J Plant Growth Regul (1986) 5:49–57

Journal of
Plant
Growth
Regulation

The Validity of Using a Single Soybean Variety to Evaluate the Growth Regulatory Activity of Chemicals

Daniel R. Nelson and Yvonne M. Muskopf

Monsanto Company, 700 Chesterfield Village Parkway, Chesterfield, Missouri 63198

Received August 30, 1985; accepted January 15, 1986

Abstract. Forty six soybean (*Glycine max* [L.] Merr.) varieties were evaluated for their vegetative response to a foliar application of abscisic acid, benzyladenine, or o-methoxyphenyl phosphinic acid (OMPA). Varietal responses to each chemical ranged from no effect to severe growth retardation. When the same varieties were evaluated for their response to water stress, the length of time required for severe wilting ranged from 48 h to 120 h. A cluster analysis was used to relate the coefficient of parentage of the varieties with their response to chemicals or water stress. The response of a variety to one of the chemicals did not predict the response to a different chemical. Further, the response of a variety to a chemical did not predict the response of a similar variety to the same chemical. The water stress rating was neither related to the chemical responses nor to the coefficient of parentage for the varieties in the test. The effects of night interruption and darkness were more pronounced on northern than on southern varieties, analogous with the pattern of reproductive regulation by photoperiod.

Plant growth regulator technology has produced commercial yield enhancement in several crops, e.g., wheat (Jung and Rademacher 1983), sugarcane (Nickell 1976), and citrus (DeWilde 1971). Despite the lack of a chemical yield enhancer for soybean, the endogenous control mechanisms that determine yield are studied extensively. The potential risks of using a single genotype for studying these interactions have not been addressed, despite the ample evidence that the response to disease (Hymowitz et al. 1976) and herbicides (Fribourg and Johnson 1955, Payne and Koszykowski 1977, Wax et al. 1974) is quite different among varieties. Studying the effects of a growth regulator is

Abbreviations: OMPA, o-methoxyphenyl phosphinic acid.

more complex because, instead of observing an injury, the plant elicits a growth response that will differ depending on the physiological and external environments.

Presenting qualitative responses of soybean varieties to the same chemical at the vegetative stage is an extension of the information available for herbicides. The use of a cluster analysis to study the relationship between the responses also quantifies the results.

Materials and Methods

Plant Propagation and Chemical Application

Seed from 46 soybean varieties (*Glycine max* [L.] Merr. cv as specified) was pregerminated on wet paper towels and then planted directly into 4-inch pots containing a mixture of coarse sand:Dupo silty clay loam:redi-earth (W. R. Grace and Co.) (50:15:35 v/v/v) that had been packed while dry, then thoroughly moistened. Plants were grown in the greenhouse at Monsanto, Chesterfield Village, Missouri. The experiments were conducted in a temperature-controlled greenhouse during May and June; light, temperature, and relative humidity were essentially optimum. The chemicals were prepared for application as follows: 50 mg of each compound was dissolved in 5 ml of acetone; 0.6 ml of the solution was added to a mixture containing 4.5 ml of acetone and 5.1 ml of water:Tween 20 (ICI Americus) (95:5 v/v). This rate of chemical application will appear in the text as 0.36 kg/ha (based on pot area). When the unifoliolates completely unfolded, three replicate plants were sprayed with ABA (Sigma), BA (Sigma), or OMPA (Sabacky 1978) using a DeVilbiss atomizer. At 2 weeks after application, any morphological response to the treatments was recorded. The types of morphological effects include leaf alteration (deviations from a normally shaped, planar leaflet), leaf inhibition (reduction in leaflet size), or stature reduction (25% or greater reduction in plant height). The morphological data were rated numerically from 1 to 7 based on the severity of the response, then entered into the computer with a variety identification code, maturity group, and coefficient of parentage for the varieties in the study. The data set was then subjected to a cluster analysis (Dixon 1983).

Water Stress

Four control plants from each of the 46 varieties were moved to a separate bench in the greenhouse at 3 weeks after planting to evaluate their response to water stress. Each of the pots was watered to saturation then permitted to dry. Daily, until the end of the experiment, plants were rated for degree of stress: level 1 = no wilt, 2 = slight wilt, 3 = moderate wilt, and 4 = complete collapse of the leaves. When plants reached stress level 4, they were rewatered. An average stress rating of the replicates was calculated at 48 and 72 h. This was converted into a stress level from 1 to 7, then entered into the computer file containing the chemical data for cluster analysis.

Darkness

Four geographically diverse soybean varieties, McCall (group 00, Northern adapted), Amsoy (group II), Cutler (group IV), and Essex (group V, Southern adapted) were treated with ABA, BA, or OMPA at 0.36 kg/ha before the expansion of the first trifoliolate leaf (see chemical application). There were 12 replicate leaves in each treatment. Controls and chemically treated plants were then placed into a cardboard box for 24 h. At the conclusion of the dark treatment, plants were removed from the box one at a time. The distance from the stem to the tip of each leaflet was measured at a right angle to the stem; this defined the pulvinar angle. The effect of darkness was calculated as the pulvinar angle of the chemical treated or control plants after treatment, as a percent of pretreatment control angle.

Night Interruption

On 20 May 1978, at the Monsanto Co., St. Charles farm, each of three varieties (Evans, Williams, and Clark, maturity groups 0, III, and IV respectively) was planted in four rows, 10 m in length. The row spacing was 45 cm and the population was 300,000 plants/ha. The rows were split into 5-m halves using four layers of 50% shade cloth stretched over steel posts. A 150 watt incandescent flood lamp with a reflector was centered between the two center rows on each side of the shade cloth. The lamps were controlled from planting until stage R-6 (Fehr and Caviness 1977) using a Paragon timer; one lamp was on from 7 P.M. until 8 P.M. and the other from 1 A.M. until 2 A.M. All plants were harvested at stage R-6. At harvest, plant height and pod number of each plant were recorded; then plant parts were separated for dry weight determination. Means, standard error, and percent control were computed.

Results

Morphological Effects

The response of each variety to BA, ABA, OMPA, and water stress was rated for severity of activity using a continuum from 1 to 7 (most severe). For reference, the individual response profiles are presented in Fig. 1 as they appeared prior to statistical analysis. A cluster analysis of these data (see Table 1) showed that no relationship existed between the response of a variety to BA and its response to ABA. The two OMPA responses were related to each other, but not to the effects of ABA or BA. When the analysis related the coefficient of parentage, maturity group, and morphological effects of these chemicals, there was no relationship.

There were important qualitative observations. There was no visible response of some varieties to 0.36 kg/ha ABA or BA, while others became severely retarded, chlorotic, or deformed. There were also various types of intermediate effects of ABA among the varieties, e.g., wavy leaf margins accom-

	Response Level*						
	1	2	3	4	5	6	7
Douglas	CDF	A	BE				
Pella	AC		B	E		D	F
Columbus	C	A	B	D	E		F
Custer		B	CEF				AD
PI 79712	A		EF		B	C	D
PI 89008	CD	A	B		E		F
HARTZ 6383	A		BCF		E		D
PI 89061-2	A		BCEF				D
Williams (1982)	A		BE	CDF			
PI 70242-2	ACD		B	EF			
PI 87631-1	CD		AB E			F	
PI 89156			B E F		CA		D
Cutler	AC		EB		F		D
Ransom	A	CB	EF		D		
Williams (1983)	AC	E	F		BD		
Dare	C	B			DEFA		
Amsoy	CEAD	F			B		
Dunfield	CD	A		F	E	B	
PI 266085A	CD	F	AE	B			
Gnome	A	E	B	FC	D		
IV 113	F	A	ECB				D
A. K. (Kansas)	C		AB E		FD		
Perry	C		BE	FD			A

	Response Level*						
	1	2	3	4	5	6	7
Centennial	AB	E	CF			D	
Union	A		E	B	C		FD
PI 91180		B	EFC				AD
Verde		F	E B		A		CD
PI 91115	AB		EF	C		D	
Peking	ACD		B		E		F
Evans	B		EC		FA		D
PI 423907	A		BEFC				D
PI 432921	AB		C EF				D
Essex	BCD				EF		A
McCall	C		BEF				AD
Sloan	ACB		E		D		F
Ross	A	B	E				FCD
Forrest	AC	BD	E		F		
Corsoy	A		EFC				D
Elf	AC		BE D				F
Lindarin				CE			FD
Calland	A		BC		D		EF
Cutler	A		EBF	C			D
PI 103091		B	F C	E			DA
H79-18009	A		EB C		F		D
PI 86482-1	CD		B				AEF
Fiskeby	CDF	A			EB		

Fig. 1. The morphological response of soybeans to chemicals and water stress. The response to 0.36 kg/ha of ABA (A), BA (B), or OMPA (E = elongation response and F = leaf alteration response) was evaluated in the greenhouse at 1 week after planting. The response to a dry-down after saturation with water was evaluated at 48 h (C) and 72 h (D). The response levels were assigned at approximately equal intervals from the weakest observed response (1) to the most severe (7).

Table 1. Correlations among varietal responses to chemicals and stress.

BA[a]	−0.22560[c] 0.1409				
48h[b]	−0.08018 0.6006	−0.10042 0.5166			
72h	−0.02623 0.8658	−0.28811 0.0610	0.64414 0.0001		
OMPA 1	0.19928 0.1894	0.03332 0.8300	−0.23717 0.1125	−0.30996 0.0383	
OMPA 2	−0.06825 0.6598	−0.19282 0.2154	−0.12724 0.4049	−0.11587 0.44895	0.42507 0.0036
	ABA	BA	48h	72h	OMPA 1

[a] BA, ABA, and OMPA were foliar applied at the fully expanded primary leaf stage at a rate of 0.36 kg/ha. There were three replicates/variety.
[b] The response to a dry-down was quantified at 48 and 72 h. There were four replicates/variety.
[c] Pearson correlation coefficient with significance level (0.01 criterion used).

panied by chlorosis, leaf distortion, or retardation without chlorosis. Williams, a variety that did not respond to 0.36 kg/ha ABA, exhibited necrosis of the leaf margins and retardation of leaf expansion after a 3.6 kg/ha application of ABA. This response was unlike that observed with any of the more sensitive varieties. OMPA exhibited much fewer types of effects and a smaller range of sensitivity. The effect of OMPA on stem elongation was recorded separately from the morphological effect on the leaves. The effects on stem elongation ranged from stature reduction to stature stimulation. Effects on leaf morphology ranged from slight leaf alteration to compaction of the trifoliolates into a terminal rosette. In addition, there was severe epinasty, or absence of it, and degrees of stem splitting and other stem effects. Evaluation of seed from different sources of Williams and Cutler showed that the effects were quite consistent within a genotype. Of the two pairs of nonidentical genotypes that had coefficients of parentage greater than 0.95, Williams and Union responded to the chemicals similarly, however, Sloan and Dunfield responded quite differently. Despite the diversity between varieties, the response of replicates within a varietal treatment was extremely uniform.

Water Stress

The degree of stress exhibited by each of the varieties at 48 and 72 h after watering appears in Fig. 1 (code letters C and D, respectively). As with the chemical effects, the replicates exhibited stress uniformly, and only 3% of the plants failed to recover after rewatering. The length of time required for severe stress varied from 48 to 120 h after watering. The varieties Ross and Verde had completely wilted at 48 h; varieties PI 89008 and PI 266085A required 120 h. Approximately one third of the varieties had not wilted at 72 h. When the water stress results were included with the chemical and genetic data in a

Table 2. Effect of darkness on leaf angle.

Variety (maturity group)	$\bar{X}$ Control distance (cm)[a]	Control % pre-dark	OMPA[b]	ABA	BA
			% Pre-dark control		
McCall (OO) pre-treat	5.9 ± 1.1	100			
post-dark	1.4 ± 0.15	23.7	21.7	20.5	19.2
Amsoy (II) pre-treat	5.7 ± 0.8	100			
post-dark	2.9 ± 0.38	50.8	54.5	51.5	16.9
Cutter IV pre-treat	5.9 ± 1.0	100			
post-dark	3.3 ± 0.53	56.0	61.5	55.6	39.0
Essex (V) pre-treat	5.9 ± 0.95	100			
post-dark	3.8 ± 0.91	63.7	70.2	23.1	29.3

[a] Leaf angle is determined by measuring the distance from the stem to the tip of the leaf at a right angle to the stem. $\bar{X}$ of 12 replications ± SE.
[b] OMPA, ABA, and BA were foliar applied at the fully expanded primary leaf stage, at a rate of 0.36 kg/ha.

cluster analysis, there was a relationship between the stress levels at 48 and 72 h, however, no relationship between the stress levels and chemical effects.

Darkness

The response of selected varieties to darkness is shown in Table 2. The effect of darkness on pulvinar angle was related to maturity group, i.e., greatest in McCall, least in Essex, and intermediate in Amsoy and Cutler. However, the influence of ABA, BA, and OMPA on this activity was neither related to maturity group nor to the morphological pattern observed in Fig. 1. OMPA inhibited and BA stimulated the pulvinar collapse of each variety similarly. Essex did not respond to BA in the morphological test, however, BA caused a pronounced pulvinal collapse in the dark. Amsoy exhibited a strong response in both tests.

Night Interruption

The Evans variety exhibited the most pronounced visible response to night interruption. The plants were tall, with thick stems and broad leaves. The inflorescences were branched and up to 6 cm in length. Maturity was delayed, however, the pods developed until stage R-6. The Evans receiving a night interruption reached stage R-6 approximately 30 days after the control plots. The Williams given night interruption reached R-6 approximately 10 days after the controls, and the treated and control plots of Clark 63 reached R-6 simultaneously.

There were also marked quantitative differences between varieties (Table 3). A mean of these differences for each variety is given in Table 3 to show the

Table 3. Response of soybeans to night interruption.

Parameter	Evans (group O)		Williams (group III)		Clark (group IV)	
	Controls ± SE[b]	Treatment[a] (% controls)	Controls ± SE	Treatment (% controls)	Controls ± SE	Treatment (% controls)
Height (cm)	100 ± 15.6	175	125 ± 19.2	120	134 ± 22.4	83
Leaf area (dm)	19 ± 2.5	245	27 ± 4.6	200	36 ± 5.8	88
Leaf number	18 ± 3.3	127	20 ± 3.1	176	30 ± 5.4	83
Leaf weight (g)	9.2 ± 1.6	249	13.5 ± 2.2	218	16.9 ± 4.2	90
Stem weight (g)	8.1 ± 1.3	460	13.4 ± 2.5	197	14.5 ± 3.6	95
Pod weight (g)	29.9 ± 3.1	183	18.5 ± 3.6	130	21.5 ± 5.1	78
Pod number	72 ± 11.3	128	71 ± 13.6	130	92 ± 18.1	78
Branch number	3.3 ± 0.5	91	4.8 ± 1.2	113	5.1 ± 2.2	124
Branch weight (g)	6.7 ± 1.0	394	11.0 ± 2.2	213	14.0 ± 3.1	66
Petiole length (cm)	27.8 ± 4.4	154	27.2 ± 5.1	102	36.4 ± 5.6	86
		$\overline{X} = 220$		$\overline{X} = 160$		$\overline{X} = 87$

[a] A 150 watt flood lamp, controlled by a timer, was on from 1 A.M. to 2 A.M. continuously from planting until harvest at R-6. Controls were given 1 h of light from 7 P.M. to 8 P.M.

[b] Data based on the mean of six replicates.

trend. Except for the lack of an effect in branch number, the response of Evans to night interruption was uniform across all growth components. There was an intermediate effect of the light treatment on the growth parameters of Williams and the lack of an effect on Clark.

Discussion

The herbicide OMPA was selected as a representative regulator because at low rates it produces morphological effects similar to 2,4-D or IAA. Unlike 2,4-D, it may be tested near other treatments without imparting formative effects; unlike IAA, it is not metabolized. ABA and a cytokinin were selected because they produce negligible morphological effects with Williams, one of the most heavily planted soybean varieties. If a physiologically active growth regulator produces no visible response with vegetative plants, another type of bioassay would be necessary to identify these activities.

OMPA activity was expressed similarly among the varieties, typical of other compounds that produce formative effects. Besides differences in sensitivity to ABA and BA, there were several distinct types of visible effects. In this context, it was not known if the visible effect produced by a sensitive variety at a low rate would be the same as that produced by an insensitive variety at a high rate. This was addressed when the Williams variety, which had exhibited no sensitivity to 0.36 kg/ha ABA, produced a distinct effect after an application of 3.6 kg/ha. Clearly, the response of a variety to one chemical does not predict the effect of a different chemical on the same variety, nor does the effect of a chemical on one variety predict the effect of the same chemical on a genetically similar variety. Similarly, there was no relationship between the insensitivity of a variety to ABA and sensitivity to water stress, despite the ABA production associated with water stress (Wright and Hiron 1969). The focus of the dry-down experiment was a rating of the stress symptoms; however, in light of the varietal differences, examination of the stress avoidance mechanism of these genotypes, e.g., root mass or diffusing resistance, as related to ABA content, would be interesting.

It has been suggested that the collapse of the pulvinus in darkness is inhibited by auxin (Williams and Raghavan 1966). Cytokinins have been shown to antagonize auxin (Vanderhoef and Stahl 1975). It was hypothesized that sensitivity of a variety to an auxin mediated environmental response would be related to the sensitivity of the same variety to an exogenously applied auxin synergist or antagonist. The effect of the dark treatment on control pulvini was related to maturity group; however, the effect of chemicals on pulvinar angle was not correlated with the profile of visible effects in Fig. 1. However, OMPA inhibited pulvinar collapse and BA stimulated it, consistent with the auxin hypothesis. Overall, the OMPA and BA results suggest that the physiological sensitivity to a chemical is not coupled with the morphological sensitivity. The results of this experiment are consistent with the lack of a relationship between chemical effects and maturity group in the cluster analysis. An alignment of light treatment effects with maturity group occurred in both the darkness and night interruption experiments, i.e., the response of the northern variety to darkness and night interruption was more pronounced than that of southern varieties. This is consistent with the well-accepted dependence of northern varieties on photoperiod for reproductive regulation.

In summary, the response of a genotype to external stimuli is complex and generally unpredictable. If there is a heritable component, it could not reliably predict the response of two similar genotypes to an exogenously applied chemical or to a water stress. The morphological effect produced by a standard compound or environmental treatment on a "representative" test variety will apparently not predict the effect of the same treatment on another variety. Furthermore, if morphological and physiological sensitivity to the environment are not coupled, the choice of genotype may potentially influence the results of any physiological research with soybean, and probably other species.

Acknowledgments. We gratefully acknowledge the assistance of Dr. L. R. Holden in the cluster analysis of the experimental results, and Dr. X. Delannay in providing the cultivars and information about their lineage.

References

DeWilde RC (1971) Practical applications of (2-chloromethyl) phosphonic acid in agricultural production. Hort Sci 6:364–370

Dixon WJ (1983) BMDP Statistical software. University of California Berkeley Press, appendix A.37, p 693

Fehr WR, Caviness CE (1977) Stages of soybean development. Special report 80, Cooperative Extension, Iowa State University

Fribourg HA, Johnson IJ (1955) Response of soybean strains to 2,4-D and 2,4,5-T. Agron J 47:171–174

Hymowitz T, Carmer SG, Newell CA (1976) Soybean cultivars released in the United States and Canada: morphological descriptions and responses to selected foliar, stem and root diseases. Internat Ag Pub, INSOY series 9

Jung J, Rademacher W (1983) Plant growth regulatory chemicals: cereal grains. In: Nickell LG (ed) Plant growth regulatory chemicals, volume I. CRC Press, Boca Raton, pp 255–261

Nickell LG (1976) Chemical growth regulation in sugar cane. Outlook on Agriculture 9:57–61

Payne RC, Koszykowski TJ (1977) Differentiation of soybean cultivars by metribuzin sensitivity. J Seed Tech 2:1–10

Sabacky MJ (1978) Herbicidal use of substituted phenyl phosphinic acids. US Patent 4, 130, 410, pp 1–6

Vanderhoef LN, Stahl CA (1975) Separation of two responses to auxin by means of cytokinin inhibition. Proc Natl Acad Sci 72:1822–1825

Wax LM, Bernard RL, Hayes RM (1974) Response of soybean cultivars to bentazon, bromoxynil, chloroxuron and 2,4-DB. Weed Sci 22:35–41

Williams CN, Raghavan V (1966) Effects of light and growth substances on the diurnal leaf movements of Mimosa pudica. JEB 17:742–749

Wright STC, Hiron RWP (1969) (+)-Abscisic acid, the growth inhibitor induced in detached wheat leaves by a period of wilting. Nature (London) 224:719–720