Role of growth inhibitors in bud dormancy in virus-infected stem cuttings of *Euphorbia pulcherrima*

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Summary. In virus-infected stem cuttings of *E. pulcherrima*, the dormancy in buds is broken in winter possibly because of the low amount of growth inhibitors, abscisic acid and phenols, as compared to healthy stem cuttings.

Euphorbia pulcherrima grows vegetatively during Summer (April-September) and becomes dormant during Winter (October-March). All plants growing on the University Campus are systemically infected by a virus tentatively identified as *Euphorbia pulcherrima* virus. Plant stocks growing in localities 10-12 km away from the Campus are uninfected. Studies on the effect of this virus on rooting stem cuttings have revealed that the buds in virus-infected stem cuttings, when planted in sand in earthern pots and periodically irrigated with water, grew into leafy shoots during winter while the buds on identically treated healthy cuttings remained dormant[1].

Induction and break in bud dormancy in plants is controlled by an interaction between growth promotors, particularly gibberellins, and growth inhibitors, abscisic acid and phenols[2,3]. The results on the estimation of growth inhibitors in healthy and diseased stem tissues during the growing as well as dormant period are reported here.

Material and methods. Branches of uniform size and approximately of the same age (since both the healthy and diseased stocks were pruned at the same time, and they gave rise to new branches at about the same time) were cut from the selected stocks and their leaves and apical parts were removed. These were then cut into 15 cm long cuttings. The term 'cuttings' refers to such leafless and truncated branches.

Phenols were extracted from the known weight of fresh stem tissues (bark) by the method of Bray and Thorpe[4]. The blank in each case consisted of water instead of the ethanol extract. The phenol content was calculated from the standard curve prepared by using different concentrations of chlorogenic acid.

Abscisic acid was extracted from 9 g of bark of the healthy and diseased stem tissues by the method of Rasmussen[5]. The dried residue so obtained was dissolved in 10 ml distilled water. Abscisic acid was bioassayed in this extract by the 'inhibition of growth of excised wheat embryo bioassay' by the method of Gabr and Guttridge[6]. The petriplates containing treated wheat embryos were incubated at 27 °C and the coleoptile length was measured in mm after 72 h. The abscisic acid activity was estimated as percentage inhibition of coleoptile length over the controls (water-treated excised embryos).

Results and discussion. Phenol content in healthy stem cuttings (table 1) was maximum in January, then it decreased continuously and was minimum in July. Thereafter it started increasing again in August and became considerably higher in September and October. Thus the phenol content in healthy cuttings seems to have a definite correlation with break and setting in of dormancy in buds of the plant; it decreases progressively with the gradual onset of the favourable season for growth to reach its minimum in July and then increases again with the advent

Table 1. Phenol content in healthy and diseased stem cuttings of *E. pulcherrima* in various months

Month	Type of cutting	Total phenol content (mg/g fresh weight of tissue)	Percentage decrease (−) in infected tissue with respect to healthy tissue
January	H	27.50	-
	D	13.70	50.18 (−)
February	H	19.00	-
	D	10.00	47.36 (−)
March	H	17.30	-
	D	7.10	58.95 (−)
April	H	8.30	-
	D	7.70	7.22 (−)
May	H	7.30	-
	D	7.00	4.10 (−)
June	H	7.10	-
	D	6.72	5.35 (−)
July	H	7.00	-
	D	6.20	11.42 (−)
August	H	9.62	-
	D	7.23	22.25 (−)
September	H	18.24	-
	D	9.53	47.86 (−)
October	H	23.10	-
	D	10.70	53.68 (−)

H = healthy; D = diseased.

Table 2. Inhibition of growth of excised wheat embryos bioassay for the estimation of abscisic acid-like activity in the purified extracts of healthy and diseased stem cuttings of *Euphorbia pulcherrima* after 72 h

Month	Treatment	Mean length of the 15 coleoptiles in mm	Percentage inhibition in coleoptile length with respect to control	Percentage decrease (−) in coleoptile length of embryos treated with diseased ABA-extract with respect to healthy extract
	Control	18.40	-	-
November	H ABA extract	11.00	40.21 %	-
	D ABA extract	12.66	31.19 %	22.43 (−)
January	H ABA extract	11.80	35.86 %	-
	D ABA extract	12.86	30.10 %	16.06 (−)
February	H ABA extract	14.00	23.91 %	-
	D ABA extract	14.83	19.40 %	18.86 (−)
May	H ABA extract	13.94	24.77 %	-
	D ABA extract	15.57	15.66 %	36.77 (−)

H = healthy, ABA extract (i.e. the ABA extract of healthy stem tissue). D = diseased, ABA extract (i.e. the ABA extract of diseased stem tissue).

of the dormant (winter) period. Phenol content was consistently less in diseased cuttings, and the difference in the phenol content between healthy and diseased stem cuttings was especially prominent in September, October and January. The phenol content in healthy stem cuttings was only marginally higher than in virus-infected stem cuttings in April, May and June, when active vegetative growth takes place.

The abscisic acid-like activity in healthy cuttings was high in November and January (the dormant period of the plant), but it decreased sharply in February before the buds resumed growth in March, and then it remained almost constant until May. The abscisic acid-like activity in diseased cuttings was also high in November and January, it decreased sharply in February and then it decreased still more in May. The abscisic acid-like activity in diseased cuttings was always less than in the healthy cuttings in all the months; but the decrease in the dormant period (November, January and February) of the plant was more than in other months, and was thus particularly significant.

It is clear from this that the amount of growth inhibitors (phenols and abscisic acid) present in diseased stem cuttings is much less than in healthy cuttings during the dormant period of the plant. This accounts for the break in bud dormancy in diseased cuttings. This conclusion is in agreement with the observation of other workers that there is an increase in growth inhibitors during the dormant period and a decrease during the growing period[7-9].

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Chemical characterization of human urine albumin in proteinuria[1]

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Summary. From the urine of a patient with proteinuria, the albumin protein component was isolated and compared with human serum albumin. By comparing the amino acid composition of the original proteins and their large cyanogen bromide fragments, peptide maps and N-terminal sequences of 33 amino acid residues, the identity of both proteins was shown.

As obvious from the published papers[2-5], the pathway of protein molecules through the blood-urine barrier is quite complicated and a large part of the proteins from the primary urine is reabsorbed by the cells of the tubuli and catabolized. In order to elucidate the mechanism of protein transfer from serum to urine at pathological states of the kidneys, our attention was primarily focussed on albumin. The determination of the complete structure[6,7] of human serum albumin (HSA), the arrangement of its disulfide bonds[8], together with progress in understanding of its molecular architecture and biosynthesis[9] have made it possible to examine the question of identity of human urine albumin (HUA) and serum albumin.

Material and methods. The albumin fraction was isolated from the urine of a patient with chronical glomerulonephritis. After dialysis of the urine for 24 h against distilled water at 24 °C, the dialysate was lyophylized and redissolved in a sodium chloride physiological solution (concentration of protein 5 g/l). HUA was isolated by alcohol fractionation according to Cohn[10], the comparison was made with HSA from ÚSOL (Prague, Czechoslovakia) by electrophoresis in polyacrylamide gel[11] and immunoelectrophoresis[12]. The amino acid analysis of HUA (one determination) was performed after oxidation of the sample by performic acid[13] and hydrolysis by 6M-HCl for 20 h at 110 °C by the method of Spackman et al.[14]. The N-terminal sequence of HUA was determined by stepwise degradation[15] in Beckman Model 890C Sequencer using the 'Fast Quadrol Program' recommended by the manufacturer. Using the same procedure as in previous studies[16,17], we isolated from the

Table 1. Amino acid composition of human urine albumin (HUA) and human serum albumin (HSA)

	Amino acids								
	Lys	His	Arg	Cys	Asp	Thr	Ser	Glu	Pro
HUA	54.1	15.9	23.4	32.3	53.4	29.0	23.8	79.3	26.1
HSA	59	16	24	35	53	28	24	82	24

	Amino acids								
	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe	Trp
HUA	16.5	56.2	38.3	6.6	9.7	61.0	18.6	31.0	+*
HSA	12	62	41	6	8	61	18	31	1

* Presence of tryptophan determined by a qualitative test. The analytical values for HUA are not corrected, the calculation is based on the content of 61 residues of leucine. The values for HSA show the number of the amino acid residues, determined in its complete sequence[7].

Fig. 1-4. Peptide maps of tryptic digests. In the left column (A) human serum albumin and its cyanogen bromide fragments, in the right column (B) human urine albumin and its cyanogen bromide fragments. 1A, B: Oxidized albumins; 2A, B: N-terminal fragments; 3A, B: middle fragments; 3A, B: C-terminal fragments. Horizontal direction: electrophoretic separation (pH 5.6, 30 V/cm). Vertical direction: chromatographic separation (n-butanol:pyridine:acetic acid:water 15:10:3:12, v/v).