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Cytokinin Regulation of Flower and Pod Set in Soybeans (*Glycine max* (L.) Merr.)

L. NAGEL†, R. BREWSTER†, W. E. RIEDELL‡ and R. N. REESE*†

†*Department of Biology/Microbiology, South Dakota State University, Brookings, SD 57007, USA and*
‡*USDA-ARS, Northern Grain Insect Research Lab., 2923 Medary Ave., Brookings, SD 57006, USA*

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Exogenous application of cytokinin to raceme tissues of soybean (*Glycine max* (L.) Merr.) has been shown to stimulate flower production and to prevent flower abortion. The effects of these hormone applications have been ascertained for treated tissues, but the effects of cytokinins on total seed yields in treated plants have not been evaluated. Our objectives were to examine the effects of systemic cytokinin applications on soybean yields using an experimental line of soybeans, SD-87001, that has been shown to be highly sensitive to exogenous cytokinin application. Soybeans were grown hydroponically or in pots in the greenhouse, and 6-benzylaminopurine (BA) was introduced into the xylem stream through a cotton wick for 2 weeks during anthesis. After the plants had matured, the number of pods, seeds per pod, and the total seed weight per plant were measured. In the greenhouse, application of $3{\cdot}4 \times 10^{-7}$ moles of BA resulted in a 79 % increase in seed yield compared with controls. Results of field trials showed much greater variability within treatments, with consistent, but non-significant increases in seed number and total yields of about 3 %. Data suggest that cytokinin levels play a significant role in determining total yield in soybeans, and that increasing cytokinin concentrations in certain environments may result in increased total seed production.

Key words: *Glycine max*, soybean, flower abortion, cytokinin, 6-benzylaminopurine, hydroponic, seed yield, wicking.

INTRODUCTION

Cytokinins play a role in regulating flower and pod development in soybeans (Reese *et al*., 1995). Carlson *et al*. (1987) and Nooden *et al*. (1990) have demonstrated a correlation between endogenous levels of cytokinins and the level of flower abortion and set. Application of exogenous benzyladenine (BA) to individual racemes has been shown to prevent abortion of flowers and/or pods (Dyer *et al*., 1988; Peterson *et al*., 1990; Mosjidis *et al*., 1993; Reese *et al*., 1995).

Abortion of a significant percentage of flowers and pods commonly occurs in soybeans (Abernethy *et al*., 1977; Dybing *et al*., 1986). The aborted flowers have generally been pollinated and fertilized, but tend to grow more slowly than the flowers that set (Huff and Dybing, 1980). Most proximal flowers set while most distal flowers abort; removal of proximal flowers can induce set of distal flowers (Huff and Dybing, 1980; Dybing *et al*, 1986; Wiebold, 1990). Abscission processes leading to flower abortion may begin very soon after anthesis (Huff and Dybing, 1980; Brun and Betts, 1984; Dybing *et al*, 1986). Compared with setting flowers, the sink intensity is lower in aborting flowers as early as 3 d after anthesis (Brun and Betts, 1984; Heitholt *et al*, 1986*a*, *b*). These observations have led to hypotheses suggesting that young, distal soybean flowers cannot compete successfully with the older proximal flowers for limited supplies of either nutrients or hormones.

Conflicting data suggest that nutritional availability (carbohydrates and nitrogen) may play a role(s) in the number of flowers formed and the total number of mature soybean fruits and seeds that develop (Sinclair and De Witt, 1976; Streeter and Jeffers, 1979; Antos and Wiebold, 1984; Dybing *et al*., 1986; Stockman and Shibles, 1986; Jiang and Egli, 1993; Dybing, 1994; Hayati *et al*., 1995). Results from these studies suggest that carbohydrate and nitrogen availability affects flower and fruit production indirectly, as these nutrient levels are most closely correlated with total shoot dry weight. However, there is strong evidence supporting a role for cytokinins in the regulation of flowering and fruit set (Huff and Dybing, 1980; Ghiasi *et al*., 1987; Peterson *et al*., 1990; Wiebold, 1990; Mosjidis *et al*, 1993; Reese *et al*., 1995).

Although available data show that cytokinins can increase flower and fruit set in individual soybean racemes, they do not clearly show whether these hormones can induce significant changes in the total production of fruits and seeds. The following experiments were conducted to test the hypothesis that cytokinins act to regulate the distribution of assimilates committed to fruit production.

MATERIALS AND METHODS

A determinate, long-racemed (approx. 15 flowers per raceme) experimental soybean line, SD-87001 (Sharma *et al*., 1990; Dybing, 1994) was grown in 22 cm pots spaced 10 cm apart, or hydroponically in the greenhouse as described by Dybing *et al*. (1986) and Dybing and Yarrow (1984). SD-87001 was chosen for pot and hydroponic greenhouse studies because it is small, early maturing (Maturity group 00) and previous work has shown it to be sensitive to exogenous cytokinin application (Dybing and

* For correspondence. Fax 00 1 605 688 5624, e-mail neil_reese@sdstate.edu

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Westgate, 1996). Two replications of the hydroponic experiments and two replications of the pot experiments were planted during the autumn and winter of 1992 and 1993 in the greenhouses in Brookings, SD, USA. Approximately 40 plants per hydroponic tube or 48 pots per bench were planted initially. Uniform, healthy plants with flowers at a nearly identical stage of development on the day of treatment (see below) were selected at the beginning of each experiment. Control and BA treatments were applied using a completely random design (approx. six plants per treatment per experiment, depending upon the number of plants at the correct stage of development on day 0). Plants showing signs of mechanical damage 24 h after treatment were removed from the trials. Greenhouse air temperature was 25 ± 5°C, and the photoperiod was maintained at 16 h by supplementing daylight with high-pressure sodium lamps.

6-Benzylaminopurine (BA) was introduced into the transpiration stream of each plant by inserting a wick through the centre of the stem below the first raceme and allowing the solution to be taken into the xylem by capillary action (Damptey *et al.*, 1978; Saini and Aspinall, 1982). Briefly, the threads (two white cotton DMC embroidery threads) were inserted through the stem with a no. 8 embroidery needle when the flowers of the bottom-most raceme had all reached anthesis. The threads were pulled through the stem forming a loop, with the ends of the thread on either side. Both thread ends were inserted into an opening in the cap of a 50 ml plastic centrifuge tube that contained water or a solution of BA adjusted to pH 4·0. The ends of the threads were dipped in paraffin prior to use to facilitate insertion through the opening in the centrifuge tube cap. The threads and top of the tubes were then sealed to the base of the plant using parafilm.

BA solutions were made by dissolving BA in a minimum of 1 M HCl to form a 10^{-3} M stock solution and then diluting with deionized water. The pH was adjusted with KOH. Water controls were acidified with HCl and adjusted to pH 4·0 with KOH in a similar manner. The hydroponically grown plants were treated with 10^{-3}, 10^{-4} and 10^{-5} M solutions, and the pot-grown plants were exposed to 10^{-4}, 10^{-5} and 10^{-6} M BA solutions. Tubes were refilled through a second opening in the top of the centrifuge tube using an 18 gauge needle. The volume of the solution added was recorded daily, and total volume was determined by subtracting the volume of solution remaining in the tube after the 2-week treatment period.

Pod fill was complete approx. 50 d after treatment and the senescent plants were harvested. Seed set was determined by counting the number of pods on each raceme and the number of seeds per pod. Total dry weight of the seeds from each plant was determined after equilibrating residual moisture to approx. 13 % by placing the seeds in a desiccator over a saturated sodium chloride solution for at least 2 weeks (Vertucci and Leopold, 1984; Vertucci and Roos, 1990).

Two field trials were conducted during the summer of 1993 at the SDSU campus farm and an additional experiment was conducted in 1995 at the USDA-ARS Experimental Farm north of Brookings, SD, USA, following the same protocols. Soybeans (SD-87001) were planted in 3 m rows, 0·75 m apart, with 30 plants m^{-1}. Two buffer rows were planted at each end of the plot. For each experiment, plants at the proper stage of development were identified and treatments were randomly applied to the selected plants. Plants growing within 0·3 m of the end of a row were not used in any experiment.

Analysis of variance of the data was made using the GLM procedures of the Statistical Analysis System (SAS Institute, Cary, NC, USA). Differences between main effects were compared using the Least Square Means (LS Means) option, to allow for differences in numbers of observations for each of the cytokinin levels. Values in the text are presented as mean ± s.d. unless otherwise noted.

RESULTS

Greenhouse studies

Cytokinin solutions were introduced into the transpiration stream continuously for 2 weeks. Preliminary studies indicated that this duration coincided with flowering of SD-87001, maximized yields, and minimized cytokinin-induced changes in morphology, which included the development of wrinkled leaves with necrotic lesions. In the hydroponic experiments, 10^{-3} M BA treatments caused death of the plants before harvest; results from these treatments were not included in the analysis.

Total uptake of water or cytokinin solution varied between individual plants, ranging from 25 to 113 ml with a mean uptake of 59·3 ± 26·2 ml. There was no significant difference in the volume taken up between water controls or the three BA treatments across the four experiments (data not shown). Variations in cytokinin uptake between the treatments did, however, create overlap in total cytokinin application between the treatments. Therefore, to examine the effects of treatments based upon actual exposure to cytokinin, the total cytokinin applied to each plant was calculated and plants were placed into one of five groups based upon an analysis of their total BA uptake (Fig. 1).

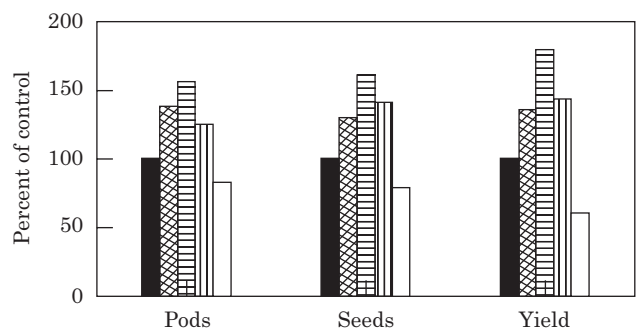

FIG. 1. Results of cytokinin treatments on soybean yields. Combined values for greenhouse hydroponic and pot experiments. Number of soybean pods per plant, number of seeds per plant and total yield (seed weight) per soybean plant, all normalized to percent of controls. Root mean square errors = 44, 44 and 61 respectively. Group 0 (■), Water controls, $n = 23$; group 1 (▩), BA = 0·038 ± 0·001 μmoles, $n = 6$; group 2 (▤), BA = 0·338 ± 0·031 μmoles, $n = 13$;. group 3 (▥), BA = 1·589 ± 0·302 μmoles, $n = 17$; group 4 (□), BA = 8·343 ± 0·521 μmoles, $n = 17$.

Solution uptake among these five groups of plants differed significantly, but correlations of solution volume to pod number ($R^2 = 0{\cdot}003$, $P = 0{\cdot}66$), seed number ($R^2 = 0{\cdot}000$, $P = 0{\cdot}97$), and seed weight ($R^2 = 0{\cdot}003$, $P = 0{\cdot}66$) were not significant. The variability in cytokinin uptake and subsequent analyses based upon total cytokinin exposure did, however, result in unequal numbers of plants per group (Fig. 1). The total amount of cytokinins applied over the 2-week period, and not the concentration of the BA application, appeared to be responsible for the observed changes in pods, seeds and yields. This was true for all BA concentrations except the 10^{-3} M BA treatment, as discussed above.

Plants in the hydroponic trials produced larger pod and seed yields than did plants in pot experiments (Table 1). Therefore, the data were normalized to percent of control-plant yields for each experiment, prior to calculating means. Figure 1 shows the mean normalized number of pods per plant, the mean normalized number of seeds per plant, and the mean normalized weight of seeds per plant for all four experiments. Mean values for pod number, seed number, and seed weight for water-control plants from each experiment are presented in Table 1.

Many of the seeds that formed in the greenhouse experiments did not develop into fully expanded mature seeds, but remained small and lenticular in shape. This was especially evident in the hydroponically grown plants (Table 1). Variation in solar radiation due to season and weather patterns contributed to the smaller seed sizes in these experiments, even though the plants were provided with supplemental light from high-pressure sodium lamps (250 μmol m^{-2} s^{-1}).

Exogenous application of $\leqslant 5 \times 10^{-6}$ moles BA (Groups 1, 2 and 3) in the greenhouse resulted in an increase in pod number and seeds per plant, and increased overall seed weight per plant (Fig. 1). Applications of 5×10^{-8} to 5×10^{-7} moles BA (Group 2) produced the greatest increase in pod number, seeds per plant and seed weight per plant in the four experiments, with values being 58, 62 and 79 % greater than controls, respectively. These values were significantly greater than controls in all experiments. Application of $>5 \times 10^{-6}$ moles BA (Group 4) caused a 27 % reduction of the number of pods, a 30 % reduction in seed numbers and a 39 % reduction in seed weight (Fig. 1). The number of seeds per pod and the average seed weight in comparison to controls were not significantly affected by the cytokinin applications.

Field Trials

Applications of cytokinin to plants growing in the field resulted in a pattern of flower set similar to that seen in the greenhouse studies (Fig. 2). Control plants in these experiments were much more productive than controls in the greenhouse studies (Table 2). Although soybean plants in group 3 (mean BA = 6·044 ± 4·58 µmoles per plant) consistently produced greater yields than the controls, the variability in field conditions and the high productivity of the controls resulted in total increases in pod number, seed number and seed weight that were not significantly higher than controls at any cytokinin application rate. Application of high rates of cytokinins (group 4, BA = 11·31 ± 0·858µmoles) resulted in a significant reduction in pod number, seed number and yield, as did low level cytokinin applications (group 1, BA = 0·042 ± 0·022 µmoles).

TABLE 1. *Pod number, seed number and seed weight per soybean plant for water controls from hydroponic (expts 1 and 2) and pot (expts 3 and 4) experiments conducted in the greenhouse*

	Hydroponics		Pots	
Variable	Expt 1	Expt 2	Expt 3	Expt 4
Pod number	82·2 ± 17·3	129·1 ± 13·4	12·0 ± 2·0	34·2 ± 5·1
Seed number	131·7 ± 32·4	249·4 ± 19·6	20·0 ± 6·0	40·4 ± 6·2
Seed weight (g)	4·67 ± 1·29	13·98 ± 2·66	1·41 ± 0·01	4·35 ± 0·44

TABLE 2. *Pod number, seed number and seed weight per soybean plant for water controls from 1993 (expts 1 and 2) and 1995 (expt 3) field experiments*

Variable	Expt 1	Expt 2	Expt 3
Pod number	36·0 ± 7·15	36·1 ± 9·04	54·1 ± 4·09
Seed number	68·6 ± 14·04	74·7 ± 18·36	122·2 ± 9·24
Seed weight (g)	10·16 ± 1·98	7·74 ± 1·71	13·14 ± 0·95

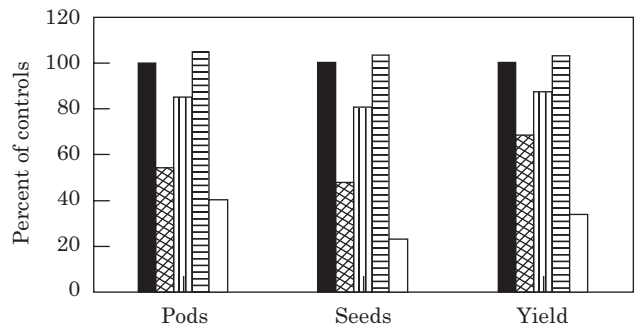

FIG. 2. Results of cytokinin treatments on field-grown soybean yields. Number of soybean pods per plant, number of seeds per plant and total yield (seed weight) per soybean plant, all normalized to percent of controls Root mean square errors =49, 53 and 50 respectively. Group 0 (■), Water controls, $n = 24$; group 1 (▩), BA = 0·042 ± 0·022 µmoles, $n = 12$; group 2 (▤), BA = 0·195 ± 0·031 µmoles, $n = 15$; group 3 (▥), BA = 6·044 ± 4·58 µmoles, $n = 23$; group 4 (□), BA = 11·31 ± 0·858 µmoles, $n = 20$.

DISCUSSION

Carlson *et al.* (1987) showed that endogenous cytokinin levels in the xylem exudate of soybean plants are relatively high at the start of anthesis and decrease as flowering progresses. Furthermore, they showed that the levels of cytokinin in the xylem sap coming from the roots were directly correlated with the percentage of flowers that set. The approach taken here supplemented the endogenous

cytokinins in the xylem stream, maintaining high levels during the entire flowering period.

Cytokinin prevention of soybean flower and pod abortion varies greatly between genotypes (Dybing and Westgate, 1996). Increased pod and seed yields in response to the BA treatments provide support for the hypothesis that cytokinin availability limits potential seed production in some soybean genotypes. Cytokinin rescue of soybean flowers and fruits (Reese *et al*., 1995) suggests that the hormone acts to redirect movement of assimilates into treated tissues, increasing sink strength and subsequent growth rates, and preventing abscission of the developing flowers and pods (Huff and Dybing, 1980; Dybing, 1994; Reese *et al*., 1995).

Although no attempt was made to measure flower production and abortion rates directly in this study, the increase in total number of pods per plant indicates that total flower set was increased by BA applications. Whether the increase in set is the result of decreased flower abortion rates, increased flower production with stable abortion rates, or some combination of these processes is still to be determined.

Cytokinin application to selected racemes has been shown to both increase flower production and reduce rates of flower abortion in those racemes (Dybing *et al*., 1986; Dyer *et al*., 1988; Reese *et al*., 1995). These earlier studies utilized single or multiple applications of BA to individual racemes and thus they are not directly comparable to this study where cytokinins were applied continuously in the transpiration stream for a 2-week period. However, results from both experimental protocols demonstrate the potential for increased soybean fruit production through an increase in the total numbers of flowers that set in response to the hormone treatments.

Increases in pod and seed production in response to cytokinin application may occur at the expense of growth in other parts of the plant, through the recommitment of the available resources from shoot and root development into fruit production. Preliminary observations support these assumptions, with BA-treated plants generally appearing smaller in size and having less well developed root systems at the time of harvest. In the field studies, wilting of plants during the heat of the day was more apparent in cytokinin-treated plants than in control plants. Changes in root and shoot development may have contributed to the differences in field and greenhouse results. Environmental stresses, especially water stress, can be controlled in the greenhouse, but in the fields where irrigation was unavailable, changes in basic plant architecture may have severely limited the potential of cytokinin application. Further examination of the effects of cytokinin treatments on plant architecture and dry weight accumulation are needed to fully assess the potential for the redistribution of dry matter into the production of seeds and the mechanisms by which BA increases seed yields in soybeans.

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