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Effects of Mycorrhiza and PGPR Application on the Growth and Yield of Waxy Corn (*Zea mays* L. var. ceratina) in a Downy Mildew-Endemic Field

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Abstract:
Waxy corn (*Zea mays* L. var. *ceratina*) yield is often limited by downy mildew (*Peronosclerospora maydis*) and unfavorable growing conditions. This study evaluated the effects of arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) on the growth and yield of waxy corn grown in a downy mildew-endemic field. The experiment was conducted from July to September 2024 in Kebonsari, Jember, Indonesia, using a randomized complete block design with four treatments: control (K), mycorrhiza 30 g per planting hole (M), PGPR 250 mL (P), and their combination (MP), all with the recommended inorganic fertilizer. Growth, flowering, biomass, and ear yield components were analyzed by ANOVA. None of the treatments significantly affected the observed variables. PGPR numerically gave the highest plant height (122.19 cm), stem diameter (1.74 cm), ear weight (37.03 g), and ear length (18.33 cm), exceeding the control by 9–110%, whereas the combined treatment gave the lowest values for most yield components. Strongly alkaline soil (pH 9.1), dry-season water deficit, and high pressure from downy mildew, corn smut, gray leaf spot, and fall armyworm limited the effectiveness of the biological agents. Mycorrhiza and PGPR should be integrated with soil amelioration, irrigation, and disease management in such fields.

Keywords:
downy mildew, mycorrhiza, PGPR, waxy corn, *Zea mays* var. *ceratina*

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INTRODUCTION

Waxy corn (*Zea mays* L. var. *ceratina*), known in Indonesia as *jagung pulut* or sticky corn, is a special type of maize whose endosperm starch consists almost entirely of amylopectin because of a mutation in the *Waxy1* gene (Yu and Moon, 2022). This starch composition gives the kernels a soft, sticky texture and a distinctive taste, so waxy corn is popular as boiled fresh corn and as a raw material for traditional foods and the starch industry. However, waxy corn generally yields less than ordinary maize, and its productivity in farmers' fields is often further reduced by pests and diseases and by unfavorable soil and weather conditions. Because waxy corn is usually harvested young and sold as fresh corn, ear size and appearance determine its market value, so any damage that reduces ear length, diameter, or kernel filling directly lowers farmers' income. In East Java, waxy corn is often grown on marginal land and in the dry season, when irrigation water is limited and the risk of stress around flowering is high. Improving the productivity of waxy corn under these conditions therefore requires practices that strengthen plant growth and tolerance to stress at low cost.

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Downy mildew, locally called *bulai*, is one of the most destructive diseases of maize in Indonesia. It is caused by several *Peronosclerospora* species, with *P. maydis* being the most prevalent on Java (Suharjo et al., 2020; Ginting et al., 2020). Infected plants show chlorotic stripes, stunting, and poor or no ear formation, and yield losses can be total in susceptible varieties when infection occurs early. The pathogen survives between seasons on maize plants and alternative hosts, so fields where the disease occurs every season are considered endemic. In such fields, the disease is mainly controlled with metalaxyl seed treatment, but reliance on fungicides raises concerns about environmental impact and declining effectiveness, and biological alternatives are being sought (Mugiastuti et al., 2023).

Other pests and diseases add to the problem. Corn smut, caused by *Ustilago maydis*, infects all above-ground organs and forms tumor-like galls on the ears, especially under hot and dry conditions (Zuo et al., 2019; Yu et al., 2023). Gray leaf spot, caused by *Cercospora* species, reduces the photosynthetic area of leaves and is favored by high humidity (Duan et al., 2022; Nsibo et al., 2024). The fall armyworm (*Spodoptera frugiperda*), an invasive pest first reported in Indonesia in 2019, has spread to nearly all maize-producing regions and can cause severe defoliation (Herlinda et al., 2022; Russianzi et al., 2021). In addition, high temperatures and drought during flowering reduce pollen viability and silk receptivity, thereby reducing kernel set (Waqas et al., 2021; Li et al., 2022).

Biological agents such as arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) offer a means to improve crop growth and resilience while reducing agrochemical use. AMF form symbiotic associations with plant roots and extend the root system through external hyphae, thereby increasing the uptake of phosphorus, zinc, and water and improving tolerance to drought and other abiotic stresses (Begum et al., 2019). A global meta-analysis showed that AMF inoculation in the field increased cereal grain yield, including maize, by an average of 16%, but the response varied widely and was modulated by soil pH (Zhang et al., 2019). Under rainfed conditions, AMF inoculation increased crop yields by an average of 23% (Wu et al., 2022).

PGPR are root-colonizing bacteria that promote plant growth through the production of phytohormones, nitrogen fixation, phosphate solubilization, and siderophore production, and protect plants against pathogens through antibiosis, competition, and induced systemic resistance (Backer et al., 2018; Basu et al., 2021; Zhu et al., 2022). Because AMF and PGPR act through partially distinct mechanisms, their combination is often expected to yield additive or synergistic effects. Co-inoculation of AMF with *Bacillus* improved nitrogen use efficiency in maize (Ganugi et al., 2022), and AMF–PGPR co-inoculation enhanced root and leaf functioning and grain yield of maize in dry soil (Khan et al., 2024). In Indonesia, combinations of biological agents with other control measures reduced the incidence of downy mildew (Mugiastuti et al., 2023).

However, most of these benefits have been demonstrated under favorable or controlled conditions, and the performance of biological agents in the field is often inconsistent. Little is known about how AMF and PGPR perform under multiple constraints, such as alkaline soil, dry-season water deficit, and high pressure from diseases and pests, which is common in downy mildew-endemic areas. Therefore, this study aimed to evaluate the effects of mycorrhiza and PGPR, applied alone and in combination with the recommended inorganic fertilizer, on the growth, flowering, and yield components of waxy corn grown in a downy mildew-endemic field, and to identify the main factors that limited their effectiveness. We hypothesized that both biological agents would improve the growth and yield of waxy corn compared with fertilizer alone, and that their combination would yield the greatest improvement due to the complementary functions of AMF and PGPR in nutrient

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uptake, stress tolerance, and plant defense. Testing this hypothesis under realistic field conditions provides information directly relevant to farmers who grow waxy corn in endemic areas, where the decision to invest in biological inputs depends on whether they provide a reliable benefit under the conditions farmers face.

METHOD

The field experiment was conducted from July to September 2024 in Kebonsari, Sumbersari District, Jember Regency, East Java, Indonesia, at an altitude of about 146 m above sea level. The site has a history of downy mildew infection in maize over several consecutive seasons and is therefore considered a downy mildew-endemic area. The experiment was carried out during the dry season, and the daily air temperature ranged from 23 to 34 °C. The soil at the site was soil type/texture with a pH of 9.1, measured with a pH meter in a 1:2.5 (soil:water solution).

The experiment was arranged in a randomized complete block design (RCBD) with four treatments and replications (blocks). The treatments were K = control (recommended inorganic fertilizer only), M = mycorrhiza 30 g per planting hole + recommended fertilizer, P = PGPR 250 mL + recommended fertilizer, and MP = PGPR 250 mL + mycorrhiza 30 g per planting hole + recommended fertilizer (Table 1). Each experimental unit was a plot containing randomly selected sample plants, which were labeled for observation. The data were analyzed according to the RCBD model $Y_{ij} = \mu + \tau_i + \beta_j + \varepsilon_{ij}$, where Y_{ij} is the observation for the *i*-th treatment in the *j*-th block, μ is the general mean, τ_i is the treatment effect, β_j is the block effect, and ε_{ij} is the experimental error.

Table 1. Treatments applied in the experiment

Code	Treatment	Description
K	Control	Recommended inorganic fertilizer (100%) without biological agents
M	Mycorrhiza	Mycorrhiza 30 g per planting hole + recommended inorganic fertilizer (100%)
P	PGPR	PGPR 250 mL + recommended inorganic fertilizer (100%)
MP	PGPR + mycorrhiza	PGPR 250 mL + mycorrhiza 30 g per planting hole + recommended inorganic fertilizer (100%)

Note: Planting spacing 70 cm × 30 cm; each treatment was replicated in blocks.

The land was plowed, harrowed, and formed into raised beds. Seeds were sown at a spacing of 70 cm × 30 cm with one/two seeds per hole and thinned to one plant per hole at days after sowing (DAS). In treatments M and MP, 30 g of mycorrhizal inoculant was placed in the planting hole at sowing so that the seed was in direct contact with the inoculum. In treatments P and MP, PGPR was applied at 250 mL per plant/per plot; the concentration of the working solution was determined by seed soaking/soil drenching. Plants were irrigated by method and frequency, but water supply was limited during part of the season due to reduced flow from the irrigation source. Weeds were controlled manually. Ears were harvested at the fresh-consumption stage.

Air temperature and relative humidity were recorded in the morning at time using a thermohygrometer. Soil temperature was measured with a soil thermometer at a depth, and soil moisture was measured with an instrument at a frequency. The occurrence of pests and diseases (downy mildew, corn smut, gray leaf spot, grasshoppers, and fall armyworm) was recorded during routine observations. The intensity of damage by grasshoppers and fall armyworm was estimated as the percentage of damaged plants/leaf area per plot, using the formula.

The observed variables are listed in Table 2. Plant height was measured from the soil surface to the tip of the highest leaf or base of the tassel; stem diameter was measured above the soil surface

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with a caliper, and the number of fully expanded leaves was counted. Male and female flower emergence were recorded as the number of days from sowing until 50% of the plants in the plot had shed pollen and had visible silks, respectively. At harvest, the ear weight with husk, ear weight without husk, ear length, and ear diameter (measured at the middle of the ear) were determined. The fresh weight of the stalk was determined immediately after harvest, and the dry weight after oven-drying. Root weight was determined after the roots were dug out, washed, and air- or oven-dried.

Table 2. Observed variables, units, and methods of observation

Variable	Unit	Method
Plant height	cm	Soil surface to tip of highest leaf, measuring tape
Stem diameter	cm	Caliper, above soil surface
Number of leaves	leaves	Fully expanded leaves counted per plant
Male / female flower emergence	DAS	Days from sowing to pollen shedding / silk emergence
Fresh / dry stalk weight	g	Weighed at harvest / after oven-drying
Root weight	g	Roots excavated, washed, and weighed
Ear weight (with / without husk)	g	Weighed at harvest
Ear length and diameter	cm	Ruler and caliper at mid-ear

The data were analyzed using analysis of variance (ANOVA) for an RCBD on a computer. The effect of the treatments was considered significant when the calculated F-value exceeded the tabulated F-value at the 5% level. Means were compared using Duncan's multiple range test (DMRT) at the 5% level when the effect was significant. Because none of the effects were significant, treatment means are presented without mean separation. To describe the direction and size of the responses, the relative change of each treatment compared with the control was calculated as $(X_t - X_k)/X_k \times 100$, where X_t is the mean of the treatment and X_k is the mean of the control. These relative changes are descriptive and should not be interpreted as significant treatment effects.

RESULTS

The experiment took place under harsh environmental conditions (Table 3). The soil was strongly alkaline (pH 9.1), the soil temperature reached 31 °C, and the soil moisture was only about 50%. Morning air temperature ranged from 25 to 31 °C, and relative humidity varied widely from 60% to 96%. Because the study was conducted during the dry season, water supply from the irrigation source declined, resulting in periods of water deficit for the crop.

Table 3. Environmental conditions at the experimental site

Parameter	Value
Altitude	146 m above sea level
Season	Dry season (July–September 2024)
Air temperature (daily range)	23–34 °C
Morning air temperature	25–31 °C
Morning relative humidity	60–96%
Soil pH	9.1 (strongly alkaline)
Soil temperature	up to 31 °C
Soil moisture	about 50%
Irrigation	Limited; decreasing water flow from the source

The ANOVA showed that the application of mycorrhiza and PGPR, either alone or in combination, had no significant effect on any of the observed variables (Table 4). Nevertheless, the

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treatment means showed consistent trends. The PGPR treatment (P) gave the highest values for plant height (122.19 cm), stem diameter (1.74 cm), number of leaves (8.36), root weight (13.22 g), dry stalk weight (91.25 g), ear weight (37.03 g), ear weight without husk (16.33 g), ear length (18.33 cm), and ear diameter (1.43). In contrast, the combined treatment (MP) produced the lowest values for most variables, including plant height (100.61 cm), root weight (9.25 g), stalk weight, ear weight (17.11 g), and ear weight without husk (5.19 g).

Table 4. Mean values of growth, flowering, and yield variables of waxy corn under mycorrhiza and PGPR treatments

Variable	K	M	P	MP
Plant height (cm)	112.08	105.72	122.19	100.61
Stem diameter (cm)	1.49	1.58	1.74	1.53
Number of leaves	7.81	7.61	8.36	7.56
Male flower emergence (DAS)	31.06	31.97	38.69	30.33
Female flower emergence (DAS)	32.42	23.86	47.39	25.37
Fresh stalk weight (g)	95.63	114.97	103.19	71.03
Dry stalk weight (g)	74.17	87.31	91.25	51.31
Root weight (g)	13.03	10.33	13.22	9.25
Ear weight with husk (g)	22.81	31.03	37.03	17.11
Ear weight without husk (g)	13.69	14.39	16.33	5.19
Ear length (cm)	8.71	12.42	18.33	8.75
Ear diameter (cm)	0.90	1.34	1.43	0.68

Note: K = control; M = mycorrhiza; P = PGPR; MP = PGPR + mycorrhiza; DAS = days after sowing. All differences among treatments were not significant (ns) according to ANOVA at the 5% level. Highlighted values should be verified (K female flowering was written as “32..42” in the original data; the unit of ear diameter was given as mm).

The flowering data showed large differences among treatments, although these were not significant. Male flowering occurred at 30.33–38.69 days after sowing, and female flowering at 23.86–47.39 days. In the PGPR treatment, both male and female flowering were later than in the other treatments.

Table 5. Relative change (%) of growth and yield variables compared with the control

Variable	M vs K (%)	P vs K (%)	MP vs K (%)
Plant height	−5.7	+9.0	−10.2
Stem diameter	+6.0	+16.8	+2.7
Number of leaves	−2.6	+7.0	−3.2
Root weight	−20.7	+1.5	−29.0
Fresh stalk weight	+20.2	+7.9	−25.7
Dry stalk weight	+17.7	+23.0	−30.8
Ear weight with husk	+36.0	+62.3	−25.0
Ear weight without husk	+5.1	+19.3	−62.1
Ear length	+42.6	+110.4	+0.5
Ear diameter	+48.9	+58.9	−24.4

The relative changes compared with the control are presented in Table 5 and Figure 1. PGPR increased all growth and yield variables compared with the control, with the smallest changes in root weight (+1.5%), number of leaves (+7.0%), and plant height (+9.0%) and the largest changes in ear weight (+62.3%), ear diameter (+58.9%), and ear length (+110.4%). Mycorrhiza alone increased stalk weight and ear traits (+5.1% to +48.9%) but slightly reduced plant height (−5.7%), number of leaves (−2.6%), and root weight (−20.7%). The combination of PGPR and mycorrhiza reduced most

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variables compared with the control, particularly ear weight without husk (−62.1%), dry stalk weight (−30.8%), and root weight (−29.0%).

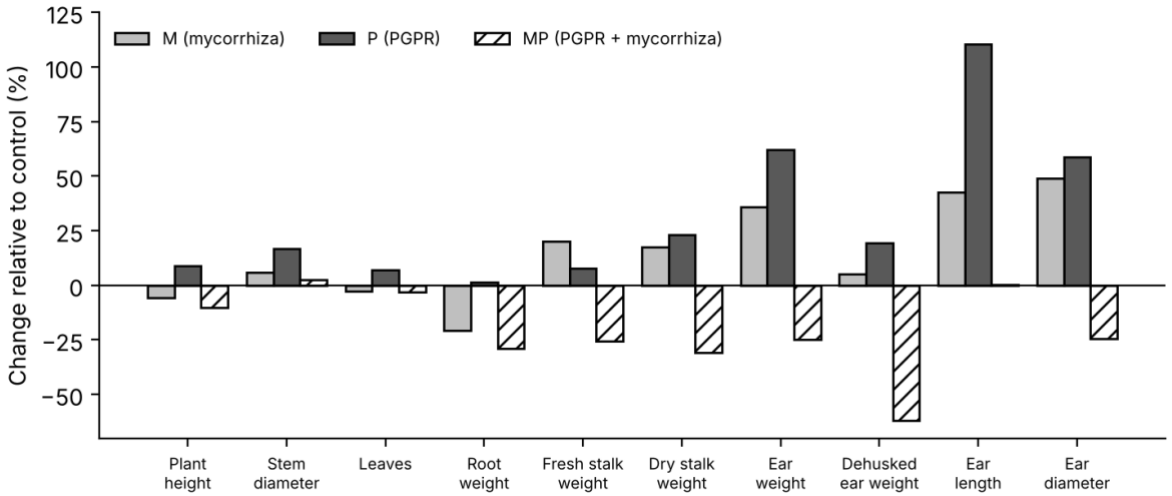


Figure 1. Relative change of growth and yield variables of waxy corn in the mycorrhiza (M), PGPR (P), and combined (MP) treatments compared with the control (K); differences were not statistically significant

All treatments were affected by several pests and diseases during the growing season (Table 6). Downy mildew caused by *P. maydis* was present in the field, as expected for an endemic area. The intensity of gray leaf spot increased as the plants grew older. Corn smut caused by *U. maydis* appeared on the ears at harvest time in all treatments and made part of the ears unmarketable. Grasshoppers and fall armyworm were the dominant insect pests, each reaching an attack intensity of about 30%. Leaves damaged by grasshoppers had ragged and torn margins, whereas fall armyworm damage was characterized by holes in the leaves and black or brown frass at the growing points.

Table 6. Pests and diseases observed in the experimental field

Organism	Type	Observation in the field
Peronosclerospora maydis	Downy mildew	Present; endemic at the site over several seasons
Cercospora sp.	Gray leaf spot	Intensity increased with plant age
Ustilago maydis	Corn smut	Galls on ears at harvest in all treatments
Grasshoppers	Leaf-feeding insects	Ragged, torn leaf margins; intensity about 30%
Spodoptera frugiperda	Fall armyworm	Leaf holes and frass at growing points; intensity about 30%

DISCUSSION

This study showed that mycorrhiza and PGPR, applied alone or in combination with the recommended inorganic fertilizer, did not significantly improve waxy corn growth or yield in a downy mildew-endemic field. Nevertheless, PGPR alone consistently yielded the highest values for growth and yield components, whereas the combination of the two agents gave the lowest. The very low ear weights in all treatments (17–37 g) show that the crop was severely stressed, and the results must be interpreted in the light of the harsh conditions under which the experiment was carried out.

The most likely explanation for the lack of significant effects is that several strong constraints acted simultaneously, limiting both crop growth and the activity of the biological agents. The soil was

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strongly alkaline (pH 9.1). At such a high pH, the availability of phosphorus, iron, zinc, and manganese is low, and the root growth and nutrient uptake of maize are reduced. Soil alkalinity is also detrimental to many AMF species: in alkaline soils of India, AMF diversity declined, and only a few tolerant taxa in the family Glomeraceae remained common (Parihar et al., 2019). A global meta-analysis likewise showed that the effect of AMF inoculation on cereal yield depends on soil pH (Zhang et al., 2019). The commercial inoculum used in this study was probably not adapted to such a high pH, which may explain why mycorrhiza alone did not improve root weight; in fact, the root weight in M was 20.7% lower than in the control.

Water deficit was another important constraint. The study was conducted during the dry season, and soil moisture was only about 50%. AMF can improve plant water status and help them tolerate drought (Begum et al., 2019), and AMF inoculation has increased crop yields under rainfed conditions (Wu et al., 2022). However, both the germination of AMF spores and the colonization of roots require adequate soil moisture, and the survival and root colonization of introduced PGPR also decline in dry soil (Basu et al., 2021). Under severe water deficit, the inoculated organisms may not have become established in sufficient numbers to influence plant growth. In addition, the soil temperature of 31 °C and air temperatures up to 34 °C were close to or above the threshold of about 30 °C above which maize pollination and kernel set are impaired (Waqas et al., 2021). Heat and drought during flowering reduce pollen viability, silk receptivity, and kernel number (Li et al., 2022), consistent with the small ears observed in this study.

The high pressure from diseases and pests further reduced crop performance. Downy mildew is a systemic disease, and plants infected at an early stage are stunted and produce small or no ears (Ginting et al., 2020). Biological agents generally act preventively by improving plant vigor or inducing resistance, and their protective effect develops gradually after colonization (Zhu et al., 2022). When disease pressure is very high, as in an endemic field during conditions favorable to the pathogen, this indirect protection may be insufficient. In Indonesia, biological control of downy mildew has been most effective when antagonistic bacteria were applied as consortia rather than as single agents (Mugiastuti et al., 2023). Corn smut appeared on the ears in all treatments at harvest. *U. maydis* infects young, actively growing tissues, and hot and dry weather during flowering favors infection (Yu et al., 2023), so the dry-season conditions in this study were favorable for the disease. Gray leaf spot increased with plant age, reducing the photosynthetic leaf area during ear filling (Nsibo et al., 2024), and defoliation by grasshoppers and the fall armyworm, each at about 30%, further reduced the assimilant source (Herlinda et al., 2022).

Although not significant, the consistent advantage of PGPR is worth noting. PGPR produce phytohormones such as auxins, gibberellins, and cytokinins, which stimulate root growth and cell elongation, and can improve nutrient availability through nitrogen fixation and phosphate solubilization (Backer et al., 2018; Basu et al., 2021). Many PGPR strains also induce systemic resistance, which primes plant defenses against pathogens and insects (Zhu et al., 2022). The higher plant height, stem diameter, and ear traits in the PGPR treatment suggest that these mechanisms operated to some extent even under stress. Bacteria multiply quickly and, when applied as a liquid inoculant near the roots, may colonize the rhizosphere faster than AMF, which need several weeks to form a functional symbiosis. This may explain why PGPR alone gave a more visible response than mycorrhiza within a short growing season.

The poor performance of the combined treatment was unexpected, because AMF and PGPR are often reported to act synergistically (Ganugi et al., 2022; Khan et al., 2024). Several factors may explain this result. When two inoculants are applied together, the organisms can compete for root exudates and colonization sites, particularly when the plant's carbon supply is limited by stress.

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Under severe stress, the carbon cost of supporting both symbionts may outweigh the benefits they provide, so the plant grows less than it would without inoculation. The success of co-inoculation depends on strain compatibility and the strain ratio; for example, the positive effect of AMF–*Bacillus* co-inoculation on maize depended on an optimal inoculation rate (Ganugi et al., 2022). In addition, the large variation in the field, caused by uneven disease and pest pressure, may have affected some plots in this treatment more strongly than others. Because the differences were not significant, the negative trend should be confirmed in further experiments before drawing firm conclusions.

The flowering data require careful interpretation. In normal maize development, male flowering precedes or coincides with female flowering, and a long interval between the two indicates stress (Waqas et al., 2021; Li et al., 2022). In the PGPR treatment, female flowering occurred about 9 days after male flowering, suggesting that the plants experienced stress around flowering. The values recorded for the mycorrhiza and combined treatments, in which female flowering appeared earlier than male flowering, are unusual and need to be verified against the field records. Accurate flowering data are important, because a delay in silking relative to pollen shedding is one of the main causes of poor kernel set under heat and drought stress.

From a practical perspective, the results show that biological agents alone cannot overcome severe abiotic and biotic constraints. In downy mildew-endemic areas with alkaline soils and limited water, mycorrhiza and PGPR should be used as part of an integrated management system. Such a system should include adequate irrigation, especially around flowering; amelioration of soil alkalinity with organic matter or acidifying amendments; the use of downy mildew-tolerant varieties and timely sowing to avoid peak disease pressure; and monitoring and control of fall armyworm and other pests. Indigenous AMF and PGPR strains adapted to alkaline and dry soils are also more likely to be effective than commercial inocula developed for other conditions (Parihar et al., 2019; Basu et al., 2021). The good agronomic value of waxy corn and its strong market demand justify further efforts to develop such integrated practices (Yu and Moon, 2022).

The results also illustrate a broader issue in the use of biological inputs. Commercial inoculants are usually evaluated under favorable conditions, and their labels rarely state the soil pH, moisture, or temperature range in which they are effective. Farmers who apply them under unfavorable conditions may therefore see little benefit and lose confidence in biological products. Field trials under realistic constraints, such as those in the present study, are thus valuable even when the effects are not significant, because they show the limits of biological agents and help define the conditions under which they should be recommended (Basu et al., 2021). Reporting such results also prevents an overly optimistic picture of biofertilizer performance based solely on successful experiments, a recognized problem in evaluating the field efficacy of AMF and PGPR (Zhang et al., 2019; Backer et al., 2018).

This study has several limitations. The experiment was conducted in only one season and one location; the number of replications was small; and root colonization by AMF and the rhizosphere population of PGPR were not measured, so it is not possible to confirm whether the inoculants became established. The incidence and severity of downy mildew and the other diseases were not quantified for each treatment, and the flowering data contain values that need verification. Future studies should include measurements of mycorrhizal colonization and PGPR populations, quantitative disease assessments, more replicates, experiments in both the dry and rainy seasons, and tests of indigenous strains adapted to alkaline soils.

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CONCLUSION

The application of mycorrhiza and PGPR, either alone or in combination with the recommended inorganic fertilizer, did not significantly affect the growth, flowering, or yield components of waxy corn grown in a downy mildew-endemic field. PGPR alone showed a consistent positive trend, yielding the highest plant height (122.19 cm), stem diameter (1.74 cm), ear weight (37.03 g), and ear length (18.33 cm), whereas the combination of mycorrhiza and PGPR produced the lowest values for most yield components. Strongly alkaline soil (pH 9.1), dry-season water deficit, high temperature, and heavy pressure from downy mildew, corn smut, gray leaf spot, grasshoppers, and fall armyworm were the main factors limiting the effectiveness of the biological agents, so their use in such fields must be integrated with soil, water, and pest management.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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