

Nodulation and nitrogen fixation on vegetative soybean plants as affected by photoperiod and gibberellic acid

P.A. Balatti, J.J. Guiamet and E.R. Montaldi

Long-day photoperiods and applications of gibberellic acid promoted shoot growth by stimulating leaf enlargement as a result of increasing available photosynthates, which was also reflected in the higher leaf, stem and nodule plant dry weights. Short-day plants had more nodules, but they were smaller (by weight) than those of long-day plants. Gibberellic acid at 1.5×10^{-6} M enhanced nodule growth without preventing nodule formation. Factors other than just gibberellic acid are concluded to be involved in the responses of nodulation and nitrogen fixation to day length.

For French summary, see next page.

The authors are affiliated to the Instituto de Fisiología Vegetal, Facultad de Agronomía, Universidad Nacional de La Plata, CC 31, La Plata (1900) Argentina. Address reprint requests to P.A. Balatti.

Introduction

The dependency of nodulation and nitrogen fixation on light is well documented. Rhizobium–legume symbiosis is affected not only by photosynthesis (Hardy & Havelka 1976) but also by day length. Mes (1959) and Balatti & Montaldi (1983) found more nodules and a higher nitrogen fixation rate respectively, in *Vicia* and soybean, when grown under short days. A 15 min exposure to red light at the end of the photoperiod reduced nodulation in rooted soybean leaves compared with those irradiated with far-red light (Balatti & Montaldi 1986).

Plant responses to both day length and light quality correlate with plant hormonal changes. Chailakyan (1972) has suggested that soybean plants grown under long-day illumination have a greater gibberellin content than those grown under short-day illumination. Railton & Wareing (1973a,b) found a higher gibberellin content and an enhanced production of gibberellin in leaves of *Solanum andigena* grown under long-day illumination. Additionally, gibberellin synthesis is known to be promoted by red light (Cooke & Kendrick 1976).

The aim of this paper is to provide evidence for the involvement of gibberellic acid in the regulation of nodulation by photoperiod. We studied the responses of inoculated soybean plants to photoperiod and to an exogenous gibberellin supply.

Materials and Methods

Seeds of soybean (*Glycine max* L. Merr), cultivar Bragg, were surface sterilized with 10% sodium hypochlorite for 15 min and germinated in darkness at 28°C. The seedlings were transplanted to 1 l plastic pots filled with vermiculite substratum previously washed and sterilized at 120°C for 2 h.

When the first trifoliolate leaf expanded to 80% of its final size, plants were inoculated with a 1×10^8 cells/ml suspension culture of *Bradyrhizobium japonicum* strain 5019 of the MIRCEN collection (Lopreto *et al.* 1973). Thereafter the pots were watered weekly with a nitrogen-free Hoagland solution. Plants were grown under short-day illumination (SD) (8 h of light + 16 h of darkness) and long-day illumination (LD) (16 h of light + 8 h of darkness). In both treatments, plants

Des photopériodes de longue journée et l'application d'acide gibbérellique a promu la croissance des pousses en stimulant l'élargissement des feuilles. Celle-ci résultait d'un accroissement de photosynthates disponibles. Cet effet s'est aussi reflété dans un accroissement de la masse de feuilles, de tiges et de nodules chez la plante. Des plants soumis à des photopériodes de courte journée présentaient plus de nodules mais ceux-ci étaient plus petits et de masse moindre que ceux de plants soumis à des photopériode de longue journée. L'acide gibbérellique à la dose de $1.5 \cdot 10^{-6}$ M a accru la croissance des nodules sans empêcher la formation des nodules. On a conclu que des facteurs autres que le seul acide gibbérellique sont mêlés aux réponses de la nodulation et de la fixation d'azote à la longueur de la journée.

were exposed to 8 h of natural daylight in a glasshouse. To avoid major differences in photosynthetic irradiance, day length in the LD treatment was extended for another 8 h with only $28 \mu\text{mol m}^{-2} \text{s}^{-1}$ of photosynthetically active radiation. The temperature during the experiment was $21 \pm 2^\circ\text{C}$. In each treatment, pots for the 12 replicates were placed at random.

Gibberellic acid (GA_3) solutions at zero, 1.5×10^{-6} M and 3.0×10^{-6} M were sprayed in each photoperiod from the moment the plants were inoculated until the end of the experiments. GA_3 was prepared daily before being sprayed onto the plants.

Plants were harvested 60 days after sowing. Organs were separated for analysis and dried at 60°C . Nodulated root systems were assessed for acetylene reduction activity by the method of Schwinghamer *et al.* (1970). The experiment was performed twice, and activities were calculated as μmol acetylene reduced/g fresh weight per h. Nitrogen content was determined by a modified microKjeldahl method (Lindner & Harley 1942). Analysis of variance and the Duncan test at 5% level of probability were used to compare the means.

Results and Discussion

Whole-plant dry weight together with leaf and stem dry weights were increased in those plants grown under long-day illumination (LD) compared with those grown under short-day illumination (SD) (Table 1). This agrees with the results of Mes (1959) for *Vicia villosa* and indicates that photoperiod affected the source-sink relationship. A larger photosynthetic area, due to leaf growth, was found in plants under LD (Table 1). Nitrogen concentration on a leaf area basis was larger under SD than under LD (Table 2). This difference can be related to differences in the ribulose-1,5-biphosphate carboxylase activities under both photoperiods (Casano *et al.* 1984).

Comparing SD and LD treatments, more nodules were found in plants grown under SD whereas nodule size was larger in those plants under LD (Table 1). Carbohydrate availability is known to limit nodule development and growth; therefore fewer nodules might be expected to be larger if the quantity of available photosynthate is the same.

Applications of gibberellic acid (GA_3) increased photosynthate accumulation and the whole-plant dry weight under either photoperiod (Table 1). This is due to GA enhancement of leaf growth and/or ribulose-1,5-biphosphate carboxylase activity (Weaver & Johnson 1985). SD plants were induced to flower and thus formed fruits.

Table 1. Effect of gibberellic acid (GA) and photoperiod upon soybean plants.

	Total plant weight (mg)	Dry weight (mg)			Leaf		Nodule	
		Stem	Leaf	Nodule	Area (dm^2)	SLA ($\text{dm}^2 \text{g}^{-1}$)	Number	Size
<i>Short days</i>								
Control (no GA)	589 (c)	121 (d)	208 (e)	34.3 (a)	1.324 (d)	0.644 (d)	96 (a)	0.3565 (b)
$1.5 \cdot 10^{-6}$ M GA	802 (b)	227 (bc)	295 (bcd)	47.8 (a)	2.055 (bc)	0.697 (bc)	97 (a)	0.4940 (a)
$3.0 \cdot 10^{-6}$ M GA	730 (bc)	215 (c)	252 (de)	38.3 (a)	1.700 (cd)	0.665 (c)	62 (b)	0.6187 (a)
<i>Long days</i>								
Control (no GA)	794 (b)	208 (c)	335 (bc)	42.8 (a)	2.440 (ab)	0.731 (bc)	78 (b)	0.5505 (a)
$1.5 \cdot 10^{-6}$ M GA	826 (ab)	257 (bc)	347 (ab)	47.5 (a)	2.700 (a)	0.783 (ab)	69 (b)	0.6921 (a)
$3.0 \cdot 10^{-6}$ M GA	961 (a)	357 (a)	396 (a)	41.2 (a)	2.770 (a)	0.751 (bc)	69 (b)	0.5971 (a)

SLA = Specific Leaf Area.

Means followed by the same letter are not significantly different. Means followed by different letters are statistically different at $P = 0.05$.

Table 2. Effect of gibberellic acid (GA) and photoperiod on %N and nitrogen content.

	%N				Nitrogen content				
	Root	Nodule	Stem	Leaf	Root	Nodule	Stem	Leaf	Whole plant
<i>Short days</i>									
Control (no GA)	1.91 (a)	6.67 (ab)	2.43 (a)	4.78 (a)	3.47 (ab)	2.32 (ab)	2.96 (bc)	9.87 (cd)	22.03 (a)
1.5 · 10 ⁻⁶ M GA	1.09 (a)	6.04 (b)	1.45 (ab)	4.20 (a)	1.82 (f)	2.86 (a)	3.30 (a)	12.21 (bc)	23.47 (a)
3.0 · 10 ⁻⁶ M GA	1.58 (a)	7.21 (a)	1.92 (a)	4.09 (a)	2.63 (cde)	2.77 (a)	4.12 (a)	10.39 (cd)	22.94 (a)
<i>Long days</i>									
Control (no GA)	1.99 (a)	6.92 (a)	1.24 (b)	4.46 (a)	4.12 (a)	2.94 (a)	2.55 (bc)	14.87 (a)	24.48 (a)
1.5 · 10 ⁻⁶ M GA	1.56 (a)	6.53 (ab)	1.01 (b)	3.98 (a)	3.03 (bc)	3.10 (a)	2.61 (bc)	13.66 (ab)	22.39 (a)
3.0 · 10 ⁻⁶ M GA	1.49 (a)	6.15 (b)	0.86 (b)	3.89 (a)	2.89 (bcd)	2.55 (ab)	3.06 (b)	14.29 (ab)	22.79 (a)

Means followed by the same letter are not statistically different. Means followed by different letters are statistically different at $P = 0.05$.

The interaction of day length and GA₃ was evident in SD plants treated with 1.5×10^{-6} M GA. These plants had the same number of nodules as control plants but the nodules were larger (Table 1). This might be related to photosynthate availability. Under LD, all the GA treated plants had fewer and larger nodules than the controls, although the differences were not always statistically significant. Mes (1959) and Ramirez & Williams (1982) found that higher levels of GA reduced nodulation in soybean plants though the experimental conditions were different.

Nitrogenase activity did not change in response to any of the treatments tested (data not shown). In contrast, Ramirez & Williams (1982) found that nitrogen fixation activity diminished in GA treated plants.

Root, leaf and nodule nitrogen content were unaffected by photoperiod but the percentage of stem nitrogen was reduced under LD (Table 2). The reduced nitrogen content might be due to a dilution effect. Photoperiod did not affect the percentage of nodule nitrogen but GA diminished it in those plants grown under LD.

It is of interest that under SD, GA provoked growth responses similar, but not equal, to those observed under LD (Vince-Prue 1985). This most likely is due to a lower gibberellin content of SD plants (Mes 1959; Chailakyan 1972; Railton & Wareing 1973a,b). Besides, GA may have acted synergistically with endogenous gibberellins of LD plants to increase whole-plant, stem and leaf dry weights more than LD alone.

We concluded that GA₃ partially replaced the LD stimulus and, from the analysis of the interaction between day length and applied GA₃, that factors other than GA₃ alone are important in nodulation and nitrogen fixation response to photoperiod.

Acknowledgements

Thanks are due to Dr A. P. Balatti for providing rhizobium culture strain 5019 (MIRCEN). This work was supported by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and by the Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC).

References

- BALATTI, P.A. & MONTALDI, E.R. 1983. Efecto del fotoperíodo sobre la nodulación y fijación de nitrógeno en plantas de soja (*Glycine max* L.Merr). *Revista de la Facultad de Agronomía, U.N.L.P.*, 3a época **57**, (1-2), 23-29.
- BALATTI, P.A. & MONTALDI, E.R. 1986. Effects of red and far red lights on nodulation and nitrogen fixation in soybean (*Glycine max* L.Merr). *Plant and Soil* **92**, 427-430.

- CASANO, L.M., VALLE, E.M., MORANDI, E.N. & NAKAYAMA, F. 1984. Ribulose biphosphate carboxylase activity of soybean (*Glycine max* L.Merr) leaves under different photoperiods. *Photosynthetica* **18**, 161–167.
- CHAILAKYAN, M.Kh. 1972. Hormonal regulation of plant flowering in different photoperiodic groups. In *Plant Growth Substances*, ed. Carr D.J., pp. 745–752. Berlin: Springer.
- COOKE, R.J. & KENDRICK, R.E. 1976. Phytochrome controlled gibberellin metabolism in etioplast envelopes. *Planta* **131**, 303–307.
- HARDY, R.W.F. & HAVELKA, U.D. 1976. Photosynthate as a major factor limiting nitrogen fixation by field grown soybeans. In *Symbiotic Nitrogen Fixation in Plants*, ed. Nutman P.S., pp. 421–439. Cambridge: Cambridge University Press.
- LINDNER, R.C. & HARLEY, C.P. 1942. A rapid method for determination of nitrogen in plant tissue. *Science* **96**, 565–566.
- LOPRETO, C.R., MAZZA, L.A. & BALATTI, A.P. 1973. Producción de inoculantes para soja. *Revista de la Facultad de Agronomía, U.N.L.P.* 3a época, **49**(2), 201–203.
- MES, M. 1959. Influence of gibberellic acid on the growth, flowering, nodulation and nitrogen assimilation of *Vicia villosa*. *Nature* **184**, 2035–2036.
- RAILTON, I.D. & WAREING, P.F. 1973a. Effects of daylength on endogenous gibberellins in leaves of *Solanum andigena*—I. Changes in levels of free-acidic gibberellin-like substances *Physiologia Plantarum* **28**, 88–94.
- RAILTON, I.D. & WAREING, P.F. 1973b. Effects of daylength on endogenous gibberellins in *Solanum andigena*—II. Gibberellin production by the leaves. *Physiologia Plantarum* **29**, 430–433.
- RAMIREZ, G. & WILLIAMS, P.M. 1982. El rol de las giberelinas en la infección y morphogenesis de nódulos de soja (*Glycine max* L.Merr). In *IX Reunión Latinoamericana de Rizobiólogos*, Lima, Peru, pp. 39–59.
- SCHWINGHAMER, E.A., EVANS, H.J. & DAWSON, M.D. 1970. Evaluation of effectiveness in mutant strains of *Rhizobium* by acetylene reduction relative to other criteria of N₂ fixation. *Plant and Soil* **33**, 192–212.
- VINCE-PRUE, D. 1985. Photoperiod and hormones. In *Encyclopedia of Plant Physiology*, Vol. 3, *Hormonal Regulation of Development*, Pharis, R.P. and Reid, D.M. pp. 308–350. Berlin: Springer.
- WEAVER, C.J. & JOHNSON, J.O. 1985. Relation of hormones to nutrient mobilization and the internal environment of the plant: the supply of mineral nutrients and photosynthates. In *Encyclopedia of Plant Physiology*, vol. 3, *Hormonal Regulation of Development*, eds Pharis, R.P. & Reid D.M. Berlin Springer.

(Received 3 October 1989; revised 6 February 1990; accepted 20 February 1990)