Sequential Action of Cytokinin and Gibberellic Acid in Wheat Aleurone Tissue

INCUBATION of de-embryoed wheat or barley grains in the presence of gibberellic acid induces α-amylase activity in the endosperm which is greater than that of tissue incubated in the absence of the hormone[1,2]. The target tissue of gibberellic acid is the aleurone layer[3], and the effects of protein synthesis inhibitors[4] have indicated that the induction involves a *de novo* synthesis of the enzyme. Induction of other hydrolytic enzymes in the aleurone tissue by gibberellic acid has also been described[5,6].

Extracts of wheat starchy endosperm also induce metabolic activity in aleurone tissue, which has been isolated as bran from the quiescent wheat grain[7]. This activity can also be induced by authentic cytokinins at concentrations between 1·0 μM and 0·001 μM, and the response can be detected within 18 h of imbibition. Bran used in previous work on the action of gibberellic acid on isolated aleurone tissue[5,6,8,9] was isolated only after the de-embryoed grain had been soaked for 1–3 days. It has therefore become imperative that the possible role of the cytokinins in the induction of α-amylase activity be investigated.

Bran segments, prepared from twenty-five sterilized, quiescent wheat grains within 4 h of imbibition, were incubated for 24 h at 25° C in 50 ml. of 0·05 M citrate buffer, *p*H 6·2, containing 0·1 μM kinetin and 20 mM $CaCl_2$. Control segments were incubated in buffer containing 20 mM $CaCl_2$ but no kinetin. Both experimental and control incubations were duplicated. After 12 h and 24 h of incubation, 1 ml. sub-samples of each medium were assayed for α-amylase content[10]. The segments were then removed from their media, washed in sterile distilled water and damp dried on filter paper. One each of the experimental and control bran samples were transferred to separate 50 ml. buffer solutions containing 10 μM gibberellic acid and 20 mM $CaCl_2$. The other two samples were transferred to separate 50 ml. buffer solutions containing 20 mM $CaCl_2$ but no gibberellic acid. The four samples were incubated for a further 48 h and sub-samples were again removed at 12 h intervals to assay α-amylase content. A fifth batch of bran segments from twenty-five quiescent grains was incubated directly in 50 ml. of buffer containing 10 μM gibberellic acid and 20 mM $CaCl_2$.

As a check on the conditions of incubation, bran segments were prepared from batches of twenty-five de-embryoed wheat grains which had imbibed water for 48 h. Bran prepared in this way responds to gibberellic acid[5,6,8,9]. These samples of bran were incubated in buffered media, either in the presence or absence of gibberellic acid.

After incubation, bran samples were homogenized and assayed for α-amylase content. In each sample a low content of α-amylase showed that the enzyme had been freely secreted into the medium.

Results are shown in Table 1. The experiment was repeated and the results were essentially the same as on the first occasion. Several significant conclusions can be drawn. The aleurone tissue isolated from quiescent grain contains a considerable quantity of α-amylase which is completely secreted when the tissue is incubated in aqueous media. The addition of either kinetin or gibberellic acid to the medium does not significantly increase the level of enzyme activity. But when the aleurone tissue is incubated first with kinetin and then with gibberellic acid, there is a two-fold increase in secreted α-amylase activity. The starchy endosperm of wheat seems to contain a compound which has cytokinin activity and which is responsible for the induction of essential metabolic processes in the aleurone cells of germinating wheat grain. It is pertinent that a cytokinin, zeatin, has been isolated from the endosperm of developing maize grain[11] and detected in extracts of germinating barley[12].

Table 1. α-AMYLASE RELEASE FROM INCUBATED BRAN

Incubation period (h)	Bran from quiescent grain						Bran from soaked endosperm	
	+Kinetin		−Kinetin		+GA_3			
0	0		0		0			
12	0·29		0·28		0·32			
24	0·62		0·61		0·62			
	+GA_3	−GA_3	+GA_3	−GA_3			+GA_3	−GA_3
0	0	0	0	0			0	0
12	0·18	0·11	0·10	0·09			0·28	0·14
24	0·35	0·20	0·21	0·20			0·45	0·20
36	0·47	0·23	0·23	0·22			0·74	0·28
48	0·61	0·26	0·25	0·23			1·08	0·36

Results are given as α-amylase units/ml. of incubation medium.

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Synthesis of Mycorrhizas in *Podocarpus* and *Agathis* with Endogone Spores

THE absorbing systems of most green plants are liable to invasion by symbiotic phycomycetes which convert them into vesicular–arbuscular mycorrhizas. This type of mycorrhiza has recently been synthesized in several angiosperms using Endogone spores[1,2]. To attempt this synthesis with members of the Podocarpaceae and Araucariaceae is desirable because they are gymnosperms, and because their root systems accommodate the endophytic mycelium in special nodules[3].

Between ten and thirty honey coloured sessile spores propagated in the roots of *Coprosma robusta* from isolations made from New Zealand soils by Dr Barbara Mosse[1] were blown into each root system from a glass pipette. Control plants received spores that had been crushed beneath a coverslip. Three soils were used: crushed rotten rock sterilized at 100° C between electrodes and stored moist for 3 yr to reduce available phosphorus, and mixtures of this inorganic soil with 5 per cent and 20 per cent (by volume) of steamed forest humus. The seedlings were *Podocarpus totara* (2 yr old) and *Agathis australis* (3 yr old) which had been raised in poor, steamed soil and had stopped growing. *P. totara* was inoculated in the spring and again in midsummer; *A. australis* was inoculated only in midsummer.

By the following summer nineteen of the twenty *P. totara* inoculated with whole spores had a typical vesicular–arbuscular infection in their nodules and had resumed growth, attaining a mean fresh weight of 1·57 g. Eleven of the plants inoculated with crushed spores were still alive but moribund, averaging 0·47 g. (A twelfth plant