GA_3 and Kinetin Stimulate the Germination of Corn and Soybean Seeds at Low Temperatures*

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Abstract There have been few studies regarding how GA and kinetin affect germination by seeds of corn and soybean under low temperature conditions. Two controlled environment experiments were conducted to examine the effect of GA_3 and kinetin on the germination rate, percent germination, and early seedling development of corn and soybean at 10, 15 and 25℃. The germination rate, percent germination and early seedling development were examined for all the treatments. The results indicated that (1) at 25℃ neither GA_3 nor kinetin affected the germination of corn and soybean seeds, (2) both GA_3 and kinetin can increase the germination rate and the percent germination of corn and soybean seeds at 10 and 15℃, (3) GA_3 and kinetin have more effect on the germination of corn and soybean seeds at 10℃ than at 15℃, (4) 0.01 mM GA_3 and 0.005 mM kinetin were the most effective concentrations for increasing germination rate, percent germination, and early seedling development of corn and soybean under low temperature conditions.

Key words Germination; *Glycine max*; Low temperature; PGRs; *Zea mays*

GA_3和kinetin在低温下对玉米和大豆种子萌发及幼苗发育影响的研究

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提　要 本文在控制温度条件下，研究了GA_3和kinetin在低温条件下对玉米和大豆种子萌发及幼苗发育的影响。结果表明，在萌发适宜温度25℃条件下两种药剂处理的效果均不显著，但在15℃和10℃较低温度条件下两种药剂对玉米和大豆种子萌发有明显促进作用，而且在10℃温度下药剂处理的效果好于在15℃温度下。在10℃低温条件下，对玉米和大豆种子萌发及幼苗发育最有效的处理是0.01 mM GA_3和0.005 mM kinetin.

关键词 萌发；大豆(*Glycine max*)；低温；植物生长调节剂；玉米(*Zea mays*)

In cool early season, crop emergence can be delayed due to slow soil warming after planting. The seed-bed temperature at the customary planting time for most field crops is typically close to the base temperature for growth, often around 10℃ for many crops of tropical and subtropical origin. Low temperature reduces the emergence rate (Mock and McNeill, 1979) and increases susceptibility to seed and seedling diseases, which reduces seedling vigour (Schulz and Bateman,

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1968). A limited amount of research has been conducted to determine the effect of plant growth regulators (PGRs) on the germination of seeds at low temperatures. Carpenter and Osmark (1992) found that the total germination of *Coreopsis lanceolata* seeds was reduced below −5℃; 44% of recently harvested seeds germinated. However 54% to 81% germination was achieved after 6 hours of soaking seeds in 1 mM GA_3, 1 mM ethephon, or 0.025 mM kinetin alone or in combination with GA_3 and ethephon. Treatments with PGRs reduced the number of days to 50% germination (T50), and the span in days between 10% and 90% germination (T90−T10).

Ralowicz et al. (1992) reported that imbibing seeds with GA at 0.7, 1.4, 2.1, and 2.8 mM enhanced total germination of curly mesquite [*Hilaria belangeri* (*Steud.*) *Nash*] seeds over the control. Low concentrations of GA (0.14, 0.28, 0.42, and 0.56 mM) did not improve germination compared to the control. These results suggested that 0.7 mM GA is the critical concentration for stimulation of germination in this species.

King and Bridgen (1990) used presowing treatments and temperature regimes to improve germination of *Alstroemeria* hybrids. They found that there was an interaction between pretreatment and environmental regime for percent germination. Germination percentages for seeds imbibed with 0.29 or 2.9 mM GA_3 solutions were higher than for the other pretreatments, but were not different from one another. The warm-cold environment yielded higher germination percentages than the other environments. The time to germination was longest for the cold-warm regime. This response depended on the genotype and the age of the seed.

Durrant and Mash (1991) investigated steeping sugar-beet (*Beta vulgaris* L. var. altissima) seeds in PGRs as a way to improve germination under cold, wet conditions. They found that adding gibberellins ($GA_{4/7}$) or an N-substituted phthalimide (AC 94377) to the seed with thiram treatment was beneficial to the germination of sugar-beet seeds under cold, wet conditions, whereas kinetin or N(6)-benzyladenine gave no improvement. Germination was even more rapid and better synchronised following a 4-day seed advancement sequence, particularly when the seeds were steeped with gibberellic acid. Overall, it was possible to increase the proportion of seeds which produced a root or hypocotyl at least 2 cm long by 9% and 14% respectively; the thermal time needed to reach 90% germination was reduced from 73 to 30 degree-days and synchrony was improved at least two-fold.

In some regions, the soil temperature in the spring season rises slowly. Corn and soybean emergence can be delayed due to slow soil warming, which may result in decreased plant density and nonuniformity among neighbouring plants. In some cases these effects may seriously decrease the grain yield. However, there are few reports about the effect of GA and kinetin on germination of plant seeds at low temperatures, and there is little information regarding the effect of GA or kinetin treatments on the seed germination and seedling development of corn and soybean at low temperatures. Therefore, we hypothesized that some PGRs, such as GA and kinetin, increase the germination and seedling development under low temperature conditions. Our objectives were to determine (1) the effect of GA_3 and kinetin on seed germination and seedling development of corn and soybean at low temperatures, (2) the most effective concentrations of GA_3

and kinetin within each temperature condition.

1 Materials and Methods

These experiments were conducted in MacDonald Campus of McGill University in Canada.

1.1 Experiment 1

The experiment was arranged as a completely randomized split-plot design with 3 replications. Temperatures were treated as main-plot units, at 25, 15, 10℃. Temperatures were controlled by incubators (Sherer, Model CEL 37-14, Controlled Environment Lab. Sherer-Gillett Co. Marshall, Michigan). Temperatures inside the incubator was monitored and recorded constantly throughout the experiment. Temperature varied no more than −0.5℃ over time. Careful measurement of temperature at a variety of locations inside the incubator were made with a Li-Cor Data logger fitted with thermocouple temperature probes in order to ensure temperature uniformity. There was no detectable spatial difference in temperature. The incubators were kept in darkness throughout the experiment.

Two cultivars of each crop, corn and soybean, and the concentrations of PGRs formed the sub-plot units. Two corn (*Zea mays* L.) hybrids, Pioneer 3921 and Hyland 2241, and two soybean [*Glycine max* (L.) Merr.] cultivars, Maple Glen and AC Bravor, were used in this experiment. These cultivars were selected as we knew they had different parentage. The two corn hybrids were from different genotypic backgrounds and the two soybean varieties were from different parents (H. Voldeng, Personal communication). All of the work reported here was conducted with the same seed lot for each hybrid and cultivar, and all the work was conducted between April and August 1995 so that seed aging was not a factor.

Another factor was PGRs at 0.001 mM for GA_3 and kinetin, respectively, and mixture of GA_3 and kinetin. No PGRs (distilled water) application was used as a control. Both GA_3 (>90% purity) and kinetin (Commercial Grade) were from Sigma Chemical Company. Four mL of each PGR solution was added to each petri plate designated for their treatment, while, further additions were made as needed. Seeds were surface sterilized for 7 minutes in sodium hypochlorite (0.5% solution containing 4 mL L-1 Tween 80) and then rinsed several times with distilled water. Ten seeds were placed on filter paper in 90 mm diameter plastic petri plates. The petri plates were kept in the incubators, which set at the different treatment temperatures.

1.2 Experiment 2

This experiment extended the range of GA_3 and kinetin concentrations tested in an effort to determine the optimum level of GA_3 and kinetin for stimulation of corn and soybean seed germination at low temperature. The concentrations of GA_3 or kinetin were 0, 0.005, 0.01, 0.015, and 0.02 mM. The corn hybrid used was Pioneer 3921, while the soybean cultivar was AC Bravor. The temperature of the incubator was set at 10℃. Other experimental conditions and procedures were the same as experiment 1.

1.3 Data collection

Observations of root and coleoptile germination and their length were made daily until no further seeds had germinated for at least 1 week. After harvesting the seedlings, root length, root fresh weight, and root dry weight were measured for all treatments of both corn and soybean. Moreover, corn shoot length, corn shoot fresh weight, and corn shoot dry weight also were measured. However, soybean shoots (above the cotyledons) were not measured as they developed very slowly during the germination period and did not achieve a size that allowed accurate measurement.

1.4 Statistical analysis

Results were analyzed statistically by analysis of variance using the Statistical Analysis System Computer Package (SAS Institute Inc., 1988). When analysis of variance showed significant treatment effects, the least significant difference (LSD) test was applied to make comparisons between the means at the 0.05 level of significance.

2 Results and Discussion

2.1 Experiment 1

The seed germination rate of corn and soybean varied among temperature treatments (Figs 1, 2). At 25℃ [in the optimal range for corn (Hope et al., 1992) and soybean (Tyagi and Tripathi, 1983)], corn and soybean seeds took less than 1 week to complete germination. The percent germination of both corn and soybean seeds reached at least 95%. At 15℃, control corn seeds needed about two weeks to complete germination. The germination percentage was 73%. At 10℃, control corn seeds began germination very late, and needed nearly four weeks to complete germination. Also, the final germination percentage were reduced to 57% for Pioneer 3921 and 77% for Hyland 2241, which were 38 and 18%, respectively lower than germination at 25℃.

At 15℃, control soybean seeds took only 1 week to complete germination (Fig. 2). The germination percentage were 80% for Maple Glen and 83% for AC Bravor, 15% and 12% lower, respectively, than at 25℃. At 10℃, soybean seeds needed 5 days to begin germination and required another 4 days to complete germination. Soybean seeds needed fewer days than corn seeds to complete germination at 10℃.

The effects of PGRs on seed germination varied among temperatures (Fig 1, 2). At 25℃, there was no difference between the applied treatments and the control. At 15℃, treatments with GA_3, kinetin, or both increased the germination speed and the final germination level of corn seeds. The highest percent germination was 90% for the GA_3 treatment, which was 17% higher than the control. The effects of treatments varied between two hybrids and among sampling periods. There were no differences among the GA_3, kinetin, or GA_3 plus kinetin treatments at 15℃.

At 10℃, adding 0.001 mM GA_3 or 0.001 mM kinetin increased the germination speed and the final percentage germination of corn seeds (Fig. 1). The rapidest and the highest percent germination was achieved by adding GA_3 alone, with a final percent germination of 87%, which was 30% higher than the control. GA_3 reduced the number of days to 50% germination (T50) by five and the number of days between 10 and 90% germination(T90—T10) by three.

Somewhat similar results were obtained by Durrant and Mash (1991), who found that adding gibberellins was beneficial to the germination of sugar-beet seeds under cold, wet conditions, whereas kinetin gave no improvement. Interestingly, we found that the GA_3 plus kinetin treatment was not different from the control treatment. The two corn hybrids responded similarly to the imposed treatments.

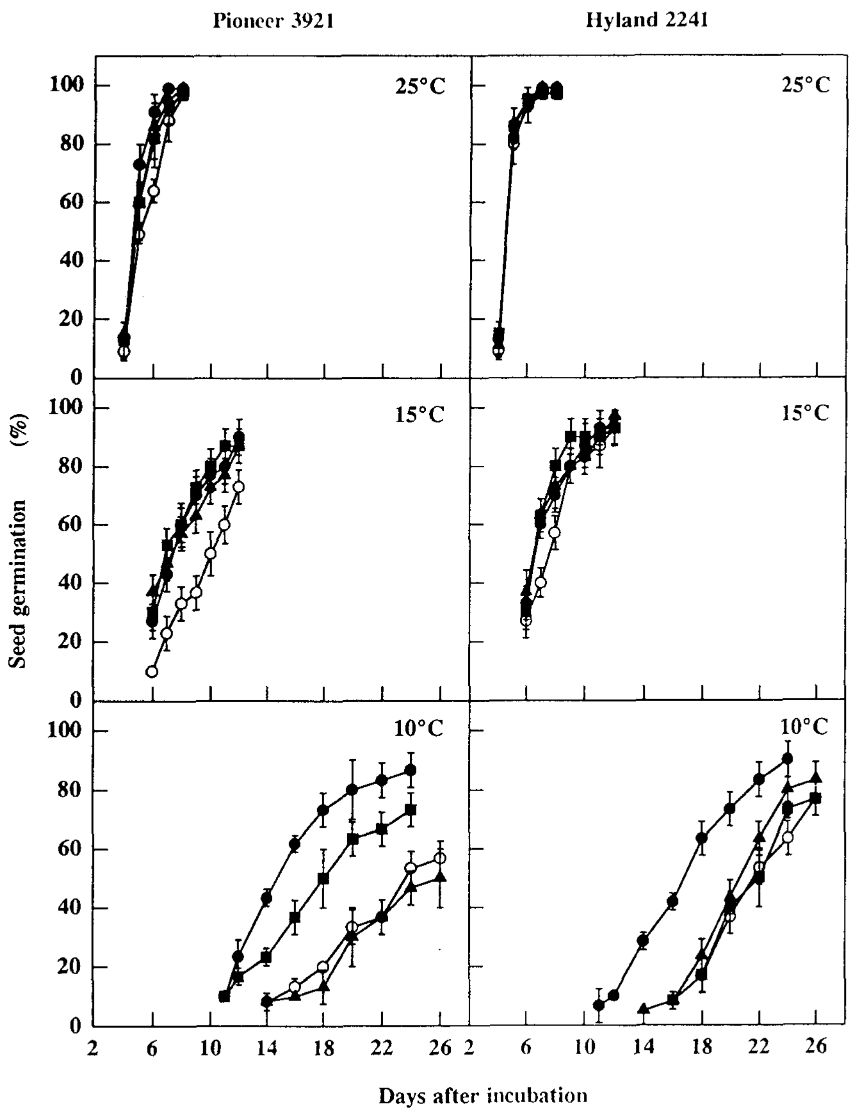

Fig. 1 The effects of GA_3 and Kinetin on the germination of corn seeds, Pioneer 3921 and Hyland 2241, exposed to 25, 15, and 10℃. The seeds were incubated with GA_3 at 0.001 mM (●), Kinetin at 0.001 mM(■), GA_3 plus Kinetin each at 0.001 mM(▲), and distilled water(○). Each value is plotted as the mean±1 SE(n=3)

Soybean responded differently from corn to the imposed PGR treatments at both 15 and 10℃(Fig. 2). Adding 0.001 mM GA_3 or kinetin or both improved the germination of soybean

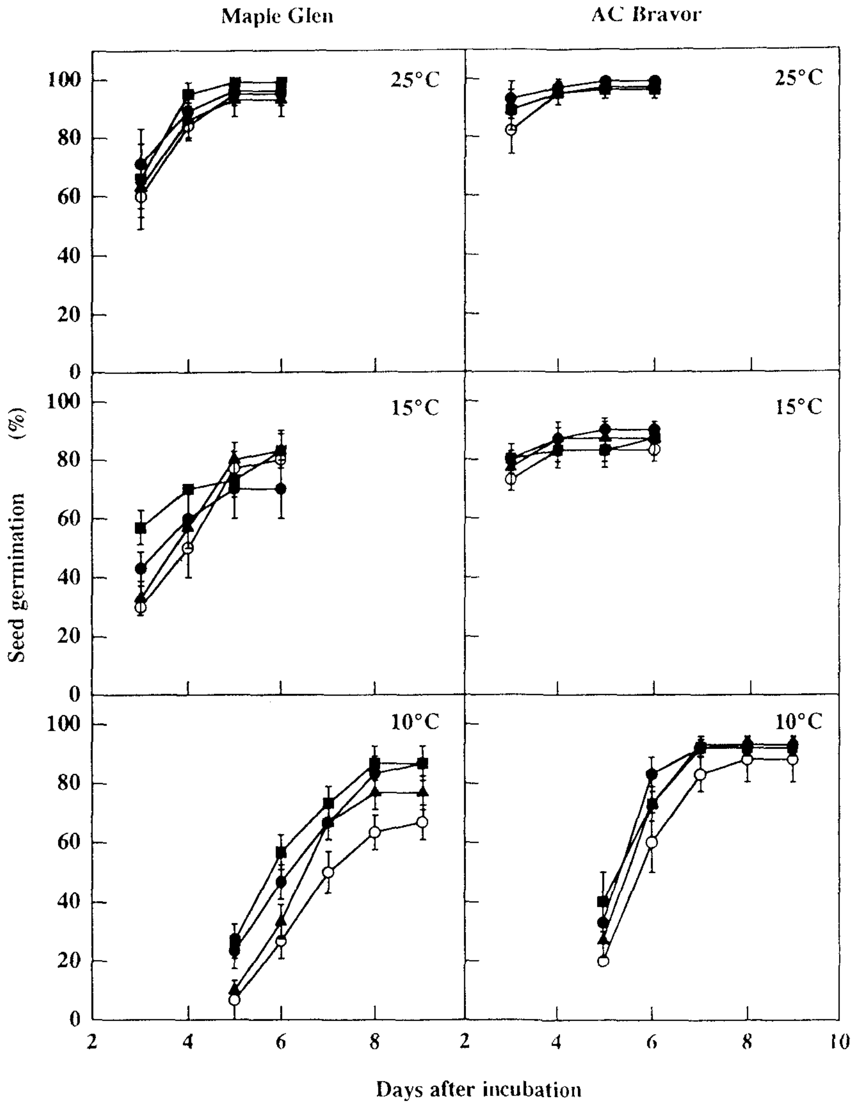

Fig. 2 The effects of GA_3 and Kinetin on the germination of soybean seeds, Maple Glen and AC Bravor, exposed to 25, 15, and 10℃. The seeds were incubated with GA_3 at 0.001 mM (●), Kinetin at 0.001 mM(■), GA_3 plus Kinetin each at 0.001 mM(▲), and distilled water(○). Each value is plotted as the mean±1 SE(n=3)

seeds. But, the response was different between two soybean cultivars. The effect of kinetin was greater than GA_3. The highest percent germination of soybean in the early germination phase was for the kinetin alone treatment. For soybean the germination differences between GA_3 or kinetin and the control were higher at 10 than at 15℃. The percent germination of GA_3 or kinetin treatments of Maple Glen soybean was 87%, which was 20% higher than the control (67%). The stimulatory effects of GA_3 on soybean were less than on corn at 10℃. For soybean the effect of GA_3 plus kinetin was less than GA_3 or kinetin alone, however, unlike corn at 10℃, the combination of GA_3 plus kinetin did cause some stimulation of soybean seed germination.

These results indicated that different crops may respond differently to GA_3 and kinetin.

Addition of 0.001 mM GA_3, kinetin or both accelerated the early development of corn and soybean roots at low temperature (Fig. 3). At the beginning of germination the root protruded first, and elongated steadily after its appearance. However, the root grew slowly at low temperatures. At 15℃, corn seeds treated with GA_3, kinetin, or both took 7 days from first watering to develop a 1 cm long root, while the control took more than 8 days to reach this stage. The rate of root growth by GA_3 treated seeds was similar to GA_3 plus kinetin, and both were higher than kinetin alone.

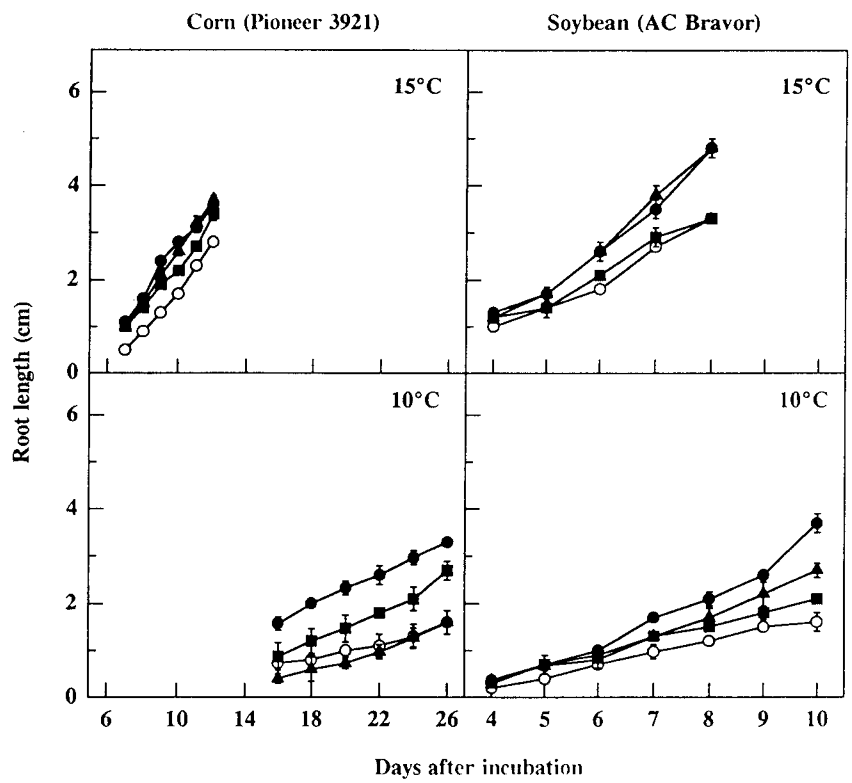

Fig. 3　The effects of GA_3 and Kinetin on the root length of corn (Pioneer 3921) and soybean (AC Bravor) seedling exposed to 15 and 10℃. The seeds were incubated with GA_3 at 0.001 mM(●), Kinetin at 0.001 mM(■), GA_3 plus Kinetin each at 0.001 mM(▲), and distilled water(○). Each value is plotted as the mean±1 SE(n=30)

The roots of all the corn seedlings, regardless of treatments, grew much more slowly at 10℃ than at 15℃ (Fig. 3). It took more than 23 days from seeding for the controls to develop a 1 cm long root. The average root length of GA_3 and kinetin treatments increased substantially faster than that of the control. Compared to the control, seeds treated with GA_3 and kinetin required only 15 and 17 days, respectively, after seeding to produce a 1 cm long root, a reduction of more than 1 week. The relative effect of GA_3 or kinetin on the early growth of roots was greater at 10 than at 15℃. As with germination rate, the GA and kinetin negated each other's effects for corn seeds and the root length was not different between the GA_3 plus kinetin treatment and the control at 10℃.

Soybean took fewer days to germinate than corn at low temperature(Fig. 3). The effects of GA_3 or kinetin or both on the early growth of roots were quite different between soybean and corn at low temperatures. At 15℃, all soybean treatments produced a 1 cm long root by 4 days after seeding. The rates of root elongation by the GA_3 and GA_3 plus kinetin treatments were higher than the control or kinetin alone treatments. Root length was not different between the kinetin treated and control seeds. Soybean roots grew more slowly at 10 than at 15℃. Seeds treated with GA_3, kinetin or both took more than 6 days after seeding to produce a 1 cm long root. However, the control required nearly 8 days to produce the same length of root. The effect of GA_3 was greater than GA_3 plus kinetin or kinetin alone at 10℃.

2.2 Experiment 2

At 10℃ final germination percentages of corn seeds treated with GA_3 or kinetin at 0.005, 0.01, 0.015 and 0.02 mM were higher than the control(Fig. 4), but were generally not different from one another. However, 0.015 mM GA_3 or kinetin caused faster germination of corn Pi-

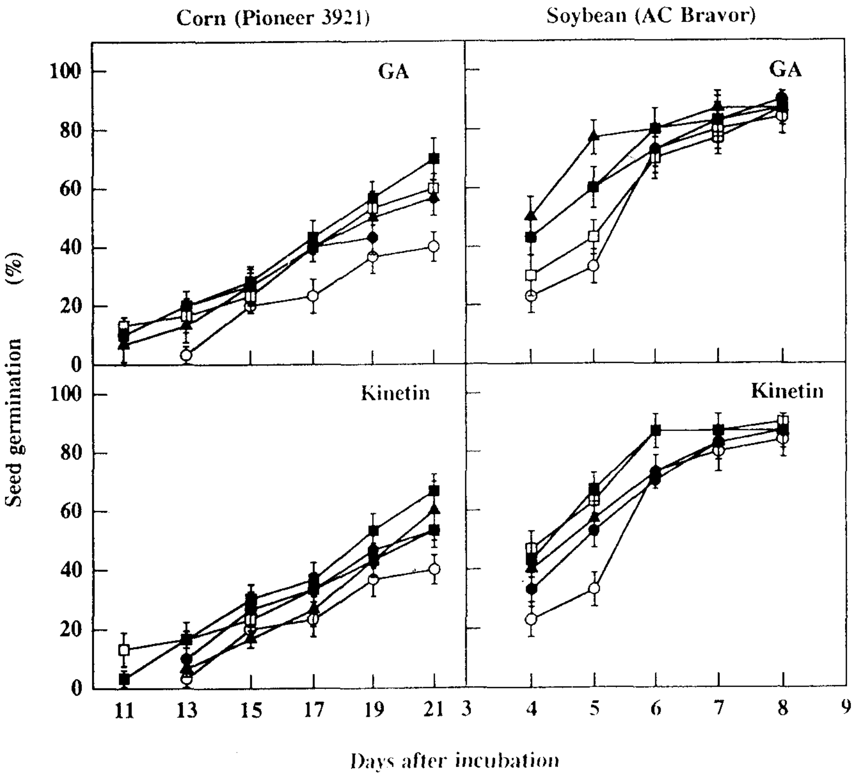

Fig. 4 The effects of different concentrations of GA_3 and Kinetin on germination of corn(Pioneer 3921) and soybean(AC Bravor) seeds exposed to 10℃. The concentrations of 0(○), 0.005(□), 0.01(●), 0.015(■), and 0.02(▲)mM were used for both GA_3 and Kinetin, respectively. Each value is plotted as the mean±1 SE(n=3)

oneer 3921 seed than other concentrations. GA_3 or kinetin treated seeds began to germinate 2 days earlier than the controls. The final germination percentages of the PGR treatments were about 20% higher than the control. At 10℃, there were differences between GA_3 concentrations for early germination of soybean seeds, with 0.02 mM being most stimulatory. There were no differences among kinetin concentrations. With the progress of the germination process, the differences among treatments became smaller and smaller, until there were no differences in the final germination percentages. By comparison, Ralowicz et al. (1992) reported that 0.7 mM GA was the critical concentration for stimulation of germination in curly mesquite[*Hilaria belangeri* (Steud.) Nash]. However, Ralowicz et al. (1992) exposed their seeds to GA only once, at the beginning of seed germination, while our seeds were exposed throughout germination. Selection of the most effective GA or kinetin concentrations will depend on the methods of application and plant species.

The effect of different concentrations of GA_3 and kinetin on the early development of corn and soybean seedlings were also evaluated at 10℃. There were differences among GA_3 and kinetin and control treatments for root fresh weight, root dry weight, root length, shoot fresh weight, shoot dry weight and shoot length (Table 1). The maximum stimulation of root growth (weight), for both corn and soybean, occurred at 0.01 mM for GA_3 and 0.005 mM for kinetin. As all the work reported here was conducted with the seeds in darkness, increased seedling weight implies increased mobilization of seed reserves into the developing seedling. There were differences in corn shoot length between different concentrations of GA_3, but there were no differences in corn root length. However, the fresh weight and dry weight of corn roots and shoots from all the kinetin treatments were higher than those of the GA_3 treatments. This was especially true at 0.015 mM kinetin which increased the fresh weight and dry weight of corn shoots more

Table 1 Effect of GA_3 and kinetin concentrations on the development of corn (Pioneer 3921) and soybean (AC Bravor) seedlings at 10℃

Hormone		Corn root			Corn shoot			Soybean root		
Type	mM	Fr. wt.[a] (mg)	Dry wt. (mg)	Length (cm)	Fr. wt (mg)	Dry wt. (mg)	Length (cm)	Fr. wt (mg)	Dry wt. (mg)	Length (cm)
	0.000	35.7	3.1	1.7	35.8	3.8	0.4	86.0	7.7	3.3
	0.005	33.4	3.7	2.1	51.7	4.8	1.0	94.7	8.5	4.0
GA_3	0.010	50.0	5.5	2.9	59.7	5.7	1.4	112.7	8.9	5.2
	0.015	44.9	4.6	2.6	55.7	4.9	1.0	114.4	8.4	4.8
	0.020	34.4	3.2	2.0	49.3	4.3	0.9	112.6	8.4	4.8
$LSD_{0.05}$		16.9	1.5	1.6	13.4	1.9	0.3	18.8	0.8	0.6
	0.000	35.7	3.1	1.7	35.8	3.8	0.4	86.0	7.7	3.2
	0.005	58.3	6.0	2.1	81.7	6.9	1.1	96.4	8.0	3.7
Kinetin	0.010	52.7	5.5	1.6	72.0	6.0	1.0	91.7	7.9	3.6
	0.015	53.7	5.6	1.8	105.0	8.7	1.1	92.3	8.1	3.1
	0.020	41.3	4.5	1.2	67.6	6.4	0.8	84.0	7.0	2.9
$LSD_{0.05}$		17.6	2.0	0.8	28.9	2.2	0.3	8.7	6.1	0.5

[a]Fr. wt. : Fresh weight.

than other concentrations, although it didn't increase the length of the corn shoot. This was also made apparent by the observation that the same concentrations of GA_3 or kinetin produced different results for root and shoot growth. Taken together, these results indicated that the concentrations of GA_3 or kinetin should be carefully selected to improve the early development of corn and soybean seedlings under low temperature conditions.

In summary, low temperature at seeding limits corn and soybean production in cool early season environments. Our results indicated that GA_3 and kinetin were effective for stimulating germination of corn and soybean seeds at low temperatures. GA_3 and kinetin were more beneficial to the germination of corn and soybean seeds at 10 than at 15℃. At 25℃, neither GA_3 nor kinetin improved seed germination. Specifically, 0.01 mM GA_3 and 0.005 mM kinetin were the most effective concentrations for increasing germination rate, percent germination, and early seedling development of corn and soybean under low temperature (10℃) conditions. The problem of low temperature and decreased germination of corn and soybean seeds under cool early season conditions can be, at least partly, solved by applying GA or kinetin as a seed pretreatment.

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