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The Role of Microbial Fertilizers on the Quantitative Traits of Sweet Corn at Different Densities

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Abstract

The effect of biofertilizers and plant density was investigated on yield and yield components of sweet corn cv. 'Sari 2802' in a split-plot field experiment based on a randomized complete block design with three replications in Lahijan, Guilan province in 2021. The main plot was assigned to biofertilizers at four levels of control (F1), using N-Fertile 1 (F2), phosphate Fertile 2 (F3), and N-Fertile 1 + Phosphate Fertile 2 (F4), and the sub-plot was assigned to plant density at three levels of 55,000 (D1), 65,000 (D2), and 75,000 plants/ha (D3). The results showed that the application of biofertilizers influenced plant height, grain number per ear, ear length, total dry matter, grain yield, and 1000-grain weight positively. The highest 1000-grain weight (383 g), the highest total dry matter (26240 kg/ha), and the highest grain yield (9146 kg/ha) were obtained from F4D2 (N-1 + P-2 and the density of 65,000 plants/ha). The results showed that the density of 65,000 plants/ha and applying N-1 and P-2 biofertilizers was the best treatment.

Key words: Biofertilizers, corn, plant density, yield, yield components

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Introduction

In recent years, the application of biofertilizers to improve soil fertility has emerged as a good alternative to chemical fertilizers and has been considered by producers as an essential method of plant nourishment to achieve sustainable agriculture goals (Asadi Rahmani & Fallah, 2001). The most important factors in maximizing seed yield and enhancing food value are nitrogen among inputs and plant density among agronomic factors (Cox & Cherney, 2001). In addition to increasing the bioavailability of minerals through biologically fixing nitrogen, solubilizing phosphorous and potassium, and suppressing pathogens, growth-promoting bacteria also influence crop yields and contribute to rooting and root expansion by synthesizing plant growth-regulating hormones (Sturz & Chrste, 2003). The application of fertilizer-2 phosphate as a phosphorus biofertilizer has been emphasized to reduce the application of chemical fertilizers in crop production (Madani et al., 2005). The bacteria of these biofertilizers can release insoluble soil phosphorus as organic phosphorus acids and light phosphorus and increase their mobility in the soil by changing acidity in the surrounding environment and helping enzymatic processes. Nitroxin is a biofertilizer that contains nitrogen-fixing bacteria, so it can be used to not only avoid the application of nitrogen fertilizers but also improve crop production owing to its various effects (Asadi Koupal & Isazadeh Lazarjan, 2009). The application of the N-1 biofertilizers and P-2 phosphorus fertilizers, alone or concurrently, in deficit irrigation conditions improved the nutritional and growth conditions of corn plants and partially moderated the inhibitory effects of the water deficit conditions on corn growth (Shirzadi & Shams, 2018). The integrated application of phosphate biofertilizers and chemical fertilizers was also found to be effective in alleviating the effects of water deficit stress (Ghasemi et al., 2011). The integrated application of biological and chemical fertilizers could also produce the maximum yield in addition to reducing the application of chemical fertilizers.

It was also reported that the non-chemical sources of plant nutrients could be a reliable alternative to chemical fertilizers in the ecological production of crops in Iran's agricultural system (Ebrahimpour et al., 2012). De Matos Nascimento et al. (2020) revealed that the maximum weighted average of corn and soil potassium content was observed in the treatment of maximum biofertilizer application. Corn seed inoculation with *Mycorrhiza* and *Azotobacter*, alone or combined, and their inoculation with mycorrhiza along with 100 kg/ha triple superphosphate resulted in the highest grain yield, plant height, and nitrogen content (Amirabadi et al., 2010). The proper plant population of corn plays a key role in plants' capability of optimally using the inputs. An optimal yield can be accomplished by selecting an optimal plant density (Farnham, 2001). It was reported that competition for moisture, radiation, and nutrients increases at higher densities, which reduces the yield (Aerts, 1999). Bahrani and Seidi (2005) observed that as the interplant spacing was increased, the plants synthesized more photosynthates due to the decrease in interplant competition, so the grain yield increased. On the contrary, the 1000-grain weight decreased at higher densities due to the decrease in the penetration of solar radiation and the availability of lower photosynthates during the grain-filling period.

The present research aimed to study the effect of biofertilizers as supplements or alternatives to chemical fertilizers on some quantitative traits of sweet corn and finally determine the most suitable plant density in Guilan province.

Materials and Methods

The research was conducted on a farm at the Flowers and Plants Research Station of Lahijan in Guilan province in 2020. The research farm was located at latitude 37.17° N and longitude 49.87° N and had an elevation of 20 meters from sea level. Before planting, the physical and chemical properties of the soil were determined through a soil analysis test on a mixture of 10-12 samples randomly taken from the 30-cm depth of the soil. The results are presented in Table 1.

The research was conducted as a split-plot experiment based on a randomized complete block

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Table 1. The physical and chemical properties of the farm soil

Depth	Electrical conductivity (EC × 10^3 Mmho/cm)	Total saturated reaction pH	Organic C (%)	Total N (%)	Neutralizing material	Absorbable P	Absorbable K	Sand (%)	Silt (%)	Clay (%)	Texture
0-30	0.129	5.5	1.6	0.15	46.0	34.7	266	51.4	21.3	27.3	Loam clay sandy

design in three replications. The main plot was assigned to biofertilizers (F1 = control; F2 = nitrogen-1 biofertilizers; F3 = phosphate-2 biofertilizers, and F4 = Nitroxin1 + phosphate2 biofertilizers), and the sub-plot was assigned to plant density at three levels (D1 = 55000, D2 = 65000, and D3 = 75000 plants/ha) randomly applied as per the experimental map. To apply the biological treatments, the target phosphorus-dissolving microorganisms were first procured from the biological laboratory of Green Biotech Company. In the treatments in which the seeds were to be inoculated with these microorganisms by impregnation, they were placed in a polyethylene sac. Then, 30 mL of sugar solution 2% was added. Then, the sac was shaken with a shaker for 30 seconds for all the seeds to get sticky uniformly. Then, a certain amount of inoculum was added to the sticky seeds to cover their whole surface. Forty-five seconds after shaking, when we were sure that the inoculum uniformly stuck to the seeds, they were spread on a clean aluminum sheet in shadow to get dried. Then, they were immediately sown.

The conventional rate of Nitroxin is 2 L/ha for corn. The Nitrogen biofertilizer-1 was applied as a foliar spray in one step. Based on the area of each plot, nearly 1 mL of Nitroxin was sprayed. Before planting, 50 kg/ha triple superphosphate and potassium sulfate were spread on the farm prior to plowing and mixed with the soil by disking. The replications and sowing rows were spaced by 3 m and 50 cm, respectively. The plots were 3 m long, and four rows were sown in each plot. The inter-row spacing was 75 cm, and the on-row inter-plant spacing was 24 cm for a density of 55,000 plants/ha, 21 cm for a density of 65,000 plants/ha, and 18 cm for a density of 75,000 plants/ha. The seeds of the cultivar 'Single Cross' were planted in April, during which they were sown in the plots by hand. After that, the plots were treated with light irrigation spaced for 3 days for the uniform emergence of the seeds. Subsequent irrigations were adjusted as per the plant requirement and based on the 50% depletion of the available moisture of the soil.

To measure the quantitative traits at the plant's physiological maturity step, 1 m2 was harvested from the middle of the plots. The grain yield and yield components, including plant height, grain yield, and grain number per ear, as well as 1000-grain weight, biomass, and harvest index, were measured on four plants from the middle rows. To measure yield components including plant height and grain number per ear, five plants were randomly selected from each experimental unit at the physiological maturity step, and the target traits were measured on them. The recordings were averaged as the observation for the experimental unit. To find out the 1000-grain weight, 200 grains were separated from the ears randomly and weighed. The result was multiplied by 5.

To determine biological yield, four middle rows were harvested from each experimental plot and stored in cotton bags. They were transferred to a laboratory and weighed after oven drying at 72°C for 24 hours. The reading was recorded as biological yield or total dry matter per unit area for each experimental plot. Then, the grains were separated and weighed to find the grain (economic) yield.

Data were analyzed in MS Excel and SAS statistical packages. The means were compared by Duncan's multiple range test at the $P < 0.05$ level.

Results and Discussion

The analysis of variance (ANOVA) showed that the effect of biofertilizers and plant density was significant on most recorded traits at the $P < 0.01$ or $P < 0.05$ level.

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Table 2. Analysis of variance of the split-plot experiment based on a randomized complete block design for the studied quantitative traits

S.O.V	df	Means of squares					
		Plant height (cm)	Number of kernels per ear	1000-grain weight (g)	Ear length (cm)	Total dry matter (g/m^2)	Grain yield (g/m^2)
Replication	2	3.111	257.444	403.361	1.361	16896115.528	69064.583
F	3	520.917**	508.333*	129.370**	5.370*	12028504.630**	3025796.963**
E1	6	1.333	99.889	42.620	1.278	140560.491	13703.991
D	2	102.194**	99.694**	349.694**	5.444*	8082724.694**	201222.583**
F×D	6	2.639**	46.583**	62.843**	4.481*	1364097.435*	36574.102**
E2	16	0.319	13.986	12.556	1.556	671400.250	9110.524
C.V		0.31%	3.02%	0.96%	6.82%	4.01%	1.15%

ns: non-significant; **: significant at $P < 0.01$; *: significant at $P < 0.05$; F: biofertilizer; D: plant density

Plant height

Based on the results of ANOVA, the simple effect of different levels of biofertilizers and plant density was significant ($P < 0.01$) on plant height as was the interactive effect of plant density and biofertilizer (Table 2). The tallest plants were 187.6 cm obtained from treatment F2 (the application of nitrogen-1 biofertilizer), significantly differing from treatment F1 (the control), whose plant height was 174.2 cm (Figure 1). Seemingly, biofertilizers provided suitable conditions for plant growth. They contributed to better vegetative growth by supplying nutrients and influencing photosynthesis and cell division. In addition, N-1 helped the development of vegetative organs and increased the height of the corn plants.

Shirzadi and Shams (2018) reported that the application of (N1) and (P2) influenced corn plant height significantly. This effect was 10.76 and 18.84% stronger in the combined application of the fertilizers than in the application of N and P fertilizers alone, respectively. The increase in plant density partially increases plant height, but a further increase in the density will reduce the height. In this regard, the elongation of internode spacing can be associated with competition and the lack of radiation interception by the lower parts of the stem (Afsharmanesh, 2006). The highest plant height was 190 cm related to the treatment of F4D3 (the application of nitrogen-1 biofertilizer + phosphate-2 biofertilizer and the density of 75,000 plants/ha), and the lowest was 171 cm related to the treatment of D1F1 (no biofertilizer application at the density of 55,000 plants/ha) (Table 4). Sangoi et al. (2002) reported that different corn hybrids responded to plant density differently, but corn hybrids with lower plant height produced better yields at higher densities. This can be related to the fact that competition for nutrients increases when plant density increases and light interception declines. Some researchers have reported that plant height and ear length, which are suitable traits for mechanized harvest, were higher at higher densities (Seyedehvand & Bankesav, 2000).

Grain number per ear

Yazdani et al. (2009) stated that the application of phosphate-solubilizing microorganisms and plant growth-promoting bacteria along with chemical fertilizers (NPK) to corn improved the ear weight, the number of rows per ear, and the number of grains per row. The difference among the treatments in the number of grains per ear might be related to their different nutritional status and the different amounts of nutrients available to plants among the treatments. If the plants are supplied with their water and nutrient requirements in adequate quantities and at the proper time during their growth and development, they can achieve proper reproductive growth by absorbing them and increasing their vegetative growth, resulting in optimal yield components.

The highest number of grains per ear was 392 obtained from the treatment of F4D2 (the application of N-1 + P-2 at the density of 65,000 plants/ha), and the lowest was 365 related to the treatment of D1N1 (no biofertilizer application at the density of 55,000 plants/ha) (Table 4). Since the number of grains per ear, which is a product of the number of grain rows per ear and the number of grains per row, represents the highest number of grains per ear, it is believed that the higher number of grains per ear was related to the availability of nutrients through the application of biofertilizers (P-2 and N-1) along with the density of 65,000 plants/ha, which finally led to higher growth and the increase in grain number per ear.

The number of grains in plants largely depends on factors that are suitable for fast growth, especially adequate nutrients and moisture. Therefore, increasing nutrients available to plants, particularly nitrogen and phosphorus, stimulates plant growth and increases chlorophyll growth and the number of grains per ear. The synthesis of growth-promoting compounds and hormones by the bacteria used, especially Nitroxin, which contained *Azotobacter*, *Azospirillum*, and phosphate-solubilizing *Pseudomonas*, might have contributed to growth stimulation and the increase in the number of pods per plant. Yazdani et al. (2009) stated that the application of phosphate-solubilizing microorganisms and plant growth-stimulating bacteria along with chemical fertilizers (NPK) improved the ear weight, the number of rows per ear, and the number of grains per row in corn plants. The difference among the treatments in the number of grains per ear might be related to their different nutritional status and the different amounts of nutrients available to the plants in different treatments. If a plant is supplied with adequate water and nutrients at suitable quantities and time, it can demonstrate better vegetable growth by absorbing them and increasing its vegetable growth, thereby producing better yield components. According to Singh and Arora (2001), when plant density is increased, the yield per plant decreases due to the decline in the plant's nutritional space and the competition over radiation. Thus, grain yield can be expected to increase by increasing plant density up to a certain level at which the increase in the number of plants per unit area can compensate for the decline in yield per plant. It is observed that the 1000-grain weight and grain number per ear decreased with increasing plant density. Indeed, the grain yield per plant was lower at higher densities. The increase in grain yield at higher densities might be expectedly influenced by the increase in the number of ears per unit area, which offsets the single-plant yield.

Table 3. The comparison of means for the effect of biofertilizers on the studied quantitative traits based on Duncan's test

Treatments	Plant height (cm)	Kernels/ear	1000-grain weight (g)	Total dry matter (g/m^2)	Grain yield (g/m^2)
F1	174.2 b	369.9 b	354.3 b	21190 b	7708 d
F2	187.6 a	373.8 ab	369.9 ab	25800 a	8027 c
F3	176.9 ab	375.8 ab	369.7 ab	24060 ab	9066 a
F4	173.3 b	386.2 a	379.9 a	25150 ab	8264 b

Similar letter(s) show the lack of a significant difference among the means.
F1 = control (no fertilizer); F2 = nitrogen-1 fertilizer; F3 = phosphate-2 fertilizer; F4 = N-1 + P-2

Table 4. The comparison of means for the effect of plant densities on the studied quantitative traits based on Duncan's test

Treatments	Plant height (cm)	Kernels/ear	1000-grain weight (g)	Total dry matter (g/m^2)	Grain yield (g/m^2)
D1	178.2 c	374.9 b	364.9 b	24270 b	8120 b
D2	181.3 b	383.6 a	375.3 a	24810 a	8343 a
D3	184 a	378.3 b	372.6 ab	24570 ab	8266 ab

Similar letter(s) show the lack of a significant difference among the means.
D1 = 55,000 plants/ha; D2 = 65,000 plants/ha; D3 = 75,000 plants/ha

Thousand-grain weight

Most photosynthates produced are used to increase grain number per plant and pod number per plant and have no significant role in increasing yield through 1000-grain weight (Salehi et al., 2007). The highest 1000-grain weight was 383 g obtained from F4D2 (the application of N-1 + P-2 and the density of 65,000 plants/ha) and the lowest was 342.7 g related to D1F1 (no biofertilizers application and the density of 55,000 plants/ha (Table 4). It seems that the biological nutritional systems alone could not meet the fertilizer need of the corn plants, but with a proper density, they increased all studied traits including 1000-grain weight.

Total dry matter

The highest total dry matter of 26,240 kg/ha was related to F4D2 (the application of N-1 + P-2 and the density of 65,000 plants/ha) and the lowest was 22180 kg/ha related to F1D1 (no biofertilizers application at the density of 55,000 plants/ha) (Table 5). The superiority of most integrated treatments in terms of most studied traits can be attributed to the fact that P-2 phosphate, zeo-organic, and N-1 nitrogen fertilizers can, in addition to possibly improving the vital processes of the soil and increasing its fertility, contribute to corn plant growth and development and increase its biological yield versus other systems through creating a proper culture medium and nutrient availability. It can therefore be inferred that phosphate-solubilizing microorganisms can increase growth by synthesizing plant hormones and thereby influence the early growth stages of the plant. Then, the root system occupies more soil volume and expands its absorption area (Rasipour & Aliasgharzadeh, 2007). In fact, the effect of increasing plant number per unit area was stronger on increasing biological yield than on partially decreasing the single-plant biological yield per unit area

Grain yield

The highest and lowest grain yields were 9146 and 7500 kg/ha obtained from the treatments of F4D2 (N-1 + P-2 and the density of 65,000 plants/ha) and F1D1 (no biofertilizers application and the density of 55,000 plants/ha), respectively (Table 4). The increased availability of P, N, and other nutrients in the combined application of biofertilizers increases their uptake by the plant, resulting in an increase in their growth, leaf area, and photosynthesis rate, so it is considered an important factor of the increase in yield and morphological traits in the integrated nutrition system. The superiority of biofertilizers for most studied traits can be ascribed to the fact that P-2 phosphate and N-1 nitrogen fertilizers resulted in the highest increase in crop yield.

The application of Nitroxin and phosphate-solubilizing bacteria significantly affected quantitative traits, like the number of main branches, the number of inflorescences per plant, flower diameter, fresh and dry flower yield, and seed yield, and the qualitative traits, such as essential oil yield and chamazulene yield in chamomile (Fallahi et al., 2009).

Research shows that the ability of *Azotobacter* to fix nitrogen and balance it in the soil depends on soil properties and plant species (Belimov et al., 1998). An increase in yield and N and P uptake was observed in sorghum and pea mostly when 50% of chemical fertilizer or manure was applied along with inoculation with growth-promoting bacteria, such as phosphate-solubilizing bacteria (Sani et al., 2004).

Conclusion

Therefore, the results showed that the application of biofertilizers influenced plant height, grain yield, biological yield, and 1000-grain weight positively. The highest 1000-grain weight (383 g), biological yield (26240 kg/ha), and grain yield (9146 kg/ha) were obtained from F4D2 (N-1 + P-2 and the density of 65,000 plants/ha). The results showed that the density of 65,000 plants/ha and the application of N-1 and P-2 biofertilizers was the best treatment. The application of a plant density higher than the optimal density can be justified by its effect on rapidly completing crop cover, which results in reducing water loss from the soil surface, increasing crop competitiveness with weeds, reducing the outbreak of pests and diseases, and finally increasing biological and corn yield per unit area.

Based on the results, the grain yield was about 10% higher in the density of 65,000 plants/ha than in the density of 75,000 plants/ha. Nitrogen-1 biofertilizers played a key role in biological yield, and phosphate-2 biofertilizers were important for grain yield.

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Table 5. The comparison of means for the interactive effect of biofertilizers and plant densities on the studied quantitative traits based on Duncan's test

Treatments	Plant height (cm)	Kernels/ear	1000-grain weight (g)	Ear length (cm)	Total dry matter (g/m^2)	Grain yield (g/m^2)
F1D1	171.0g	365c	342.7f	17.33ab	22180d	7500f
F1D2	177.3e	368bc	359.0e	18ab	23610cd	7854de
F1D3	f 174.3	376.7abc	361.3e	18.33ab	23790cd	7771e
F2D1	185.3c	377.3abc	365.7de	17.67ab	24090bc	7868de
F2D2	189.7a	385ab	371.0cd	17b	23840cd	8022cd
F2D3	188.7ab	389a	373bcd	18ab	24240bc	8191c
F3D1	171.0g	375abc	375abc	18.33ab	24520abc	8995a
F3D2	174.0d	376abc	380a	18ab	24950abc	8117c
F3D3	174.7f	376abc	382a	18.67ab	25970ab	8444b
F4D1	185.3c	382b	376abc	ab6518.	25280abc	8230bc
F4D2	187.3b	392a	383a	19.68a	26240a	9146a
F4D3	190a	384b	379abc	19.62a	25890ab	9058a

Similar letter(s) show the lack of a significant difference among the means.
F1 = control (no fertilizer); F2 = nitrogen-1 fertilizer; F3 = phosphate-2 fertilizer; F4 = N-1 + P-2
D1 = 55,000 plants/ha; D2 = 65,000 plants/ha; D3 = 75,000 plants/ha

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