Phytochemistry, Vol 23, No 11, pp 2695–2696, 1984
Printed in Great Britain

0031–9422/84 $3 00 + 0 00

EFFECT OF DROUGHT ON PHYTYL WAX ESTERS IN *PHASEOLUS* LEAVES

WAYNE H ANDERSON, JOANNE L GELLERMAN and HERMANN SCHLENK

The Hormel Institute, University of Minnesota, 801 16th Avenue NE, Austin, Minnesota 55912, U S A

(*Received* 31 *January* 1984)

Key Word Index—*Phaseolus acutifolius*, *P vulgaris*, Leguminosae, drought, phytyl wax esters

Abstract—The small amount of phytol which is bound as wax ester in mature bean leaves is increased 10–20 fold by drought Watering the plants before permanent wilt reverses this trend Maximum amounts of phytyl wax esters in plants still viable are higher in the drought resistant tepary bean (*Phaseolus acutifolius*) than in the less resistant garden bean (*P vulgaris*)

INTRODUCTION

Phytol of leaf lipids is bound mainly in chlorophyll (Ph_{chl}) but occurs in much smaller amounts also in wax esters where phytol (Ph_w) is esterified to long chain fatty acids Accumulation of Ph_w has been reported for leaves after abscission [1] and after frost damage [2] Such observations suggested a study of the response of Ph_w to stress in mature leaves Specifically, we are reporting on the effect of drought on Ph_w in resistant and non-resistant *Phaseolus* species

RESULTS AND DISCUSSION

Amounts of Ph_w were *ca* 0 1 % in leaf lipids of garden bean (*P vulgaris*) and several other angiosperms under our standard conditions Consistently, these concentrations markedly increased when water was withheld for several days The amounts of free phytol hardly changed during such treatment When watering was resumed before the time of permanent wilt, the trend to high Ph_w values was reversed within a few days Such responses could be demonstrated also for heat and cold stress

For a detailed study of these phenomena, we chose drought as the stress factor, using garden bean and, for comparison, the more drought resistant tepary bean (*P acutifolius* A Gray) [3] Table 1 shows typical data from one of several experiments Samples were taken daily during the drought period and 7 days later during which the plants were watered daily to check for recovery Water potentials were measured to characterize the stressed condition of the plants

In garden bean, Ph_w increased within 7 days drought from 13 to 245 μmol/100 g dry wt of leaves, at ψ_w − 16 7 bars Subsequent watering reduced Ph_w by 75 % or more, with ψ_w back at the initial range After 8 days drought, garden bean did not survive In these plants, Ph_w had increased to 355 μmol and watering reduced this value only by 20 %, while ψ_w remained unchanged at − 22 bar

In the drought resistant tepary bean, plants stayed viable for 10 days of drought and Ph_w reached concentrations of 500 + μmol Upon watering, Ph_w was reduced by 75 % or more in the plants that had been dry up to 6 days The high levels of Ph_w reached during dry days 7–10 were reduced only by *ca* 50 % after the recovery period. Recovery of these plants was obvious, but after 11 days of drought terminal wilt had occurred

When comparing the amounts of Ph_w in the two species, it is apparent that maximum values before terminal wilt are in tepary bean *ca* twice as high as those in garden bean (Table 1) The accumulation of Ph_w to higher concentrations and over a longer period of stress in tepary bean was observed in all comparative experiments

Apparently, the phytol waxes are inside the tissue since they are extractable only by disintegration of the leaves It is suggestive that the phytol moiety, Ph_w, originates in chloroplasts This was supported by some experiments with [2-^{14}C]mevalonate Mature chloroplasts are known to be self-sufficient with regard to synthesis of Ph_{chl} and *in vitro* they do not take up mevalonate [4–7] In our experiments, the labelled precursor was infused into normal and drought stressed tepary plants Two to seven days later, the steroids of total leaf lipids were labelled in radioactive yields of 4–17 %, while under all conditions Ph_w as well as Ph_{chl} were not significantly labelled With such indication for its origin in chloroplasts, it remains undecided if Ph_w accumulates from *de novo* synthesis or is derived from chlorophyll The origin of the phytol accumulating as wax ester during stress is under further investigation

EXPERIMENTAL

Tepary bean was obtained from the Plant Science Department, University of Arizona, Tucson, AR Other seeds were from commercial sources Pots with two bean plants were grown under fluorescent and incandescent lights (16 500 lx at pot ht) with a 14/10 hr light/dark and a corresponding 27/20° temp cycle, in a rel humidity of 50–70 % Plants were watered daily and, in the stress expts, H_2O was withheld 3–4 weeks after planting when they were *ca* 30 cm high The experimental dry/wet periods were completed before blooming

Water potentials were measured by the pressure chamber technique [8] using the second or third trifoliate leaves All leaves of a bean plant were collected for analysis, omitting those which were in normal senescence or obviously damaged beyond recovery The leaves were immediately weighed, boiled in MeOH for 10 min and stored at − 17°

Lipids were obtained by two successive extractions with $CHCl_3$–MeOH (2 1) in an Omnimixer Dry wts of the total samples were determined with aliquots from the suspension of the first extraction Lipids were recovered [9] and taken up in

Table 1 Phytol wax esters in bean leaves during drought*

	Dry period (days)						
	0	2	4	6	7	8	10
Garden bean							
ψ_w, bars	−2 5	−1 5	−11 0	−12 9	−16 7	−22 8	—
		(−2 3)	(−4 3)	(−2 3)	(−2 5)	(−22 1)	
% Lipid in dry wt†	7 6	6 7	10 7	7 6	12 1	10 5	—
		(4 5)	(8 2)	(9 5)	(8 4)	(2 4)	
μmol Ph_w/100 g dry wt	13	23	145	231	245	355	—
		(6)	(28)	(64)	(57)	(268)	
Tepary bean							
ψ_w, bars	−1 1	−2 1	−12 3	−17 5	−17 2	−14 5	−16 6
		(−2 6)	(−4 6)	(−0 7)	(−3 2)	(−4 9)	(−2 8)
% Lipid in dry wt†	6 5	6 6	9 6	6 7	6 3	7 1	4 0
		(8 3)	(7 9)	(8 5)	(9 0)	(7 8)	(9 1)
μmol Ph_w/100 g dry wt	11	22	160	430	360	575	500
		(28)	(53)	(57)	(182)	(211)	(277)

*Figures in parentheses indicate values after 7 days watering subsequent to the respective dry periods
†Including chlorophyll

5 ml of the same solvent for analysis

Heptadecyl hexadecanoate and heptadecanol were added to the total lipids as int standards for the quantification of Ph_w and of free Ph, respectively Wax esters and free alcohols were isolated by prep TLC on silica gel H, 0 5 mm thick, 3–20 mg lipid, solvent petrol (30–60°)–Et_2O (19 1) Alcohols of waxes were prepared by alkaline trans esterification and isolated by TLC, solvent petrol (60–70°)–Et_2O–EtOH–aq NH_3 (70 30 1 0 3) TMSi ethers were prepared using Sylon HTP (Supelco) and Ph_w quantified by GC (column 240 × 0 3 cm i d, 10% Silar 5C on Chrom-WAW, 180–240° at 3°/min, inj and FID at 250°), referring to heptadecyl hexadecanoate as int standard At concns of 0 3% Ph_w, the reproducibility of the analyses was ± 0 03% Ph of the total lipids was determined by similar procedures, applied to the respective fraction of the first prep TLC

DL-[2-^{14}C]Mevalonate was prepared from commercial lactone About 0 5 μmol mevalonate (10 × 10^6 dpm) was infused [10] into the stem of normal or stressed plants between the lowest leaves and the first trifoliate leaf After 2–7 days with or without H_2O supplied, leaves were harvested and ^{14}C in lipid fractions measured after the first prep TLC (see above) by scintillation counting Ph_w was checked for radioactivity by collection as TMSi ether from GC and by HPLC of non-derivatized Ph_w (200 μg samples, 5 μm ODS column 4 6 × 150 mm, RID, solvent MeOH–H_2O, 19 1, 1 5 ml/min)

Acknowledgements—We are grateful to Dr L R Parsons for helpful discussions and to Ms S Diggins for very competent technical assistance This work was supported in part by NSF Award PCM 77-20170, by USPHS grant HL 08214 from the Program Projects Branch, Extramural Programs, NHLB Institute and by The Hormel Foundation

REFERENCES

1 Csupor, L (1970) *Planta Med* **19**, 37
2 Gellerman, J L, Anderson, W H and Schlenk, H (1975) *Lipids* **10**, 656
3 National Academy of Sciences (Report) (1979) in *Tropical Legumes Resources for the Future*, p 92 Washington, DC, and literature quoted therein
4 Rogers, L J, Shah, S P J and Goodwin, T W (1967) *Biochemistry of Chloroplasts*, Vol II (Goodwin, T W, ed) p 283 Academic Press, New York
5 Treharne, K J, Mercer, E I and Goodwin, T W (1966) *Biochem J* **99**, 239
6 Wellburn, A R and Hampp, A R (1976) *Biochem J* **158**, 231
7 Schneider, M M, Hampp, R and Ziegler, H (1977) *Plant Physiol* **60**, 518
8 Scholander, P F, Hammel, H T, Bradstreet, E D and Hemmingsen, E A (1965) *Science* **148**, 339
9 Folch, J, Lees, M and Sloane-Stanley, G A (1957) *J Biol Chem* **226**, 497
10 Gellerman, J L and Schlenk, H (1969) *Lipids* **4**, 484