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Growth and Protein Content of *Cassia senna* L. Seedlings

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Abstract. Water uptake by seeds, and the growth and protein contents of cotyledons, radicles and hypocotyls of *Cassia senna* seedlings were studied during the first 8 days after soaking.

Water uptake increased rapidly for 4 hours after soaking. The lengths and fresh weighs of the radicle and hypocotyl increased up to 6 days, while the fresh weight and protein content of the cotyledons decreased rapidly during the first 3 days and thereafter decreased slowly.

The protein contents of the cotyledons and axis were analyzed by sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDS-PAGE). The dry seeds contained several major bands and numerous minor bands and as germination progressed there was a reduction in the protein content and number of these components. Also, there was a decline in the number of bands in extracts from the radicle during germination.

Introduction

Seed germination of desert plants of Saudi Arabia has been studied by several investigators [1–5]. However, most of the work has been concerned with the effect of temperature and salinity on the germination process. Less is known about the effect of high temperature and salt stress on the physiology and metabolism of resulting seedlings.

This work was undertaken as a part of a program to study the growth and metabolism of seedlings of plants adapted to the arid environment of Saudi Arabia.

Materials and Methods

C. senna seeds were collected from Makka Road in 1982 and were stored at 25°C. Seeds were treated with concentrated sulphuric acid to break seed dormancy [1]. Water uptake was measured as described in a previous paper [2]. The seeds were germinated at 30°C in the dark [1, 3].

About 40 seedlings were harvested 1, 3, 4, 6 and 8 days after soaking and were separated into cotyledons, radicle and hypocotyl. On each occasion 27 seedlings were used to measure fresh weight and the length of the radicles and hypocotyls. The fresh weight of the separate organs was taken for the 27 seedlings collectively.

Seedling parts were wrapped in aluminium foil frozen at – 8°C for 48 h and then freeze-dried and ground to a fine powder and stored at – 8°C until they were used for anlaysis.

Soluble protein which was used for polyacrylamide gel analysis under dissociating conditions (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) was extracted with 0.2 M tris/HC1 buffer, pH 6.8, containing 20% (w/v) sucrose and 2% (w/v) sodium dodecyl sulphate (SDS) as described before [6]. Protein that was used for protein content determination was extracted with 0.2 M tris/HC1 buffer, pH 6.8, containing 2% (w/v) ascorbic acid and 2% (w/v) polyvinylpyrrolidone. Protein contents were determined according to the Lowry method as described by Stenesh [7] using albumin as a standard.

Soluble amino acids within the cotyledons were extracted with 60% ethanol at 4°C overnight and the extract was centrifuged at 4000 rpm for 5 m. The supernatant was used for amino acid determination as described by Yemm and Cocking [8], using L-glutamic acid as a standard.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described previously [6] using phosphorylaseb(94000 daltons), albumin (67000), ovalbumin (43000), carbonic anhydrase (30000), trypsin inhibitor (20000) and α-lactoalbumin (14000) for molecular weight measurement.

Results

Seedling growth

Upon soaking the seeds in water their water content increased up to 45% of the original seed weight in the first hour and after that it increased linearly with time (Fig. 1).

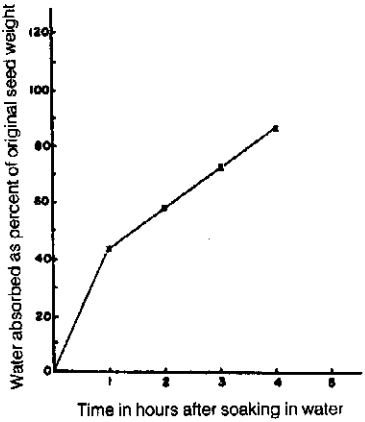

Fig. 1. Imbibition of water by seeds
***Note:* Each figure is the mean of three measurements**

Radicle elongation was rapid in the first 3 days and there was a slight increase in length between the 3rd and 4th day but there was no apparent elongation after that (Fig. 2). The hypocotyl started to elongate after the radicle and increased in length linearly with time at least until 6 days (Fig. 2).

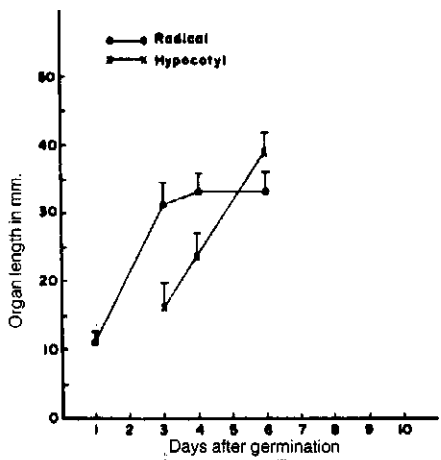

Fig. 2. Change in lengths of radicle and hypocotyl axis during germination

Cotyledon fresh weight decreased by 45% between the 1st and 3rd day, after germination, after which there was little further loss in fresh weight (Fig. 3). By contrast the fresh weight of the radicle and hypocotyl increased with time (Fig. 3).

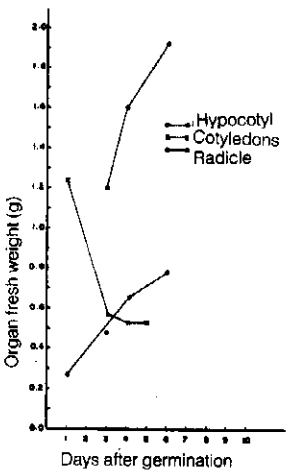

Fig. 3. Change in fresh weight of the cotyledons, radicle and hypocotyl after germination

Protein and soluble amino acid contents

Dry seeds of *C. senna* contained about 425 mg soluble protein /g dry weight; that is, protein made up about 42% of their dry weight (Fig. 4). This soluble protein content declined very rapidly in the cotyledons between the 1st and 3rd day; cotyledons lost about 58% of their original protein content in this period (Fig. 4). After that, the rate of protein depletion was slower.

Dry seeds contain relatively low content of soluble amino acids and amino acid content increased during germination (Fig. 4).

Fig. 5 shows the sequence of changes in cotyledonary protein patterns during the germination period. The extract from the dry seeds consisted of several major bands with a high protein content and of numerous minor bands.

The cotyledonary protein patterns of one-day-old seedlings were almost identical to those of dry seeds. The staining intensity of two of the major bands (mol. wt.

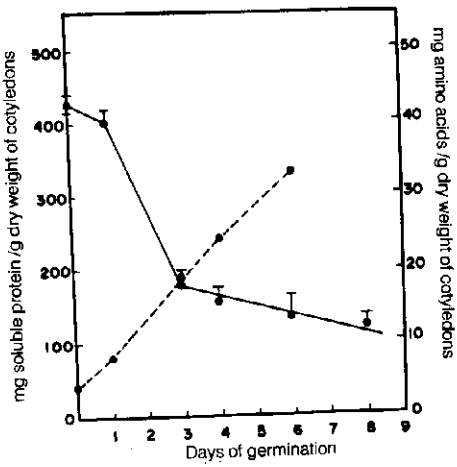

Fig. 4. Protein and free amino acids content of the cotyledons after germination
•——• protein content,
•....• amino acids content
***Note.* Each figure is the mean of three measurement**

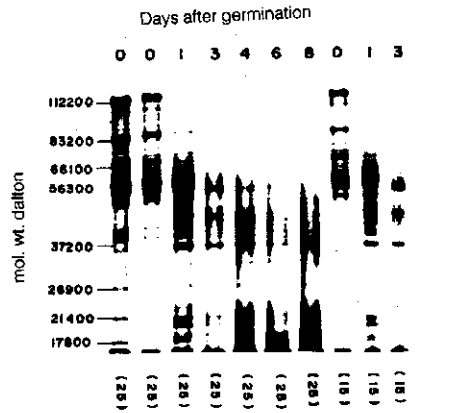

Fig. 5. 17% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of cotyledonary protein at various stages of germination
***Note.* Numbers between parentheses represent volume (μl) of sample loaded of 1/50 ratio between tissue and extraction buffer**

112200 and 83200 daltons) decreased. Also, the results demonstrate the apparent increase in staining intensity of a component having mol. wt. about 37200 daltons.

By the third day, most of the major components had been hydrolyzed and as germination progressed further, the protein content of all subunits decreased. The cotyledonary extract of 8-day-old seedlings had a limited number of bands with a low protein content.

The data in Fig. 6 show the electrophoretic patterns of axis protein at various stages of germination. The extract from the radicle of one-day-old seedlings had several minor components and, as germination progressed, there was a decline in the number of bands and in the staining intensity of the bands. The extract from the hypocotyl resolved into one major band and several minor bands and there was no apparent change in protein patterns during germination.

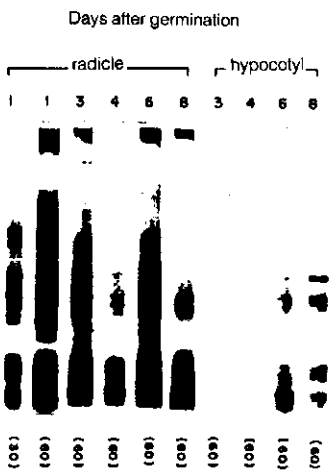

Fig. 6. 17% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of protein of the radicle and hypocotyl axis at various stages of germination
Note: **Number between parentheses represent volume (μl) of sample loaded of 1/50 for radicle and 1/75 for hypocotyl, the ratio between tissue and extraction buffer**

Discussion

The data presented in this paper show that dry seeds of *C. senna* contain high levels of reserve protein, making up about 42% of their dry weight. This result con-

firms and extends the previous observation [9] that protein is the major reserve food of desert plant seeds.

The germination of *C. senna* seeds is characterized by a rapid decline in the fresh weight of the cotyledons which is accompanied to a decrease in their protein content.

The loss of proteins from the cotyledons could be due to the transport of amino acids to the growing axes [10, 11] or to respiratory loss, or it might result in the accumulation of free amino acids in the cotyledons.

The analysis of soluble protein in the radicle and hypocotyl did not suggest the accumulation of soluble protein in the axes, but there was, instead, a decrease in soluble protein in the radicle. However, this does not exclude the possibility that the hydrolyzed protein from the cotyledons might be used for the synthesis of insoluble protein in the axes.

Also, the analysis of free amino acids and protein content in the cotyledons during the various stages of germination showed the increase in free amino acids in the cotyledons, but at a much lower rate than the hydrolysis of protein. This might suggest the rapid utilization of hydrolysis products of protein and/or the rapid translocation of amino acids to the growing axes.

The analysis of cotyledonary protein showed that there was no apparent modification of protein prior to the rapid utilization as has been suggested for various species [9, 12, 13, 14]. Also, the results suggest that the different components were degraded at different rates as has been shown for various species [10, 11].

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نمو وبروتين بادرات العشرق

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ملخص البحث. لقد تم دراسة معدّل امتصاص الماء للبذور والنمو والمحتوى البروتيني للفلقات والجذير والسويقة الجنينية السفلى لبادرات العشرق لمدة ثمانية أيام بعد النقع.

يزداد امتصاص البذور للماء بشكل سريع خلال الساعات الأربع الأولى من النقع. يزداد الطول والوزن الرطب للجذير والسويقة الجنينية السفلى أثناء الإنبات إلى اليوم السادس وينقص الوزن الرطب والمحتوى البروتيني للفلقات بشكل سريع في خلال الأيام الثلاثة الأولى ثم يكون النقص بشكل بطيء فيما بعد.

كذلك لقد تم تحليل المحتوى البروتيني للفلقات والجذير والرويشة بواسطة التفريد الكهربائي SDS-PAGE المستخلص من البذور الجافة يحتوي على عدة حزم ذات محتوى بروتيني مرتفع بالإضافة إلى عدة حزم ذات محتوى بروتيني منخفض، وأثناء المراحل المختلفة من الإنبات ينقص كل من عدد الحزم والمحتوى البروتيني للحزم الأخرى، كذلك أثناء الإنبات يحدث نقص في عدد الحزم البروتينية في مستخلص الجذير.