

## IMPACT OF LOW-INPUT MANAGEMENT AND MICROBIAL BIOSTIMULANTS ON YIELDS OF TRADITIONAL PEPPER VARIETIES

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### Abstract

*Traditional varieties are good candidates to be grown under sustainable conditions. In this research we evaluated the effect of reducing fertilization and irrigation dose, and the use of microbial biostimulants (N-fixing + K and P-solubilizing bacteria + mycorrhizal endomycorrhizae fungi) on yield parameters of two traditional pepper varieties (BGV13004, BGV5126) and one commercial variety (Cabañeros). The treatments consisted of two fertilisation levels (100% F and 50% F) combined with two irrigation levels (100% I and 75% I). Furthermore, the effect of microbial biostimulants (B) was studied at the lowest fertilisation rate using the commercial products Bactogreen® and Agromic®. Reduced fertilisation decreased total yield in varieties BGV13004 and Cabañeros, as a consequence of reduced fruit set. On the contrary, in variety BGV5126, the decrease in the concentration of the nutrient solution (100% F vs. 50% F) led to an increase in yield, probably due to a greater sensitivity of this variety to salinity. The effect of B was highly dependent on the variety studied, with both an increase (BGV13004) and a decrease (BGV5126) or no impact (Cabañeros) on total production.*

**Key words:** PGPB, landrace, local varieties, sustainability, *Capsicum*.

### INTRODUCTION

Agriculture has an important role in today's economy, but also in maintaining the environment. In recent years, there has been a growing interest in sustainable agriculture.

Pepper (*Capsicum* spp.) is one of the most economically important horticultural crops in Spain (FAOSTAT, 2021). The genus *Capsicum* consists of numerous species. Thanks to this, there is a great genetic diversity of pepper in colour, size, shape and use of its fruits (Moscone et al., 2007). In Spain, a variety of ecotypes of peppers adapted to the local conditions can be found, where they are still cultivated today. These varieties are called traditional varieties or landraces (Pereira-Dias et al., 2019). Modern agriculture's focus on economically productive varieties has increased genetic erosion leading to a loss of genetic diversity in crops. In this sense, the cultivation of local and traditional varieties can help to

protect agricultural diversity and mitigate the effect of genetic erosion (Fita et al., 2015).

Plant nutrition, especially nitrogen (N), has a direct influence on crop yield. The correct management of the plant nutrition is crucial for the proper development of crops and for the sustainability of the environment (Kanneh et al., 2017). Thus, a deficiency of NPK has been correlated with significant losses in crop production (Abdelaziz et al., 2008). As a consequence of the close correlation between N fertilization and yield, over-application of N is a widespread practice that leads to increased nutrient leaching and environmental pollution (da Silva et al., 2020). To achieve a sustainable agriculture, different breeding approaches should be considered, such as optimizing low-input crop management or using microbial biostimulants (B). Traditional varieties are the result of a selection process by farmers under local agro-climatic conditions of low fertilization and irrigation (Fita et al., 2015). In

this regard, these materials offer a great opportunity for low-input cultivation.

Another aspect, in which traditional and modern varieties seem to differ, is in their interaction with soil microorganisms used as biostimulants. It has been described in cereals, that landraces interact better with mycorrhizae than modern commercial varieties (Londoño et al., 2019). Among these potential microbial biostimulants, the arbuscular mycorrhizal fungi (AMF) and the plant growth promoting bacteria (PGPB) are promising (Glick, 2014; Bona et al., 2015; Ruzzi & Aroca, 2015). The AMF establishes a symbiosis with plant roots that helps to enhance nutrient uptake by the host plant and resist external abiotic factors and pathogens (Emmanuel & Babalola, 2020). The PGPBs promotes plant growth through different mechanism, including nitrogen fixation and solubilization of phosphorus and potassium from the soil (Glick, 2012). The co-inoculation of both organisms seems to combine their benefits to achieve higher productivity. PGPBs have been shown to stimulate the beneficial role of AMF and vice versa (Xie et al., 2018).

The objective of this work was to evaluate the yield of traditional pepper varieties in response to a low-input crop management, and its combination with the use of microbial biostimulants (AMF and PGPB).

## MATERIALS AND METHODS

The experiment was carried out under greenhouse conditions, in the experimental farm 'Torreblanca', located in Torre Pacheco (Murcia), SE Spain. Two varieties of traditional pepper were cultivated (BGV13004 and BGV5216) belonging to the Germplasm Bank of COMAV-UPV in Valencia, and one commercial variety, Cabañeros, as control

(Table 1 and Figure 1). Transplants were established in December 2020 following a randomized block design. Sixty plants were distributed in the six treatments, with two random blocks each, and five plants per block (ten plants per treatment) for each variety. The distance between the rows was 1.0 m and the distance between adjacent plants within a row was 0.4 m.

Chemical fertilization at 100% consisted of 175 kg·ha<sup>-1</sup> of N, 180 kg·ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub>, 276 kg·ha<sup>-1</sup> of K<sub>2</sub>O, 125 kg·ha<sup>-1</sup> of Ca and 30 kg·ha<sup>-1</sup> of Mg. The irrigation at 100% consisted of 96 L/plant (0.24 m<sup>3</sup>/m<sup>2</sup>). Both conditions were chosen following standard production practice for the region. The products used as the microbial biostimulant treatment were Bactogreen® (*Azospirillum brasilense*, *Pseudomonas fluorescens*, *Bacillus megaterium* and *Bacillus circulans*), applied every month from the transplant, and Agromic® (*Rhizophagus intraradices*, *Funneliformis mosseae* and *Funneliformis caledonium*), applied in the seedbed and in the transplant. The treatments consisted of two levels of fertilisation (F), 100% F and 50% F, combined with two levels of irrigation (I), 100% I and 75% I, resulting in four treatments (100F+100I, 100F+75I, 50F+100I, 50F+75I). In addition, the effect of biostimulants (B) was studied at the 50% fertilization level, both 100% and 75% of irrigation, which resulted in four treatments for comparison (50F+100I, 50F+100I+B, 50F+75I, 50F+75I+B).

To determine the yield, the number and mean weight of the fruits, each fruit was collected and weighed individually (from 28<sup>th</sup> January to 30<sup>th</sup> July). The results were statistically analysed using the IBM SPSS Statistics 25 software through analysis of variance (ANOVA) and Duncan's test to determine differences between means.

Table 1. Accession, species, type of variety, origin, type of pepper and shape of fruit of the three varieties used

Accession	Species	Type of variety	Origin	Common name	Shape of the fruit
BGV5126	<i>Capsicum annuum</i> L.	Traditional	Valencia, Spain	Valenciano	Triangular
BGV13004	<i>Capsicum annuum</i> L.	Traditional	Euskadi, Spain	Pimiento de asar	Triangular
Cabañeros	<i>Capsicum annuum</i> L.	Commercial	Ramiro Arnedo S.A.	Lamuyo	Rectangular

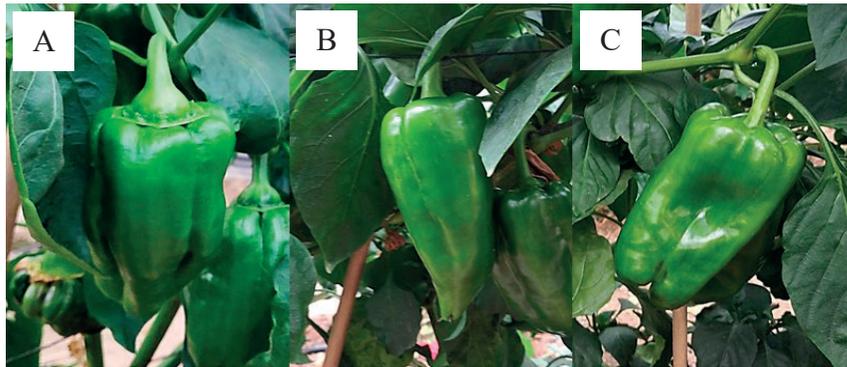


Figure 1. Pictures of accessions BGV5126 (A), BGV13004 (B) and Cabañeros (C)

## RESULTS AND DISCUSSIONS

Considering the yield, the number of fruits and the mean fruit weight, the response of each variety to the treatments presented great variation. A decrease in fertilizer dose from 100% to 50% significantly reduced total yield in the traditional variety BGV13004 ( $p \leq 0.05$ ) and in the commercial variety Cabañeros ( $p < 0.01$ ), regardless of irrigation dose (Table 2). In both varieties, this reduction was attributed to a lower number of fruits ( $p \leq 0.05$ ). While in BGV13004 the mean fruit weight was not significantly affected by the level of fertilization at any of the irrigation doses, in Cabañeros it increased as the fertilizer dose decreased in the reduced irrigation treatment (75% I). Contrary to that observed in these two varieties, in BGV5126 the reduction of the fertilization dose produced an increase in yield ( $p \leq 0.05$ ) regardless of the irrigation dose (Table 2). This higher yield was triggered by an increase in the number of fruits ( $p < 0.01$ ) which, in the case of the 100% irrigation treatment, was associated with a decrease in fruit weight.

Pepper yield is highly sensitive to the amount of fertilizer (Rodríguez et al., 2020). According to Aminifard et al. (2012), vegetative growth characters, which form the basis of flowering and fruiting, are affected by the amounts of nitrogen present in the plant. Similarly to that observed for BGV13004 and Cabañeros, previous studies describe that an increase in NPK dose leads to an increase in pepper yield (Tumbare et al., 2004; Law-Ogbomo & Egharevba, 2009; Coolong et al., 2019).

However, a high concentration of nutrients can cause an increase in salinity in the root zone, which could eventually lead to reduced crop

growth and productivity (Padilla et al., 2017). In previous studies, cultivars with different salt tolerances have been reported depending on their ability to activate a series of physiological responses, such as the control of water and ion homeostasis, the regulation of salt ion uptake/accumulation or the detoxification of reactive oxygen species (ROS) (Giorio et al., 2020). Although some research suggests that these mechanisms are less efficient in commercial cultivars compared to landraces (Fita et al., 2015), the lower yield observed in the treatment with higher nutrient concentration could be attributed to a higher sensitivity of the BGV5126 variety to salinity.

Variability in response to irrigation dose reduction was also found among the three varieties. While in the traditional varieties BGV13004 and BGV5126, the total yield was not affected by a reduction in irrigation (75% I), Cabañeros showed a decrease ( $p \leq 0.05$ ) in total yield due to this reduction (Table 2). A significant interaction between fertilization and irrigation dose (F $\times$ I) on mean fruit weight was observed for Cabañeros ( $p < 0.01$ ) and BGV5126 ( $p \leq 0.05$ ). While in 100% F conditions the mean fruit weight decreased with decreasing irrigation dose, in 50% F the weight, was not affected in BGV5126 or increased in the case of Cabañeros. In our study, an increase in salinity in the root zone is to be expected both in the treatments with a higher nutrient dose (100% F) and in the treatments with a reduced irrigation dose where the net nutrient supply is maintained (75% I). Therefore, the decrease in production observed in Cabañeros due to the reduction in irrigation dose and the decrease in fruit weight obtained in Cabañeros and BGV5126 by reducing irrigation in the treatment with the highest

fertilisation dose (100F+75I), can be attributed to an increase in salinity that would affect yield parameters (Chartzoulakis & Klapaki, 2000).

Regarding the use of microbial biostimulants, differences in their effect on yield were observed according to pepper variety. The yield of BGV13004 increased significantly ( $p \leq 0.05$ ) after the addition of the biostimulants, due to a higher number of fruits ( $p < 0.01$ ) (Table 3). According to these results, the microbial biostimulants seem to compensate for the reduction in the yield caused by the decrease in fertilizer dose. On the contrary, in BGV5126, the addition of biostimulants produced a significant decrease in total yield ( $p \leq 0.05$ ) (Table 3). This decrease in total yield seems to be due to a decrease in the number of fruits, although no significant differences were obtained for this parameter. Finally, in Cabañeros, the effect of B on total pepper yield was not significant but a reduction in mean fruit weight ( $p < 0.01$ ) and an increase in fruit number ( $p < 0.01$ ) were observed after B addition (Table 3). Therefore, B seems to cause more fruits but smaller, thus maintaining yield in the commercial variety.

Microbial biostimulants affect pepper yield (Ruzzi & Aroca, 2015; Pereira et al., 2016; Bona et al., 2017) and differences in response to microbial biostimulants between pepper varieties have been found (Sensoy et al., 2007). According to Glick (2012), the main direct effect of PGPB is to provide plants with resources that are not available in the soil such as nitrogen, phosphorus, and potassium. As well, AMF form symbiotic associations with the plant and increase the ability of roots to absorb nutrients (Emmanuel & Babalola, 2020).

The increased production in BGV13004 after B addition found in this study makes sense with what is found in the literature (Sensoy et al., 2007; Turkmen et al., 2008; Bona et al., 2017). However, the beneficial effect of the microbial biostimulants on production was not observed in Cabañeros (no effect) and in BGV5126 (detrimental effect). The observed effect of the treatments on the yield depends on two opposing factors: the nutrient requirements and

the sensitivity to salinity. Both the fertilization dose and the irrigation dose, and the addition of B, modify these two aspects and their effect depends largely on the variety studied. Therefore, the increase in nutrient concentration can cause both an increase and a decrease in production due to increased nutrient availability and increased salinity, respectively. In the same way, the reduction of the irrigation dose, maintaining the level of nutrients, can affect the varieties to a different extent depending on their tolerance to salinity. Moreover, microbial biostimulants increase the nutrients available in the soil, in contact with plant roots (Glick, 2012). Thus, B application can also affect salinity in the root zone and consequently affect varieties differently. The higher nutrient concentration could lead to an increase in salinity in the root zone and, consequently, a decrease in yield in more sensitive varieties (Aminifard et al., 2012), as observed in BGV5126.

## CONCLUSIONS

In conclusion, the studied varieties presented variability with regard to the response to the dose of fertilizer, irrigation and the application of microbial biostimulants. Contrary to that observed in the commercial variety Cabañeros, the traditional varieties BGV13004 and BGV5126 tolerated the limitation of the irrigation dose (75% of the control), so it could contribute to the sustainability of the crop through a management that implies water saving.

Regarding fertilization management, the highest production in variety BGV5126 was obtained in treatments with lower fertilizer concentration, probably due to a low tolerance to salinity. Therefore, this variety is of great interest for low-input. Finally, the response of the variety BGV13004 to the application of PGPB and AMF suggests that the nutrient demand of this variety can be supplemented with microbial biostimulants. All these results show the suitability of the traditional varieties studied to be cultivated under sustainable management guidelines.

Table 2. Effect of fertilization and irrigation on total yield, mean fruit weight and number of fruits in the commercial variety Cabañeros and the traditional varieties BGV13004 and BGV5126

Accession	Treatment		Yield (kg·plant <sup>-1</sup> )	Fruit weight (g)	Number	
Cabañeros	Fertilization (F)	100	3.46 ± 0.34	204 ± 10	18 ± 1	
		50	2.42 ± 0.24	204 ± 8	13 ± 1	
		<i>p-value</i>	*	ns	**	
	Irrigation (I)	100	3.54 ± 0.33	216 ± 6	17 ± 1	
		75	2.42 ± 0.26	193 ± 9	14 ± 1	
		<i>p-value</i>	*	*	ns	
	Interaction (F×I) Fertilization (F)	100	Irrigation (I) 100	4.17 ± 0.52	229 ± 4 <sup>b</sup>	19 ± 2
				75	2.75 ± 0.39	183 ± 15 <sup>a</sup>
		50	100	2.90 ± 0.29	203 ± 10 <sup>ab</sup>	15 ± 1
				75	2.00 ± 0.33	205 ± 12 <sup>b</sup>
		<i>p-value</i>		ns	**	ns
BGV13004	Fertilization (F)	100	2.00 ± 0.15	135 ± 6	18 ± 2	
		50	1.25 ± 0.16	119 ± 6	10 ± 1	
		<i>p-value</i>	*	ns	*	
	Irrigation (I)	100	1.641 ± 199	125 ± 7	15 ± 2	
		75	1.567 ± 163	129 ± 6	13 ± 2	
		<i>p-value</i>	ns	ns	ns	
	Interaction (F×I) Fertilization (F)	100	Irrigation (I) 100	2.03 ± 0.30	138 ± 9	19 ± 3
				75	1.97 ± 0.15	135 ± 10
		50	100	1.31 ± 0.26	115 ± 9	11 ± 2
				75	1.17 ± 0.19	123 ± 6
		<i>p-value</i>		ns	ns	Ns
BGV5126	Fertilization (F)	100	1.79 ± 0.19	125 ± 9	17 ± 2	
		50	2.78 ± 0.27	114 ± 5	24 ± 2	
		<i>p-value</i>	**	ns	*	
	Irrigation (I)	100	2.45 ± 0.29	132 ± 7	20 ± 2	
		75	2.12 ± 0.22	106 ± 5	21 ± 2	
		<i>p-value</i>	ns	**	ns	
	Interaction (F×I) Fertilization (F)	100	Irrigation (I) 100	1.78 ± 0.33	145 ± 12 <sup>b</sup>	15 ± 2
				75	1.76 ± 0.25	99 ± 7 <sup>a</sup>
		50	100	3.25 ± 0.40	116 ± 5 <sup>a</sup>	27 ± 4
				75	2.40 ± 0.34	112 ± 7 <sup>a</sup>
		<i>p-value</i>		ns	*	ns

\*and \*\*represent  $p \leq 0.05$  and  $p < 0.01$ , respectively ns: not significant. Values are the means of ten replicates. Values followed by the same letter within a column are not significantly different at the 0.05 level of probability according to the Duncan test. Data are means ± SE.

Table 3. Effect of irrigation and biostimulants on total yield, mean fruit weight and number of fruits in the commercial variety Cabañeros and the traditional varieties BGV13004 and BGV5126

Accession	Treatment		Yield (kg·plant <sup>-1</sup> ),	Fruit weight (g)	Number	
Cabañeros	Irrigation (I)	100	3.15 ± 0.29	194 ± 7	17 ± 1	
		75	2.10 ± 0.23	193 ± 10	12 ± 1	
		<i>p-value</i>	*	ns	*	
	Biostimulants (B)	0	2.42 ± 0.24	204 ± 8	13 ± 1	
		1	2.94 ± 0.34	182 ± 9	17 ± 2	
		<i>p-value</i>	ns	*	*	
	Interaction (IxB) <i>Irrigation (I)</i>	100	<i>Biostimulants (B)</i>			
			0	3.09 ± 0.26	205 ± 12	16 ± 1
			1	3.58 ± 0.46	194 ± 7	19 ± 2
		75	0	2.00 ± 0.33	205 ± 12	11 ± 1
			1	2.26 ± 0.33	176 ± 17	15 ± 3
			<i>p-value</i>	ns	ns	ns
BGV13004	Irrigation (I)	100	1.64 ± 0.20	114 ± 5	15 ± 2	
		75	1.47 ± 0.16	124 ± 6	14 ± 2	
		<i>p-value</i>	ns	ns	ns	
	Biostimulants (B)	0	1.25 ± 0.16	119 ± 6	10 ± 1	
		1	1.84 ± 0.17	119 ± 6	18 ± 2	
		<i>p-value</i>	*	ns	**	
	Interaction (IxB) <i>Irrigation (I)</i>	100	<i>Biostimulants (B)</i>			
			0	1.27 ± 0.29	115 ± 10	11 ± 2
			1	2.09 ± 0.29	118 ± 4	19 ± 3
		75	0	1.17 ± 0.19	123 ± 6	10 ± 2
			1	1.71 ± 0.21	125 ± 9	18 ± 3
			<i>p-value</i>	ns	ns	ns
BGV5126	Irrigation (I)	100	2.65 ± 0.30	118 ± 6	23 ± 3	
		75	2.15 ± 0.26	109 ± 7	21 ± 2	
		<i>p-value</i>	ns	ns	ns	
	Biostimulants (B)	0	2.78 ± 0.27	114 ± 5	24 ± 2	
		1	1.90 ± 0.24	114 ± 9	18 ± 2	
		<i>p-value</i>	*	ns	ns	
	Interaction (IxB) <i>Irrigation (I)</i>	100	<i>Biostimulants (B)</i>			
			0	3.22 ± 0.46	117 ± 6	27 ± 4
			1	2.05 ± 0.36	121 ± 10	19 ± 3
		75	0	2.40 ± 0.36	112 ± 7	23 ± 3
			1	1.65 ± 0.27	104 ± 15	17 ± 3
			<i>p-value</i>	ns	ns	nNs

\* and \*\*represent  $p \leq 0.05$  and  $p < 0.01$ , respectively ns: not significant. Data are means ± SE

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