Short Communication

Marked Inhibition of Floral Bud Initiation by Optically Active Absciscic Acid in *Pharbitis nil*

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Since the first isolation of abscisic acid from young fruit of the cotton plant,¹⁾ its biological activity has been extensively investigated in various plant species.²⁾ Absciscic acid is a plant hormone that regulates stomatal closure and dormancy, and these characteristic activities distinguish it from all the other plant hormones. Absciscic acid also produces a flowering response, although it does not appear to be a major determinant in floral transition (see the review by Bernier³⁾). Most research into the

activities of abscisic acid has been conducted on the racemic compound, which has been made commercially available by organic synthesis. A natural type of (S)-(+)-abscisic acid has also been synthesized chemically,⁴⁻⁶⁾ as well as having been produced by microbial fermentation with *Cercospora rosiicola*;⁷⁾ however, the very high cost of producing an optically active sample by fermentation has rendered it prohibitive for use in routine research into plant science and technology.

We have found that (S)-(+)-abscisic acid was produced by the fungus *Botrytis cinerea* when cultured on a nutrient agar medium.⁸⁾ As large-scale production now ensures high yields, the use of natural abscisic acid for agricultural purposes has become practical. We now report that (S)-(+)-abscisic acid completely inhibited floral bud initiation in *Pharbitis nil*. Interestingly, complete inhibition of floral bud initiation never occurred when racemic abscisic acid was applied to the plant, even at higher concentrations.

Germinated seeds of *Pharbitis nil* (Violet strain) were sown in wet vermiculite and grown under a long-day regimen with a photoperiod of 16 hr (from 4 a.m. to 8 p.m.) in a greenhouse

Table I. EFFECTS OF (S)-(+)- AND RACEMIC FORMS OF ABSCLSCIC ACIDS ON FLOWER BUD INITIATION IN *Pharbitis nil*

Method of application	Conc. (ppm)	Flower buds/plant		Plant height (cm)	
		(S)-(+)-	Racemic	(S)-(+)-	Racemic
Drench	25	0	0.8	16.2	17.9
	5	0	1.6	18.1	20.2
	1	0.8	2.2	22.6	23.8
	0	2.6		24.3	
Spray	500	0	1.2	17.7	19.2
	125	0	1.4	19.5	20.4
	30	1.2	1.8	21.8	22.9
		2.8		26.8	

Seeds were sown singly in plastic pots (4 cm diameter, 4 cm deep) that contained vermiculite, and grown in a greenhouse. The temperature was kept above 18°C. Aqueous solutions of abscisic acid were applied by the drench (20 ml/pot) or spray (10 ml/10 seedlings) method. Ten plants were used for each application. Applications were made 5 hr before the short-day treatment was begun. On the day after the hormone application, the plants were transplanted singly to larger pots (7 cm diameter, 7 cm deep) that contained soil. The number of flower buds was counted 14 days after the hormone treatment.

with a supplemental light source from white fluorescent lamps (Hitachi FL40 SSD/37-D). The photosynthetic photon flux (PPF) was $45 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the top of the plants. Five-day-old seedlings were transferred to a short-day regimen with a dark period of 15 hr (from 6 p.m. to 9 a.m.). An aqueous solution of abscisic acid was applied to the seedlings by the drench or spray method before, or after, the short-day treatment (see the footnotes to Tables 1 and 2). On the day after this application, the plants were transferred back to the long-day regimen. The number of floral buds produced was counted 14 days after the application.

The inhibitory activity of (*S*)-(+)-abscisic acid on floral bud initiation is compared with that of racemic abscisic acid in Table I. With both the drench and spray methods, (*S*)-(+)-abscisic acid showed much stronger activity than the racemic compound. By the drench application, (*S*)-(+)-abscisic acid completely inhibited floral bud initiation at 5 ppm, whereas the racemic compound allowed floral buds to form even at 25 ppm. A similar marked difference in activity between the two compounds was found with the spray method. However, a much greater amount (125 ppm) of (*S*)-(+)-abscisic acid, compared to the drench method, was required for the complete inhibition of flower bud initiation, with the racemic compound (125 ppm) only showing a slight inhibitory effect.

The timing of the application was an important factor for the most effective inhibition of floral bud initiation (Table II). The greatest inhibition took place when (*S*)-(+)-abscisic acid was applied just before the short-day treatment, whereas, when applied just after that treatment, it tended to promote initiation. The present studies show that natural (*S*)-(+)-abscisic acid obtained from a fermentation broth of *Botrytis cinerea* completely inhibited floral bud initiation in *Pharbitis nil*.

The results from previous investigations of *Pharbitis nil*, in which racemic abscisic acid was used, did not show any clear inhibition of flowering.⁹⁻¹¹ The maximum concentration

Table II. TIMING FOR THE APPLICATION OF (*S*)-(+)-ABSCISIC ACID AND ITS EFFECT ON FLOWER BUD INITIATION IN *Pharbitis nil*

Conc. (ppm)	Flower buds/plant			
	Timing of application			
	10 hr before	5 hr before	just before	just after
200	1.1	0	0	3.0
100	1.7	0.9	0.6	2.9
0	2.6			

The methods of cultivation and hormone application are described in the footnote to Table I. Test solutions (10 ml/10 plants) of abscisic acid were sprayed on the plants 10 or 5 hr before, or just before or after the short-day treatment.

tested in the previous investigations was 4 ppm by root or 100 ppm by foliar application, and in these concentrations for the racemic compound, our own results also showed no inhibitory activity. There have been several comparative studies reported for the *S*- and *R*-enantiomers of abscisic acid; the natural *S*-enantiomer was much stronger in its closure of the stomata.¹² In our experiments, the flower bud initiation was markedly inhibited by the (*S*)-(+)-enantiomer. Therefore, the natural *S*-enantiomer seems to be physiologically important for high activity in the regulation of stomata closure and flower bud initiation. On the other hand, in such other bioassay systems as a growth inhibition test, the activity of the *S*-enantiomer was equal to, or slightly stronger than that of the *R*-enantiomer.¹³ We show, in Table 2, that the growth inhibitory activity towards the plant height of *Pharbitis nil* was not much different between the *S*-enantiomer and the racemic compound. We would like to emphasize that the (*S*)-(+)-abscisic acid used in the present studies has been shown to be optically pure on the basis of a chiral HPLC analysis of the sample. The enantiomeric purity of the chiral sample used in previous studies, however, could not be confirmed (a chiral chromatographic column for the enantiomeric analysis of abscisic acid

was only developed in 1988¹⁵⁾). The results of our present investigation indicate that the natural type of (*S*)-(+)-absciscic acid markedly inhibited the floral bud initiation induced by a short-day treatment in *Pharbitis nil*. Since the racemic mixture of absciscic acid showed only very weak inhibition of flower bud initiation, even at high concentrations, the results of previous studies should be re-evaluated for optically pure (*S*)-(+)-absciscic acid in order to establish the actual activity of natural absciscic acid. The biological activity of the unnatural *R*-enantiomer also should be examined, and our chiral synthesis of it is now being conducted.

References

- 1) K. Ohkuma, J. L. Lyon, F. T. Addicot and O. E. Smith, *Science*, **142**, 1592 (1963).
- 2) L. A. D. Zeevaart, *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, **39**, 439 (1988).
- 3) G. Bernier, *Ann. Rev. Plant Physiol.*, **39**, 175 (1988).
- 4) F. Kienzle, H. Meyer, R. E. Minder and H. Thommen, *Helv. Chim. Acta*, **61**, 2616 (1978).
- 5) M. Soukup, T. Lukac, B. Lohri and E. Widmer, *Helv. Chim. Acta*, **72**, 361 (1989).
- 6) K. Sakai and K. Takahashi, Proceedings of the 33rd Symposium on the Chemistry of Terpenes, Essential Oils and Aromatics, Japan, 1989, p. 87.
- 7) G. Assante, L. Merlini and G. Nasini, *Experientia*, **33**, 1556 (1977).
- 8) S. Marumo, M. Katayama, E. Komori, Y. Ozaki, M. Natsume and S. Kondo, *Agric. Biol. Chem.*, **46**, 1967 (1982).
- 9) S. Nakayama and T. Hashimoto, *Plant & Cell Physiol.*, **14**, 419 (1973).
- 10) H. Harada, T. K. Bose and J. Cheruel, *Z. Pflanzenphysiol. Bd*, **64**, s. 267 (1971).
- 11) H. M. M. El-Antably, P. F. Wareing and J. Hillman, *Planta*, **73**, 74 (1967).
- 12) W. R. Cummins and E. Sondheimer, *Planta*, **111**, 365 (1973).
- 13) B. V. Milborrow, *Ann. Rev. Plant Physiol.*, **25**, 259 (1974).
- 14) The optical purity of (*S*)-(+)-absciscic acid was analyzed by HPLC with Chiralcel OD L (4.6 mm i.d. × 250 cm) and *n*-hexane-2-propanol-trifluoroacetic acid (80:20:1, v/v/v) as the solvent; retention time: (*S*)-(+)-ABA, 9.23 min; (*R*)-(-)-ABA, 12.15 min.
- 15) Y. Okamoto, R. Aburatani and K. Hatada, *J. Chromatogr.*, **448**, 454 (1988).