by atomic absorption (PerkinElmer, Model 2308, USA) according to Allen [29].

3. Mineral and element contents

After cessation by acid, investigated of N, P, K, Mg and Ca contents. Total nitrogen (N) was dignified by using Kjeldahl method

4 Total Soluble Sugars (TSS) assessed by anthrone method [30]. Protein was determined by AOAC [31]. In addition, folic acid was estimated according to the method of Ruengsitagoon and Hattanat

3. Results and Discussion

1. Yield parameters

Results presented in Tables (1) demonstrate the effect of water stress and different microorganisms plus their interaction on dry yield and yield components of common bean seeds in the expression of no. of pods plant-1, pod length, weight of 100 seed, weight of pods, weight of seed plant-1, yield ton/fed, and WUE ton/m3. It is clear from the results presented in Table (1) that inoculation of common bean seeds with the different types of studied microorganisms combined under different water stress investigated gave a high value for yield characters and its component of common bean plant than those obtained for the un-inoculated ones (at all treatments). The highest mean values were obtained with the treatment of AMF+EM under WS1. These observations in good agreement with Utkhede [33] indicated that inoculation of tomato plants by AMF and EM produced

significantly higher fruit number and fruit yield compared to other plants.

2. Quality parameters of common bean seeds

Quality parameters of common bean seeds.

Table (2) show that the mean values of crude protein, crude fiber, total soluble sugar, and folic acid in seeds of common bean as influenced by water stress and different microorganisms alone or in acombination are presented in **Table (2).** The co-inoculation of common bean seeds with the mixture of microorganisms under all water stress levels significantly increased the average values of crude protein, total carbohydrates, crude fiber, folic acid and Vitamin B1 in seeds of common bean than those in control plants under the same water stress. The most suitable treatment which realized the highest values were connected with the treatment of AMF+EM under second water stress (WS_1)**.** Biofertilizers can exert positive effect on plant growth including synthesis of phyto-hormones, N_2-fixation, and nitrate reductase activity and enhancing minerals uptake [34]. Our results also were in covenant with Abdelmoneim et al. [35] who stated that protein content enhancement is related to relative increase in nitrogen fixation due to biofertilizer application.

The use of mycorrhizal fungi and EM application play a significant role with water stress these results in accordance with [36, 37] who reported that the using of EM encourage plant growth production of hormones and rise nitrogen fixation

and phosphorous intake that cause cumulative of protein content in the seeds. Moreover, it can raise crop yield, develop soil fertility and crop quality, help to accurate nutritional and functional crop complaints, increase nutrient availability and plant nutrient uptake [38]. Furthermore, many authors recommend that changes in sugar content are related to greater production of assimilates in plants inoculated with AMF and EM. This is caused by better mineral nutrition of plants and the creation of phytohormones by symbiotic microorganisms, it is expected that the improvement in mineral intake by plants results in yield quality enhancement [39, 40].

3. Mycorrhizal colonization levels

Mycorrhizal colonization parameters were articulated as rate of root colonization (F), intensity of cortical colonization (M) and arbuscules rate in roots (A) **Table (3).** The data revealed that the level of AMF root colonization continued to increase with the growth in plant age in all treatments. At the similar time, the AMF colonization was significantly reduced by increasing the water stress level at WS_2 while significantly increased at WS_1 particularly at the interaction between AMF and EM. Thus, it can be concluded that under water stress (WS_1) the AM fungi were able to increase the root infection significantly. In this present study, mycorrhizal levels of root infection were significantly reduced in common bean root tissues with WS_1 than with WW plants, and this result was more caused by the topmost water stress level (WS_2). While, the WS_1 recorded highly significant. Our data are in covenant with Asrar *et al.* [41] who stated that, both strengths of AMF colonization (M) and arbuscular occurrence (A) were innocent with WS_1 than with WW plants, and this result was more caused with the highest water stress level decreased in snapdragon root tissues. The result is in harmony with the verdict of Kaya *et al.* [42] who described that water stress significantly reduced the AM colonization by *G. clarum* in watermolen plants. In dissimilarity, preceding studies have revealed that the % of root colonized by AMF wasn't affected by water stress [43, 12].

2. Nutrient contents

Contents of nitrogen, phosphorus, potassium, calcium and magnesium of common bean plants are affected by either inoculation by different microorganisms and different levels of water stress are presented in **Table (4).** A positive effect was observed on the mean contents of all Nutritional elements under study due to using the combination. In this respect, the highest values; (3.13, 0.400, 2.99, 2.87 and 2.29%) for N, P, Ca, K and Mg%, respectively in common bean seeds AMF plants [45]. Thus, it is marked that the AMF fungal association propositions a number of benefits to the plants.

Table (1): Interaction between water stress and different microorganisms on yield parameters of common bean seeds

Treatments		No. of pods/plant	Pod length cm	Weight of 100 seed g	Weight of pods g	Yield ton/ Fed	WUE ton/m^3
WW	**Control**	16.67^{rs}	10.55^{s}	36.64^{s}	111.54^{s}	22.98^{s}	2.836^{r}
	AMF	27.00^{jk}	13.32^{k}	55.41^{k}	155.51^{k}	30.36^{k}	4.413^{j}
	EM	23.00^{mn}	12.15^{n}	48.45^{n}	138.92^{n}	27.60^{n}	3.814^{m}
	Endophy	21.67^{no}	11.83^{o}	45.80^{o}	133.69^{o}	26.66^{o}	3.622^{n}
	EM+Endo	25.67^{kl}	13.11^{l}	52.79^{l}	149.81^{l}	29.42^{l}	4.214^{k}
	AMF+EM	31.00^{gh}	14.26^{h}	62.54^{h}	171.63^{h}	33.13^{h}	4.825^{h}
	AMF+Endo	29.67^{hi}	13.95^{i}	59.55^{i}	166.70^{i}	32.19^{i}	4.791^{h}
	Mixture	28.33^{ij}	13.65^{j}	57.64^{j}	160.71^{j}	31.27^{j}	4.605^{i}
WS_1	**Control**	11.33^{vw}	9.27^{w}	27.55^{w}	89.45^{w}	19.30^{w}	2.043^{v}
	AMF	36.00^{cd}	15.54^{d}	71.58^{d}	193.68^{d}	36.83^{d}	5.777^{d}
	EM	33.67^{ef}	14.92^{f}	66.63^{f}	182.41^{f}	34.96^{f}	5.384^{f}
	Endophy	32.00^{fg}	14.55^{g}	64.50^{g}	177.41^{g}	34.04^{g}	5.189^{g}
	EM+Endo	34.67^{de}	15.23^{e}	69.37^{e}	188.56^{e}	35.90^{e}	5.573^{e}
	AMF+EM	40.00^{a}	16.53^{a}	78.56^{a}	210.89^{a}	39.62^{a}	6.354^{a}
	AMF+Endo	38.67^{ab}	16.18^{b}	76.64^{b}	204.33^{b}	38.68^{b}	6.161^{b}
	Mixture	37.67^{bc}	15.86^{c}	73.48^{c}	199.51^{c}	37.75^{c}	5.957^{c}
WS_2	**Control**	10.00^{w}	8.96^{x}	25.57^{x}	84.19^{x}	18.41^{x}	1.847^{w}
	AMF	17.67^{qr}	10.86^{r}	39.31^{r}	117.93^{r}	23.90^{r}	3.043^{q}
	EM	14.00^{tu}	9.92^{u}	32.32^{u}	100.88^{u}	21.14^{u}	2.434^{t}
	Endophy	12.67^{uv}	9.56^{v}	29.49^{v}	95.22^{v}	20.23^{v}	2.238^{u}
	EM+Endo	15.33st	10.25^{t}	34.35^{t}	106.25^{t}	22.07^{t}	2.644^{s}
	AMF+EM	24.33^{lm}	12.46^{m}	50.61^{m}	144.31^{m}	28.49^{m}	4.016^{l}
	AMF+Endo	20.33^{op}	11.53^{p}	43.40^{p}	127.73^{p}	25.75^{p}	3.426^{o}
	Mixture	19.00^{pq}	11.19^{q}	41.23^{q}	122.21^{q}	24.84^{q}	3.224^{p}
	LSD at 5%	1.71	0.12	1.61	2.18	0.12	0.10

Table (2): Effect of water stress levels and different microorganisms on quality parameters of common bean seeds.

Treatments		Curdeprotien%	TSS %	Folic acid (mg/100g)
WW	Control	13.31^{p}	4.83^{p}	184.50^{o}
	AMF	16.85^{h}	5.46^{i}	194.47^{k}
	EM	16.29^{i}	5.13^{l}	191.27^{m}
	Endophy	15.92^{j}	4.98^{n}	189.73^{n}
	EM+Endo	16.54^{i}	5.28^{k}	192.87^{l}
	AMF+EM	17.52^{fg}	5.97^{f}	198.73^{h}
	AMF+Endo	17.35^{g}	5.79^{g}	197.33^{i}
	Mixture	17.23^{g}	5.60^{h}	195.70^{j}
WS1	Control	12.79^{q}	4.74^{q}	171.47^{v}
	AMF	18.54^{c}	6.28^{d}	225.27^{d}
	EM	17.98^{de}	5.97^{f}	222.43^{f}
	Endophy	17.79^{ef}	5.80^{g}	220.90^{g}
	EM+Endo	18.21^{d}	6.12^{e}	223.53^{e}
	AMF+EM	**19.54^{a}**	**6.82^{a}**	**229.93^{a}**
	AMF+Endo	19.33^{ab}	6.61^{b}	228.37^{b}
	Mixture	19.13^{b}	6.46^{c}	226.53^{c}
WS2	Control	12.27^{r}	4.26^{t}	169.60^{w}
	AMF	14.75^{l}	4.91^{o}	175.43^{s}
	EM	14.02^{n}	4.57^{r}	172.50^{u}
	Endophy	13.69^{o}	4.43^{s}	171.17^{v}
	EM+Endo	14.35^{m}	4.74^{q}	174.27^{t}
	AMF+EM	15.73^{jk}	5.37^{j}	180.30^{p}
	AMF+Endo	15.44^{k}	5.26^{k}	178.67^{q}
	Mixture	15.00^{l}	5.06^{m}	177.13^{r}
LSD $_{at\ 5\%}$		**0.05**	**0.09**	**0.07**

Table (3): Mcorrhizal levels of common bean plants under different water stress levels

Treatments		*Mycorrhizal levels*		
		F%	*M%*	*A%*
WW	AMF	70.00^{g}	43.00^{g}	27.00^{i}
	AMF+EM	95.00^{b}	56.00^{b}	39.00^{c}
	EM+Endo	85.00^{d}	50.00^{d}	35.00^{e}
	Mixture	75.00^{f}	45.00^{f}	30.00^{g}
WS1	AMF	85.00^{d}	48.00^{e}	43.00^{b}
	AMF+EM	100.00^{a}	64.33^{a}	49.27^{a}
	EM+Endo	91.00^{c}	54.00^{c}	43.33^{b}
	Mixture	85.00^{d}	50.00^{d}	37.00^{d}
WS2	AMF	65.00^{h}	39.00^{h}	23.13^{k}
	AMF+EM	80.67^{e}	49.67^{d}	30.67^{f}
	EM+Endo	75.00^{f}	45.00^{f}	28.00^{h}
	Mixture	70.00^{g}	40.00^{h}	26.00^{j}
LSD $_{at\ 5\%}$		1.34	1.06	0.50

Table (4): Interaction between water stress levels and different microorganisms on N, P, K, Mg and Ca % of common bean seeds.

reatments		N%	P%	K%	Ca%	Mg%
WW	Control	2.13^{p}	0.275^{p}	1.78^{r}	1.65^{r}	1.16^{q}
	AMF	2.70^{h}	0.344^{h}	2.36^{j}	2.32^{i}	1.79^{h}
	EM	2.61^{i}	0.334^{i}	2.23^{k}	2.24^{jk}	1.73^{i}
	Endophy	2.55^{j}	0.329^{j}	2.18^{k}	2.20^{k}	1.68^{j}
	EM+Endo	2.65^{i}	0.336^{i}	2.27^{k}	2.28^{ij}	1.77^{hi}
	AMF+EM	2.80^{fg}	0.356^{f}	2.52^{h}	2.49^{f}	1.97^{e}
	AMF+Endo	2.78^{g}	0.351^{g}	2.47^{i}	2.44^{g}	1.90^{f}
	Mixture	2.76^{g}	0.348^{gh}	2.40^{j}	2.37^{h}	1.85^{g}
WS1	Control	2.05^{q}	0.261^{q}	1.67^{s}	1.54^{s}	1.08^{r}
	AMF	2.97^{c}	0.379^{c}	2.77^{d}	2.72^{c}	2.11^{d}
	EM	2.88^{de}	0.366^{e}	2.61^{f}	2.60^{e}	2.02^{e}
	Endophy	2.85^{ef}	0.362^{e}	2.57^{g}	2.57^{e}	2.00^{e}
	EM+Endo	2.91^{d}	0.372^{d}	2.68^{e}	2.66^{d}	2.07^{d}
	AMF+EM	3.13^{a}	0.400^{a}	2.99^{a}	2.87^{a}	2.29^{a}
	AMF+Endo	3.09^{ab}	0.390^{b}	2.94^{b}	2.82^{b}	2.22^{b}
	Mixture	3.06^{b}	0.383^{c}	2.83^{c}	2.77^{b}	2.17^{c}
WS2	Control	1.96^{r}	0.199^{r}	1.49^{t}	1.43^{t}	0.97^{s}
	AMF	2.36^{l}	0.306^{m}	2.01^{o}	1.88^{n}	1.38^{n}
	EM	2.24^{n}	0.297^{n}	1.93^{pq}	1.77^{p}	1.26^{p}
	Endophy	2.19^{o}	0.290^{o}	1.90^{q}	1.70^{q}	1.23^{p}
	EM+Endo	2.30^{m}	0.301^{n}	1.95^{p}	1.82^{o}	1.32^{o}
	AMF+EM	2.52^{jk}	0.324^{jk}	2.17^{l}	2.09^{l}	1.59^{k}
	AMF+Endo	2.47^{k}	0.320^{k}	2.11^{m}	2.01^{m}	1.51^{l}
	Mixture	2.40^{l}	0.312^{l}	2.07^{n}	1.97^{m}	1.44^{m}
LSD $_{at\ 5\%}$		0.05	0.005	0.04	0.05	0.04

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