Article

Use of Bioinoculants Affects Variation in Snap Bean Yield Grown under Deficit Irrigation

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Abstract: The use of beneficial microorganisms, such as plant growth promoting rhizobacteria (PGPR) and mycorrhizal fungi, for organic farming could improve the productivity and the resilience of vegetable crops. Both PGPR and PGPF are allowed for organic farming, and they represent new important tools for regenerating poor and marginal soils in transition to environmentally friendly farming. In the experiment, the effects of PGPM-based products were evaluated on snap bean in combination with two irrigation regimes. The experimental design adopted was split-plot, with the main plot represented by the irrigation regime (reintegration of 100 and 60% of the ETc), the sub-plot by the microbial consortia, and finally the sub-sub-plot by genotype ('Domino' and 'Maxi'). Seeds were sown in a cold greenhouse and the growing cycle finished after 86 days from sowing. The results showed a significant increase of the yield due to the application of PGPM compared to the control. The deficit irrigation applied (ETc 60%) affected plants growth in the two genotypes and their related production differently (in average 2.20 kg m^{-2} for Domino and 3.63 kg m^{-2} for Maxi), showing a positive effect of PGPM on yield (in average 2.47 kg m^{-2} without PGPM and 3.36 kg m^{-2} with PGPM) and product quality. Furthermore, an interesting negative correlation between the number of nodules and the yield was also observed, as a consequence of their early outcome which increased plant productivity in relation to the experimental factors.

Keywords: PGPM; drought stress; nodules; organic farming; sustainability

1. Introduction

Nowadays, sustainable agricultural methodologies based on ecological principles and natural rules is of primary importance in order to respond to the intensification of agriculture based worldwide on the efficient use of available resources [1]. The key challenge is to increase the production of foods and feeds with minimal environmental impacts in terms of nutrient leaching, biodiversity loss, greenhouse gas emissions, and resource exhaustion [2]. This frame-low input in farming practices represents a primary goal of enhancing the sustainability of cropping schemes in order to cope with climate change and achieve high yields in more environmentally friendly conditions [3–6]. To meet what was mentioned—besides a number of approaches which can be adopted, such as low nitrogen supply [7], cropping in soilless conditions [8,9], and overall breeding for resistance [10–14]—deficit irrigation, where possible, represents a sustainable way to save water [15–21]. Although deficit irrigation represents a limiting factor in horticulture, researchers' interest in assessing protocols to save water in agriculture has increased [17,22]. To this aim, the use of helpful microorganisms, such as plant-growth-promoting rhizobacteria (PGPR), added in the rhizosphere, has been shown to increase plants' potential resistance to abiotic stresses such as water shortage in a number of crops [11], including tomato wheat, rice [23], and common bean [24]. The naturally occurring soil-dwelling microbiota, in fact, represents a useful way to establish long-term resilient farming systems [25]. The rhizosphere is the soil region that is adhered to plant roots and represents

the area with the highest microbial activity where chemical, physical and microbiological interactions take place with intensive feedbacks on plant growth [26]. The rhizosphere includes plant-growth-promoting rhizobacteria (PGPR) which exert their enhancing roles through both direct and indirect pathways [27]. Direct mechanisms involve phytohormone production, atmospheric nitrogen fixation, iron sequestration, and inorganic phosphate solubilization [28]. On the other hand, PGPR indirectly promote plant growth by inducing anti-phytopathogen compound production and, as a consequence, they develop abiotic stress tolerance abilities such as drought and salinity resistance [29–32].

According to scientific literature, [23,24,33], common bean, *Phaseolus vulgaris* L., has a high agronomic interest worldwide [34]. It belongs to the Fabaceae family, and similar to other legume crops, it has a key role in improving soil fertility by boosting nitrogen input by symbiotic N fixation [35]. Common bean represents 50% of grain legumes used for direct human consumption and remains the most important grain legume and vegetable crop in the twenty-first century [36]. Originating from two different gene pools, Mesoamerican and Andean, its broad adaptation, consumer preference, and easiness of production for both dry seeds and green pods allows it to keep an edge over the other legumes [37]. Common bean presently offers a distinctive opportunity to understand how both the host and the environment contribute to rhizosphere microbiome assembly and vice versa, due to the pre-existing genetic differences in each gene pool followed by divergent breeding history [25,38].

Within the framework of the H2020 BRESOV project, of which the overall target is to increase plants' tolerance to biotic and abiotic stresses and to adapt varieties to the specific requirements of organic and low-input production processes, we evaluated the effects of a commercial product based on PGPM (*Trichoderma* spp., *Bacillus* spp., *Pseudomonas* spp., etc.) on two different green bean cultivars under a deficit irrigation regime.

2. Materials and Methods

2.1. Plant Material

The experiment was carried out during spring 2021. The seeds of snap beans were sown directly into the soil in a cold greenhouse at the experimental farm of ITAKA s.r.l. company located in Comiso — South East of Sicily— (37°00′09.7″ N, 14°.34′45.4″ E) on 11 March 2021. Two commercial varieties, 'Maxi' (*Phaseolus vulgaris* L. var *nanus*) from Hild company and 'Domino' (*Phaseolus vulgaris* L. var *vulgaris*) from De Bolster, were adopted in the experiment. The two varieties are both present on the market for green pod consumption and were part of a much larger seed set used in the contest of the BRESOV project by different partners.

Seeds were sown in rows with a space of 50 cm between rows and 40 cm along the row. For each spot, a total of 5 seeds were sown with a density of 20 plants m^{-2}.

2.2. Irrigation Regimes

Before experiment onset, the field was prepared with a tiller and abundantly watered during the first week of March [39]. The irrigation system was arranged by using driplines with drippers at a distance of 0.20 m from each other, and served by a reservoir located near the greenhouse. Water counters were installed upstream the dripline, one for each of the two main plots. A weather station (Watchdog 2500 series) was installed in the field provided by Ecosearch s.r.l. (Montone, Italy) From the weather station, 6 probes were installed—2 for each repetition, at 0.05 and at 0.25 m of depth, respectively—in order to monitor the percentage of humidity in the soil. For the determination of water stress, crop reference evapotranspiration (ET_0) provided by the weather station was taken into account daily, and it was assumed that ET_c 100 was the gradient corresponding to soil saturation.

For the first 3 weeks from experiment onset, plants were irrigated with the same water volumes until the unfolding of the 4th leaf; after this period, the irrigation was differentiated considering 100% of water requirement (ET_c 100) and a deficit irrigation corresponding to 60% of ET_c (ET_c 60).

2.3. Microorganisms Treatments

Exactly one week before sowing the snap bean seeds, the first treatment with PGPM (MO) was carried out according to the protocol provided by ITAKA s.r.l.; to this purpose, the commercial product Maxi Soil® was used. This formulate consists of a microbial consortium containing three species of *Trichoderma*(*T. harzianum, T. asperelum, T. atroviride*) and *Bacillus amylofiquefaciens, B. azotoformans, B. megaterium, B. pumilus, B. subtilis, Pseudomonas lurida, P. fluorescence, Streptomyces griseus,* and *S. lydicus*.

One week after sowing, the second treatment with MO was carried out. Maxi Soil was diluted in water at a rate of 0.5 g L^{-1} (0.5 g m^{-2}) and applied to the soil by fertigation.

2.4. Morpho-Physiological Parameters

Pods were harvested at commercial maturity after 65, 72, 79, and 86 days from sowing. At every harvest, the total yield and the number of pods for each plant were recorded; moreover, starting from the second harvest, three pods per plant were randomly collected in order to analyze the pod's weight and diameter. At the end of the cropping cycle, ten entire plants per plot were removed from the field in order to register the fresh and dry weight of both epigeal and hypogeal portions. To calculate the percentage of dry matter, the epigeal and hypogeal portions of the plants were dried in a heater for 72 h at 68 °C until constant weight, then the dry weight was weighted and the percentage of dry matter was calculated. Before the destructive assay, the number of the ramification of the first order and the number of root nodules were recorded.

During the cropping cycle, 55 days after sowing, the SPAD (Soil Plant Analysis Development) index was registered using a "SPAD 502 Plus Chlorophyll Meter" (Spectrum technologies, Inc., Aurora, IL, USA).

2.5. Experimental Design

The experimental design was a "Split-plot" with 3 factors (Figure 1). The main factor was represented by the two different irrigation regimes based on crop evapotranspiration (ET_c). ET_c was calculated according to the Penman–Monteith formula [39]. The sub-plot was represented by application or no application of PGPMs (MO or NMO); the sub-sub-plot was represented by the two adopted genotypes (GE) of snap bean. Each repetition was divided into 2 equal plots corresponding to the two different water regimes. Each plot was divided into 4 sub-plots, 1.00 m equidistant between each other, and representing the combination of the 3 experimental factors. Each elemental plot was 4.60 m long and consisted of 3 rows with 0.50 m equidistant between them. Each row was divided in half in such a way to obtain 6 sub-sub-plots within, in which a randomization of the 2 varieties of snap bean with 3 repetitions for each was arranged.

2.6. Statistical Analysis

The data obtained were subjected to statistical analysis with the Student-Newman-Keuls ANOVA 1 test performed with the software CoStat version 6.451(CoHort Software, Birmingham, England). Correlation and PCA were performed by using IBM SPSS Statistics for Windows, Version 28.0 (IBM Corp: Armonk, NY, USA).

2.7. Climatic and Soil Conditions

During the experiment, climatic conditions were stable over the whole cultivation season, with the relative humidity of the air ranging from a minimum of 30% to a maximum of 80% (Figure 2). The night and day shift in air temperature during the growing period varied from 3 °C to 18 °C at night and from 22 °C to 46 °C during the daytime (Figure 2). Concerning the soil temperatures, at 25 cm of depth, the temperatures ranged from a minimum of 7.8 °C (Tmin) to a maximum of 29.4 °C (Tmax), while on the surface, at 5 cm of depth, it ranged from a minimum of 18.8 °C (Tmin) to a maximum of 46.1 °C (Tmax), as shown on Figure 1. Concerning the relative humidity (R.U.) of the soil, it varied from 11 to 54% and from 63 to 93% for the minimum and the maximum R.U., respectively (Figure 2).

The greenhouse temperature was maintained on a range between 10 °C Tmin and 35 °C Tmax (Figure 2) by opening or closing both the windows and doors.
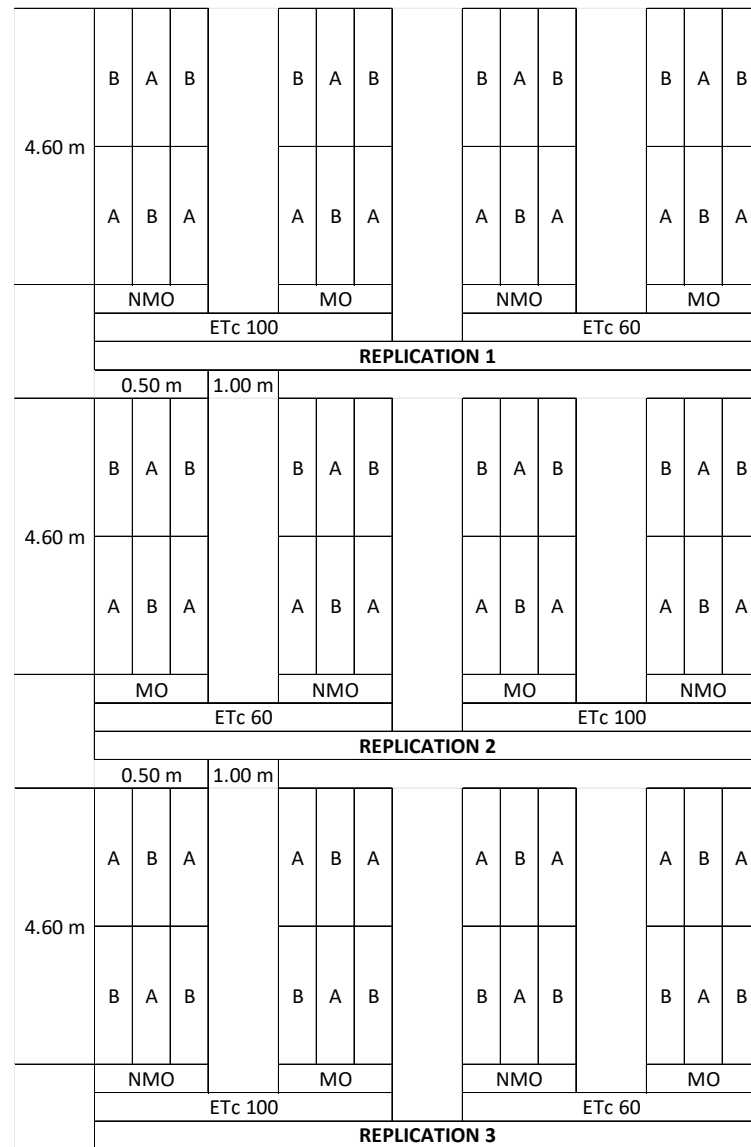

Figure 1. Experimental design. The experimental field was arranged as "split plot" with three experimental factors and three replications. The two different irrigation regimes were based on ETc (ETc 100 and ETc 60). The sub-plot was represented by application or not of PGPMs (MO or NMO); the sub-sub-plot was represented by the two genotypes of snap bean adopted: Domino (A) and Maxi (B).

In order to evaluate the soil characteristics for the snap bean cultivation, soil samples were collected at 30 cm depth and uniformed in bulk. The soil characteristics were uniform among the field and belonged to the sandy-loamy typology. These kinds of soil characteristics are optimal for snap bean cultivation [40–42], and are shown in Table 1.
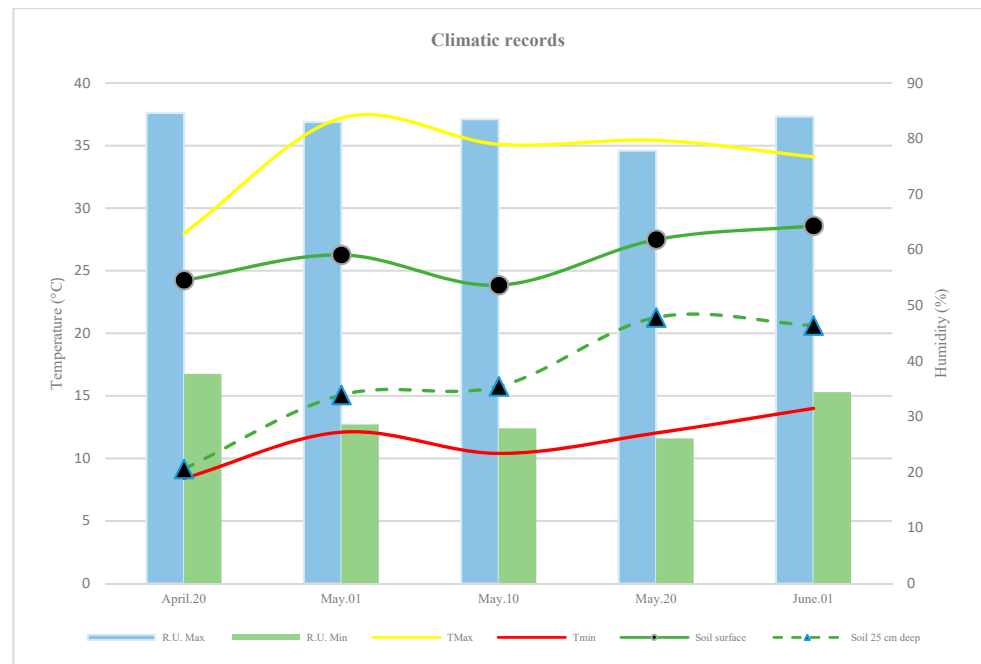
Figure 2. Records of air and soil temperatures (°C) and relative humidity (%) in greenhouse during the growing period.

Table 1. Soil analysis of the experimental field.

Soil Analysis		
Fine ground (<2 mm)	989	g/kg
Sand (0.02–2 mm)	856	g/kg
Silt (0.002–0.02 mm)	53	g/kg
Clay (<0.002 mm)	91	g/kg
Total Limestone	5	g/kg
Total Nitrogen (N)	1	g/kg
Organic carbon	6.7	g/kg
C/N Ratio	6.7	
Assimilable phosphorus (P_2O_5)	144	mg/kg
Exchangeable potassium (K_2O)	706	mg/kg
pH	7.6	
specific conductivity (25 °C)	3.63	dS/m
Cation exchange capacity (CSC)	11.5	meq/100 g
Degree of saturation in bases (GDB)	100	%
Exchangeable Calcium	7.9	meq/100 g
Exchangeable Magnesium	1.7	meq/100 g
Exchangeable sodium	0.4	meq/100 g
Exchangeable potassium (saturated extract)	1.5	meq/100 g
Calcium	68.89	%
Magnesium	14.45	%
Sodium (ESP)	3.63	%
Potassium	13.03	%
K/Mg Ratio	0.9	
Mg/K Ratio	1.11	

3. Results

3.1. Production and Plants Characteristics

The yield in pods (kg m^{-2}) was significantly affected by ET$_c$, MO, and GE.

Considering the effect of ET$_c$, the yield ranged from 2.92 to 4.05 kg m^{-2} for ET$_c$ 60 and ET$_c$ 100, respectively. Regarding the influence of MO, the yield varied from 3.08 to 3.89 kg m^{-2} for NMO and MO, respectively. Different yield was also observed due to GE, as B was observed to have a higher yield (4.04 kg m^{-2}) compared to A (2.92 kg m^{-2}) (Table 2 and Figure 3).

Table 2. Table with all characters analyzed with statistical analysis (ANOVA—Student-Newman-Keuls).

| | ET$_c$ 100 | | | | | | ET$_c$ 60 | | | | | | MEAN | | | | | | |
| | NMO | | | MO | | | NMO | | | MO | | | | | | | | | |
	A	B	$\bar{x}$	A	B	$\bar{x}$	A	B	$\bar{x}$	A	B	$\bar{x}$	ET$_c$ 100	ET$_c$ 60	NMO	MO	A	B	TOT
Yield kg m^{-2}	3.45	3.91	3.68	3.84	5.00	4.42	2.00	2.95	2.47	2.40	4.32	3.36	4.05	2.92	3.08	3.89	2.92	4.04	3.48
Pod N° m^{-2}	820.0	335.9	578.0	803.6	504.6	654.1	651.2	341.4	496.3	747.1	548.9	648.0	616.0	572.1	537.1	651.0	755.5	432.7	594.1
Pod Ø (mm)	5.88	6.88	6.38	6.71	9.00	7.86	6.09	5.78	5.93	6.49	8.76	7.63	7.12	6.78	6.16	7.74	6.29	7.61	6.95
Pod length (cm)	11.7	11.3	11.5	12.0	14.3	13.1	10.8	9.0	9.9	12.4	13.1	12.8	12.3	11.3	10.7	12.9	11.7	11.9	11.8
Pod weight (g)	4.3	12.4	8.4	5.0	10.8	7.9	3.1	7.9	5.5	3.2	7.8	5.5	8.1	5.5	6.9	6.7	3.9	9.8	6.8
N° Branch	4.3	4.0	4.2	5.3	4.7	5.0	6.3	5.7	6.0	6.0	5.0	5.5	4.6	5.8	5.1	5.3	5.5	4.8	5.2
E.F.W. (g)	252.0	170.7	211.3	278.7	282.0	280.3	125.0	146.7	135.8	163.3	210.0	186.7	245.8	161.3	173.6	233.5	204.8	202.3	203.5
I.F.W. (g)	27.3	14.0	20.7	20.7	16.0	18.3	10.0	12.5	21.7	16.7	16.7	19.2	19.5	15.8	16.6	18.8	21.2	14.2	17.7
E.D.M. (%)	16.9	17.3	17.1	16.0	15.9	16.0	31.7	40.1	35.9	34.2	33.0	33.6	16.5	34.7	26.5	24.8	24.7	26.6	25.6
I.D.M. (%)	19.6	24.1	21.8	53.3	46.8	50.0	51.4	52.3	51.9	60.3	70.5	65.4	35.9	58.6	36.9	57.7	46.2	48.4	47.3
N° nodules	85.0	40.7	62.8	80.0	26.3	53.2	114.7	56.0	85.3	70.3	17.7	44.0	58.0	64.7	74.1	48.6	87.5	35.2	61.3
SPAD	43.5	44.4	44.0	45.2	45.5	45.3	43.6	45.2	44.4	46.3	48.2	47.3	44.6	45.9	44.2	46.3	44.6	45.8	45.2

Analysis of variance—Student-Newman-Keuls

	ET$_c$	MO	GE	ETc × MO	ETc × GE	MO × GE	ETc × MO × GE
Yield kg m^{-2}	***	***	***	n.s.	n.s.	n.s.	n.s.
Pod N° m^2	n.s.	*	***	n.s.	n.s.	n.s.	n.s.
Pod Ø (mm)	n.s.	**	*	n.s.	n.s.	*	n.s.
Pod length (cm)	n.s.	**	n.s.	n.s.	n.s.	n.s.	n.s.
Pod weight (g)	***	n.s.	**	n.s.	**	n.s.	n.s.
N° Branch	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
E.F.W. (g)	**	*	n.s.	n.s.	n.s.	n.s.	n.s.
I.F.W. (g)	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.
E.D.M. (%)	***	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
I.D.M. (%)	***	***	n.s.	*	n.s.	n.s.	n.s.
Nod N°	n.s.	**	***	n.s.	n.s.	n.s.	n.s.
SPAD	*	***	*	n.s.	n.s.	n.s.	n.s.

n.s.: not significant; *: *p* value = 0.05%; **: *p* value = 0.01%; ***: *p* value = 0.001%.

Furthermore, the number of pods per m^{-2} was significantly affected by MO and GE. Concerning the effect of MO, the values ranged from 537.1 to 651.0 for NMO and MO, respectively, whereas GE ranged from 432.7 to 755.5 for B and A, respectively (Table 2). The pod diameter was affected by the interaction of MO × GE. Among A, the values varied in average from 5.99 to 6.60 mm for NMO and MO, respectively, and among B from 6.33 to 8.88 for NMO and MO, respectively (Table 2). The pod length was significantly affected by MO; longer pods were observed for MO (12.9 cm) than NMO (10.7 cm) (Table 2). The pod weight was statistically influenced by the interaction of ET$_c$ × GE. Among A, the values fluctuated from 3.2 to 4.6 g for ETc 60 and Etc 100, respectively, and values for B ranged from 7.9 to 11.6 g for ETc 60 and ETc 100, respectively (Table 2).

Significant variations were noted in the snap bean development between the different treatments among the cultivars. Plant epigeous fresh weight (E.F.W.) was significantly affected by ET$_c$ and MO, ranging from 161.3 g to 245.8 g for ETc 60 and ETc 100, respectively, and from 173.6 to 233.5 g for NMO and MO, respectively (Table 2). Otherwise, plants' hypogeous fresh weight (I.F.W.) was significantly affected by GE, with values ranging from 14.2 to 21.2 g for B and A, respectively. The plant epigeous dry matter (E.D.M. %) was significantly influenced by ETc, ranging from 16.5 to 34.7% for ETc 100 and Etc 60, respectively, whereas the ipogeous dry matter (I.D.W.) significantly differed according to the interaction between ET$_c$ × MO. Among ET$_c$ 100, the values ranged from 21.84 to 50.0% for ET$_c$ 100 NMO and MO, respectively, whereas among ET$_c$ 60, values ranged from 51.9

to 65.4% for NMO and MO, respectively (Table 1). The number of first-order branches was significantly influenced only by ETc, with values ranging from 4.6 to 5.8 for ETc 100 and ETc 60, respectively. Regarding the nodulation expressed by the number of nodules, it was significantly affected by MO and GE. Concerning the effect of MO, the number of nodules varied from 48.6 to 74.1 for MO and NMO, respectively, and regarding GE, the values fluctuated from 35.2 to 87.5 for B and A, respectively (Table 2 and Figure 4).
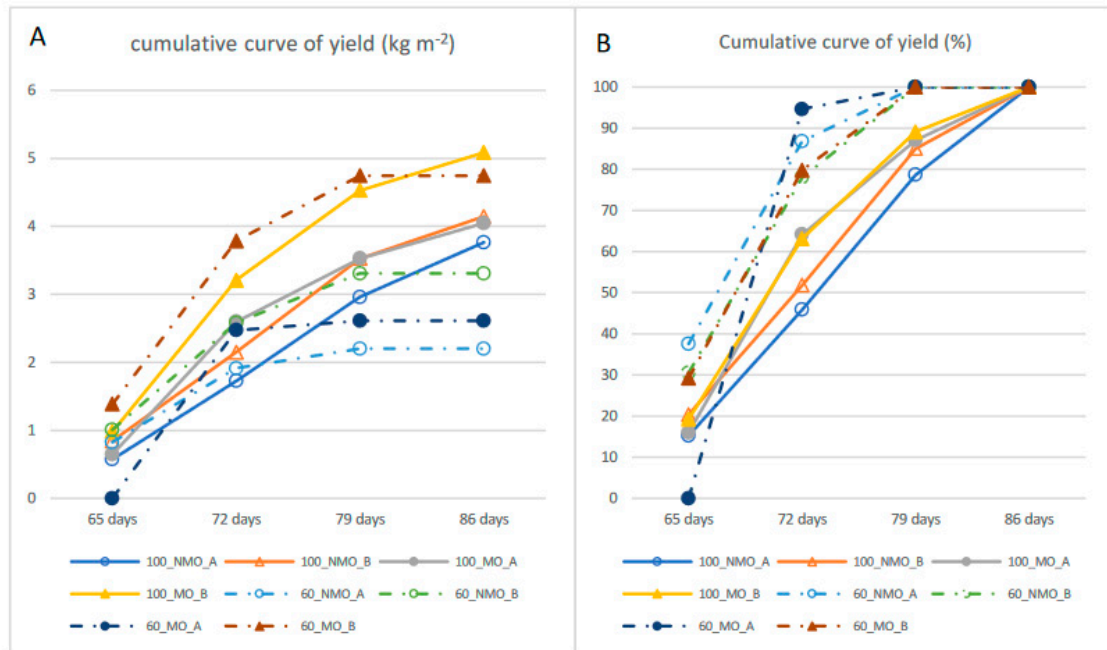

Figure 3. Yield cumulative curves. (**A**) yield of all harvest expressed in kg m^{-2}; (**B**) percentage of yield collected per harvest.
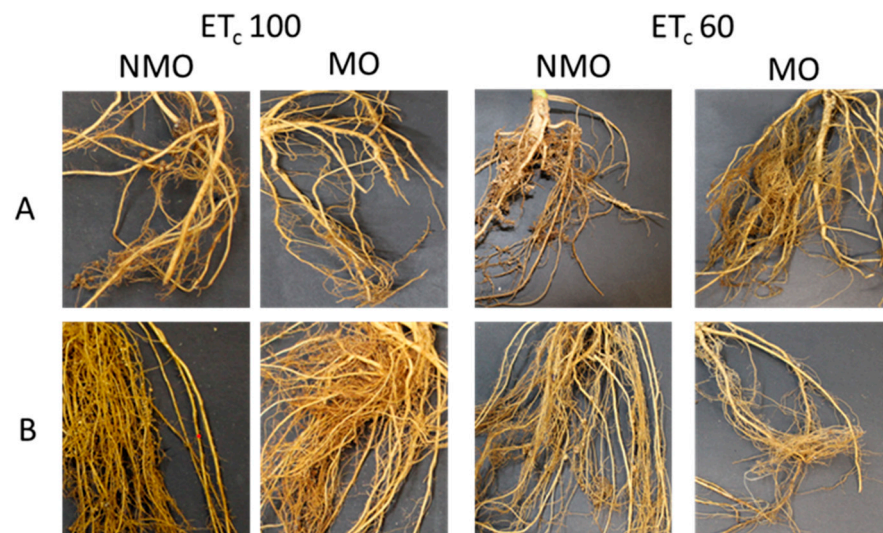

Figure 4. Details of roots and nodules among the thesis. Differences in nodulation can be observed between the two different irrigation regimes (ETc 100 and ETc 60), by application of PGPMs (MO or NMO) and between the two genotypes of snap bean adopted: Domino (**A**) and Maxi (**B**).

The SPAD was significantly influenced by ET$_c$, MO, and GE. Regarding ETc, the values ranged from 44.6 to 45.9 for ETc 60 and ETc 100, respectively. Concerning the MO application, the values varied from 44.2 to 46.3 for NMO and MO, respectively. Regarding GE values, the range fluctuated from 44.6 to 45.8 for A and B, respectively (Table 2).

3.2. Correlations

The Pearson's correlations determined among the experimental factors highlighted some parameters for better understanding and distinguishing the effect of the microbial treatment in deficit regime conditions between the two genotypes studied (Table 3). The yield was positively correlated with pod diameter, pod length, and the pod weight, which indicates how the pod's characteristics influenced the yield. It is interesting that the yield was also positively correlated to the E.F.W. and negatively correlated to the N° of branches and the N° of nodules. Regarding the pod number, it was positively correlated with the I.F.W and the N° of nodules, and negatively correlated with the pod weight. The pod diameter was positively correlated with the pod length and the pod weight, and was otherwise negatively correlated with the N° of nodules. The pod length was positively correlated with E.F.W. instead, and the pod weight was negatively correlated with the N° of branches and the N° of nodules. The N° of branches was positively correlated with E.D.M., I.D.M., and the N° of nodules. Concerning E.F.W., it was positively correlated with I.F.W. and negatively correlated with E.D.M; otherwise, E.D.M. was positively correlated with I.D.M.

Table 3. Pearson's correlations of the characteristics analyzed.

	Yield kg m^{-2}	Pod N° m^2	Pod Ø (mm)	Pod Length (cm)	Pod Weight (g)	N° Branch	E.F.W. (g)	I.F.W. (g)	E.D.M. (%)	I.D.M. (%)	N° Nodules	SPAD
Yield kg m^{-2}	1											
Pod N° m^2	−0.092	1										
Pod Ø (mm)	0.582 **	−0.177	1									
Pod length (cm)	0.506 *	0.257	0.730 **	1								
Pod weight (g)	0.670 **	−0.745 **	0.475 *	0.169	1							
N° Branch	−0.520 **	0.229	−0.163	−0.150	−0.523 **	1						
E.F.W. (g)	0.413 *	0.221	0.355	0.415 *	0.102	−0.035	1					
I.F.W. (g)	−0.050	0.608 **	−0.137	0.317	−0.391	0.182	0.502 *	1				
E.D.M. (%)	−0.472 *	−0.189	−0.142	−0.260	−0.269	0.423 *	−0.598 **	−0.353	1			
I.D.M. (%)	−0.050	0.076	0.359	0.168	−0.233	0.432 *	−0.116	−0.318	0.589 **	1		
N° nodules	−0.622 **	0.518 **	−0.604 **	−0.261	−0.691 **	0.464 *	−0.161	0.398	0.045	−0.226	1	
SPAD	0.444 *	−0.068	0.432 *	0.230	0.207	−0.039	−0.172	−0.301	0.299	0.583 **	−0.446 *	1

*: Correlation significative at 0.05; **: correlation significative at 0.01.

Interesting correlations were also found regarding the SPAD, which was positively correlated with yield, pod diameter, and I.D.M, whereas it was negatively correlated with the number of nodules.

3.3. Principal Component Analysis (PCA)

From the analysis of the data by principal component analysis, a total of 12 principal components (PC) were observed, and among them, the first two were responsible for 70.39% of the total variance registered. The first two PC were used to describe the distribution in a two-dimensional space limited by the principal detected components (Figure 5). The PCA analysis showed that the PC1 is positively correlated with yield, pod Ø, pod length, pod weight, and E.F.W., and was negatively correlated with N° of branches, E.D.M., and nodule N°, representing 44.70% of the total variance (Table 4, Figure 5). Concerning the PC2, it was positively correlated to Pod N° and I.F.W., and it represented 29.09% of the total variance (Table 4). The distribution of the studied parameters can be subdivided into two main blocks, one represented (in the space at the bottom) by genotype B while genotype A is distributed in the space at the top (Figure 5). The PCA clearly shows different responses to MO under the different ETcs; the distance between the MO and NMO sample is higher in B than A in both ETc 60 and 100. Interestingly, there is poor distance between 60_MO_B and 100_MO_B.
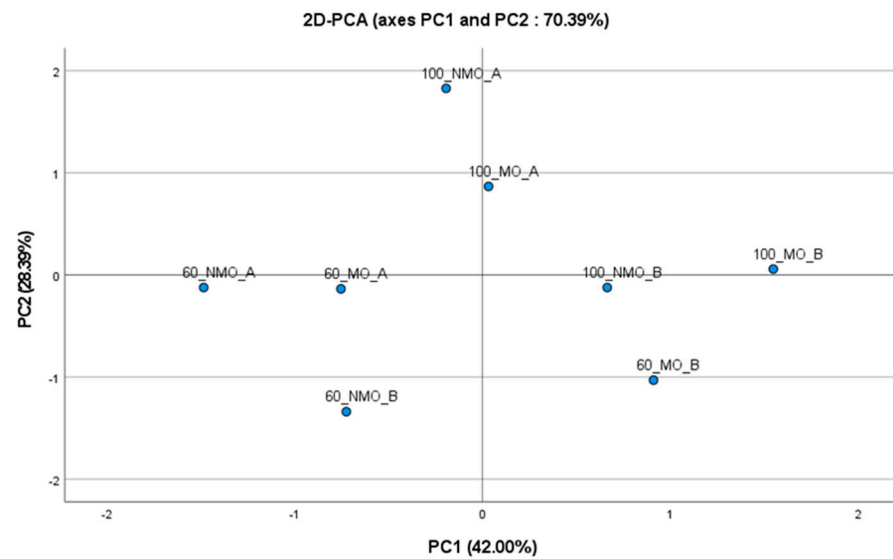
Figure 5. Two-dimensional principal component analysis (2D-PCA) that showed the characteristics analyzed.

Table 4. PCs matrix related to the characteristics analyzed.

	Component Scores	
	PC1	**PC2**
Yield kg m^{-2}	0.976	0.068
Pod N$^{\circ}$ m^{-2}	−0.299	0.722
Pod Ø (mm)	0.849	−0.246
Pod length (cm)	0.705	0.229
Pod weight (g)	0.762	−0.335
N$^{\circ}$ Branch	−0.745	−0.353
E.F.W. (g)	0.668	0.576
I.F.W. (g)	−0.032	0.844
E.D.M. (%)	−0.547	−0.750
I.D.M. (%)	−0.076	−0.624
N$^{\circ}$ nodules	−0.850	0.458
SPAD	0.417	−0.570
% of Variance	42.00	28.39

Extraction Method: Principal Component Analysis with two components extracted.

Extraction Method: Principal Component Analysis with two components extracted.

4. Discussion

Abiotic stresses are hostile to plant growth and development. In particular, water deficiency is a severe constraint that affects growth and limits agricultural productivity on a global scale, a reason why several authors focused their attention on the optimization of strategies to ameliorate water deficit [43–46].

Plant-growth-promoting microbe (PGPM) treatment may be advantageous in the contest of water deficit regimes; it is demonstrated, in fact, that both PGPR and PGPF guarantees the survival of the plant during a drought through a variety of processes including osmotic adjustments, improved phytohormone synthesis, and antioxidant activity, among others, and these mechanisms also promote the plant's development while improving crop yield [27,47–50].

Farmers and companies now recognize the usefulness of PGPM in promoting plant growth and yield. In fact, several PGPM-based formulates are commercialized and widespread [27,51]. On the basis of the recent literature [52–54], the hypothesis to verify

is that this type of formulation could be useful in overcoming drought stress by improving plants' growth and final yield. The results obtained from the present experiment are compatible with what has already been reported in literature [54–57], and confirm the usefulness of the formulation in improving yields both in optimal water supply and in case of drought stress. In literature, it is reported that the factors influencing the efficacy of microbial treatments are complex and genotype dependent [27]. Consequently, a different effectiveness of the consortium was found between the two cultivars used in the experiment (Domino and Maxy). Despite this, for both genotypes, an increase in terms of yield, pod diameter, and fresh weight were observed for the plants where the PGPM-based product was applied in both irrigation regimes. Between the two genotypes, Maxy benefited more from the treatment than Domino (Table 2), confirming the hypothesis that applying soil microorganisms to cropping schemes is genotype dependent. In particular, comparing the data obtained in ET_c 100 in the untreated control theses with the ET_c 60 theses treated with MO, it's clear that the values are comparable (Figure 5), thus observing compensation of the stress by the treatment. This result is of high importance when concerning water shortages in many environments and/or the need to save water for a better approach to the sustainability of farming practices.

Another interesting point of discussion concerns the nodulation. The nodules of legumes are of particular interest for the scientific community, as the site of nitrogen fixation by means of symbiotic nitrogen-fixing bacteria. Root nodules of legumes are the product of a highly specific interaction between the bacteria involved (rhizobia) and plants' roots or stems [58]. The inverse correlations observed between yield, pod size, and number of nodules are interesting. The number of nodules observed on the roots was lower in plants with higher yield and pod diameter, both in relation to ETc, GE, and to the Maxy Soil application. However, as reported in literature and as confirmed by the cumulative yield curve (Figure 2), this can be explained by assuming a greater metabolic activity of the plant, which close to the end of the cropping cycle, has reinvested its resources by subtracting nutrients from the nodules to reinvest them in the growth of the pods. In fact, the literature reports how the plant can regulate nodulation according to its specific needs [59]. Obviously, in order to better clarify the effect of this type of commercial microbial formulations on nodulation, more specific and in-depth studies are needed, focusing on the interaction between applied PGPMs, symbiotic rhizobia, and the plant response.

5. Conclusions

The PGPM based products mentioned in the present paper has brought an increase in yield both in optimal irrigation conditions and in deficit water conditions. The increase of yield was observed in both genotypes, but between these, the cultivar "Maxy" took the greatest advantage of the treatment, observing an almost complete compensation of the water stress. Furthermore, the inverse correlation between nodulation and yield suggests a reinvestment of the plant's nutritional resources in the last phases of the cropping cycle, which leads to the detriment of the nodules, and benefits the pods' growth.

According to what is reported in literature, the study confirms the effectiveness of PGPM applications in improving the growth and yield of crops both under optimal conditions and under stress, while taking into account the variability found between genotypes.

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