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ACTIVITY OF BRANCHING ENZYME AS A CARDINAL FEATURE OF THE Ra LOCUS IN *PISUM SATIVUM*

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Key Word Index—*Pisum sativum*; Leguminosae; pea; embryos; starch; Ra locus; 1,4-α-glucan branching enzyme.

Abstract—The aim of this work was to discover whether the Ra locus in *Pisum sativum* affected the maximum catalytic activities of: starch synthase, ADPglucose pyrophosphorylase, alkaline inorganic pyrophosphatase, α-glucan phosphorylase and 1,4-α-glucan branching enzyme. These enzymes were measured at four stages during the development of the embryos of cv. Birte (RaRa) and Greenshaft (rara). No significant differences between the two varieties were found for the first four enzymes; each increased during development. In Birte the activity of branching enzyme was appreciable and increased in parallel with those of the other enzymes during development. In Greenshaft no activity of branching enzyme was found until the embryos exceeded 200 mg fresh weight, thereafter it increased but even at the 400 mg stage was only 14% of that in Birte. Embryos of Greenshaft contained five times more ADPglucose than those of Birte. It is suggested that the Ra locus determines branching enzyme activity and, through this, the amount and composition of the starch.

INTRODUCTION

The varieties of pea, Birte and Greenshaft, henceforth called round and wrinkled, differ at the Ra but not the Rb locus. The former is RaRa RbRb, the latter is rara RbRb [1]. Previous studies [2, 3] of the developing embryos of the two varieties have established the following. First, there is a marked difference in starch content: the developing embryos of round seeds contain five times as much starch as those of wrinkled seeds. Second, this difference in starch content is associated with similar differences in the partitioning of sucrose between starch synthesis and respiration, and in the rate of starch accumulation. Third, the difference in starch content is not related in any obvious way to differences in the immediate metabolism of sucrose. The aim of the present work was to discover if the embryos of the two varieties differed in their capacity to catalyse the reactions most closely concerned with starch synthesis. Accordingly, we measured the maximum catalytic activities of ADPglucose starch synthase (EC 2.4.1.21), ADPglucose pyrophosphorylase (EC 2.7.7.27), 1,4-α-glucan branching enzyme (EC 2.4.1.18), alkaline inorganic pyrophosphatase (EC 3.6.1.1) and α-glucan phosphorylase (EC 2.4.1.1). The first four enzymes were chosen because of their close association with starch synthesis [4, 6]. α-Glucan phosphorylase was measured because of the possibility that the lower starch content of wrinkled peas was due to more rapid turnover caused by a greater capacity to break down starch.

This work was done with embryos. We took developing seeds from growing pods and removed the testa. The whole of the material within the testa, which at the stages of development studied consisted almost entirely of cotyledons with a small radicle, was used without further dissection. Our measurements were made during the period of rapid growth of the embryos when fresh weight and starch content increase almost linearly [2]. We define the developmental stages according to embryo fresh weight, as 100, 200, 300, and 400 mg. As we could not always harvest embryos of these precise weights, for each stage we took embryos that were within 40 mg of the specified weight. For example for the 100 mg stage we used embryos between 60 and 140 mg for each sample: for each sample within this range values for enzyme activity or metabolite content are expressed per 100 mg to give the value at the 100 mg stage. We stress that the procedures we used for growing and sampling the embryos were those used in our previous work [2, 3] and that data in the present paper are directly comparable to our earlier measurements.

RESULTS

Synthesis of 1,4-α-*glucan*

Estimates of the maximum catalytic activities of enzymes involved in the synthesis of 1,4-α-glucan are given in Table 1. We investigated the reliability of the assays represented in Table 1. First, using extracts of mixtures of both types of embryos, we optimized the concentration of each component, and the pH of the reaction mixtures, and showed that activity was linearly related to time and amount of extract. Next we completed recombination experiments to see if differential loss of activity occurred during extraction of the embryos. For each test we prepared three samples of plant material: one was a mixture of the two varieties of embryo, one was a γ stage club of *Arum maculatum* [7], and one was a mixture of *Arum* club

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Table 1. Maximum catalytic activities of enzymes of starch metabolism in developing embryos of round and wrinkled peas

Enzyme	Variety	Enzyme activity nmol/min per embryo in embryos of fr.wt 100	200	300	400 mg
Starch synthase	Round	12.4 ± 1.2	23.7 ± 1.3	24.7 ± 2.6	30.8 ± 0.6
	Wrinkled	12.0 ± 1.5	27.3 ± 2.6	46.6 ± 2.2	32.0 ± 4.8
ADPglucose pyrophosphorylase	Round	114 ± 11	400 ± 8	587 ± 34	707 ± 84
	Wrinkled	70 ± 4	217 ± 10	444 ± 32	973 ± 88
Inorganic pyrophosphatase	Round	229 ± 48	719 ± 55	743 ± 133	719 ± 134
	Wrinkled	344 ± 30	1071 ± 53	1110 ± 87	1361 ± 75
α-Glucan phosphorylase	Round	12 ± 0.4	29 ± 1.4	78 ± 11.4	113 ± 17.9
	Wrinkled	15 ± 6.4	13.5 ± 0.9	58 ± 14.0	89.5 ± 10

Values are means ± s.e.m. of estimates from at least four samples.

and the two varieties of pea embryo. For ADPglucose starch synthase, ADPglucose pyrophosphorylase and α-glucan phosphorylase, the activities in the third sample were 108, 119 and 100%, respectively, of those predicted from the measurements made on the separate components (the first two samples) of the mixtures. For pyrophosphatase we prepared duplicate samples of tissue; one was extracted in the usual way, the other was extracted in buffer that contained a measured amount of the pure enzyme. The latter activity was comparable to that found in the sample of embryos. Our estimate of the recovery of the pure pyrophosphatase was 111%.

The maximum catalytic activity of ADPglucose starch synthase (Table 1) increased during the early stages of embryo development and then either increased more slowly (round) or declined (wrinkled). Only at the 300 mg stage was there a significant difference between the two varieties, and here the higher activity was found in the variety with less starch. The activity of ADPglucose pyrophosphorylase rose sharply throughout embryo development so that in both varieties there was an increase in activity per g fresh weight as well as per embryo. The activities in the two varieties were similar. Inorganic pyrophosphatase activity rose initially in both varieties, and then more slowly in wrinkled peas or not at all in round peas. After the 100 mg stage the activity in wrinkled peas was greater than that in round peas ($P < 0.01$). Finally, α-glucan phosphorylase activity, which was similar in both varieties, rose after an initial lag.

Previously [2] we have estimated the rate of starch synthesis in the embryos from our measurements of starch accumulation. These estimates are compared with the enzyme activities (Table 2) and the following conclusions drawn. First, the activities of the enzymes held to be directly involved in starch synthesis, ADPglucose starch synthase, ADPglucose pyrophosphorylase, and pyrophosphatase, were sufficient to account for the rate of starch accumulation observed *in vivo*, and increased during development in a way that suggested direct involvement in starch synthesis. Second, ADPglucose starch synthase was the only enzyme whose maximum catalytic activity approached the rate of starch accumulation *in vivo*. Activity at the 100 mg stage was too low to support the rate of starch synthesis observed in the more mature embryos. Thus the increase in activity that took place during development was a prerequisite for the final rate of starch synthesis achieved and is an example of coarse control of metabolism [8]. Third, the lower starch con-

Table 2. Comparison of rates of starch accumulation and maximum catalytic activities of enzymes of starch metabolism in developing embryos of round and wrinkled peas

Measurement	Stage of development	Rate (nmol/min per embryo) Round	Wrinkled
Starch accumulation*	200–400 mg	22	8
	300–400 mg		14
ADPglucose starch synthase	100 mg	12	12
	300 mg	25	47
ADPglucose pyrophosphorylase	300 mg	587	444
Inorganic pyrophosphatase	300 mg	743	1110
α-Glucan phosphorylase	300 mg	78	58

*Data are from Edwards and ap Rees [2] and are based on estimates from four different samples of embryos.

Table 3. Comparison of maximum catalytic activity of branching enzyme and starch accumulation in developing embryos of round and wrinkled peas

Fr.wt of embryo (mg)	Branching enzyme* (μmol glucose transferred/min per embryo)		Starch content† (mg/embryo)	
	Round	Wrinkled	Round	Wrinkled
100	1.14 ± 0.09 (7)	0.15 ± 0.10 (13)	4.0 ± 0.7	0.8 ± 0.1
200	3.09 ± 0.14 (8)***	0.01 ± 0.01 (8)	20.0 ± 2.8	2.2 ± 0.4
300	4.20 ± 0.33 (6)**	0.56 ± 0.24 (9)*	60.1 ± 3.2	5.4 ± 0.4
400	7.82 ± 0.59 (6)***	1.16 ± 0.51 (6)	86.0 ± 2.5	16.5 ± 2.2

*Values are means ± s.e.m from the number of separate samples of embryos shown in parentheses: differences between the mean and that of the preceding stage are indicated by: no superscript, not significant ($P > 0.05$); *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

†Data from ref. [2], means ± s.e.m of values from at least four different samples.

tent of the wrinkled peas was not due to a lower capacity of ADPglucose starch synthase, inorganic pyrophosphatase, or ADPglucose pyrophosphorylase. Finally, there is no evidence that the low starch content of the wrinkled peas was due to the possession of a greater capacity to catalyse phosphorylysis of 1,4-α-glucan.

Branching enzyme

The routine assay that we used for the branching enzyme measured the extent to which pea extracts increased the ability of pure α-glucan phosphorylase, from rabbit muscle, to incorporate [^{14}C]glucose 1-phosphate into 1,4-α-glucan. For separate extracts of each variety, we optimized the pH, and the concentration of each component, of the assay mixture, and showed that activity was linearly related to time and amount of extract. Table 3 relates measurements of the maximum catalytic activity of branching enzyme to our previous estimates of starch accumulation [2]. There is a marked difference between the two varieties. In the round peas the activity was appreciable in relation to the activities of the other enzymes measured (Table 1) and the rate of starch accumulation (Table 2). Further, activity increased during development in a manner comparable to other enzymes involved in starch synthesis. In the wrinkled peas no activity could be demonstrated consistently until the later stages of development and even here it never amounted to more than 15% of that found in the round peas of comparable weight. Of the 21 embryos from the 100 and 200 mg stage that we assayed, 13 showed no detectable activity and the values found for the rest varied widely (Table 3). A remarkably close correlation was found between branching enzyme activity and the accumulation of starch.

Our assay for branching enzyme is prone to interference when used with unfractionated extracts. The following experiments were done to see if differential interference, i.e. by extracts of wrinkled but not round peas, accounted for the differences in branching enzyme activity shown in Table 3. First we checked that extracts of wrinkled peas did not contain substances that seriously interfered with the assay for branching enzyme. We prepared mixed samples of embryos of round and wrinkled peas. Two such mixtures were of embryos at the 100 mg stage and a third was of round peas at 100 mg and wrinkled peas at 300 mg. When these mixtures were homogenized the activities found in the extracts were, respectively, 97, 79 and 93% of the values predicted from measurements made on the separate components of the mixtures. Variation of the pH and the composition of the assay mixture revealed no consistently demonstrable activity in extracts of wrinkled peas up to 200 mg fresh weight and did not increase the activity found in the extracts of older embryos. Comparable differences in activity between the two types of extract were found when the enzyme was assayed by following the decrease in absorption of an iodine–amylose complex. Further, using the latter assay, we demonstrated that the differences in activity between Birte and Greenshaft were also characteristic of round and wrinkled genotypes derived from JI 430 as described by Hedley *et al.* [9]. The latter are near-isogenic except at the Ra locus.

The low values for branching enzyme in extracts of wrinkled peas could reflect the presence in these extracts, but not in those of round peas, of high activities of enzymes that rapidly hydrolysed the labelled glucan formed in the assay. Thus we assayed α-amylase (EC 3.2.1.1) in extracts of embryos of 200–400 mg fresh weight. We found no activity for either Birte or Greenshaft. For the following enzymes we compared the two types of extract under the conditions used to assay branching enzyme. The possibility that the two varieties differed in respect of other amylases, for example a heat-labile endoamylase comparable to that described for *Arum maculatum* [10], was checked by measuring the total amount of maltose plus glucose produced when extracts, prepared as for the assay of branching enzyme, were incubated with soluble starch. Extracts of embryos of 300 mg fresh weight gave values of 0.252 ± 0.063 and 0.288 ± 0.046 μmol glucose equivalents/min per embryo (mean ± s.e.m from 5 extracts) for round and wrinkled peas, respectively. When mixtures of round and wrinkled embryos were assayed the values obtained for the above activities were within 10% of those predicted from measurements made on the separate components of the mixture. For the de-branching enzyme (EC 3.2.1.41), measured in extracts of 400 mg fresh weight, we obtained a value of 0.08 μmol/min per embryo for round peas but found no activity in extracts of wrinkled peas.

We made one further check that the low activities for branching enzyme were not due to preferential hydrolysis

of labelled glucan by extracts of wrinkled peas. An extract of round peas was assayed for branching enzyme in the usual way and then the labelled glucan formed in the assay was isolated by centrifugation and washed free of [^{14}C]glucose 1-phosphate. This labelled glucan was then added to assay mixtures for branching enzyme that contained glucose 1-phosphate in place of [^{14}C]glucose 1-phosphate. These assay mixtures were then incubated in the usual way with extracts of round (407 mg fresh weight) and wrinkled (340 mg fresh weight) embryos. After 10 min the extent to which labelled glucose had been released from the [^{14}C]glucan during the assay for branching enzyme was determined. No such release was detected with extracts of embryos of round peas: release by extracts of wrinkled peas amounted to less than 0.9% of that initially present in the labelled glucan. These results strongly suggest that during the assay of branching enzyme there was no significant breakdown of the labelled glucan by either type of extract.

Measurements of ADPglucose

We investigated whether the differences in branching enzyme activity and starch content were accompanied by differences in the content of ADPglucose. We measured the latter by isolating it by HPLC and assaying it by measuring A_{254}. The following is evidence that this method is reliable. The peak ascribed to ADPglucose from the embryos had a retention time that was within 0.2 min of that found with authentic ADPglucose. We found no component of the extracts, except UDPglucose, that had a retention time approaching that of ADPglucose. The method separates ADPglucose and UDPglucose satisfactorily [4]. We subjected the peak attributed to ADPglucose to acid hydrolysis. When the products were re-analysed by HPLC, the original ADPglucose had disappeared completely. The amounts of adenine and glucose in the hydrolysate from round peas were 93 and 51%, respectively, of those expected if the peak was assumed to be entirely ADPglucose. The corresponding figures for wrinkled peas were 83 and 96%. We attribute the low value for glucose for round peas to the relative insensitivity of the assay for glucose, compared to that for adenine, and the small amount of ADPglucose in the round peas. Losses during extraction and analysis were investigated by preparing duplicate samples, each a mixture of the two varieties of embryo. One sample was freeze-clamped and extracted in the usual way. The other was treated similarly except that 100 nmol ADPglucose was added to the freeze-clamped sample. Comparison of the amounts of ADPglucose found in the two extracts (mean ± s.e.m of 6 separate experiments) showed that 86 ± 6% of the added ADPglucose was recovered.

The amounts of ADPglucose in embryos of round and wrinkled peas at the 300 mg stage were 67.6 ± 10.8 (10) and 365 ± 11.7 (7) nmol/g fresh weight, respectively; values are means ± s.e.m of estimates from the number of samples shown in parentheses. Thus embryos of wrinkled peas contain up to five times more ADPglucose ($P<0.001$) than do those of round peas. This suggests very strongly that lack of branching enzyme affects consumption of ADPglucose. In pea leaves ADPglucose behaves as if there is no major regulatory step in starch synthesis beyond the synthesis of ADPglucose. Cessation of starch synthesis on transfer of a leaf to the dark results in the virtual disappearance of ADPglucose [11]. We investigated whether ADPglucose in pea embryos behaved in the same way when starch synthesis was reduced by incubating excised developing embryos in a medium that lacked sugar. There was a pronounced fall in ADPglucose after 4 hr and almost all of it had disappeared after 23 hr (Table 4).

DISCUSSION

We suggest that our measurements of enzyme activities reflect the maximum catalytic activities of the embryos. All assays were optimized and all tests for preferential loss during extraction gave negative results. The possibility that our low values for branching enzyme in extracts of wrinkled peas was due to rapid breakdown of the labelled glucan formed in the assay is made unlikely by three observations. First, we detected no significant differences between extracts of round and wrinkled peas in respect of starch-degrading enzymes. Second, no significant breakdown of labelled glucan could be detected during the assay of branching enzyme. Finally, the differences in branching enzyme correlated with differences in ADPglucose and the content and composition of starch, differences that we will suggest could arise specifically from a lack of branching enzyme.

From their study of round and wrinkled peas Matters and Boyer [12] concluded that the embryos of the wrinkled peas that they investigated were characterized by a delay in the appearance of starch synthase activity and by low activity of branching enzyme. We found no evidence, for any stage of development, that the activity of starch synthase was lower in extracts of wrinkled peas than in those of round peas (Table 1). In addition, our data for branching enzyme differ from those of Matters and Boyer. We found an almost qualitative distinction between round and wrinkled embryos until they passed the 200 mg stage, after which low activity of branching enzyme appeared in the wrinkled embryos (Table 3). Matters and Boyer reported little difference in activity between the two types of embryo during early development and that a discrepancy appeared only later on. Further, they reported that embryos with the greatest difference in branching enzyme activity had comparable amounts of

Table 4. ADPglucose content of excised embryos of round and wrinkled peas incubated in the absence of sucrose

Variety of pea	Initial content	ADPglucose (nmol/g fr.wt) content after incubation for 2 hr	4 hr	23 hr
Wrinkled	235	158		
	241	187		
	256		126	
	223		126	
	461			23
	384			36
Round	75	40		
	70	44		
	77	46		
	39			27
	45			20

starch, whilst at the stage when starch content differed most, the activities of branching enzyme were comparable. It is not clear whether these differences are due to the use of different genotypes or to the fact that Matters and Boyer do not report having authenticated their measurements of enzyme activity.

We conclude that of all the enzymes of starch and sugar metabolism reported in this and our previous work [2, 3], only branching enzyme showed any significant difference between Birte and Greenshaft. We also obtained evidence for this difference in varieties that were more nearly isogenic except for the Ra locus. Thus we suggest that greatly reduced maximum catalytic activity of branching enzyme is a key characteristic of embryos that are genotypically rara. Our data do not reveal whether this is due to a structural or a regulatory gene. Recent studies [13] provide convincing evidence that maize kernels contain two branching enzymes and there is evidence that this may be true of peas [12]. The changes in branching enzyme activity shown in Table 3 suggest that the simplest explanation of our results is that the Ra locus determines the activity of one of the two branching enzymes, that this is the dominant of the two enzymes during embryo development, and that it appears first during this development.

The possibility [12, 14] that all the phenotypic characteristics of the Ra locus stem from a difference in starch content and quality raises the question of how lack of branching enzyme affects starch synthesis. The latter can clearly account for the decreased branching of amylopectin and the increased content of amylose that are characteristics of the starch of wrinkled peas [12]. Studies *in vitro* have revealed marked stimulation of α-glucan 4-glucosyl transferase by branching enzyme [15, 16]. Further, highly branched glycogen molecules have been shown to be superior to amylose and maltosaccharides as acceptors for mammalian glycogen synthase [17, 18]. Studies of glycogen synthase from rabbit muscle [19] showed a progressive decrease in activity as the outer chains of glycogen were lengthened. Further, conversion of glycogen to its component maltosidic chains by debranching enzyme greatly reduced its efficiency as an acceptor for the synthase. This loss in efficiency was progressive and paralleled the extent of debranching. Thus it may be argued that branching enzyme is required to produce the substrate with which glycogen synthase reacts most efficiently. A similar situation has been described for α-glucan phosphorylase [20]. As there is, in this respect, a close similarity between these two different transglucosylases, it seems highly likely that starch synthase, which is very closely related to glycogen synthase, will also require branching enzyme for optimal activity. Thus lack of branching enzyme *in vivo* would be expected to diminish greatly the activity of starch synthase and thus reduce the total amount of starch formed. Evidence for such restriction of starch synthase in the embryos of wrinkled peas is provided by the marked accumulation of ADPglucose.

The high activity of alkaline pyrophosphatase in plastids [5, 21] will ensure that the synthesis of ADPglucose is irreversible *in vivo*. Thus this compound is unlikely to restrict its own synthesis by a mass action effect and some form of feedback inhibition must operate at, or before, the last irreversible step. It is likely that the high amounts of ADPglucose restrict the activity of ADPglucose pyrophosphorylase by competing with ATP. The fact that the amounts of glucose 1-phosphate, glucose 6-phosphate and fructose 6-phosphate, all of which are in equilibrium, are significantly higher in embryos of wrinkled than in embryos of round peas strongly supports this suggestion [3].

We suggest that the Ra locus determines the activity of branching enzyme that can restrict starch synthesis by reducing the activity of starch synthase and that this, in turn, causes ADPglucose to accumulate and inhibit ADPglucose pyrophosphorylase. The extent to which branching enzyme limits starch synthesis in wild-type peas is not known. The fact that ADPglucose drops markedly when starch synthesis is reduced suggests that under normal circumstances the main control may be on production rather than use of ADPglucose.

EXPERIMENTAL

Materials. Substrates, enzymes and cofactors were from Boehringer, except that alkaline pyrophosphatase, glucose-1,6-bisphosphate and ADPglucose were from Sigma, and isotopes from the Radiochemical Centre, Amersham. Seeds of *Pisum sativum* L. cv. Birte (JI 1068, round seeded), cv. Greenshaft (JI 430, wrinkled seeded) were generously supplied by the John Innes Institute, Norwich. Plants were grown and embryos were harvested as in ref. [2].

Methods. Embryos were homogenized for enzyme assays as in ref. [2]: extraction medium was 50 mM glycylglycine buffer, pH 7.5, except that 30 mM sodium citrate, pH 7.2, 1 mM dithiothreitol was used for branching enzyme, debranching enzyme and the measurement of heat-labile endoamylase. Extracts for measurement of α-amylase were made as in ref. [10]. For starch synthase, alkaline inorganic pyrophosphatase and α-amylase, samples of unfractionated homogenate were assayed. For ADPglucose pyrophosphorylase, the unfractionated homogenate was centrifuged at 100 000 g for 30 min and the supernatant was assayed; a similar procedure was followed for the remaining enzymes, except that centrifugation was at 60 000 g. Enzymes were assayed at 25°, unless shown otherwise, according to the following references, in the reaction mixtures listed: ADPglucose starch synthase [4], 50 μl extract, 2.63 mM ADP [U-^{14}C]glucose (0.05Ci/mol), 100 mM bicine–25mM K acetate–0.5 M citrate (pH 8.6), 0.1 mg amylopectin in 150 μl at 30°; ADPglucose pyrophosphorylase [4], 10 μl extract, 100 mM HEPES (pH 7.0), 10 mM $MgCl_2$, 50 μg bovine serum albumin, 3.5 mM 3-phosphoglycerate, 1.5 mM ATP, 0.33 μg inorganic pyrophosphatase, 2.5 mM [U-^{14}C]glucose 1-phosphate (0.5 Ci/mmol) in 200 μl at 37°; α-glucan phosphorylase [22], 100 μl extract, 20 mM Na_2HPO_4–KH_2PO_4 buffer (pH 7.0), 25 mM $MgCl_2$, 2 μM glucose-1, 6-bisphosphate, 0.85 mM NAD, 4 units phosphoglucomutase, 2.8 units glucose-6-phosphate dehydrogenase (NAD-dependant from *Leuconostoc*), 37.5 mg amylopectin in 3.0 ml; inorganic pyrophosphatase [6], 0.5 ml extract, 50 mM Tris–HCl (pH 8.0), 7.5 mM $MgCl_2$, 1.5 mM $Na_4P_2O_7$ in 2.0 ml; α-amylase [23], 13 mM sodium acetate, pH 5.6, 10 mM NaCl, 3 mg soluble starch, 100 μl extract in 400 μl; heat labile endoamylase [10], 100 mM sodium citrate buffer, pH 6.0, 1 mg soluble starch, 20 μl extract in a total volume of 50 μl and incubated for 1 hr; samples of the reaction mixture were made up to 1.0 ml with 50 mM sodium acetate, pH 6.0, and 100-μl portions of the diluted solution were incubated with 2 units maltase at 25° for 1 hr and assayed for glucose as in ref. [2]; debranching enzyme [24], 0.14 mM phosphate–citrate buffer, pH 5.0, 50 mg pullulan, 0.5 ml extract in 5.0 ml. Branching enzyme was assayed routinely as in ref. [25] at 30° in a reaction mixture that contained, in 50 μl, 20 μl extract, 100 mM sodium citrate, pH 6.0, 1 mM AMP,

50 mM [U-^{14}C]glucose 1-phosphate (0.1 Ci/mol) and 0.25 unit α-glucan phosphorylase (from rabbit muscle, Sigma); incubation was for 15 or 20 min. For the alternative assay [26], the reaction mixture contained 40 mM sodium citrate, pH 7.0, 1.6 mg amylose and 500 μl extract in 2.0 ml at 30°.

ADPglucose and its components were measured as in ref. [4]. For the experiments reported in Table 4, embryos (200–300 mg fr.wt) were divided into their two cotyledons. One was freeze-clamped and killed at once to give the initial content of ADPglucose, the other was incubated as indicated (Table 4) and then killed. The incubation medium was the chase medium from ref. [2] except that sucrose was replaced with 0.83 M mannitol, and the asparagine and the amino acids were omitted. Each cotyledon was incubated in 5 ml medium in a 60-ml container at 25° in the dark with shaking. ^{14}C was measured as in ref. [2].

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