Effect of Pretreatment of Corn Seed with 6-(Substituted)purines on Growth of Plants

Donald Tuttle and Charles G. Skinner

Corn seeds were presoaked in five different 6-(substituted)purine solutions, and the resultant germinated seedlings were grown in hydroponic media. An augmentation in both whole weight and primary root growth was observed during early stages of growth; however, a field study which involved carrying the plant through to maturity and harvest did not indicate an increased crop yield over that of nontreated seeds.

Numerous types of physiological responses have been reported in a variety of plant systems treated with kinetin or related 6-(substituted)purines (Miller, 1961). For example, stimulation of the rate of seed germination has been observed (Miller, 1956; Skinner *et al.*, 1956a), as well as stimulation of growth of intact plants (Hillman, 1957). Most of these experiments have been concerned with short-time growth studies, or the systems were utilized as bioassay tools to indicate molecular structure specificity of analogs. At the molecular and cellular level of development, the 6-(substituted)purines have been demonstrated to augment the rate of cell division (Miller *et al.*, 1956). Treatment with kinetin was found to maintain a higher protein level in detached *Xanthium* leaves (Richmond and Lang, 1957), and kinins were subsequently found to increase the rate of RNA and protein synthesis in several systems (Olszewska, 1959; Sugiura *et al.*, 1962). Recently, a 6-(substituted)aminopurine was isolated from corn kernels which was termed zeatin (Letham, 1964), and it was later shown to be 6-(4-hydroxy-3-methylbut-2-enyl)aminopurine (Letham *et al.*, 1964) in the trans configuration (Shaw and Wilson, 1964). Thus, 6-(substituted)-purine derivatives appear to be biologically functional in the growth and/or development of corn, and since a number of synthetic purine derivatives were available (Gorton *et al.*, 1957; Henderson *et al.*, 1962; Skinner and Shive, 1955; Skinner *et al.*, 1956b) which had previously been demonstrated to possess physiological activity in a number of biological systems, it was of interest to determine if pretreatment of corn seeds by these analogs would affect the growth and development of the resulting plants. In one instance, a study was carried through to determine the effect of yield of harvest.

EXPERIMENTAL

Hydroponic Studies. Mexican June corn was washed with 1% bromine-water for about 5 minutes, drained, and rinsed with distilled water, and about 50 seeds were placed in 50-ml. samples of 10 μg. per ml. solutions of the 6-(substituted)purine derivatives for 12 hours of presoaking. The seeds were drained, placed in Petri dishes containing filter paper wet with the corresponding purine solutions, and permitted to germinate in the dark at 30° C. for 4 days. The resulting plants were then transferred to crocks containing 3.5 liters of nutrient solution (Machlis and Torrey, 1956). The seeds were held in place (seven to a crock) by wrapping the grain section in cotton, and permitting the primary root section to come in contact with the nutrient solution which was continuously aerated. After growing in a greenhouse for 10 days, the resulting plants were recovered, and the length of the primary root was measured along with the total weight of the air-dried whole plant (Table I).

Harvest Studies. These experiments were carried out under the same pretreatment conditions described above using 125 seeds per 100 ml. of the appropriate 6-(substituted)purine solution. The presoaked seeds were drained, blotted dry, and divided into groups of 20 seeds each for immediate planting.

A rectangular area of plowed field (approximately 40 × 60 feet) was treated with 400 pounds of 16–20–0 fertilizer and divided into 36 plots. For each treatment, five hills of corn containing three seeds each, planted about 2 inches deep, were symmetrically located in each of six plots. The planting procedure was determined through a set of random numbers so that each plot contained treated seeds distributed in a nonpredetermined sequence. After approximately 3 months, the corn, which appeared to be mature (as evidenced by possessing a dry silk), was harvested, and the yields from plants produced from the treated seeds were combined and compared with the water-treated controls. A second harvest was obtained 2 weeks later. The corn production of the purine-treated seeds was compared as to total number of ears, and total weight of crop as determined immediately after harvest (Table II).

Table I. Effect of 6-(Substituted)aminopurine Pretreatment on Growth of Corn Seeds in Hydroponic Media

Pretreatment Solution, 10 μg./Ml., Substituent Group	Average Growth Response after 10 Days: Primary root length, mm.	Total plant wt., grams	Weight difference, % of water control
Water blank control	21.7	1.78	...
Benzyl-	20.7	2.08	+17
Phenethyl-	26.9	2.38	+34
Phenylpropyl-	24.3	2.35	+32
Phenylbutyl-	30.2	3.07	+72
Phenylpentyl-	27.0	2.44	+37
Phenylheptyl-	20.5	1.70	−4

Department of Chemistry, North Texas State University, Denton, Tex. 76203.

Table II. Field Test of Corn Seeds Pretreated with 6-(Substituted)purines

Purine Pretreatment, 10μg./Ml.[a]	First Harvest[b]		Second Harvest[c]		Total Harvest		Av. Wt. Corn per Ear, Lb.
	No. ears	Wt. ears, lb.	No. ears	Wt. ears, lb.	No. ears	Wt. ears, lb.	
Water Control	60	61	23	13.5	83	74.5	0.90
Kinetin	65	54	19	10.5	84	64.5	0.77
6-Benzylamino-	61	48	17	8.5	78	56.4	0.72
6-Thienylamino-	60	54	21	11.8	81	65.8	0.81
6-Phenylbutylamino-	70	60	14	9.3	84	69.3	0.82
6-Benzylthio-	54	46	26	15.3	80	64.3	0.77

[a] Presoaked 12 hours in the dark at 20° C.
[b] 3 months after planting.
[c] 3 months and 2 weeks after planting.

RESULTS AND DISCUSSION

Pretreatment of seeds with 6-(substituted)purines augments the rate of germination of certain species; however, with corn seed no significant stimulation of rate of germination was observed over that of the water control. The seeds were presoaked for 12 hours using three different concentrations of 6-(ω-phenylalkyl)aminopurines, 1, 10, and 30 μg. per ml., containing alkyl groups from methyl to heptyl. After 48 hours, the water control seeds were about 70% germinated, whereas the treated seeds were between 65 and 85% germinated. In the experiments utilizing germinated corn seeds for additional growth studies, the young seedlings selected from a group of germinated seeds were those with the more advanced growth; thus, the slower-germinating seeds, which might have some different genetic characteristics, were eliminated.

To examine chemical pretreatment effects of the corn seeds on subsequent plant growth, selected groups of comparable size of the germinated seedlings were placed in a hydroponic medium, and allowed to grow for an additional 11 days under normal greenhouse conditions. The total weight gain was determined as well as the length of the primary root. The results of one set of experiments are summarized in Table I, and repeats of this experiment gave a series of comparable data. Plants grown from seeds pretreated with 6-(4-phenylbutyl)aminopurine had a total weight gain of about 70% greater than the water control in this time period, and an increase in primary root growth was also evident. Three other compounds produced similar results as indicated in Table I. Thus, pretreatment of corn seeds with selected 6-(substituted)purines appears to stimulate an early plant development; however, as subsequently indicated, these effects are essentially eliminated by the time the plant has reached maturity.

Since seedlings grown under hydroponic conditions will not unequivocally demonstrate the effect of the chemical treatments on subsequent whole plant development in a field system, and since facilities were not available in the hydroponic assay system to determine the effect of the pretreatment on the ultimate crop yield, the study was repeated under typical growing conditions. No satisfactory procedure was readily available for measuring the root growth of the mature plants under these field conditions; however, the seed production from the harvest could be easily assayed, and is, of course, the more important demonstrable variable. In view of the time required for such an extended study, several of the kinetin analogs were examined concurrently for comparative purposes (Table II). One month after planting, one plant from each hill was recovered and weighed in an effort to establish any apparent growth differentials at this stage of development. The average weight of plants from the water-treated seeds was 20.7 grams, the kinetin and 6-thionylaminopurine were comparable, but the plants presoaked in 6-benzylthiopurine and 6-(4-phenylbutyl)aminopurine were 30% heavier than the water control.

Early stimulation of growth induced by pretreatment with 6-(substituted)purines does not carry through to harvest; actually, the reverse is true. None of the treated plants produced as well as those from seeds presoaked in water alone. Even the seeds pretreated with the more active analog 6-(4-phenylbutyl)aminopurine merely approached the value of the water-treated seeds, but did not exceed it. It is tempting to speculate that pretreatment of seeds with the naturally occurring purine, zeatin, might augment the growth and ultimate yield of harvest, and attempts are now under way to synthesize sufficient quantities of this compound to carry out such a study.

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