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Effects of growth substances on non-induced and *Botrytis cinerea* culture filtrate-induced phaseollin production in *Phaseolus vulgaris* cell suspension cultures

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Suspension cultures of *Phaseolus vulgaris* produced the phytoalexin phaseollin in the absence of added inducers. The concentrations of plant growth substances in the culture medium affected growth of the cultures and the extent of phaseollin production, both in untreated cultures and in response to treatment with sterile culture filtrates from *Botrytis cinerea*. 2,4-Dichlorophenoxyacetic acid was inhibitory to induced production at concentrations of around 2×10^{-6} M and above, whereas 1-naphthylacetic acid only inhibited significantly at 2×10^{-4} M. Gibberellic acid and abscisic acid both stimulated non-induced production, abscisic acid also inhibiting growth of the cultures. High kinetin concentrations were inhibitory to non-induced production, but partially relieved inhibition of induction in culture filtrate-treated cells caused by growth in high concentrations of 2,4-dichlorophenoxyacetic acid. This increased response was associated with higher phenylalanine ammonia-lyase activity during the induction period than observed in cultures grown in low kinetin concentrations. However, as phenylalanine ammonia-lyase activity was often higher in control than in induced cultures, it is unlikely that this enzyme plays a regulatory rôle in phaseollin biosynthesis in this system.

INTRODUCTION

The responses induced in plant cells by interaction with fungal pathogens are very complex, and results of biochemical and physiological studies are often difficult to interpret in view of the physical presence of the invading micro-organism and the several different cell types which may be interacting. In an attempt to simplify the system, several workers have investigated the possibilities of using plant tissue cultures as a source of more homogeneous material for studying disease resistance responses, and the recent demonstrations of fungal metabolites with phytoalexin-inducing activity [1, 2, 6] may lead to the development of induced culture systems which avoid the use of infecting micro-organisms but which may still approximate to the *in vivo* situation.

Although early work has tended to concentrate on the overall expression of disease resistance in tissue cultures [15, 16, 25], the more specific response of phytoalexin production has now been demonstrated in such systems. Thus, callus cultures of soybean produced the phytoalexin glyceollin in response to infection by *Phytophthora megasperma* var. *sojae*, although there was no direct correlation between the extent of induction and the known resistance responses of the varieties from which the cultures were isolated [17]. Suspension cultures of soybean exhibited a marked increase in phenylalanine ammonia-lyase (PAL) activity associated with glyceollin biosynthesis on treatment with a purified phytoalexin elicitor from cell walls of *P.*

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megasperma var. *sojae* [11]. In pea suspension cultures, the phytoalexin pisatin was formed in response to coconut milk in the culture medium [4].

We have recently described the production of the isoflavonoid phytoalexin phaseollin in bean (*Phaseolus vulgaris* var. Canadian Wonder) suspension cultures growing in fully defined media in the absence of added inducing agents, and have shown it to be markedly dependent on the synthetic auxin concentration in the culture medium [9]. Components present in culture filtrates from the grey mould *Botrytis cinerea* stimulated phaseollin synthesis in the bean cultures [10]. However, the levels of production in response to culture filtrate treatment were low under the growth conditions used for the plant cultures, and we were thus prompted to investigate further the effects of culture medium composition on the capacity for phaseollin synthesis in both induced and non-induced cultures. It was hoped that such investigations would lead to the establishment of culture conditions optimal for phytoalexin production, and thus provide a useful system for the study of isoflavonoid biosynthesis.

It is important to note that in the present paper we define induction as stimulation of phaseollin synthesis by culture filtrate above the level occurring in untreated cultures. Increased synthesis as a result of altered growth regulator levels is regarded as a change in the background, non-induced rate. This classification of the response as induced and non-induced is not meant to imply mechanistic differences; our present lack of knowledge of both the enzymology of isoflavonoid phytoalexin biosynthesis and the mechanism by which the pathway is switched on by elicitors makes it impossible at this stage to determine the site of action for the hormonal effects.

MATERIALS AND METHODS

Growth and maintenance of tissue cultures

Callus cultures were initiated from radicle explants of *Phaseolus vulgaris* var. Canadian Wonder as described previously [9]. Stock callus cultures were maintained on medium B [9], a modified Schenk & Hildebrandt medium [21] containing the growth substances *p*-chlorophenoxyacetic acid at a concentration of 10^{-5} M, 2,4-dichlorophenoxyacetic acid (2,4-D) at 2×10^{-6} M and 6-furfurylaminopurine (kinetin) at 5×10^{-7} M.

The initiation and growth conditions for suspension cultures have been described elsewhere [10].

In the experiments investigating the effects of synthetic auxin and kinetin levels, callus lumps (approximately 8 g total fresh wt, 21 days after subculture onto fresh semi-solid medium B) were used as inocula into 250 ml of test media in 1 l flasks, and were agitated by reciprocal shaking. The Schenk & Hildebrandt medium with the omission of the growth regulators present in medium B is termed medium A, and was used as the basic medium for the 2,4-D, 1-naphthylacetic acid (NAA) and kinetin additions. In the remaining experiments, 30 ml of stock suspension, 28 days after subculture into medium B, were used as inoculum into 220 ml of test medium (medium B plus required additions).

Preparation of fungal culture filtrates

Culture filtrates were prepared from 12-day-old shake cultures of *Botrytis cinerea* (grown in Czapek–Dox medium) as described previously [10].

Determination of phaseollin

Phaseollin was extracted from suspension cultured cells according to the method of Cruickshank & Perrin [7], and was determined quantitatively from its extinction at 280 nm after separation by thin-layer chromatography [9].

Phaseollin induction in response to a *Botrytis cinerea* culture filtrate was estimated by measuring the extent of incorporation of L-[U¹⁴C]-phenylalanine into the phytoalexin as described previously [10]. All results are expressed as ct/min in the phaseollin extracted from two pooled, replicate 8 ml batches of culture. The percentage of the total radioactivity applied that became incorporated into phaseollin was between 0.005% (lowest control value) and 1.43% (highest induced value). From isotope dilution measurements, these values correspond to levels of phaseollin production of between 0.3 and 90 µg per g fresh wt respectively over the 60 h induction period.

Assay of phenylalanine ammonia-lyase

PAL was assayed radiochromatographically as described previously [9]. Enzyme activities are expressed as nmol cinnamic acid produced min⁻¹ mg protein⁻¹.

Determination of protein

Protein precipitated from supernatants by treatment with equal volumes of 10% trichloroacetic acid was determined by the method of Lowry *et al.* as modified by Leggett-Bailey [18].

Measurement of packed cell volumes

Packed cell volumes were measured by centrifugation of 10 ml aliquots of suspension culture in graduated centrifuge tubes for 5 min at 1200 g. Values are expressed as percentages.

Measurement of fresh wt

Fresh wts of suspension cultured cells were measured by weighing after centrifugation of suitable aliquots of suspension through pre-weighed sintered glass centrifuge filters.

RESULTS

Effect of growth substances on non-induced production

The effects of altered 2,4-D and NAA levels on non-induced phaseollin production have been described elsewhere [9]. In the present work, we were hoping to find conditions giving lower background rates of production than we had previously observed, whilst maintaining growth and capacity for induction by culture filtrate.

The basic medium used when studying the effects of altered synthetic auxin concentrations contained the cytokinin kinetin at a concentration of 5×10^{-7} M, this low concentration being necessary to prevent re-differentiation of the cultures. The effect on the growth of the cultures of the omission of kinetin, and its inclusion in the medium at concentrations of 5×10^{-6} and 5×10^{-5} M, is shown in Fig. 1, for suspensions growing in two different 2,4-D concentrations. In cultures from which kinetin was omitted, green embryo-like structures appeared after about 14 days.

These persisted throughout the growth period, with little sign of further development, in media containing 2×10^{-5} M 2,4-D. However, at the lower 2,4-D concentration, star-shaped clusters of pointed root-like organs (up to 1 cm in length) appeared in the cultures after 27 days, and this coincided with the disappearance of the green cell aggregates.

FIG. 1. Effects of kinetin on the growth of *P. vulgaris* cell suspension cultures. Callus lumps were transferred to 250 ml batches of liquid medium A with the addition of 2,4-D at 2×10^{-6} M (a) or 2×10^{-5} M (b). Levels of kinetin in the medium were zero (○—○), 5×10^{-6} M (■—■) and 5×10^{-5} M (●—●). Results are the average of four determinations.

Variation in the levels of kinetin affected phaseollin production during the growth period of these cultures (Fig. 2). This was most noticeable in cultures growing in the presence of 2×10^{-6} M 2,4-D, where increased kinetin levels markedly depressed production. Although the higher 2,4-D concentration was itself inhibitory to production, there was still some evidence of further inhibition by kinetin.

Inclusion of gibberellic acid (GA_3) in the culture medium (medium B) at concentrations of 10^{-7} M to 10^{-5} M had no effect on the growth (packed cell volume increase) of the bean suspensions. Increased GA concentrations brought about increased phaseollin production in the absence of culture filtrate (Table 1).

Inclusion of abscisic acid (ABA) in the culture medium at concentrations from 10^{-7} to 10^{-4} M resulted in decreased growth of the cultures associated with increased phaseollin production as a function of increasing concentration of the growth inhibitor (Fig. 3). Phaseollin production in these treatments was associated with a greater degree of cellular browning than observed in the other experiments. The dotted lines in Fig. 3 show the growth and phaseollin production of cultures growing in medium B alone, and thus serve as controls for the ABA and GA data.

Effects of growth substances on Botrytis cinerea culture filtrate-induced production

In *B. cinerea* culture filtrate-treated suspensions, growth in the presence of increased kinetin concentrations at least partly overcame the inhibition of phaseollin induction caused by the presence of high 2,4-D levels (Fig. 4), although the cytokinin had

FIG. 2. Effects of kinetin on phaseollin production by *P. vulgaris* cell suspension cultures. Callus lumps were transferred to 250 ml batches of medium A with the addition of 2,4-D at 2×10^{-6} M (a) or 2×10^{-5} M (b). Levels of kinetin in the media were zero (○—○), 5×10^{-6} M (■—■) and 5×10^{-5} M (●—●). Results are the average of two determinations.

TABLE 1

Effects of growth in medium B alone, or with the addition of various levels of gibberellic acid, on phaseollin production by *P. vulgaris* cell suspension cultures

Gibberellic acid concentration (M)	Packed cell volume (%)	Phaseollin (µg per g fresh wt)
0	11.5 ± 2.5	1.36 ± 0.4
10^{-7}	14.6 ± 2.7	8.15 ± 2.6
10^{-6}	12.0 ± 3.0	12.30 ± 2.9
10^{-5}	10.4 ± 1.2	39.60 ± 5.7

Packed cell volumes and phaseollin levels were determined after 21 days growth in the test media. Results are the averages and standard deviations of four determinations.

little effect at the lowest 2,4-D concentration tested. The lack of effect of kinetin in the controls is presumably due to the use in these experiments of suspensions grown for 21 days in liquid medium after initiation. In such cultures, the effects of kinetin on non-induced production are not significantly manifest until about day 28 (Fig. 2).

Figure 4 also shows the extent of response to culture filtrate in suspensions grown in the presence of 5×10^{-7} M kinetin with NAA as synthetic auxin. In agreement with our previously reported results on non-induced production [9], NAA was also

FIG. 3. Effects of abscisic acid on growth (a) and phaseollin production (b) by *P. vulgaris* cell suspension cultures. Thirty ml aliquots taken from suspensions which had been through two serial subcultures in liquid medium B were transferred to 220 ml batches of medium B alone (---), or with abscisic acid present at 10^{-7} M (□—□), 10^{-6} M (■—■), 10^{-5} M (●—●) or 10^{-4} M (▲—▲). These concentrations refer to the biologically active (+) enantiomer. Packed cell volume results are the average of four determinations, phaseollin levels the average of two.

less inhibitory to culture filtrate-induced production than was 2,4-D. A concentration as high as 2×10^{-4} M was required for significant inhibition of induction.

The results in Table 2 indicate that growth in the presence of high kinetin concentrations resulted in higher PAL activities in the cells after 21 days' growth

TABLE 2
Effects of 2,4-D and kinetin levels on PAL activity during phaseollin induction in P. vulgaris cell suspension cultures

Additions to medium A 2,4-D (M) Kinetin (M)	Treatment	PAL activity at time <i>t</i> (h)		
		<i>t</i> =0	<i>t</i> =9	<i>t</i> =24
2×10^{-6} 5×10^{-7}	Induced	2.05	3.05	5.50
	Control	2.05	5.60	2.68
2×10^{-6} 5×10^{-5}	Induced	7.65	7.90	5.00
	Control	7.65	12.95	7.55

Twenty-one days after transfer of callus lumps to liquid medium A with the 2,4-D and kinetin additions shown, 8 ml aliquots of the resulting cell suspensions were transferred to flasks containing 2 ml of sterile *B. cinerea* culture filtrate (induced) or Czapek-Dox medium (control). PAL activities at various times after transfer are expressed as nmol cinnamic acid produced min^{-1} mg protein $^{-1}$.

FIG. 4. Effects of synthetic auxin and kinetin concentrations on the response of *P. vulgaris* cell suspension cultures to treatment with a sterile *B. cinerea* culture filtrate (unshaded plus shaded portions of histograms). Shaded areas alone represent values for control (Czapek-Dox medium-treated) cultures. Callus lumps were transferred to 250 ml batches of liquid medium A with kinetin at the concentrations shown and synthetic auxin at 2×10^{-7} M (a), 2×10^{-6} M (b) and 2×10^{-5} M (c). Histograms with filled-in bottom areas represent cultures grown with 2,4-D as synthetic auxin, those with striped bottom areas representing NAA-grown cultures. Radioisotope incorporation into phaseollin was measured after 21 days growth in the test media. Results are the average of two determinations.

(zero time for the induction experiments) and during the subsequent period of phytoalexin synthesis in response to culture filtrate than in cultures grown with the cytokinin present at only 5×10^{-7} M. However, PAL activity in control cultures was generally higher than that in cultures of identical origin which had been treated with the culture filtrate.

In spite of its effect on non-induced phaseollin production, growth in the presence of gibberellic acid had little effect on the response to culture filtrate.

DISCUSSION

The present results demonstrate that phaseollin synthesis in bean cell suspensions as a result of treatment with culture filtrates from *Botrytis cinerea* is affected by synthetic auxin levels in a similar manner to the non-induced production we had previously observed [9].

Variations in synthetic auxin concentration in the culture medium had considerable effect on the growth rates of the bean cell suspensions [9], but did not appear to determine patterns of re-differentiation, although low-frequency differentiation of roots occurred when callus cultures were transferred to low auxin, semi-solid media. The only condition leading to differentiation of cell cultures in the present work was the omission of kinetin, and this was associated with higher levels of background, non-induced production of phaseollin than occurred in cultures containing the cytokinin. Although re-differentiation has been shown to restore the capacity for production of tropane alkaloids in cultures of *Atropa belladonna* [24], and for biosynthesis of furanocoumarins and furanoquinoline alkaloids by suspension cultures of *Ruta graveolens* [12], the situation with respect to isoflavonoid phytoalexin production in the bean suspensions is clearly more complex; although cultures undergoing differentiation (kinetin omitted from the medium) had increased background rates of phaseollin synthesis, they were less responsive to treatment with *Botrytis* culture filtrates when grown in high auxin media than were cultures grown in the presence of the cytokinin (Fig. 3).

Treatment of broad bean plants with the cytokinin 6-benzylaminopurine in field tests has been shown to reduce significantly infection by *Botrytis cinerea* and *B. fabae*, both of which often attack senescent tissue [19], and treatment of pea leaf discs with the same compound reduced senescence and maintained the capacity for pisatin induction [3]. It is therefore possible that the differential effects of kinetin on induced and non-induced phaseollin production by the bean cultures might be explained similarly, with kinetin retarding ageing responses of the cells (i.e. maintaining cell divisions and decreasing cellular browning and the rate of background phaseollin synthesis) whilst maintaining their ability to respond to inducers. Such an explanation is also in accord with the stimulation of phaseollin production by ABA, whose endogenous levels generally rise in ageing or stressed tissues [27].

There are several reports on the inhibitory effect of high synthetic auxin concentrations on PAL activity in plant tissue cultures [8, 9, 22, 26], and it has recently been shown that kinetin slightly stimulates PAL in bean callus [13]. Similar results are reported here, but any direct correlation between PAL activity and phaseollin biosynthesis must be seriously questioned in view of the lower activities of the enzyme in induced than in control cultures. A similar lack of correlation between PAL activity and isoflavonoid biosynthesis has been described in cowpea hypocotyls responding to heavy metal ions or actinomycin D [20] and in pea endocarp tissues treated with poly-L-arginine [14]. The levels of PAL activity in the bean suspensions were always in excess of those required to account for the measured rates of phaseollin production [9]. Although the possibility of the existence of PAL

isoenzymes specific for different areas of phenolic metabolism cannot yet be dismissed, it would appear more likely that the rate limiting steps in phaseollin biosynthesis are at some later and as yet uncharacterized stage of the pathway.

If plant cell cultures are to be of value in the study of the biosynthesis of isoflavonoid phytoalexins, high rates of overall production are clearly desirable. However, if background, non-induced rates of synthesis are also high, such systems may be less suitable for studying the mechanism of altered gene expression in response to elicitors, bearing in mind the very low rates of synthesis occurring in healthy, uninfected plant tissues. The ideal tissue culture system should therefore show low background rates of synthesis, and produce phytoalexin in response to elicitors at rates comparable to those observed in infected, whole plant tissue. We conclude that the bean culture system can be manipulated to partially fulfil these requirements; during growth in a culture medium supplemented with a synthetic auxin (2,4-D at around 2×10^{-7} M or NAA at concentrations of up to 2×10^{-5} M) and a kinetin concentration of around 5×10^{-6} M, background rates of phaseollin synthesis are low, and rates of synthesis in response to culture filtrate treatment are of the order of 60 to 90 μ g per g fresh wt over 60 h, a value approaching the rates observed in tissues of the whole plant in response to fungal infection [5]. This rate of synthesis is a considerable improvement on that reported for culture filtrate-treated suspensions growing in medium B [10].

We must also conclude, however, that the non-induced production of phaseollin occurring under certain hormonal conditions does not present a good system for further study, the response being too low and occurring over too long a time period. Furthermore, the recent demonstration of the toxicity of phaseollin to bean suspension cultures [23] rules out the possibility of continued growth and subculture of suspensions in media permitting high rates of background synthesis.

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REFERENCES

1. ANDERSON-PROUTY, A. J. & ALBERSHEIM, P. (1975). Host-pathogen interactions. VIII. Isolation of a pathogen-synthesized glucan that elicits a defence response in the pathogen's host. *Plant Physiology* **56**, 286-291.
2. AYERS, A. R., EBEL, J., FINELLI, F., BERGER, N. & ALBERSHEIM, P. (1976). Host-pathogen interactions. IX. Quantitative assays of elicitor activity and characterization of the elicitor present in the extracellular medium of cultures of *Phytophthora megasperma* var. *sojae*. *Plant Physiology* **57**, 751-759.
3. BAILEY, J. A. (1969). Phytoalexin production by leaves of *Pisum sativum* in relation to senescence. *Annals of Applied Biology* **64**, 315-324.
4. BAILEY, J. A. (1970). Pisatin production by tissue cultures of *Pisum sativum*. *Journal of General Microbiology* **61**, 409-415.
5. BAILEY, J. A. (1973). Phaseollin accumulation in *Phaseolus vulgaris* following infection by fungi, bacteria and a virus. In *Fungal Pathogenicity and the Plant's Response*, Ed. by R. J. W. Byrde & C. V. Cutting, pp. 337-353. Academic Press, London.
6. CRUICKSHANK, I. A. M. & PERRIN, D. R. (1968). The isolation and partial characterization of monilicolin A, a polypeptide with phascollin-inducing activity from *Monilinia fructicola*. *Life Sciences* **7**, 449-458.
7. CRUICKSHANK, I. A. M. & PERRIN, D. R. (1971). Studies on phytoalexins. XI. The induction, antimicrobial spectrum and chemical assay of phaseollin. *Phytopathologische Zeitschrift* **70**, 209-229.

8. DAVIES, M. E. (1972). Effects of auxin on polyphenol accumulation and the development of phenylalanine ammonia-lyase activity in darkgrown suspension cultures of Paul's scarlet rose. *Planta* **104**, 66-77.
9. DIXON, R. A. & FULLER, K. W. (1976). Effects of synthetic auxin levels on phaseollin production and phenylalanine ammonia lyase (PAL) activity in tissue cultures of *Phaseolus vulgaris* L. *Physiological Plant Pathology* **9**, 299-312.
10. DIXON, R. A. & FULLER, K. W. (1977). Characterization of components from culture filtrates of *Botrytis cinerea* which stimulate phaseollin biosynthesis in *Phaseolus vulgaris* cell suspension cultures. *Physiological Plant Pathology* **11**, 287-296.
11. EBEL, J., AYERS, A. R. & ALBERSHEIM, P. (1976). Host-pathogen interactions. XII. Response of suspension cultured soybean cells to the elicitor isolated from *Phytophthora megasperma* var. *sojae*, a fungal pathogen of soybeans. *Plant Physiology* **57**, 775-779.
12. GAMBORG, O. L., CONSTABEL, F., LA RUE, T. A. G., MILLER, R. A. & STECK, W. (1970). The influence of hormones on secondary metabolite formation in plant cell cultures. In *Les Cultures de Tissus de Plantes*, p. 335. Colloques Internationaux du C.N.R.S. No. 193, Paris.
13. HADDON, L. & NORTHCOLE, D. H. (1976). Correlation of the induction of various enzymes concerned with phenylpropanoid and lignin synthesis during differentiation of bean callus (*P. vulgaris* L.). *Planta* **128**, 255-262.
14. HADWIGER, L. A. & SCHWOCHAU, M. E. (1970). Induction of phenylalanine ammonia-lyase and pisatin in pea pods with polylysine, spermidine or histone fractions. *Biochemical and Biophysical Research Communications* **38**, 683-688.
15. HELGESON, J. P., KEMP, J. D., HABERLACH, G. T. & MAXWELL, D. P. (1972). A tissue culture system for studying disease resistance: the black shank disease in tobacco callus cultures. *Phytopathology* **62**, 1439-1443.
16. INGRAM, D. S. (1967). The expression of *R* gene resistance to *Phytophthora infestans* in tissue cultures of *Solanum tuberosum*. *Journal of General Microbiology* **49**, 99-108.
17. KEEN, N. T. & HORSCH, R. (1972). Hydroxyphaseollin production by various soybean tissues; a warning against the use of unnatural host-parasite systems. *Phytopathology* **62**, 439-442.
18. LEGGETT-BAILEY, J. (1962). In *Techniques in Protein Chemistry*, Chapter 11. Elsevier Publishing Co., Amsterdam.
19. MOORE, K. G. & LEACH, R. (1968). The effect of 6-benzylaminopurine (benzyladenine) on senescence and chocolate spot (*Botrytis fabae*) of winter beans (*Vicia faba*). *Annals of Applied Biology* **61**, 65-76.
20. MUNN, C. E. & DRYSDALE, R. B. (1975). Kievitone production and phenylalanine ammonia lyase activity in cowpea. *Phytochemistry* **14**, 1303-1307.
21. SCHENK, R. H. & HILDEBRANDT, A. C. (1971). Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. *Canadian Journal of Botany* **50**, 199-204.
22. SHAH, R. R., SUBBIAH, K. V. & MEHTA, A. R. (1976). Hormonal effects on polyphenol accumulation in *Cassia* tissues cultured *in vitro*. *Canadian Journal of Botany* **54**, 1240-1245.
23. SKIPP, R. A., SELBY, C. & BAILEY, J. A. (1977). Toxic effects of phaseollin on plant cells. *Physiological Plant Pathology* **10**, 221-227.
24. THOMAS, E. & STREET, H. E. (1979). Organogenesis in cell suspension cultures of *Atropa belladonna* L. and *Atropa belladonna* cultivar *lutea* Döll. *Annals of Botany* **34**, 657-669.
25. WARREN, R. S. & ROUTLEY, D. G. (1970). The use of tissue cultures in the study of single gene resistance of tomato to *Phytophthora infestans*. *Journal of the American Society of Horticultural Science* **95**, 266-269.
26. WESTCOTT, R. J. & HENSHAW, G. G. (1976). Phenolic synthesis and PAL activity in suspension cultures of *Acer pseudoplatanus* L. *Planta* **131**, 67-74.
27. WRIGHT, W. L. & HIRON, R. W. P. (1972). The accumulation of abscisic acid in plants during wilting and under stress conditions. In *Plant Growth Substances*, Ed. by D. J. Carr, pp. 291-298. Springer-Verlag, Berlin.