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Changes in the levels of wheat- and barley-germ agglutinin during embryogenesis in vivo, in vitro and during germination

P.C. Morris[1], S.E. Maddock[2], M.G.K. Jones[2] and D.J. Bowles[1]

[1] Department of Biochemistry, University of Leeds, Leeds LS2 9JT, and
[2] Department of Biochemistry, Rothamsted Experimental Station, Harpenden, Herts. AL5 2JQ, UK

Abstract. Radioimmunoassay has been used to measure levels of wheat-germ agglutinin and barley-germ agglutinin during embryogenesis and germination. The two lectins exhibited similar patterns of accumulation during grain maturation in vivo and both decreased to low levels after imbibition of harvest-ripe grains for 3 d. Precocious germination of immature wheat and barley embryos excised and cultured in vitro could be prevented either by inclusion of abscisic acid or mannitol in the culture medium. Changes in the level of wheat-germ agglutinin induced by in vitro culture depended on the maturation stage of the embryo. No direct correlation was found between application of exogenous abscisic acid and accumulation of the lectin.

Key words: Abscisic acid and precocious germination – Embryogenesis – *Hordeum* (lectin) – Lectin – *Triticum* (lectin).

Introduction

Members of the Hordeae tribe of cereals contain the two structurally similar agglutinins: wheat-germ agglutinin (WGA) and barley-germ agglutinin (BGA) (Peumans et al. 1982; Stinissen et al. 1983; Miller and Bowles 1983). Wheat-germ agglutinin in particular has been extensively characterized (reviewed by Grivet et al. 1983), but despite the detailed information available concerning the physical and chemical properties of the protein, its function remains unknown. Results of a study by Triplett and Quatrano (1982) indicated that synthesis of WGA was regulated by abscisic acid (ABA), in that excised, immature embryos cultured in the presence of this growth regulator accumulated the lectin to a greater extent than those embryos left attached to the mother plant. Immature embryos cultured in the absence of exogenous ABA did not synthesize WGA and germinated precociously into plantlets. These results have led to suggestions that the lectin could be involved in developmental arrest.

The present study was designed to investigate these possibilities further, using embryos at different stages of maturation, and also to examine the parallels between BGA and WGA in terms of their accumulation during embryogenesis and their disappearance during germination and early seedling development.

Abbreviations: ABA = abscisic acid; BGA = barley-germ agglutinin; dpa = days post authosis; RIA = radioimmunoassay; SDS-PAGE = polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate; WGA = wheat-germ agglutinin

Materials and methods

Plant material. Mature barley (*Hordeum vulgare* cv. Igri) grains were obtained from the University of Leeds, Headingley Hall Farm, Tadcaster, West Yorkshire, UK. Barley (cultivars Golden Promise and Sundance) and wheat (*Triticum aestivum* cv. Timmo) were cultivated under glasshouse conditions at Rothamsted Experimental Station. The plants were tagged at anthesis and grains removed throughout early and mid stages of maturation (10–36 days post anthesis; dpa). Embryos were excised (Maddock et al. 1983) and either frozen rapidly and stored at -20° C prior to analysis or cultured, scutellum-down, under sterile conditions on a basal medium containing Murashige and Skoog's (1962) nutrients (minus hormones) and 3% (w/v) sucrose, plus selected additives. Chemicals were obtained from Sigma Chemical Co., Poole, Dorset, UK unless otherwise stated.

Preparation of BGA. Barley embryos were isolated from mature grains (cultivar Igri) by briefly grinding the seed in a coffee grinder for 5 s. and manually recovering individual freed embryos. The lectin was isolated from these embryos by a similar protocol to that described for WGA (Bouchard et al. 1976; Miller and Bowles 1982), using the immobilised affinity absorbent: *p*-aminobenzyl-1-thio-2-acetamido-β-glucopyranoside. Polyacrylamide-gel electrophoresis in the presence of

sodium dodecyl sulfate (SDS-PAGE) and under reducing conditions, using gradient gels, and rocket immunoelectrophoresis were as described by Miller and Bowles (1982, 1983).

Preparation of embryo extracts. Two replicates of fifteen embryos pooled from specific stages of development were homogenised at 4° C in phosphate-buffered saline (PBS; 10 mM KH_2PO_4/K_2HPO_4, 145 mM NaCl, ph 7.0) containing 0.1% (w/v) Triton X-100, 100 mM N-acetyl-D-glucosamine and, after sonication (Dawe instruments, London UK; microprobe attachment) for 3 × 10 s the particulate debris was removed by centrifugation at 4° C and 30000 *g* for 30 min. The supernatant was assayed in duplicate for protein (Lowry et al. 1951) and for lectin by radioimmunoassay (RIA). Protein concentrations in extracts assayed by RIA were generally in the range 50–400 µg. For determination of lectin content in excised embryos cultured in vitro, tissue was also subjected to an alternative extraction protocol involving acid treatment as described by Triplett and Quatrano (1982).

Radioimmunoassay. Wheat-germ agglutinin was iodinated using the chloramine-T procedure as described by Miller and Bowles (1985) to a specific activity of 814 kBq·mol^{-1}. Monospecific antiserum to WGA was raised in rabbits and used in RIA (Miller and Bowles 1983, 1985). Preformed, double antibody complexes were prepared by addition of 10 µl of rabbit anti-WGA IgG (ammonium-sulphate cut) at 5 mg·ml^{-1} to 40 µl of goat anti-rabbit IgG (Behringwerke AG, Marburg, FRG) at 1/100th dilution, and 50 µl of 20 mg·ml^{-1} cytochrome c in radioimmune buffer [PBS containing 0.1% (w/v) Triton X-100, 100 mM N-acetyl-D-glucosamine]. The antibody mix was incubated for 2 h at 37° C and left for at least 4 h at 4° C. Radioimmunoassay, using 10 µl ^{125}I-labelled WGA at 24 µg·ml^{-1} as tracer was carried out using a standard curve of cpm versus log concentration, fitted using a spline function on a Rack Gamma (LKB, Wallac Oy, Turku, Finland) and operating in the range of 2–200 pmol lectin ± 1 pmol.

Results

Preparation of BGA. Barley embryos excised from dry grains were used as the starting material for lectin purification. Typically, 600 µg lectin could be isolated from 1 g embryos using a protocol that involved the affinity adsorbent originally devised by Monsigny and coworkers for the purification of WGA (Bouchard et al. 1976). After SDS-PAGE, BGA was found to have an M_r of 18 000 and the lectin cross-reacted with antisera raised to WGA in rocket immunoelectrophoresis (Fig. 1). Radioimmunoassay on given amounts of BGA yielded results equivalent to those given by WGA (Fig.2) enabling the direct use of the RIA system employed for WGA to determine endogenous levels of BGA.

Lectin levels in maturing and germinating embryos. Lectin levels were determined by RIA in maturing zygotic embryos (10–36 dpa; plants grown in the glasshouse in the spring) from two barley cultivars (Golden Promise and Sundance) and one wheat cultivar (Timmo). Results are shown in Fig. 3.

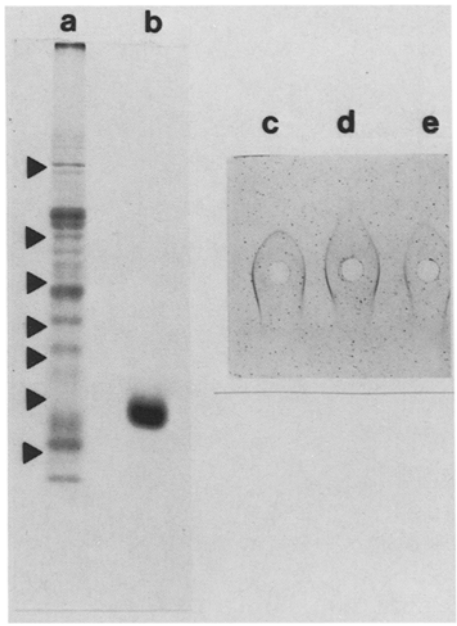

Fig. 1. Characterization of barley-germ agglutinin. Both BGA and WGA were isolated as described in Materials and methods. *a, b* SDS-PAGE under reducing conditions, 10–15% (w/v) polyacrylamide gradient gels. *Track a*, barley embryo extract (100 µg); *track b*, BGA (30 µg). *Arrows* mark positions of M_r markers: BSA, 66 kDa; ovalbumin, 36 kDa; glyceraldehyde 3-phosphate dehydrogenase, 24 kDa; soybean trypsin inhibitor, 20 kDa; α-lactalbumin 14.2 kDa. *c–e* Rocket immunoelectrophoresis, antibody window contains 25 µl anti-WGA ml; antigens at 1.2 µg: *c*, WGA; *d*, ^{125}I-WGA, *e*, BGA. Protein visualized by Coomassie-Blue

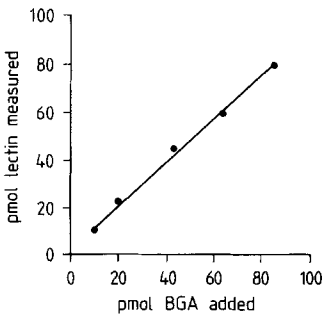

Fig. 2. Calibration of RIA for assay of BGA. Antisera raised to WGA was used in an RIA to estimate levels of BGA as described in Materials and methods

Barley-germ agglutinin first appeared between 16 and 20 dpa in both barley cultivars and increased steadily in amount with development until, by 36 dpa, values had reached approx. 50% of that found in mature desiccated embryos (e.g. for Sundance, 89 pmol/embryo, 295 pmol/mg protein). Wheat-germ agglutinin appeared slightly later than BGA at 22 dpa, but also increased in amount with development. The levels of WGA at 36 dpa were

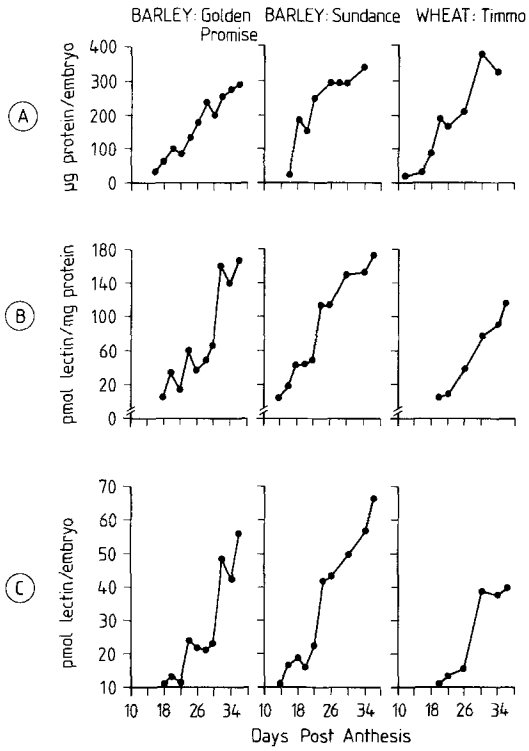

Fig. 3A–C. Changes in protein (**A**) and lectin (**B,C**) content of wheat and barley embryos during maturation. Embryos were harvested from plants grown in the glasshouse in the spring at intervals after anthesis. Protein and lectin levels were measured as described in the text

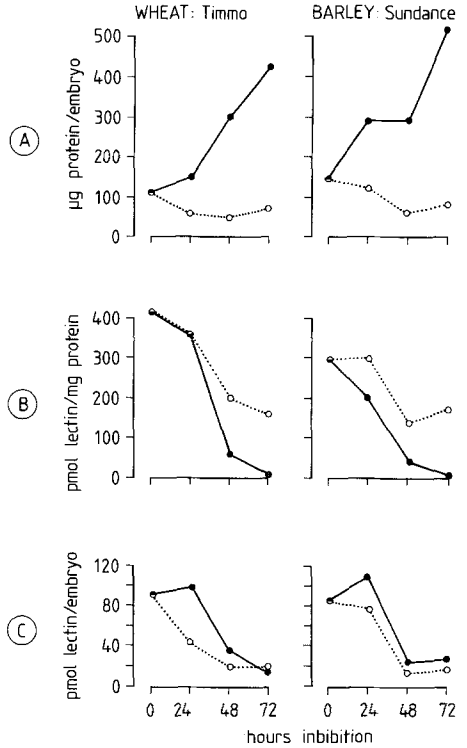

Fig. 4A–C. Changes in protein (**A**) and lectin (**B,C**) content of wheat and barley embryos during germination and early seeding development. Surface-sterilized harvest-ripe grains and excised embryos were imbibed in H_2O on filter paper under sterile conditions for periods up to 72 h. ●–●, Embryos imbibed whilst attached to the grain; ○–○, excised embryos

only approx. 40% of that found in the mature embryo (e.g. for Timmo, 92 pmol/embryo, 410 pmol/mg protein), and this probably reflects the longer maturation cycle of wheat than barley. No lectin was found in the endosperm of either cereal during the time-course of grain development investigated.

Surface-sterilised mature grains and excised mature embryos of wheat and barley were placed on moistened filter paper at 20° C and allowed to imbibe and germinate. Lectin levels in embryos were assayed by RIA throughout germination and by 72 h, levels of both lectins decreased substantially, irrespective of whether the embryos were excised or within the intact grain (Fig.4).

Lectin levels in immature embryos cultured in vitro. Immature wheat embryos (Timmo) were excised 14 dpa from plants grown in the glasshouse in the spring, and either assayed for protein and WGA immediately or cultured, scutellum-down, in the presence or absence of 10^{-6} M ABA for 5 d before assaying. Grains were also allowed to remain on the parent plant until 19 dpa, when embryos were excised and lectin and protein levels measured. In-vitro culture conditions had a profound influence on the morphology and physiology of the immature embryos. As shown in Table 1, embryos cultured on basal medium in the absence of ABA, germinated precociously within the 5-d period to yield small plantlets (mean fresh weight approx. 3 mg), showing a 15-fold increase in fresh weight and 17-fold increase in protein content. In the presence of ABA, precocious germination of the immature embryos was totally arrested although both the fresh weight and protein content of the embryos increased (four-fold and 11-fold, respectively). Control embryos, which matured for the same time period attached to the grain on the mother plant, increased less in fresh weight and protein over the 5 d (four-fold and seven-fold, respectively).

Wheat-germ agglutinin was absent in the 14-dpa immature embryos, although by 19 dpa low but measurable levels had begun to accumulate. When lectin levels were measured in the excised immature embryos cultured in vitro, very similar amounts of WGA were found in both those that

Table 1. Influence of in-vitro culture conditions on immature wheat embryos excised at 14 dpa

Days in culture	Treatment	Morphology	FW per embryo-plant (mg)	Protein per embryo-plant (μg) (RIA extract)	pmol WGA	
					per mg protein	per embryo-plant
0		Embryo	0.2	6.7	0	0
5		Young plant	2.9	117	53	6.2
5	10^{-6}M ABA	Embryo	0.8	77	71	5.5
5	Left on plant	Embryo	0.8	48.3	8.6	< 1.0

Table 2. Influence of in-vitro culture conditions on immature wheat embryos excised in 26 dpa.

Days in culture	Treatment	Morphology	FW per embryo-plant (mg)	Protein per embryo-plant (μg)		pmol WGA			
						per mg protein		per embryo-plant	
				Acid extract	RIA extract	Acid extract	RIA extract	Acid extract	RIA extract
0		Embryo	1.5	96	160	69	59	6.6	9.4
6		Young plant	47	483	916	19	8.7	9.2	8.0
6	10^{-4}M ABA	Embryo	3.7	325	375	109	106	35.4	39.8
6	10^{-6}M ABA	Germinating embryo	14	472	633	44	32	20.8	20.3
6	9% Mannitol	Embryo	3.5	229	383	109	96	25.0	36.8
6	Left on plant	Embryo	2.2	207	307	136	119	28.3	36.5

had precociously germinated and those that had been arrested from germination by inclusion of 10^{-6}M ABA in the growth medium.

These results indicated that excision of small, immature embryos from the grain-mother plant preceding the appearance of endogenous lectin, caused an increase in WGA levels in the tissue irrespective of culture conditions.

The effect of the size of the immature embryos on subsequent lectin levels was investigated and the protocol was extended to include a wider range of culture and extraction conditions. The results from this second set of experiments are shown in Table 2. Larger, immature embryos (26 dpa) were used which had a greater fresh weight (7.5-fold) and protein content (24-fold) than those studied previously. As before, excised embryos were cultured, scutellum-down, in the presence or absence of ABA. The effect of inclusion of mannitol in the growth medium was also investigated. After 6 d of in-vitro culture or continued development on the parent plant, embryos were extracted either in radioimmune buffer as before, or using the acid-extraction protocol of Triplett and Quatrano (1982). At this later developmental stage, higher levels of ABA (10^{-4}M) were required to completely arrest precocious germination although inclusion of ABA at 10^{-6}M still greatly inhibited germination. Mannitol at 9% (w/v) was also found to inhibit the precocious germination of the embryos.

In the absence of either ABA or mannitol, 26-dpa embryos germinated and had developed into large seedlings (average fresh weight 47 mg) after 6 d in culture, showing increases in fresh weight (31-fold) and protein content (sixfold). Both 10^{-4}M ABA and 9% (w/v) mannitol induced the same morphological effect in arresting precocious germination and the same increases in fresh weight and protein content (two-fold and 2.4-fold). The intermediate effect of 10^{-6} M ABA on these embryos was also reflected in the changes in fresh weight and protein content. Control embryos matured for 6 d on the parent plant increased only slightly in size and protein content (1.4-fold and 1.9-fold, respectively), indicating that by this later stage the growth rate was slowing down. Extraction of the tissue in radioimmune buffer always yielded higher protein than the acid extraction, presumably as a result of the inclusion of solubilized membrane proteins in the extract.

By this later stage of maturation, WGA was already present in the immature embryos. After 6 d, levels of the lectin had increased to an almost identical extent in embryos matured on the grain-mother plant or excised and cultured on 10^{-4}M

Table 3. Influence of in-vitro culture conditions on immature wheat embryos excised at 20 dpa

Days in culture	Treatment	Morphology	FW per embryo-plant (mg)	Protein per embryo-plant (μg) (RIA extract)	pmol WGA	
					per mg protein	per embryo-plant
0		Embryo	0.7	68	0	0
7		Young plant	36.1	480	6.5	3.1
7	10^{-6} ABA	Embryo	2.7	325	43.7	14.2
7	9% Mannitol	Embryo	1.8	235	38.7	9.1
7	Left on parent plant	Embryo	1.3	144	5.2	< 1.0

Table 4. Influence of in-vitro culture conditions on immature barley embryos excised at 14 dpa

e b Days in culture	Treatment	Morphology	FW per embryo-plant (mg)	Protein per embryo-plant (μg) (RIA extract)	pmol BGA	
					per mg protein	per embryo-plant
0		Embryo	0.25	10	0	0
8		Young plant	11	333	4.0	1.3
8	10^{-6} ABA	Embryo	3.4	280	13.0	3.6
8	9% Mannitol	Embryo	2.0	150	13.0	2.0
8	Left on parent plant	Embryo	2.1	140	18.0	2.5

ABA or 9% mannitol. This increase in WGA occurred if calculated as either lectin per mg protein or per embryo, although the latter increases were much higher. In seedlings arising from precociously germinated embryos, lectin content was maintained at the day-0 level if calculated per embryo, but was reduced per mg protein. Very similar results were obtained whether the tissue was acid-extracted or extracted in radioimmune buffer. The lack of direct involvement of WGA in causing developmental arrest was also confirmed when embryos were excised at 34 dpa. At this stage they already contained substantial levels of lectin (147 pmol WGA·mg^{-1} protein), yet produced well-established plants within 8 d of culture on basal medium (results not shown).

As a further confirmation that the age of the immature embryos affected their response to in-vitro culture, an intermediate size of wheat embryos (average fresh weight 0.7 mg) was also studied. ABA and mannitol were used to inhibit precocious germination and the tissue was extracted with radioimmune buffer. As shown in Table 3, increases in fresh weight and protein and changes in lectin content during in-vivo development and in-vitro culture were intermediate to those shown in Tables 1 and 2. Inclusion of mannitol, either at 4.5% or 9%, prevented precocious germination. Lectin was absent from 20-dpa embryos and only low levels had begun to accumulate if they were left for a further 7 d on the mother plant. After in-vitro culture, WGA was present in the young plants but greater amounts of the lectin had accumulated in embryos prevented from precocious germination.

For comparison, the effect of excision and in-vitro culture of immature barley embryos (14 dpa) was also investigated. Results are shown in Table 4 and indicate that barley shows an identical morphological response to wheat, in that the small immature embryos (average fresh weight 0.25 mg) germinated precociously on basal medium but were arrested from germination by inclusion of 10^{-6}M ABA or 9% (w/v) mannitol in the medium. The lectin was absent in 14-dpa embryos but low levels began to accumulate by 22 dpa, irrespective of culture conditions. When barley embryos were excised at 34 dpa and cultured for 8 d on 10^{-4} M ABA or 9% (w/v) mannitol, still only relatively low levels of BGA had accumulated: 23 pmol BGA·mg^{-1} protein (+ ABA), 13 pmol BGA·mg^{-1} protein (+ mannitol).

Discussion

The close structural similarity between the lectins of wheat and barley has enabled us to estimate levels of BGA using an RIA characterized initially for WGA. Our results indicate that both lectins appear at very similar developmental stages during

embryogenesis in vivo, are found in almost identical amounts in harvest-ripe grains and decrease in parallel during germination. In contrast to these similarities, differences were found between the lectins during in-vitro culture of immature embryos, when BGA levels were consistently observed to be much lower than WGA and to fluctuate less in response to culture conditions. A similar pattern was found during long-term culture of wheat and barley tissues under a variety of hormonal regimes (data not shown). Observations have also indicated a slightly different cellular location of BGA and WGA in the germinating embryos of 24-h-imbibed grains (Mishkind et al. 1983).

We have confirmed earlier observations that precocious germination of excised immature barley and wheat embryos can be prevented by inclusion of exogenous ABA in the culture medium or by conditions of osmotic stress, such as inclusion of high levels of mannitol (for a review, see Norstog 1979). It was found necessary to culture the excised embryos scutellum-down to obtain an 100% consistency in the effect of the additives, presumably because uptake of media components is most efficient in this orientation, and increased concentrations of additives were required to arrest the germination of more mature embryos.

Changes in the level of WGA induced by excision and in-vitro culture depended on the age of the embryo. If embryos were excised at very early stages (0.2 mg fresh weight), similar amounts of the lectin were found both in excised material that had precociously germinated and embryos that had been prevented from doing so by exogenous ABA. The level of WGA was greater in cultured material than in embryos that had been left on the parent plant. In contrast, at a much later maturation stage (1.5 mg fresh weight) when WGA was already present in appreciable amounts in the embryos, the lectin continued to accumulate at the same rates during in-vitro and in-vivo culture, providing germination was prevented. Thus, levels of the lectin were nearly identical in embryos left associated with the parent plant or excised and cultured in the presence of ABA or mannitol. Removal of the embryo from the mother plant and culture on basal medium allowed germination and caused WGA accumulation to cease, so that although lectin levels were maintained per plant, they decreased per mg. protein. An intermediate response was found when embryos of 0.7 mg fresh weight were cultured.

Although these results indicate no specific correlation between WGA accumulation and the application of exogenous ABA, the observations could be explained by changes in the level of endogenous growth regulator. It has been reported by King (1976), that the more rapid the grain growth during early maturation, the greater the content of ABA. In this study, the increase in protein content of embryos cultured in vitro at 14 dpa was substantially greater than that shown by embryos left associated with the parent plant, perhaps reflecting nutrient availability in the basal medium. Abscisic acid is known to rapidly attain maximal levels by approx. 30 dpa, thereafter decreasing sharply and remaining in low amounts for the remainder of maturation and grain desiccation (King 1976, 1979; King et al. 1979). In the older embryos used in this study (26 dpa), it is possible that endogenous ABA was kept at high levels either by continued attachment to the parent plant or by osmotic stress or by application of exogenous growth regulator. Under those conditions, lectin accumulation would continue since the usual maturation pathway of the embryo was maintained. Recent studies on precocious germination in *Brassica napus* have indicated that it represents a complex developmental sequence that exhibits characteristics of both embryogeny and germination (Finkelstein and Crouch 1984). The lack of a simple switch-over from the maturation to germination pathways may explain why WGA accumulation occurs in very young embryos even under conditions that permit precocious germination.

Although the effects of exogenous ABA on germination of cultured barley and wheat embryos described here are apparently identical to those reported for wheat by Triplett and Quatrano (1982) and rice (Stinissen et al. 1984), it is surprising that we do not observe the same effects of the growth regulator on the endogenous lectin. Technically, this study differs considerably from that carried out on rice embryos, since the effect of ABA on the synthesis of affinity-purified proteins including the rice lectin was determined by in-vivo labelling. The rice embryos were excised at 12 dpa and cultured for only 2 d with inclusion of [^{35}S] cysteine for the final 12 h of each time point. Incorporation of cpm/embryo into affinity-purified protein (time 12 h: 300 cpm/embryo) decreased irrespective of culture conditions although exogenous ABA affected the rate of decline (time 48 h:ABA [+] 50 cpm/embryo, ABA [–] no incorporation). Our study provides data on the amount of lectin rather than lectin synthesis, but nevertheless our conclusion about a direct effect of exogenous ABA clearly differs from that discussed by Stinissen and

coworkers. However, WGA levels were also measured by RIA in the study carried out by Triplett and Quatrano (1982) when the amount of lectin present was expressed as percent of acid-soluble protein extracted. We included acid extraction in our protocol to ascertain whether the different techniques used had caused the anomolies between their observations and ours. Since the extraction method did not appreciably alter our results, the anomolies in lectin content may have arisen from use of different antisera, minor differences in the conditions used to culture immature embryos or reflect different physiological ages of embryos used. For example, King (1979) observed that even in a controlled environment, a shift of up to 5 d in the timing of changes of ABA content and the ability of the embryo to germinate was routinely observed between experiments. To clarify the potential relationship between levels of endogenous growth regulator and lectin accumulation, experiments are currently in progress to determine levels of WGA and ABA during embryogenesis using immunological techniques.

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