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Plant-growth-promoting rhizobacteria and kinetin as ways to promote corn growth and yield in a short-growing-season area

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Abstract

The base temperature for germination of corn is approximately 10°C, which results in slow germination and emergence of corn crops sown into cool soils. The effects of plant-growth-promoting rhizobacteria (PGPR) and kinetin on grain and sweet corn emergence, plant growth and yield were studied under short season conditions in 1996 and 1997. Two PGPR strains (*Serratia proteamaculans* 1-102 and *Serratia liquefaciens* 2-68) were used. The kinetin concentrations were 0, 1 and 5 μM. The experiment was structured as a randomized complete block design with four replicates. The plant growth responses were variable and depended on the PGPR strain, harvest date and growth parameters evaluated. There were interactions among PGPR, kinetin and corn hybrid. PGPR provided a greater stimulation of seedling emergence than kinetin. PGPR strain 1-102 was best at promoting emergence. One month after planting, both PGPR and kinetin increased plant growth, and PGPR strain 2-68 resulted in a greater growth than that of strain 1-102. PGPR strain 2-68 plus 1 μM kinetin was the best treatment for promoting plant growth. The plant height and root dry weight of sweet corn were less affected than those of grain corn. The effects of PGPR on plant growth decreased as the plants developed. Two months after planting, there were no effects of kinetin on plant growth, however, PGPR still had positive effects on the leaf area of grain corn, but they decreased the leaf area of sweet corn. The plant dry weight of grain corn was increased by PGPR strain 2-68. The grain corn yield was increased by PGPR strain 2-68 in both years. In 1997, PGPR strain 2-68 increased the sweet corn yield. Kinetin alone had no effects on yields in either year for the two cultivars studied.

Keywords: Corn; Kinetin; Plant-growth-promoting rhizobacteria

1. Introduction

Corn is a major crop in eastern Canada, an area where the growing period is short, and spring temperatures are low. Corn has been widely used for grain and forage in this area (Bureau de la Statistique du Québec, 1994). Intensive corn production has resulted in increasing energy consumption and potential for groundwater pollution (Liang et al., 1991). Alternatives are being sought by the combination of a gradual reduction of the use of fertilizers, on the one hand, and a greater use of plant biological and genetic potential, and microbial effects that can increase corn production on the other hand.

In short season areas, crops are seeded into cool soils to maximize the length of the production season. Low temperature reduces the emergence

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rate (Mock and McNeill, 1979) and increases susceptibility to seed and seedling diseases, which results in poor stand establishment (Dubetz et al., 1962). It can also make the locally adapted weeds very competitive early in the season.

Free-living soil bacteria beneficial to plant growth are usually referred to as 'PGPR' (Kloepper et al., 1989) or 'yield-increasing' bacteria (Piao et al., 1992). PGPR have the potential to contribute to the development of sustainable agricultural systems (Schippers et al., 1995). PGPR can affect plant growth directly or indirectly. Indirect promotion of plant growth occurs when PGPR lessen or prevent the deleterious effects of one or more phytopathogenic organisms. The direct promotion of plant growth by PGPR generally entails either the bacterium providing a compound that acts as a plant growth regulator or facilitating the uptake of certain nutrients from the environment (Glick, 1995).

Suslow and Schroth's study (Suslow and Schroth, 1982) showed that sugar beet seedling emergence was not affected by PGPR treatment. Later work on soybean and canola showed that PGPR increased seedling emergence when soil temperatures were below 20°C, and they termed such bacteria as 'emergence-promoting rhizobacteria' (Kloepper et al., 1986). Biocoating of seeds, such as radish, with PGPR could also improve plant establishment (Schippers et al., 1995).

Co-inoculation of PGPR with *Bradyrhizobium* has been shown to increase nodulation and nitrogen fixation by soybean at suboptimal root zone temperatures (Zhang et al., 1997). The effect of different strains varied with root zone temperature. The increases in soybean nodule number and level of activity due to PGPR were largely due to improved overall physiological performance and growth of the plants.

Kinetin regulates cell division and differentiation in plants. Kinetin has been shown to improve germination and emergence of crop seeds at low temperatures (Carpenter and Osmark, 1992; Wang et al., 1996). It may also play a vital role in situations during which plants suffer from inadequate fertilization (Kuiper and Staal, 1987). Kinetin increased plant height, stem diameter, branching, and caused early flowering of *Adonis automnalis* (Abdalla et al., 1985). Work in our laboratory, under controlled environment conditions, showed that corn and soybean seedlings were healthier with kinetin than with GA_3 treatments, and 5 μM kinetin was the most effective concentration for increasing germination rate, percentage germination and early seedling development of corn under low temperature (Wang et al., 1996). The effects of kinetin on crop yield have not been studied thoroughly (Frankenberger and Arshad, 1995), and the combined effects of kinetin and PGPR on corn emergence and yield under field conditions have not been tested.

The objective of this work was to determine the ability of kinetin and PGPR to improve corn emergence, plant growth and final yield under field conditions in a short-growing-season area.

2. Materials and methods

The experiments were carried out in 1996 and 1997 at the Emile A Lods research centre of McGill University (Ste Anne de Bellevue, Québec). A completely randomized block design was used for both experiments. Kinetin and PGPR experiments were conducted separately in 1996 on a sandy loam soil and each had four replicates. In 1997, kinetin and PGPR were combined factorially into one experiment on a loam soil with three replicates. The surface of each plot was 22.5 m^2. Corn plant populations were thinned to 50 per plot for sweet corn and 120 per plot for grain corn.

The same two corn hybrids were used in both years. The grain corn hybrid was Pioneer 3978, and the sweet corn hybrid was Combo (WH Perron, Quebec).

Two PGPR strains were used, *Serratia proteamaculans* 1-102 (Yellowknife, NWT) and *Serratia liquefaciens* 2-68 (James Bay soil, NWT). These were selected because of their previously demonstrated beneficial effects on soybean (Zhang et al., 1997). PGPR were cultured in *Pseudomonas* media (Polonenko et al., 1987) in 250-ml flasks shaken at 250 rpm at 25°C for 1.5 days. A 1-day-old PGPR subculture was diluted to an OD_{420} of 0.1 (cell density approximately 10^8 ml^{-1}) and used as inoculant. Inoculation was applied evenly along

the row at a rate equal to 1 ml per seed using a 50-ml syringe.

Kinetin (from Sigma, Ontario) concentrations were 0, 1 and 5 μM in both years. Kinetin concentrations were selected according to previous indoor work (Wang et al., 1996). To estimate the amount of kinetin to be applied to each of the seeds, 100 seeds of each corn hybrid (from the same sources as the seed used in the field study) were weighed before and after complete imbibition. The difference in weight provided an estimate for the amount of water imbibed by the 100 seeds. The amount of kinetin to be applied to each seed for the 1 and 5 μM concentrations was calculated based on the amount that would have been contained in the amount of water taken up for 1 and 5 μM solutions. A 1% solution of carboxymethyl cellulose sodium salt was used as a sticker for the kinetin and was added to kinetin solutions and then mixed with seeds before sowing. Equal amounts of water and sticker were applied to the seeds of the control plots.

2.1. Data collection and analysis

The time of 50% seed emergence and the final seedling number [24 days after planting (DAP)] were recorded in 1996. The number and time of seedling emergence (1 cm above the soil surface) were recorded successively until the maximum seedling number was reached in 1997. Corn was sown on 17 May 1996 and 22 May 1997. Plants were harvested three times during the growing season: approximately 1 month after planting (V4 stage, four-leaf; Iowa State University of Science and Technology, 1986), approximately 2 months after planting (V12 stage, 12-leaf), and final harvest. For sweet corn, the final harvests (R3, milk stage) were on 14 August 1996 and 16 August 1997; for grain corn (R6, physiological maturity) 26 September 1996 and 5 October 1997. At each harvest, plant height, leaf area and plant dry weight were recorded. SPAD readings (Chlorophyll metre SPAD-502, 1989 Minolta Camera Co., Ltd, Japan) were used to monitor leaf chlorophyll concentration changes (Earl and Tollenaar, 1997) and were taken every week during the growing season.

The results were analysed statistically by analysis of variance using the Statistical Analysis System Computer Package (SAS Institute) using an alpha value of 0.05. Preplanned comparisons between kinetin or PGPR treatments and the control means were also made using single degree of freedom of contrasts (Steel and Torrie, 1980). When differences were significant at levels between 0.1 and 0.05, the P values are given.

3. Results and discussion

3.1. Corn emergence

Different responses of seedling emergence to PGPR and kinetin were found in the two year-experiments. In 1996, 5 μM kinetin increased early seedling emergence for both grain corn and sweet corn. PGPR strain 2-68 also increased early seedling emergence (Table 1). There were no effects of kinetin and PGPR on the final seedling number. Thus, whereas both PGPR and kinetin accelerated corn emergence, neither increased final stand.

In 1997, PGPR improved seedling emergence, but no differences could be found at the last two countings for strain 1-102 (Table 2). Strain 1-102 alone had the greatest effect on early seedling development of all treatments. Strain 2-68 alone or combined with kinetin also stimulated early seedling development. The final number of seedlings established was increased by strain 1-102 or

Table 1
Fifty per cent seed emergence time for sweet corn and grain corn in 1996[a]

	Treatment	Days after planting
Kinetin	Water	13.0
	1 μM	12.0
	5 μM	11.5*
PGPR	Water	14.1
	1-102	13.5
	2-68	12.4*

[a] No interaction was found for the two corn species, with kinetin or PGPR treatments. Data were the combination of the two corn species. Means are compared with water control within kinetin or PGPR treatment. * Means different at 0.05 level of probability.

Table 2
Corn seedling number at four measurement times with different PGPR and kinetin treatments in 1997[a]

PGPR	Kinetin (μM)	5 June	9 June	11 June	13 June
Water	Water	45.85	80.67	84.00	85.00
Water	1	39.67*	83.33	87.16	87.83
Water	5	48.50	81.00	85.67	86.83
1-102	Water	52.50*	88.33**	90.00**	91.33**
1-102	1	45.50	83.17	84.17	86.50
1-102	5	44.83	81.00	84.17	86.17
2-68	Water	49.00	86.50**	89.17*	89.17*
2-68	1	47.67	84.67*	88.33*	88.50
2-68	5	47.83	86.00*	89.50*	89.83*
Contrast					
1-102 vs. water		**	*	ns	ns
2-68 vs. water		*	**	*	0.07
1 μM kinetin vs. water		ns	*	ns	ns
5 μM kinetin vs. water		ns	0.08	ns	ns

[a] Means in the same column are compared with the first mean (pre-planned comparison with the control). *, ** Means different at the 0.05 and 0.01 levels of probability.

2-68 alone, and strain 2-68 in combination with 5 μM kinetin. The improvement in seedling emergence by PGPR was also found in work with soybean and canola at low soil temperature (Kloepper et al., 1986), where it was found that some PGPR induced increases in seedling emergence, in some cases achieving increases up to 100% greater than controls. The only effect of kinetin on seed emergence in 1997 was at the second counting, when it decreased emergence.

3.2. Corn growth

In 1996, 1 μM kinetin increased grain corn leaf area and decreased root dry weight at 35 DAP (Table 3). The decrease in root dry weight by kinetin is in agreement with its physiological role on plant morphogenesis (Skoog and Armstrong, 1970). The above-ground shoot dry weight was decreased by 5 μM kinetin for grain corn at 64 DAP. For sweet corn, no effects were found at 35 DAP. One micromolar kinetin increased sweet corn leaf area, and 5 μM kinetin increased sweet corn shoot dry weight at 64 DAP. PGPR strain 1-102 increased grain corn leaf area and root dry weight at 35 DAP (Table 4). Leaf area and shoot dry weight were increased by PGPR strain 2-68 for both corn hybrids at 64 DAP. Plant height and chlorophyll concentration were not affected by the applied treatments (data not shown).

Table 3
Kinetin effects on corn leaf area, shoot dry weight and root dry weight at 35 and 64 days after planting in 1996[a]

	Treatment	35 days after planting			64 days after planting	
		Leaf area (cm^2)	Shoot dry weight (g)	Root dry weight (g)	Leaf area (cm^2)	Shoot dry weight (kg)
Grain corn	Water	398.2	3.03	0.73	2254.5	0.479
	1 μM	420.6*	2.90	0.65*	2069.3*	0.467
	5 μM	387.5	3.28	0.73	2247.3	0.433*
Sweet corn	Water	297.1	1.90	0.30	1837.0	0.324
	1 μM	272.8	1.75	0.30	2037.0*	0.327
	5 μM	304.4	2.13	0.31	1860.0	0.416**

[a] Means in each column of the same cultivar are compared with the control (water treatment). *, ** Means different at 0.05 and 0.01 levels of probability.

Table 4
PGPR effects on corn leaf area, shoot dry weight and root dry weight at 35 and 64 days after planting in 1996[a]

		35 days after planting			64 days after planting	
		Leaf area (cm^2)	Shoot dry weight (g)	Root dry weight (g)	Leaf area (cm^2)	Shoot dry weight (kg)
Grain corn	Water	354.6	3.03	0.67	2008.5	0.381
	1-102	429.8**	3.30	0.78*	2131.0	0.403
	2-68	375.9	3.45	0.67	2315.2**	0.481**
Sweet corn	Water	300.1	2.21	0.27	1910.3	0.336
	1-102	315.7	2.17	0.30	1989.4*	0.358
	2-68	320.8	2.34	0.31	2053.7*	0.387*

[a] Means in each column of the same cultivar are compared with the control (water treatment). *, ** Means different at 0.05 and 0.01 levels of probability.

In 1997, data collected at 1 month after planting showed that both PGPR and kinetin increased plant growth compared to the controls (Table 5), as shown by the contrasts. Kinetin had stimulating effects on some of the measured variables, but these effects were infrequent and not consistent across the corn hybrids (Table 5). Generally speaking, PGPR were more effective than kinetin in promoting plant growth. PGPR strain 2-68 plus 1 μM kinetin was the best treatment for promoting plant growth at this time. Plant height and root dry weight of sweet corn were less affected by this treatment than grain corn. Corn leaf area was the variable most increased by the combination of PGPR and kinetin (Table 5).

Two months after planting, the relative effects of PGPR on plant growth were less than at the first harvest, and there were no differences due to kinetin alone (Table 6). There would be less available exogenous kinetin in the rhizosphere as the plants grow. However, larger plants might be less sensitive to PGPR or kinetin. Enhanced plant

Table 5
Plant height, leaf area, shoot and root weight 32 days after planting in 1997[a]

PGPR	Kinetin (μM)	Grain corn				Sweet corn			
		Plant height (cm)	Leaf area (cm^2)	Shoot dry weight (g)	Root dry weight (g)	Plant height (cm)	Leaf area (cm^2)	Shoot dry weight (g)	Root dry weight (g)
Water	0	25.0	299.3	2.15	0.26	24.7	185.3	1.86	0.19
Water	1	30.3**	378.3**	2.86*	0.29	24.3	237.0**	1.62*	0.18
Water	5	28.0**	350.7**	2.10	0.29	25.3	285.3**	1.96	0.23
1-102	0	28.3**	346.3**	2.66	0.34*	25.7	279.7**	1.90	0.20
1-102	1	27.7*	331.3	2.09	0.30*	25.0	258.3**	2.14*	0.19
1-102	5	27.7*	356.3**	2.61	0.31*	24.7	308.3**	2.01	0.23
2-68	0	29.7**	362.7**	3.58**	0.31*	27.0*	285.7**	1.92	0.17
2-68	1	28.7**	376.3**	2.98**	0.39**	27.0*	289.3**	2.28*	0.24*
2-68	5	28.3**	357.3**	2.68	0.32**	27.3**	268.7**	1.99	0.21
Contrast									
1-102 vs. water		ns	ns	ns	**	ns	**	**	ns
2-68 vs. water		0.06	*	**	**	**	**	**	ns
1 μM kinetin vs. water		*	*	ns	0.09	ns	ns	0.08	ns
5 μM kinetin vs. water		ns	0.055	*	ns	ns	**	ns	*

[a] Data were collected on 24 June 1997. Treatment without PGPR and kinetin was the control. Means in the same column are compared with the control.*, ** Means different at 0.05 and 0.01 levels of probability.

Table 6
Plant height, leaf area and above ground plant dry weight 64 days after planting in 1997[a]

	Kinetin (μM)	Grain corn			Sweet corn		
		Plant height (cm)	Leaf area (cm^2)	Plant dry weight (g)	Plant height (cm)	Leaf area (cm^2)	Plant dry weight (g)
Water	0	196.3	4310	0.500	168.0	3262	0.430
Water	1	216.3	4504	0.481	159.0	3062*	0.361**
Water	5	200.0	4384	0.497	170.3	3022*	0.381
1-102	0	220.7	4874**	0.481	181.0	3070*	0.439
1-102	1	192.7	4782**	0.541	178.3	2924**	0.446
1-102	5	189.3	4762*	0.531	183.0	2790**	0.358*
2-68	0	204.3	4950**	0.570**	187.3	3050*	0.446
2-68	1	192.7	4520	0.549*	183.7	2918**	0.410
2-68	5	195.0	4806**	0.526	184.3	3220	0.459
Contrast							
1-102 vs. water		ns	**	0.07	0.08	**	ns
2-68 vs. water		ns	**	**	*	ns	*
1 μM kinetin vs. water		ns	ns	ns	ns	**	ns
5 μM kinetin vs. water		ns	ns	ns	ns	**	*

[a] Plants were harvested on 24 June 1997. Treatment without PGPR and kinetin was the control. Means in the same column are compared with the control.*, ** Means different at 0.05 and 0.01 levels of probability.

growth by PGPR was also observed only during the early part of the growing season by other researchers (Kloepper et al., 1986). No differences were found for plant height. PGPR had a positive effect on the leaf area of the grain corn hybrid, but decreased the leaf area of sweet corn. The plant dry weight of grain corn was increased by PGPR strain 2-68 alone or when combined with 1 μM kinetin. Sweet corn plant dry weights were decreased by 1 μM kinetin alone and by PGPR strain 1-102 combined with 5 μM kinetin.

Analysis of variance showed that for most growth variables, there were interactions between PGPR and kinetin. The causes of these interactions are not known. The functions attributed to kinetins may be overlapped by other phytohormones and may be the net result of their interaction (Frankenberger and Arshad, 1995). Höflich (1992) observed stimulated growth and yield of several crops in pot and field experiments in response to inoculation with *Pseudomonas fluorescens* and attributed the effects to the phytohormone production ability of the inoculant. It is not known whether the two PGPR strains used here produce phytohormones. Generally speaking, PGPR can excrete phytohormones, such as auxin and cytokinin [for a review see, Arshad and Frankenberger (1998)], and these can be active in promoting or inhibiting plant growth. Corn plants can also excrete zeatin and derivatives, which promote cell division (Miller, 1965). Exogenous application of kinetin to corn seed could have disrupted the phytohormone balance, such as the kinetin/auxin ratio, in the micro-environment of the soil, and therefore directly affected PGPR and/or plant growth. Interactions between PGPR and soybean genotypes were previously observed (Dashti et al., 1997). In our work, plant height was less affected than other variables, and no effect on plant height could be seen 2 months after planting. Differences between grain corn and sweet corn were obvious, and their responses to PGPR and kinetin differed in 1997, although not in 1996 (Tables 1–6). Work on lentil and pea (Chanway et al., 1989) showed differences in the plant-growth-promoting ability of two PGPR strains, which were at least in part attributable to plant genotype effects.

There were no effects of PGPR or kinetin on

SPAD readings. Again, the only detectable difference was between the two hybrids (data not shown).

3.3. Corn yield

There was an increase in grain corn yield by PGPR strain 2-68 in 1996 ($P=0.09$, Fig. 1A). Kinetin had no effect on grain corn yield. No effects of either PGPR or kinetin could be detected on sweet corn yield in 1996.

In 1997, PGPR strain 2-68 again increased grain corn yield (Fig. 1A). No synergistic effect of PGPR and kinetin on grain corn yield was found. As in 1996, no difference was found for grain corn with kinetin treatments. A PGPR-by-kinetin interaction existed for sweet corn yield in 1997 (Fig. 1B). PGPR strain 2-68 alone increased sweet corn yield compared to the water control. There was an increase ($P=0.065$) in yield with 5 μM kinetin for both corn types. No effect could be seen with 1 μM kinetin application.

Differences in yields between the two years may have been due to differences in soil type and/or climate at the two sites. In 1996, the corn was grown on a sandy-loam soil, and the previous crop was pasture. In 1997, the corn was grown on a loam soil, and the previous crop was soybean. In 1996, drought conditions occurred during the grain-filling period, with only 22.5 mm of rainfall in August. As pointed out by Schippers et al. (1995), optimal functioning of PGPR strains is strongly influenced by environmental factors including soil characteristics, plant species and even plant genotypes within a species, and other rhizosphere microflora. Less than optimal or unfavourable conditions may lead to little or no synthesis of photohormones in the root zone, or other beneficial effects, resulting in the failure of PGPR to promote plant growth.

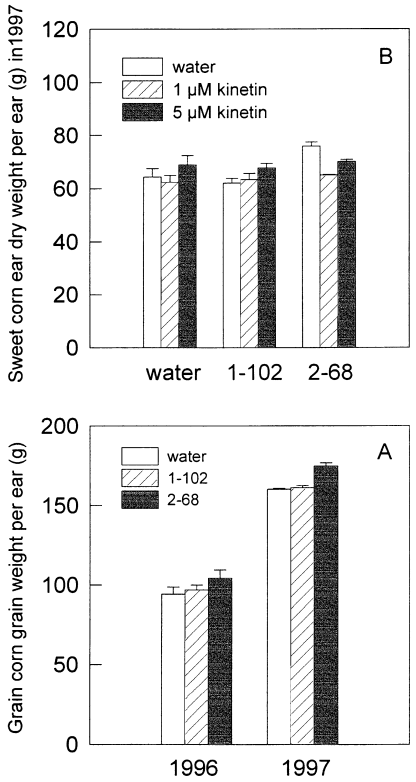

Fig. 1. PGPR and kinetin effects on grain corn grain weight and sweet corn ear dry weight. '⊤': standard error.

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