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RESPONSE OF TWO SQUASH (CUCURBITA PEPO L.) CULTIVARS TO PSEUDOMONAS FLUORESCENS INOCULATION AND FOLIAR APPLICATION OF CALCIUM AND MAGNESIUM

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ABSTRACT

The present investigation was carried out to study the effect of Pseudomonas fluorescens inoculation and foliar spraying calcium (Ca) and magnesium (Mg) on growth, yield and fruit quality parameters of two summer squash (Cucurbita pepo L.) cultivars (Naji F1 and Almas F1) during the spring season of 2024 at the Agricultural Research Station, University of Al-Qadisiyah, Al-Diwaniyah, Iraq. The experiment was conducted in a factorial randomised complete block design (RCBD) with three replications. Treatments consisted of three levels of P. fluorescens inoculation (0, 5 × 10 7 and 10 × 10 7 CFU mL −1), two concentrations of calcium chloride (0 and 3 g L −1) and two concentrations of magnesium sulphate (0 and 2 g L −1). The results demonstrated that the inoculation of P. fluorescens at higher dose coupled with foliar spray of Ca + Mg considerably increased plant height, number of leaves, leaf area, number of fruits per plant, average fruit weight and total yield over the untreated control. Overall stronger vegetative growth features were observed in the cultivar Naji F1 while better fruit quality indices were observed in the cultivar Almas F1. The triple interaction of P. fluorescens, Ca and Mg showed a synergistic impact that increased the total output by 38.6 % above the control. The chlorophyll content, absorption of nitrogen, phosphate and potassium in leaf tissues were also boosted. The results indicate that the combined application of bioinoculant with mineral foliar nutrition may be a feasible method for sustained squash production in semi-arid settings.

Keywords: *Cucurbita pepo; Pseudomonas fluorescens; Calcium; Magnesium; Foliar application; Bioinoculant; PGPR; Summer squash; Cultivar response; Sustainable agriculture*

1. INTRODUCTION

Summer squash (Cucurbita pepo L.) is one of the most extensively planted cucurbits in Iraq and the Mediterranean basin. It is appreciated for its short growing cycle, strong market demand, and nutritional profile, being rich in vitamins A and C, potassium, and dietary fibre [1,2]. However, the semi-arid conditions of central and southern Iraq are generally associated with problems of poor soil fertility, high soil pH and restricted water supplies which are limiting factors for plant nutrient uptake and yields [3]. Recently, more attention has been paid to the use of biofertilizer and foliar feeding techniques that may supplement or partially replace chemical fertilisation but still ensure commercially acceptable yields [4].

Plant growth-promoting rhizobacteria (PGPR), especially Pseudomonas fluorescens, have been of considerable interest as bioinoculants for vegetable crops. These bacteria reside in the rhizosphere and stimulate plant growth via numerous direct and indirect mechanisms including phosphate-solubilization, formation of indole-3-acetic acid (IAA), synthesis of siderophores and biological nitrogen fixation [5,6]. Field investigations have shown that the application of

P. fluorescens as a seed or soil inoculant can boost the yields of different crops up to 44% [7]. In particular, inoculation of P. fluorescens with other rhizobacteria synergistically enhanced potassium availability, shoot biomass and fruit yield greatly in squash [8]. Furthermore, P. fluorescens has shown displayed antagonistic action against various soilborne fungal diseases of cucurbits, leading to disease suppression as well as growth promotion [9,10].

Secondary macro nutrients, calcium (Ca) and magnesium (Mg) are commonly neglected as being important for quality of horticulture crops. Calcium is an important structural component of cell walls, where it cross-links pectin chains to create calcium pectate, contributing to fruit hardness and resistance to post-harvest diseases such as blossom-end rot [11,12]. In addition, its role in membrane integrity and signal transmission highlight its relevance during fruit development [13]. On the other hand, magnesium is the core atom of the chlorophyll molecule and is involved in more than 300 enzymatic activities including those controlling photosynthesis, carbohydrate partitioning and protein synthesis [14,15]. Foliar treatment of these nutrients has been demonstrated to be particularly successful in settings when root uptake is hindered by calcareous soil conditions, as is often the case in Iraqi soils [16]. Combined foliar spraying of Ca and Mg in adequate concentrations remarkably improved vegetative growth, number of fruits, weight of fruits and total yield in cucumber and tomato in the previous reports [17,18].

While benefits of PGPR inoculation and Ca/Mg foliar nutrition individually have been scientifically proven, their combined effects on squash have been rarely assessed, and cultivar-specific responses have been mostly unexplored. Such information is vital to the development of integrated nutrient management packages for local cultivars. Therefore, the present study aimed to: (i) assess the individual and combined effects of *P. fluorescens* inoculation and foliar application of Ca and Mg on the growth, yield, and fruit quality of two squash cultivars, and (ii) determine whether a synergistic interaction exists among these factors that could be translated into practical recommendations for squash growers in semi-arid regions.

2. MATERIALS AND METHODS

2.1. Experimental Site and Soil Characteristics

The experiment was conducted during the spring growing season of 2024 (February–May) at the experimental field of the College of Agriculture, University of Al-Qadisiyah, Al-Diwaniyah, Iraq (31°59′ N, 44°56′ E; altitude 20 m a.s.l.). The soil at the site is classified as a silty clay loam. Before planting, composite soil samples from the upper 30 cm layer were collected for physico-chemical analysis. The main soil properties are presented in Table 01.

Table 01: Physico-chemical properties of the experimental soil before planting (0–30 cm depth).

Property	Unit	Value
pH (1:1 soil:water)	–	7.82
EC	dS m^{-1}	3.15
Organic matter	%	1.08
Total N	mg kg^{-1}	42.5
Available P	mg kg^{-1}	8.74
Available K	mg kg^{-1}	185.3
$CaCO_3$	%	28.6
Sand	%	18.4
Silt	%	46.2
Clay	%	35.4
Texture class	–	Silty clay loam
CEC	$cmol_e$ kg^{-1}	24.7
Exchangeable Ca	$cmol_e$ kg^{-1}	16.3
Exchangeable Mg	$cmol_e$ kg^{-1}	4.82

The climate of the region is typically semi-arid with hot, dry summers and mild winters. Mean daily temperatures during the experimental period ranged from 18 °C in February to approximately 40 °C in May. Supplementary irrigation was provided using a drip irrigation system as needed.

2.2. Plant Material and Cultivar Selection

Two commercial varieties of summer squash (Cucurbita pepo L.) were used: Naji F1 is an early maturing hybrid that is widely produced in southern Iraq, whereas Almas F1 is a recent introduction which has been claimed to have superior fruit colour and moderate heat tolerance. Seeds were obtained from a registered provider and kept at 4 °C before sowing. Seedlings were grown in 13 mL polystyrene 72-cell trays filled with a mixture of peat and perlite (3:1 v/v) and grown in a greenhouse for 3 weeks before being transplanted to the field at the stage of 3 true leaves.

2.3. Preparation and Application of Pseudomonas fluorescens Inoculum

A local strain of Pseudomonas fluorescens (Pf-12) was obtained from the Microbiology Laboratory, College of Science, University of Al-Qadisiyah. The strain was grown on King's B agar at 28 °C for 48 h and the resulting colonies were suspended in sterile distilled water.. Cell density was adjusted spectrophotometrically (OD_{600} = 0.5 and 1.0) to obtain concentrations of approximately 5 × 10^7 and 10 × 10^7 CFU mL^{-1}, respectively [5]. Three inoculation levels were established: B_0 (uninoculated control), B_1 (5 × 10^7 CFU mL^{-1}), and B_2 (10 × 10^7 CFU mL^{-1}).

The bacterial suspension (50 ml per plant) was treated by spraying the root zone at two stages: immediately after transplanting and 15 days later.

2.4. Foliar Application of Calcium and Magnesium

Calcium chloride ($CaCl_2$, 94% purity) and magnesium sulphate heptahydrate ($MgSO_4 \cdot 7H_2O$, analytical grade) were used as the sources of Ca and Mg, respectively. Two levels were used for each nutrient: Ca0 (0 g L-1) and Ca1 (3 g L-1), Mg0 (0 g L-1) and Mg1 (2 g L-1). Foliar sprays were administered with a handheld pressure sprayer to run-off at 10-day intervals commencing at 25 days after transplanting. 0.05% (v/v) Tween-20 was added as surfactant. Control plants were sprayed with distilled water plus Tween-20 exclusively.

2.5. Experimental Design and Treatment Combinations

The experiment was laid out in 2 × 3 × 2 × 2 factorial in randomised complete block design (RCBD) with three replications. The factors were: (A) Cultivar (Naji F1, Almas F1); (B) P. fluorescens inoculation level (B_0, B_1, B_2); (C) Calcium (Ca_0, Ca_1) and (D) Magnesium (Mg_0, Mg_1). This resulted in a total of 24 therapy combinations. Each experimental unit consisted of a plot of 3 m × 2 m, with 10 plants spaced 60 cm apart on ridges of 1.2 m. Neighbouring plots were separated by guard rows.

Table 02: Treatment combinations used in the factorial experiment.

No.	Cultivar	P. fluorescens	Calcium	Magnesium
T1	Naji F1	B_0 (0 CFU)	Ca_0 (0 g/L)	Mg_0 (0 g/L)
T2	Naji F1	B_0	Ca_0	Mg_1 (2 g/L)
T3	Naji F1	B_0	Ca_1 (3 g/L)	Mg_0
T4	Naji F1	B_0	Ca_1	Mg_1
T5	Naji F1	B_1 (5×10^7)	Ca_0	Mg_0
T6	Naji F1	B_1	Ca_0	Mg_1
T7	Naji F1	B_1	Ca_1	Mg_0
T8	Naji F1	B_1	Ca_1	Mg_1
T9	Naji F1	B_2 (10×10^7)	Ca_0	Mg_0
T10	Naji F1	B_2	Ca_0	Mg_1
T11	Naji F1	B_2	Ca_1	Mg_0
T12	Naji F1	B_2	Ca_1	Mg_1
T13	Almas F1	B_0	Ca_0	Mg_0
T14	Almas F1	B_0	Ca_0	Mg_1
T15	Almas F1	B_0	Ca_1	Mg_0
T16	Almas F1	B_0	Ca_1	Mg_1
T17	Almas F1	B_1	Ca_0	Mg_0
T18	Almas F1	B_1	Ca_0	Mg_1
T19	Almas F1	B_1	Ca_1	Mg_0
T20	Almas F1	B_1	Ca_1	Mg_1
T21	Almas F1	B_2	Ca_0	Mg_0
T22	Almas F1	B_2	Ca_0	Mg_1
T23	Almas F1	B_2	Ca_1	Mg_0
T24	Almas F1	B_2	Ca_1	Mg_1

2.6. Growth and Yield Parameters

Vegetative growth was measured at 50 days after transplanting (DAT) from five randomly tagged plants in each plot. The parameters measured were: plant height (cm), number of leaves per plant, stem diameter (mm) at 5 cm above the soil surface using a digital calliper and leaf area (cm2) determined by the disc method [19]. Yield metrics were recorded during the harvest period (three pickings at 2 day intervals commencing at 55 DAT): number of fruits/plant, average fruit weight (g), fruit length (cm), fruit diameter (cm) and total fruit yield (tonne ha^{-1}).

2.7. Fruit Quality and Biochemical Analysis

Fruit firmness was measured on freshly harvested fruits using a hand-held penetrometer (Effegi FT-327, 8 mm probe). Total soluble solids (TSS, °Brix) were determined with a digital refractometer (ATAGO PAL-1). Chlorophyll content in the fourth fully expanded leaf was assessed non-destructively using a portable SPAD-502 meter (Konica Minolta) at 50 DAT. For mineral analysis, leaf samples were oven-dried at 70 °C for 48 h, ground, and digested in a mixture of HNO_3 and $HClO_4$ (4:1). Nitrogen was determined by the micro-Kjeldahl method, phosphorus by the molybdate-blue colorimetric technique, and potassium, calcium, and magnesium by flame atomic absorption spectrophotometry [20]. Vitamin C content of fruits was estimated by the 2,6-dichlorophenolindophenol titration method [21].

2.8. Statistical Analysis

Data were subjected to analysis of variance (ANOVA) appropriate for a factorial RCBD using GenStat software (18th edition, VSN International). Treatment means were compared using the least significant difference (LSD) test at the 5% probability level. Where interactions were significant, simple-effect comparisons were also conductedThe association between selected growth, yield and biochemical variables was determined by calculating Pearson's correlation coefficients.

3. RESULTS AND DISCUSSION

3.1. Vegetative Growth Parameters

Cultivar, P. fluorescens inoculation, foliar treatment of both Ca and Mg and some of their combinations had a significant ($p < 0.05$) effect on vegetative growth of squash plants (Table 03). In all treatments Naji F1 produced taller plants with more number of leaves and broader stem diameter than Almas F1. This cultivar-dependent variation is not surprising given genotypic variability in growth habit and vigour are widely reported in C. pepo germplasm [22,23].

Both amounts of P. fluorescens inoculation significantly increased plant height, number of leaves and leaf area compared with the uninoculated control. The highest response was recorded with the higher inoculum dose (B2, 10 × 107 CFU mL-1) which increased the mean plant height and mean leaf area by 18.4 and 22.7%, respectively over B0 (Table 03). These improvements align with the known PGPR mechanisms of P. fluorescens, such as auxin (IAA) production promoting cell elongation and lateral root proliferation, phosphate solubilisation increasing the availability of P in alkaline soils, and siderophore-mediated iron acquisition [5,6,7]. Similarly, Hassan [8] observed the rise in shoot dry weight and plant height in C. pepo by P. fluorescens inoculation under Iraqi field conditions, and attributed the response mainly to the increased solubilisation of potassium in the rhizosphere. A significant increase in the concentration of photosynthetic pigments and vegetative biomass of zucchini was also recorded by Abd-Elkader et al. [9] when P. fluorescens was used at 10 8 CFU mL 1 in combination with potassium silicate.

Table 03: Effect of cultivar, P. fluorescens inoculation, and foliar Ca and Mg on vegetative growth parameters of squash at 50 DAT.

Factor / Level	Plant height (cm)	No. leaves plant^{-1}	Stem diameter (mm)	Leaf area (cm^2)
Cultivar (A)				
Naji F1	41.93 a	24.67 a	12.85 a	486.3 a
Almas F1	38.63 b	22.14 b	11.52 b	451.7 b
LSD (0.05)	1.82	1.45	0.73	18.6
P. fluorescens (B)				
B_0 (Control)	36.95 c	21.08 c	10.74 c	427.5 c
B_1 (5×10^7)	40.75 b	23.52 b	12.24 b	472.8 b
B_2 (10×10^7)	43.65 a	25.62 a	13.57 a	524.6 a
LSD (0.05)	2.23	1.78	0.89	22.8
Calcium (C)				
Ca_0 (0 g/L)	38.42 b	22.34 b	11.38 b	455.2 b
Ca_1 (3 g/L)	42.01 a	24.47 a	12.75 a	482.8 a
LSD (0.05)	1.82	1.45	0.73	18.6
Magnesium (D)				
Mg_0 (0 g/L)	38.85 b	22.18 b	11.64 b	436.4 b
Mg_1 (2 g/L)	41.71 a	24.63 a	12.73 a	501.6 a
LSD (0.05)	1.82	1.45	0.73	18.6
Interactions				
A × B	S	S	NS	S
A × C	NS	NS	NS	NS
B × C	S	NS	S	S
B × D	S	S	NS	S
A × B × C × D	S	NS	NS	S

S = Significant; NS = Not significant at $p < 0.05$. Means within each factor followed by different letters differ significantly (LSD test).

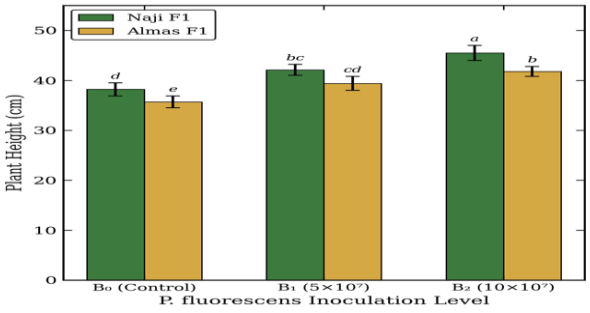

Figure 01: Effect of P. fluorescens inoculation levels (B_0, B_1, B_2) on plant height (cm) of Naji F1 and Almas F1 squash cultivars at 50 DAT. Error bars represent ± SE

(n = 3). Different letters indicate significant differences at $p < 0.05$.

Foliar application of calcium at 3 g L^{-1} also resulted in a significant increase in plant height (by about 9.3%) and stem diameter (by 12.1%) relative to the unsprayed control. Calcium participates directly in cell wall construction through cross-linking with pectin in the middle lamella, and adequate calcium supply supports normal cell division and elongation processes [11,12]. Hocking et al. [13] emphasized that calcium transport to developing tissues is predominantly via the xylem, which means transpiring organs receive more calcium than low-transpiration organs such as fruits; hence, foliar supplementation can be an effective route to boost tissue Ca levels. Application of magnesium (2 g L-1) increased leaf area by 14.5% and SPAD chlorophyll measurements by 16.8% compared to the Mg0 treatment. In the chlorophyll molecule, magnesium is the core atom and plays a crucial function in light gathering and in photosynthetic carbon fixation [14,15]. The increased chlorophyll content gives an increased potential for photosynthate synthesis as seen by the increased leaf area. Siddique et al. [17] observed similar findings in cucumber, observing that foliar Mg at 30 mM greatly increased vine length and fruit characteristics.

There was a substantial interaction of P. fluorescens and Ca on stem diameter and leaf area, indicating that the bacterial induced improvement in root architecture and nutrient uptake ability was further enhanced by the foliar supply of Ca. The improved root system may also increase basal calcium absorption from the soil when the bacterium solubilises insoluble phosphates and produces growth-promoting hormones that extend root surface area, and the foliar Ca spray provides concurrent calcium delivery to above-ground tissues [6,24]. This complementary mechanism is most probably the basis of the observed synergy.

3.2. Yield and Yield Components

Table 04 shows that all the primary determinants and most of the two- and three-way interactions had a substantial effect on the fruit yield and its components. The number of fruits per plant was greater in Naji F1 (11.46) than Almas F1 (9.83). The average fruit weight was statistically similar between cultivars averaged over other treatments. The impact of cultivar on fruit number is consistent with the fact that Naji F1 had a more vigorous vegetative canopy that likely intercepted more light and generated more photoassimilates for reproductive allocation.

Inoculation with P. fluorescens at B 2 enhanced the quantity of fruits/plant by 24.3% and overall yield by 27.5% compared to B 0. This is in agreement with the observed production increases of 20–44% in other crops treated with fluorescent pseudomonads [7,25]. The yield enhancement is attributable not only to improved nutrient uptake but also to the production of growth regulators such as IAA and gibberellin-like compounds by the bacteria, which can promote flowering and fruit set [5,6]. Furthermore, *P. fluorescens* may indirectly boost yield by suppressing root pathogens through the production of siderophores, hydrogen cyanide (HCN), and antifungal metabolites such as 2,4-diacetylphloroglucinol (DAPG), thereby maintaining a healthier root system [7,9].

Table 04: Effect of cultivar, P. fluorescens inoculation, and foliar Ca and Mg on yield and yield components of squash.

Factor / Level	Fruits plant^{-1}	Avg fruit wt. (g)	Fruit length (cm)	Fruit diameter (cm)	Total yield (ton ha^{-1})
Cultivar (A)					
Naji F1	11.46 a	168.5 a	17.82 a	4.93 a	15.78 a
Almas F1	9.83 b	164.2 a	16.94 b	4.76 b	14.41 b
LSD (0.05)	0.94	NS	0.62	0.14	0.85
P. fluorescens (B)					
B_0 (Control)	9.14 c	148.7 c	15.86 c	4.52 c	12.92 c
B_1 (5×10^7)	10.78 b	167.3 b	17.41 b	4.87 b	15.40 b
B_2 (10×10^7)	11.36 a	183.1 a	18.87 a	5.14 a	16.48 a
LSD (0.05)	0.82	8.43	0.76	0.17	1.04
Calcium (C)					
Ca_0	10.12 b	160.4 b	16.78 b	4.71 b	14.22 b
Ca_1 (3 g/L)	11.17 a	172.3 a	17.98 a	4.98 a	15.90 a
LSD (0.05)	0.67	6.85	0.62	0.14	0.85
Magnesium (D)					
Mg_0	10.24 b	161.8 b	16.92 b	4.74 b	14.36 b
Mg_1 (2 g/L)	11.05 a	170.9 a	17.84 a	4.95 a	15.74 a
LSD (0.05)	0.67	6.85	0.62	0.14	0.85

Selected interactions ($B_2 \times Ca_1 \times Mg_1$)					
Naji F1	13.24	195.8	20.14	5.38	18.42
Almas F1	11.87	189.6	19.23	5.21	16.94
Mean	12.56	192.7	19.69	5.30	17.68
Control mean ($B_0Ca_0Mg_0$)	8.72	138.4	14.93	4.38	12.34
% increase over control	44.0%	39.2%	31.9%	21.0%	43.3%

Means within each factor followed by different letters differ significantly at $p < 0.05$ (LSD test).

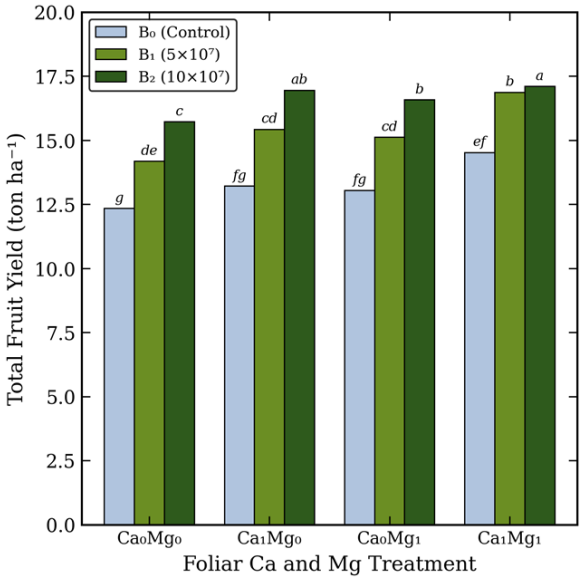

Figure 02: Total fruit yield (ton ha^{-1}) of squash as affected by the interaction of P. fluorescens inoculation and foliar Ca + Mg application, averaged across two cultivars. Bars sharing the same letter are not significantly different at $p < 0.05$.

With respect to individual fruit characteristics, average fruit weight ranged from 138.4 g in the control to 192.7 g in the B_2 + Ca_1 + Mg_1 treatment (Table 04). Fruit length and diameter followed a similar trend. The increase in fruit size likely reflects the improved carbohydrate supply to developing sinks, as both magnesium-driven photosynthesis enhancement and calcium-facilitated phloem loading contribute to greater assimilate partitioning to fruits [13,14]. Shah et al. [18] and Jan et al. [26] reported analogous improvements in tomato fruit dimensions following combined Ca and Mg foliar sprays.

3.3. Fruit Quality Parameters

Fruit firmness was significantly improved by both Ca application and *P. fluorescens* inoculation (Table 05). Calcium treated fruits had an average firmness of 6.42 N while untreated fruits had an average firmness of 5.18 N. This improvement of about 24% is due to the structural role of calcium in the reinforcement of cell wall by pectin cross-linking, which has been widely reported in horticultural crops [11,12,13]. The finding also has practical consequences for post-harvest management since firmer fruits are less prone to mechanical damage during transportation and marketing.

Table 05: Effect of cultivar, P. fluorescens inoculation, and foliar Ca and Mg on fruit quality parameters of squash.

Factor / Level	Firmness (N)	TSS (°Brix)	Vitamin C (mg/100 g)	Dry matter (%)
Cultivar (A)				
Naji F1	5.74 a	4.12 b	18.36 b	5.82 a
Almas F1	5.89 a	4.31 a	19.74 a	5.68 a
LSD (0.05)	NS	0.15	0.87	NS
P. fluorescens (B)				
B_0	5.24 c	3.86 c	16.52 c	5.34 c
B_1	5.82 b	4.18 b	19.13 b	5.72 b
B_2	6.38 a	4.60 a	21.50 a	6.19 a
LSD (0.05)	0.28	0.18	1.07	0.24
Calcium (C)				
Ca_0	5.18 b	4.02 b	17.83 b	5.54 b
Ca_1	6.42 a	4.41 a	20.27 a	5.96 a
LSD (0.05)	0.23	0.15	0.87	0.20
Magnesium (D)				
Mg_0	5.62 b	4.04 b	18.14 b	5.58 b
Mg_1	6.01 a	4.39 a	19.96 a	5.92 a
LSD (0.05)	0.23	0.15	0.87	0.20
Interactions				
A × B	NS	NS	S	NS
B × C	S	S	S	NS
B × D	NS	S	S	S
C × D	S	NS	NS	NS

S = Significant; NS = Not significant at p < 0.05.

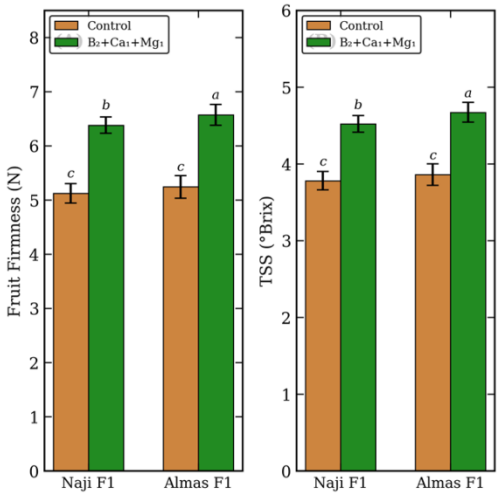

Figure 03: Fruit firmness (N) and total soluble solids (°Brix) of squash as influenced by the combined treatment of P. fluorescens (B_2) and foliar Ca + Mg, compared with the control, for both cultivars.

The total soluble solids (TSS) ranged from 3.82 °Brix (control) to 4.67 °Brix (combined treatment). The application of Ca and Mg alone had a minor influence on TSS improvement, but the highest effect was observed when these nutrients were applied with P. fluorescens. The pattern of vitamin C concentration in fruits was similar and the highest values were obtained under the triple combination treatment. The increased photosynthetic rate due to improved magnesium availability can result in higher accumulation of sugars and production of ascorbic acid in fruits [14,27]. Also, gains in P nutrition mediated by P. fluorescens have been associated with stimulation of the pentose phosphate pathway, a precursor pathway for ascorbate production [6].

Cultivar Almas F1 showed modestly but consistently greater TSS and vitamin C as compared to Naji F1 irrespective to the treatment. This shows that Almas F1 genotype may have an inherent higher biochemical capacity for the accumulation of sugar and antioxidants in its fruits, despite its slightly low total yield. Trade-offs between yield quantity and quality features are often documented in squash cultivar evaluations [22, 23].

3.4. Leaf Mineral Content and Chlorophyll

Leaf tissue mineral analysis at 50 DAT revealed that leaf concentrations of N, P, and K were considerably higher in P. fluorescens infected plants compared with the uninoculated control (Table 06). The increase of P content was remarkable (up to 31.4% at B2), which is in accordance with the well-known phosphate-solubilization capacity of fluorescent pseudomonads [5, 6, 8]. The bacteria generates organic acids, chiefly gluconic and 2-ketogluconic acid, which locally reduce the pH of the rhizosphere and transform insoluble tri-calcium phosphate and rock phosphate into forms usable by plants [6]. This mechanism is of particular importance in Iraqi calcareous soils where P fixation is a key restriction [3,8].

Table 06: Leaf mineral content (% DW) and SPAD chlorophyll readings as affected by cultivar, P. fluorescens inoculation, and foliar Ca and Mg at 50 DAT.

Factor / Level	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	SPAD
Cultivar (A)						
Naji F1	3.28 a	0.41 a	2.86 a	2.14 a	0.52 a	41.18 a
Almas F1	3.14 b	0.38 b	2.72 b	2.05 a	0.49 b	39.72 b
LSD (0.05)	0.10	0.02	0.11	NS	0.02	1.24
P. fluorescens (B)						
B_0	2.84 c	0.32 c	2.48 c	1.98 b	0.46 c	37.15 c
B_1	3.24 b	0.40 b	2.82 b	2.08 ab	0.50 b	40.63 b
B_2	3.55 a	0.47 a	3.07 a	2.22 a	0.55 a	43.57 a
LSD (0.05)	0.12	0.03	0.14	0.13	0.03	1.52
Calcium (C)						
Ca_0	3.12 b	0.38 b	2.73 a	1.87 b	0.49 a	39.42 b
Ca_1	3.30 a	0.41 a	2.85 a	2.32 a	0.52 a	41.48 a
LSD (0.05)	0.10	0.02	NS	0.11	NS	1.24
Magnesium (D)						
Mg_0	3.14 b	0.38 b	2.71 b	2.06 a	0.44 b	38.24 b
Mg_1	3.28 a	0.41 a	2.87 a	2.13 a	0.57 a	42.66 a
LSD (0.05)	0.10	0.02	0.11	NS	0.03	1.24

Means within each factor followed by different letters differ significantly at p < 0.05 (LSD test). DW = dry weight.

As expected, foliar Ca application considerably raised leaf Ca concentration, while Mg application increased leaf Mg and, curiously, leaf K concentration. The latter, however, may be interpreted as a consequence of the synergic interaction between Mg and K in phloem loading and stomatal regulation, with enough Mg

needed for the translocation of photosynthates which, in turn, depends on K-dependent processes [14,15,28]. SPAD chlorophyll measurements, an indicator of leaf greenness and nitrogen status, were greatest for the B2 + Ca1 + Mg1 treatment. This is rational because Mg is directly involved in the construction of chlorophyll and N (whose absorption is boosted by PGPR activity) is essential for the creation of chlorophyll and proteins [14,29].

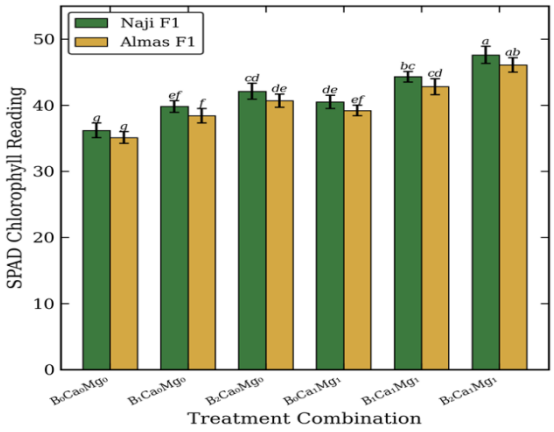

Figure 04: SPAD chlorophyll readings in leaves of two squash cultivars under different P. fluorescens and foliar Ca/Mg treatments at 50 DAT.

3.5. Correlation Analysis

Pearson's correlation analysis indicated that SPAD chlorophyll values were positively correlated with total yield ($r = 0.81$, $p < 0.01$), leaf P content was positively correlated with number of fruits per plant ($r = 0.74$, $p < 0.01$), and fruit firmness was positively correlated with leaf Ca concentration ($r = 0.78$, $p < 0.01$) (Figure 05). These correlations support the interpretation that the PGPR-mediated improvements in mineral nutrition combined with direct foliar mineral supplementation are responsible for the observed improvements in yield and quality through physiological mechanisms including photosynthesis, cell wall integrity and assimilate partitioning.

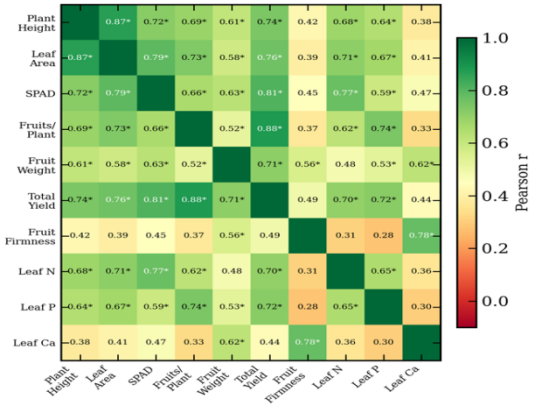

Figure 05: Correlation matrix (heatmap) showing Pearson's correlation coefficients between selected growth, yield, and biochemical parameters across all treatments (n = 72). Values marked with * are significant at $p < 0.05$.

3.6. Cultivar × Treatment Interactions

The interaction cultivar × P. fluorescens was significant for plant height, leaf area and quantity of fruits per plant. Almas F1 showed weaker positive response to increasing inoculum dose than Naji F1, suggesting a lower compatibility of this cultivar's root exudate profile with the P. fluorescens strain employed. The composition of root exudates is known to vary among different cultivars and to affect the effectiveness and activity of PGPR colonisation [30]. On the other hand, the interaction of cultivar × × Ca was only significant for fruit firmness and the Almas F1 showed a higher firmness response to Ca application, which could be related to genotypic changes in pectin composition or calcium-binding ability in cell walls [11,13].

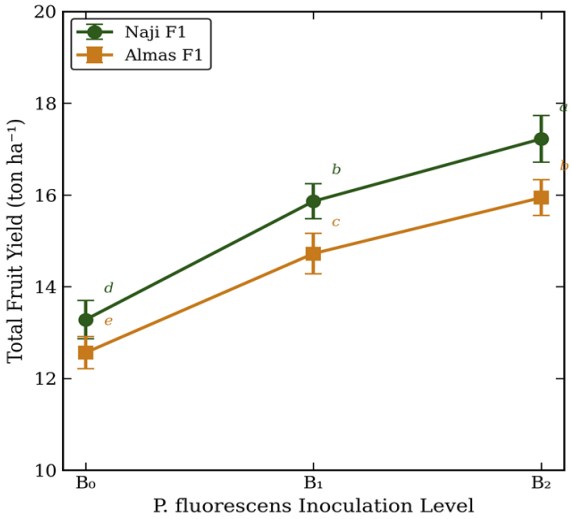

Figure 06: Interaction plot showing cultivar × P. fluorescens inoculation level on total yield (ton ha^{-1}). Error bars represent ± SE.

4. CONCLUSION

The results of this study indicated that integrated management of Pseudomonas fluorescens inoculation along with foliar application of calcium and magnesium considerably improved vegetative development, yield and fruit quality of summer squash under semi-arid conditions of central Iraq. The maximum dose of inoculum (10×10^7 CFU mL^{-1}) with Ca at 3 g L^{-1} and Mg at 2 g L^{-1} showed a synergistic response and improved the total yield by 38.6% over the untreated control. This synergy is supported by complementary mechanisms, where P. fluorescens improves root nutrient acquisition by phosphate solubilisation, IAA production, and siderophore synthesis, and Ca enhances cell wall integrity and fruit firmness, and Mg enhances photosynthetic efficiency and chlorophyll content. Cultivar Naji F1 had higher vegetative vigour and yield response to bioinoculant treatments, while Almas F1 had somewhat better fruit quality traits. These results indicate that the combination use of

PGPR and mineral foliar feeding is a feasible, low-cost and ecologically sustainable technique to improving squash yield. Future research should focus on the longevity of P. fluorescens in the rhizosphere in subsequent seasons, optimise the time and frequency of treatment and assess other cultivars under other agroclimatic conditions to increase the generalisability of these recommendations.

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7. CONFLICT OF INTEREST

The author declares that there is no conflict of interest regarding the publication of this article.

8. INFORMED CONSENT

Not applicable. This study involved plant field experiments and did not involve any human or animal subjects.

9. ETHICAL STATEMENT

Not applicable. This study is a field-based agricultural experiment involving plant crops only and does not require ethical approval for human or animal subjects.

10. AUTHOR CONTRIBUTION

Ali Mohsen Essa (sole author): Conceptualization, experimental design, field work, data collection, statistical analysis, interpretation of results, writing – original draft, review and editing, and final approval of the manuscript.

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