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Some metabolic changes in germinated *Acacia farnesiana* L. seeds

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ABSTRACT

The changes in fresh weight, dry weight, starch content, amylase and pullulanase activities and the electrophoretic protein and amylase pattern changes of cotyledons were studied during different days of germination. Cotyledon fresh weight increased linearly between 3 and 9 days, the dry weight declined rapidly between 1 and 3 days and linearly with time thereafter. Mature seeds contained 13.2 mg starch g dry weight with starch levels increasing up to 23.3 mg/g dry weight by the third day post germination. Amylase and pullulanase activities increased linearly between 1 and 5 days. Mature seed extract resolved into one major band and two minor bands of amylase activity. The slowest migrating band disappeared at day 5 and a new band appeared at day 7 of germination. The analysis of protein by sodium-dodecyl-sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) showed that protein patterns underwent marked changes in the third day, while the pattern of 1 day old seedlings was almost identical to that of the ungerminated seeds. The two major subunits almost completely disappeared and three bands of lower molecular weight appeared in the extract. As germination progressed, the protein content of the larger subunits declined, and new minor bands appeared.

INTRODUCTION

It is well established that seeds contain an abundant reserve of starch, protein and lipid, which are of great importance for seedling establishment. This reserve food is stored in storage tissues which are usually found in the endosperm of monocots or cotyledons of dicotyledon plants (Ashton 1976).

The process of seed germination is accompanied by mobilization of reserve protein (Abbot & Matheson 1972, Basha & Beevers 1975, Wilson *et al.* 1986, Al-Helal 1989, 1992) and carbohydrates, in the storage tissues to provide the essential soluble products for seedling growth (Sprent 1968).

During the hydrolysis of reserves, the activities of certain hydrolyses in the cotyledons rise from low levels to extraordinarily high levels (Sprent 1968, Yomo & Varner 1973, Basha & Beevers 1975, Koshiba & Minamikawa 1981). Despite the considerable amount of research work available on food mobilization of seeds, the hydrolysis of substances in seeds of plants adapted to arid environments is less

well understood. Therefore, the present investigation was initiated to study the hydrolysis of food reserves and the activities of carbohydrates in cotyledons of *Acacia farnesiana.*

Acacia farnesiana, originally a native of tropical America but widely distributed and usually cultivated in warm countries, is seen occasionally as cultivated or self-sown plants in almost all parts of Saudi Arabia.

MATERIALS AND METHODS

Seeds of *A. farnesiana* were collected from the desert of Saudi Arabia in 1997 and were stored at 4°C for 2 years.

Germination

Seeds were treated with concentrated sulphuric acid to break seed dormancy. They were then rinsed several times with distilled water and distributed over two layers of filter paper moistened with 10 ml water in petri dishes. The dishes were kept in a dark growth cabinet at 30°C. After 24 h incubation all the ungerminated seeds were removed. The cotyledons were collected after 1, 3, 5, 7 and 9 days of germination and weighed. Twenty cotyledons were collectively wrapped in aluminum foil and placed in a freezer at −30°C for 24 h, they were then freeze-dried and ground to a fine powder, weighed and stored at −30°C prior to analysis.

Extraction and assay of starch and reducing sugars

Samples (20 mg) of finely dried ground seeds or freeze-dried etiolated germinated cotyledons were extracted with 10 ml of 80% aqueous ethanol at 4°C overnight. After centrifugation at 1300×g for 5 min, the supernatant was used for reducing sugar determination as decribed below. The residues were suspended in 10 ml distilled water in a glass tube and incubated in a boiling water bath for 2 h. After cooling to room temperature, the gelatinized starch produced was hydrolyzed by adding 2 mg of α-amylase powder (BDH Chemicals) at room temperature for 1.5 h. After centrifugation, the liberated reducing sugars were determined according to the method of Bernfeld (1955) using maltose as a standard. Since the amylase powder contained reducing sugars, a control tube was prepared by dissolving 2 mg α-amylase powder in 10 ml of water, the reducing sugar content was measured and the experimental readings were corrected. In addition, before hydrolysis by α-amylase, the starch extract was analyzed for the possible presence of reducing sugars. The data for starch content were subjected to analysis of variance and LSD test at 95%.

Extraction of enzymes

Enzymes were extracted by homogenizing 20 mg of seeds or freeze-dried cotyledons in 1 ml 0.1 M tris/HCl buffer pH 6.8 containing 1 mM $CaCl_2$ and 0.5 mM of phenyl-methyl-sulphonyl-fluoride (at 4°C overnight to minimize the possible effect of protease on isoenzyme patterns). After centrifugation at 1300×g for 7 min,

the supernatant was used for enzyme assays. Sucrose (20% w/v) was included in the extraction buffer for samples used for electrophoretic analysis, and the ratio between tissue and extraction buffer volumes was 1 to 40.

Assay of enzymes

The pullulanase activity was measured at pH 5.0. The assay mixture contained 50 μl enzyme extract, 1% pullulan and 50 mM sodium acetate buffer. The assay mixture for amylase contained 100 μl enzyme extract, 100 mM sodium acetate, pH 5.0 and 0.5% w/v starch. The mixtures were incubated at room temperature for 30 min. The liberated reducing sugars were determined according to the method of Bernfield (1955).

Electrophoresis

Discontinuous, vertical polyacrylamide gel electrophoresis (PAGE) was performed according to the method of Davis (1964) using LKB equipment (Sweden). Development was carried out at 10°C at constant current (25 mA). For SDS-PAGE, the SDS gel contained 1% w/v sodium-dodecyl-sulphate and the developing buffer contained 0.1% (w/v) SDS. Enzyme extraction, SDS-protein extraction, eletrophoresis and gel staining were carried as described in Al-Helal (1988).

Staining

Gels used for amylase study were incubated for 2 h at 25°C in a solution of 1% soluble starch containing 0.1 M acetate buffer, pH 5.0. The gels were washed with acidified water and were stained in acidified iodine-potassium solution according to the method of Brewbaker *et al.* (1968).

Protein bands were made visible by staining in Coomassie Blue R-250 dissolved in ethanol: acetic acid:water (100:15:85) and destaining was carried out in a mixture of the same solvent.

RESULTS AND DISCUSSION

The cotyledon fresh weight increased linearly between days 3 and 7 (Fig. 1). This increase was due to water uptake, which increased with germination time (Table 1). In contrast, cotyledon dry weight declined rapidly between 1 and 3 days of seed germination due to protein hydrolysis.

Protein is the major reserve food in desert plant seeds. Our results show that hydrolysis of storage food especially protein in the cotyledon lead to the dry weight decline during seed germination. Similar results previously have been reported for several species (Al-Helal 1992).

The cotyledon extract contained no reducing sugars. The data presented in Table 2 shows that ungerminated mature seeds contained a very low level of starch, which made up 1.32% of seed dry weight. This low level starch in the seeds was in good agreement with that reported for *Cassia senna* (Al-Helal 1994).

Cotyledonary starch content increased slightly up to day 3 of the germination period and thereafter declined. The slight increase in starch content in the first days of germination and its hydrolysis thereafter are in good agreement with the results

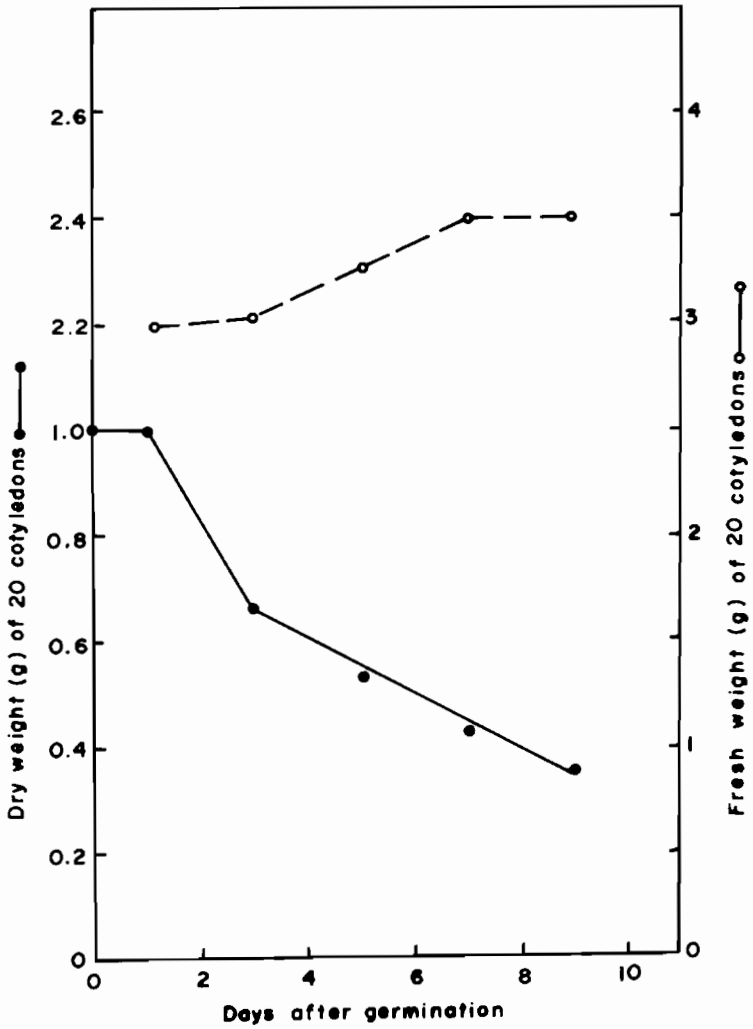

Fig. 1. Change in fresh weight and dry weight of twenty cotyledons during germination.

Table 1. Percentage of water content of collective twenty cotyledons during nine days of seed germination.

Days after germination	% Water content
0	68
1	78
3	84
5	87
7	89
.9	—

Table 2. Cotyledonary starch content during the various stages of seed germination.

Days of germination	mg starch /g dry weight	% starch content/dry weight
0	13.2 ± 1.3^a	1.32
1	18.4 ± 1.7^b	1.84
3	23.3 ± 1.5^c	2.33
5	11.8 ± 0.9^a	1.18
7	12.0 ± 0.8^a	1.20
9	12.0 ± 1.1^a	1.20

Note: Each number is the mean of three readings ± standard error
Values without a common letter are significantly different at 95% level.

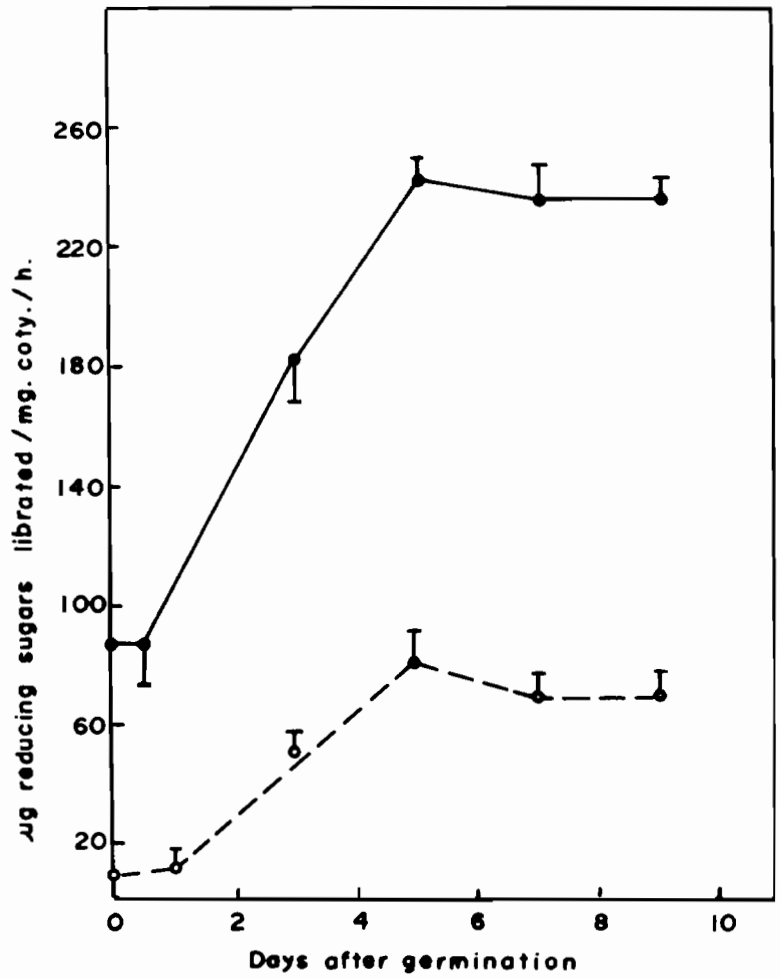

Fig. 2. Amylase and pullulanase activities of the cotyledons after germination.
●—● amylase activity
○—○ pullulanase activity
Note : Each point is the mean of three readings ± standard error.

reported for soybean (Adams *et al.* 1981) and *C. senna* seeds (Al-Helal 1994), but in contrast to results that have been reported for other species (Sprent 1968, Tanaka & Akazawa 1970) in which starch content declined from early stages of germination.

Dry seeds had relatively low levels of amylase and pullunase activities (Fig. 2). The activities of both enzymes increased linearly between days 1 and 5, and then reached a plateau. Previously no work has been conducted on the physiological activities of amylase and pullulanase during seed germination of *Acacia farnesiana* and their effect on starch hydrolysis. Analysis of starch in the cotyledons during the various stages of seed germination has been conducted and showed a low level of starch that decreased during the last days of germination due to the increase of amylase and pullulanase activities. These results are in agreement with those reported for other species (Sprent 1968, Thevenot *et al.* 1992, Al-Helal 1994).

The electrophoretic pattern of amylase activity in the cotyledonary extracts of dry seeds, one day old seedlings and 3 day old seedlings resolved into one fast migrating component (Rf 0.66) which had the highest enzyme activity and two minor, slow moving bands (Rf 0.13 and 0.19) (Fig. 3). The slower moving bands (Rf 0.13 and 0.19) disappeared from cotyledon extracts of 5 and 7 day old seedlings. An

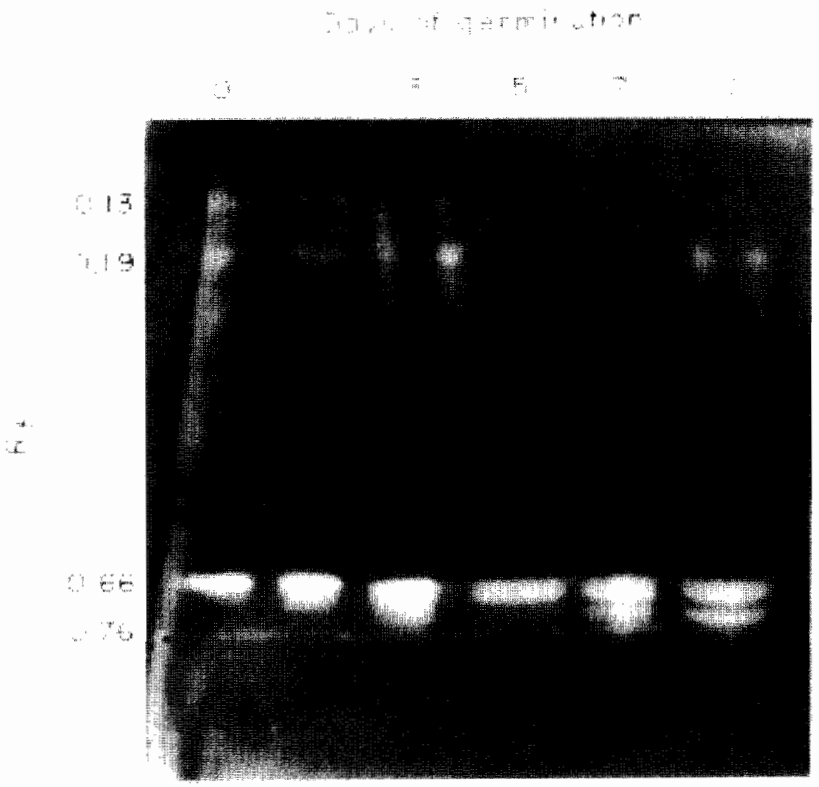

Fig. 3. Polyacrylamide gel electrophoresis (PAGE) of amylase of cotyledons at various stages of germination.

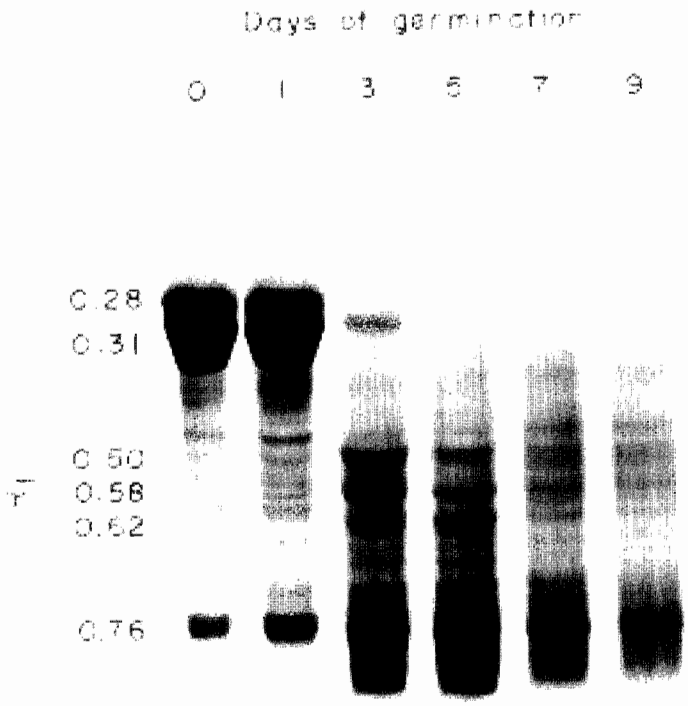

Fig. 4. Sodium-dodecyl-polyacrylamide gel electrophoresis of cotyledons at various stage of germination.

additional fast moving band (Rf 0.76) appeared in the cotyledonary extracts of 7 day old seedlings. These results indicate that this period was characterized by an increase in the amylolytic activity of the two fast moving components which might suggest the important role of these components in starch hydrolysis. This work indicates the importance of the relationship between the electrophoretic pattern change in amlyase activity and starch metabolism in this species.

The cotyledonary proteins of dry seeds resolved into three major bands (Rf 0.28, 0.31 and 0.76) with high protein concentration, plus several minor bands of low concentration. (Fig. 4). The electrophoretic pattern of cotyledonary proteins of one day old seedlings is almost identical to that of ungerminated seeds. These results are almost in agreement with those reported for other Acacia species of Saudi Arabia (Al-Helal 1989, 1994).

On the third day of germination, the cotyledonary protein pattern underwent marked changes. The slower migrating subunits (Rf 0.28 and 0.31) almost disappeared, and three new bands (Rf 0.50, 0.58 and 0.62) of lower molecular weight appeared. These results might suggest the degradation of the major storage protein from the early days of germination, as indicated by the disappearance of two major higher molecular weight bands. From Fig. 4, it is clear that as germination progressed, there was a rapid decline in content of most protein subunits except for

the fastest migrating components (Rf 0.76) which appear to resist hydrolysis. New minor bands appeared during germination, which supports protein hydrolysis. The electrophoretic change in protein patterns observed for this species is identical to that reported for *Acacia laeta* (Al-Helal 1989). These results suggest that the large protein subunits observed on the third day of germination degraded to smaller subunits. Protein modification prior to rapid hydrolysis has been suggested for peas (Basha & Beevers 1975), soybeans (Wilson *et al.* 1986), french beans (Nielsen & Liener 1984) and other leguminous plants.

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بعض التغيرات في الأنشطة الأيضية للبذور المنبتة لنبات الأكاسيا فارنسيانا

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خلاصة

تم القيام بدراسة التغير في الوزن الجاف والرطب والمحتوى النشوي والبروتيني ونشاط كل من إنزيمي الأميليز و البلليلينيز في فلقات بادرات نبات الأكاسيا فارنسيانا في مراحل مختلفة من الإنبات.

أوضحت نتائج الدراسة أن الوزن الرطب لفلقات البادرات يزداد طردياً من اليوم الثالث إلى اليوم التاسع ، كما وسجل تناقص في الوزن الجاف بين اليومين الأول والثالث ، وكان المحتوى النشوى النشوي في الفلقات الجافة مرتفعاً (13.2 ملجرام/جرام) ازدادت كميته في الأيام الأولى من الإنبات حتى وصلت (23.3 ملجرام/جرام) ثم أخذت في التناقص بشكل سريع فيما بعد.

كما وجد زيادة في نشاط كل من إنزيمي الأميليز والبلليلينيز مع زيادة عدد أيام الإنبات . تم تحليل المحتوى البروتيني للفلقات بواسطة SDS-PAGE وجد أن مستخلص الفلقات الجافة يحتوي على حزم نات محتوى بروتيني مرتفع بالإضافة إلى عدة حزم صغيرة ذات محتوى بروتيني منخفض ، اختفت الحزم الصغيرة في اليوم الخامس وظهرت حزم عديدة بدلاً منها.