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Mobilization of Starch and Protein in Germinating Rice Grains

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Abstract. The protein patterns of albumin, globulin, prolamin and glutelin fractions extracted from dry grains of rice (*Oryza sativa* L. CV. Al-Ahssa) were analyzed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). No bands were detected in albumin and prolamin fractions. The globulin fraction contained several bands, while the glutelin fraction resolved into two major bands and few minor bands. Also, two glycoprotein bands were detected in the glutelin fraction. Grains dry weight declined linearly throughout the germination period. Starch content decreased at a linear rate in the first 9 days and more rapidly thereafter.

A study was made of the biochemical changes taking place during germination of grains over a period of 11 days. The grains contain a high amount of reserve starch, which made up about 50% of grain dry weight, and a low amount of SDS-extracted protein that made up 2% of grain dry weight. The electrophoretic pattern changes of protein during germination showed that protein components differ with respect to the rate of protein hydrolysis. Ungerminated grains had no invertase activity and a low level of total amylase activity. Amylase activity increased rapidly during germination reaching a maximum of 30-fold in day 11. Invertase activity increased slowly up to day 9.

The extract of the ungerminated grains resolved into two electrophoretic bands of amylase activity and a third band appeared three days after germination.

Introduction

Seeds contain an abundant reserve of starch, protein and lipid which are of a great importance for seedling establishment. The reserve food is stored in the storage tissues which are usually found in the endosperm of monocots or cotyledons of dicots [1].

During germination the seed rapidly changes from a quiescent state to a metabolically active state. During germination storage protein and starch are hydrolyzed to their consistents amino acids and soluble sugars, respectively [2-7]. The solubilized derivatives of hydrolysis are then transported to the growing embryonic axis where they are used for the synthesis of protoplasm, structural components and enzyme protein, and as an energy source [8; 9].

The mobilization of storage constituents during germination has been studied in several species by different investigators [2; 5; 7]. Metabolic and structural changes in starch have been reviewed by Juliano [10, pp. 175-205]. It appears from the literature that the patterns of hydrolysis vary with species and with varieties within the same species.

At the onset of reserve mobilization the activities of certain hydrolases such as amylase, protease, peptidase and phosphorylase rise from an initially low level to a high level [2; 7; 10-12].

Despite the considerable amount of literature available on the metabolism of germinating rice grain information concerning protein and starch metabolism in rice grain native to Saudi Arabia are lacking. Therefore, the aim of the present study was to obtain information concerning breakdown of storage proteins and starch and synthesis of certain enzymes.

Materials and Methods

Materials

Brown rice grains (*Oryza sativa* CV. Al-Ahssa Cultivar) were obtained commercially from Al-Ahssa in 1992 and were stored in a closed container at 4°C.

Germination

Grains were germinated at 30°C in the dark in Petri dishes on filter paper moistened with 15 ml distilled water. Hundred germinated grains were collected daily after 1, 3, 5, 7, 9 and 11 days of germination. The grains were frozen at 30°C for 24 h, freeze-dried, ground to a fine powder, weighed and stored at -30°C until analysis.

Analysis

Extraction and assay of starch were carried out as described in [7].

Total protein was extracted in 0.2M Tris-HCl buffer, pH 6.8, containing 2% (w/v) sodium dodecyl sulphate as described in [13]. Protein content was measured according to the method of Peterson [14] using albumin (bovine serum) as a standard. Protein content was either measured directly in the extract or after precipitation with trichloroacetic acid at a final concentration of 10% (w/v). All protein was precipitated by TCA, since no protein was detected in the supernatant.

Protein fractionation

The testa was removed manually from dry seeds and the seeds ground to a fine powder on a grinder. A sample of ground seed (0.9 g) was incubated in 9 ml 0.2 M NaCl at 4°C overnight. After centrifugation for 7 min at 1300 xg, the precipitate which contained the prolamin and glutelin fractions was removed from the supernatant. The supernatant was dialyzed against distilled water for 48 h at 4°C and centrifuged at 1300 xg for 7 min. the supernatant contained the albumin fraction. The precipitate which contained the globulin fraction was dissolved in 4.5 ml 0.2 M Tris/HCl buffer, pH 6.8.

The precipitate that contained the prolamin and glutelin fractions was suspended in 4.5 ml 80% (v/v) ethanol overnight at 4°C and centrifuged for 7 min at 1300 xg. The supernatant contained the prolamin fraction. The precipitate was suspended in 4.5 ml 0.05 M NaOH at 4°C overnight and centrifuged to yield a supernatant which contained glutelin fraction.

Assay of enzyme activity

Enzymes were extracted in chilled 0.03 M Tris-HCl, pH 6.8, as before [7].

Total amylase (α and β) activity was measured at pH 5.0 by the method of Bernfield [15, pp. 149-158] using maltose as a standard. The assay mixture contained 50 µl enzyme extract, 0.5% (w/v) starch and 30 mM sodium acetate buffer, pH 5.0. The mixture was incubated at room temperature for 30 min.

The assay mixture of invertase contained 100 ml enzyme extract, 50 mM sodium acetate, pH 5.0, and 3.5% (w/v) sucrose. Assays were carried out as in [7].

Protein extraction from germinated grains

Protein from germinated grains was extracted with 0.2 M tris-HCl buffer, pH 6.8, containing 20% (w/v) sucrose and 2% (w/v) sodium dodecyl sulphate (SDS) as described before [13].

Protein used for amylase isoenzymes analysis was extracted as above except that SDS was omitted from the extraction buffer.

Electrophoresis

To each protein fraction from dry seeds, sodium dodecyl sulphate and sucrose were added in a final concentration of 2% and 20% (w/v), respectively. Under reducing conditions β-mercaptoethanol was added to each extract in a final concentration of 10 mM.

Discontinuous vertical sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out in 17% gels according to the Laemmli procedure [16]. Electrophoresis was carried out at 10°C and at a constant current (25 mA). The standard protein markers for moleclar weight measurement were phosphorylase b (94000 dalton), albumin (bovin serum, 67000), ovalbumin (43000), carbonic anhydrase (30000), trypsin inhibitor (20000) and α-lactoalbumin (14000), obtained from BDH chemicals.

Amylase isoenzymes (α and β) were separated on a discontinuous 7.5% polyacrylamide gel electrophoresis (PAGE) at 10°C and at a constant current (25 mA).

Staining

Protein bands were made visible by staining in 0.05% Coomassie Brilliant Blue R dissolved in ethanol: acetic acid: water (100: 15:85). Destaining was carried out in a mixture of the same solvent.

Gels were stained for glycoprotein as described in [17].

Gels used for the amylase study were incubated for 2h at 25°C in 0.1 M acetate buffer, pH 5.0, containing 1% (w/v) soluble starch. The gels were washed with acidified water and were stained in acidified iodine-potassium iodine solution according to the method of Brewbaker, *et al.* [18].

Results and Discussion

Protein patterns of dry grains

Figure 1 shows the electrophoretic patterns of protein of the different fractions under non-reducing conditions. No protein bands were observed in either the albumin and

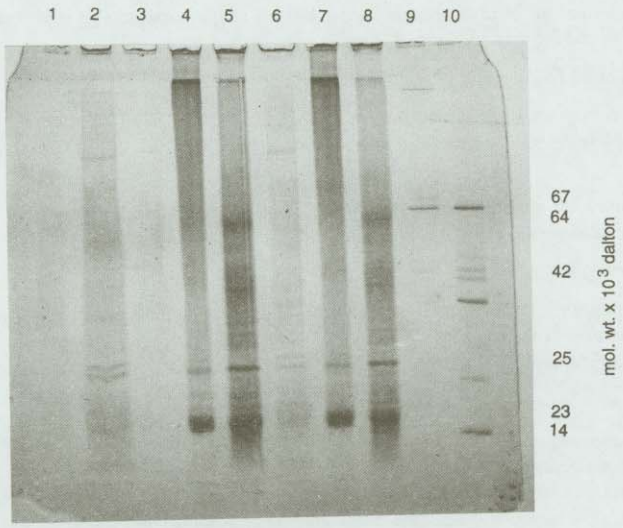

Fig. 1. 17% SDS-polyacrylamide gel Electrophoresis of the different protein fractions under non-reducing Conditions.

Track	:	1 albumin (100 µl)	:	6 albumin (50 µl)
	:	2 globulin (120 µl)	:	7 glutelin (20 µl)
	:	3 prolamin (100 µl)	:	8 SDS-extracted protein
	:	4 glutelin (30 µl)	:	9 and 10 standard protein
	:	5 SDS-extracted protein (30 µl)	:	10 standard protein (30 µl)
Note	:	Numbers between brantheses were volume of sample loaded.		

prolamin fractions which might indicate that these fractions either had no protein or they might have bands of very low protein content which cannot be visualized by the stain used in this study. The globulin fraction resolved into several minor bands with a low protein content and a large volume of extract was used to visualize the bands on the gel. The glutelin fraction was resolved into two major electrophoretic bands (mol.wt. 25,000 and 23,000) and the fastest migrating component (mol. wt. 23,000) appeared to be more intense. The SDS-extracted protein contained about four major bands and several minor bands (Figs 1 and 2).

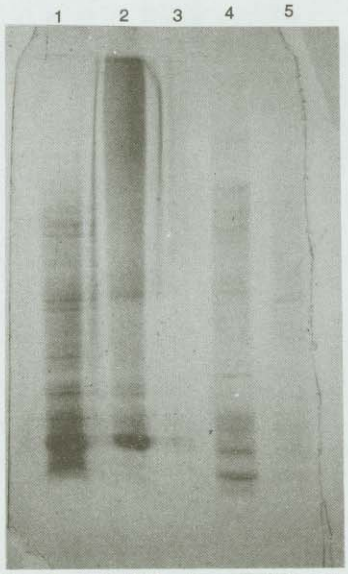

Fig. 2. 17% SDS-polyacrylamide gel electrophoresis of the different protein fraction under reducing conditions

Track : 1 SDS-extracted protein
: 2 glutelin
: 3 prolamin
: 4 globulin
: 5 albumin

Sample loaded was:

Tracks : 1 and 2 each 20 µl
: 3 and 5 each 100 µl
: 4 : 120 µl

Figure 2 shows the electrophoretic patterns of protein of the different fractions under reducing conditions. The patterns of protein under reducing conditions was similar to that under non-reducing conditions except that in the globulin fraction the two fastest moving bands appeared to be stained better in the presence of the reducing agent. Also, an additional band (the fastest moving band) appeared in the SDS-extract.

Two glycoprotein bands were detected in the glutelin fraction only (Fig. 3).

Fig. 3. Zymogram patterns of glycoprotein of the glutelin fraction. Sample loaded was about 25 μg protein.

Changes during germination

The change in the dry weight of 100 grains during the period of grain germination is presented in Fig. 4. The weight of grains decreased at a constant rate from the first day of germination to the end of the germination period (11 days). Grains dry weight declined in the final day of germination to a ninth of its original value.

The results in Fig. 4 show the mobilization of storage starch during the germination period. It can be seen that the ungerminated rice grains contain high level of starch (500 mg/g dry weight). That is, starch made up about 50% of grain dry weight. Starch content decreased at a constant rate between day 1 and 9 of germination and declined more rapidly thereafter reaching a very low level on day 11; starch made up about 6% of grain dry weight.

Dry ungerminated grains contained low levels of SDS-extracted protein; it made up about 2% of grain dry weight. Figure 5 shows the electrophoretic pattern changes of total

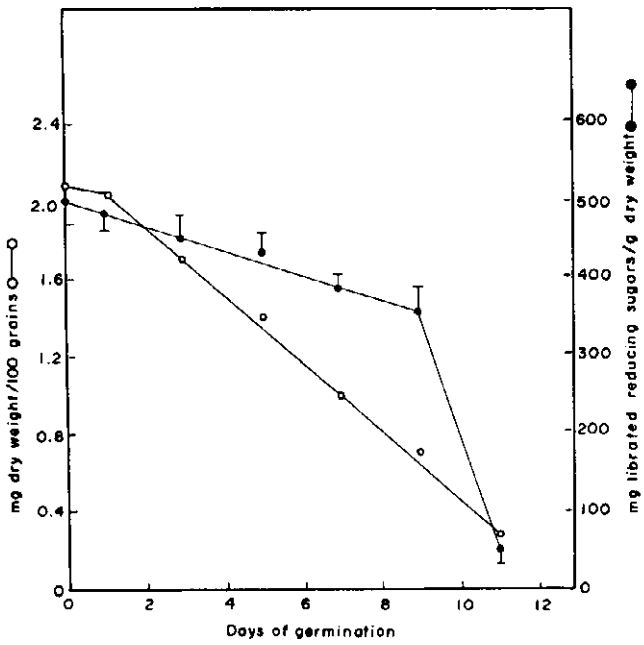

Fig. 4. Dry weight and starch content of grains during germination. For starch content each point is the mean of 4 readings ± standard error.

protein during the period of germination. It is clear from the change in the staining intensity of the bands that the protein content of the major bands declined during germination. Each band differs with respect to the rate of protein hydrolysis and the fastest moving component (mol.wt. 23,000) appears to be the most resistant to hydrolysis.

Ungerminated grains had a low level of total amylase activity (Fig. 6). In the extracts of germinated grains, amylase activity increased slowly but linearly between day-1 and day-3 of germination. Thereafter amylase activity increased more rapidly; enzyme activity increased up to 30 fold, at day-11 compared to the original level.

The extracts from ungerminated grains and from one-day germinated grains had no invertase activity. Invertase activity increased between 3rd and 9th days of germination (Fig. 6).

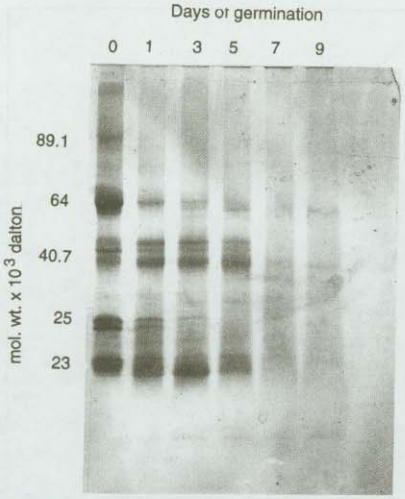

Fig. 5. Sodium dodecyl sulphate polyacrylasmide gel electrophoresis (SDA-PAGE) of total protein of grains at the various stages of germination.

Note: Sample loaded was 100 µl of 20 mg/100 µl extraction buffer.

The electrophoretic patterns of amylase activity throughout the germination period is presented in Fig. 7. The extracts from ungerminated grains and one-day germinated grains each resolved into two distinct electrophoretic bands of amylase activity. A third band of amylase activity appeared in the grain extracts from the 3rd day of germination. The fastest moving component had the highest amylase activity and its activity increased during the germination period.

General Discussion

The analysis of the different protein fractions of dry rice seeds by SDS-PAGE showed that the major protein was detected in the glutelin fraction. This result is similar to that reported by Horikosh, *et al.* [19]. Also, the detection of two major subunits in the glutelin fraction (Fig. 1) is similar to the result of Went and Luthe [20].

The results of this investigation demonstrated that ungerminated rice grain had a high amount of reserve starch (50% of dry weight) and a very low amount of SDS-extracted protein (2% of dry weight). Similarly it has been shown that starch is the major abundant reserved food in rice; it makes up more than 50% of grain dry weight, while protein makes up small percent of dry weight [21; 22].

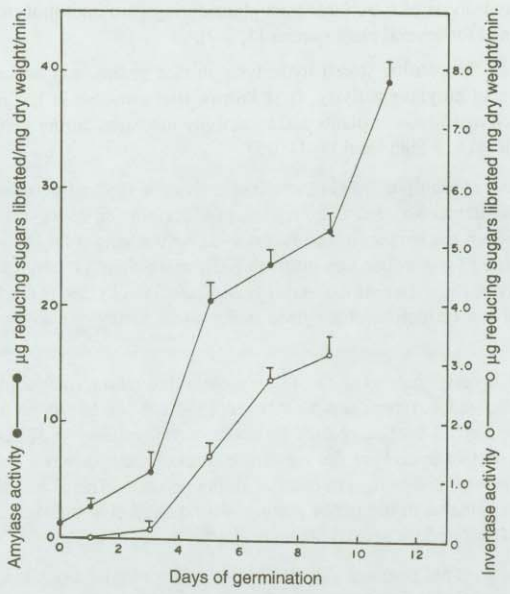

Fig. 6. Amylase and invertase activities of the grains during germination. Each point is the mean of three readings ± standard error.

Fig. 7. Polyacrylamide gel electrophoresis (PAGE) of amylase of grains at various stages of germination.

Note: Sample loaded was 50 µl of 20 mg/100 µl extraction buffer.

The rapid hydrolysis of starch that took place during germinaitonof rice is similar to the results obtained for several plant species [5; 7-9].

As shown in the results, starch hydrolysis in rice grains was accompanied by a marked increase in amylase activity. It is known that amylase is the major enzyme involved in starch hydrolysis in plants and its activity increases during germination from an initially low level to a high level [7; 11; 12].

The detection of multiple forms of amylase activity in dry and germinated grains of rice (Fig. 7) is similar to what has been reported for germinated seeds of several species [7; 21; 22]. However, the number of bands observed for this cultivar is in disagreement to the results obtained for another rice cultivar [22]; more than 11 amylase forms were detected. Moreover, the nature of the multiple amylase form in rice is not known. It has been shown that the formation of amylase isoforms in barley due to post-transitional modification [23].

The electrophoretic analysis of rice grain protein throughout the germination period by SDS-PAGE showed that there was no apparent modification of protein that took place prior to utilization as has been suggested for seeds of various species [2; 24; 25]. It may be assumed that protein hydrolysis during germination of seeds depends on plant species and on various factors such as the structure of storage protein. The results also suggested that different components of the major protein were degraded at different rates. Similar results have been reported for several plant species [2; 3; 21; 25].

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References

[1] Ashton, F.M. " Mobilization of Storage Proteins of Seeds." *Ann. Rev. Pl. Physiol.*, 27 (1976), 95-117.

[2] Basha, S.M. and Beevers, L. "The Development of Proteolytic Activity and Protein Degradation During the Germination of *Pisum sativum* L." *Planta* (Berl.), 124 (1975), 77-78

[3] Al-Helal, A.A. "Growth and Protein of *Cassia senna* L. Seedling." *J. King Saud Univ. (Science)*, 4, No. 1 (1992), 5-13.

[4] Nielsen, S.S. and Liener, I.E. "Degradation of the Major Protein of *Phaseolus vulgaris* During Germination, Role of Endsgenous Proteases and Proteases Inhibitors." *Pl. Physiol.*, 74 (1984), 498-499.

[5] Abbot, I.R and Matheson, N.K. "Starch Depletion in Germinating Wheat, Wrinkled-Seeded Peas and Senescing Tobacco Leaves." *Phytochem.*, 11 (1972), 126-127.

[6] Koops, A.J. and Groenevelld, H.W. "Mobilization of Endosperm Reserves and Uptake of Sucrose and Valine by the Cotyledons of *Euphoria lathyris* L." *J. Exp. Bot.*, 41 (1990), 1279-1285.

[7] Al-Helal, A.A. "Starch Content and Activities of Three Enzymes of Carbohydrate Metabolism in *Cassia senna* L. Seedings." *J. King Saud Univ. (Science)*, 6, No. 1 (1994), 13-22.

[8] Beevers, L. and Spitoesser, W.E. "Protein and Nucleic Acid Metabolism in Germinating Peas." *J. Exp. Bot.*, 19 (1968), 698-711.

[9] Kern, R. and Chrispeels, J. "Influence of the Axis on the Enzymes of Protein and Amide Metabolism in the Cotyledons of Mung-Bean Seedlings." *Pl. Physiol.*, 62 (1973), 815-819.

[10] Juliano, B.O. "Biochemical Properties of Rice." In: B.O. Juliano (Ed.), *Rice Chemistry and Technology*.

St. Paul, Minnesota: Amer. Assoc. of Serial Chemist Incorburated, 1985.

[11] Tanaka, Y. and Akazawa, T. "Amylase Isozymes in Gibberellic Acid-Treated Barley Seeds." *Pl. Physiol.*, 46 (1970), 686-691.

[12] Koshiba, T. and Minamikaura, T. "Purification by Affinity Chromatography of α-Amylase, Main Amylase in Cotyledons of Germinating *Vigna mungo* Seeds." *Pl. and Cell Physiol.*, 22 (1981), 978-979.

[13] Al-Helal, A.A. "Amylase Isoenzymes and Protein of Date Palm (*Phoenix dactylifera* L.) Fruit." *Bot. Bull Acad. Sinica.*, 29 (1988), 239-244.

[14] Peterson, G.L. "A Simplification of the Protein Assay Method of Lowry *etl al.* Which is More Generally Applicable." *Ann. Bioch.*, 83 (1977), 346-356.

[15] Bernfield, P. "Amylases, α and β." In: Colowick, S.P. and Kaplan,(Eds.), *Methods in Enzymology I.* New York: Academic Press, 1955.

[16] Laemmli, U.T. "Cleavage of Structural Proteins During the Assembly of the Head Bacteriophage." *Nature*, 227 (1970), 680-685.

[17] Glossmann, I.I. and Neville, D.M. "Glycoproteins of Cell Surfaces. A Comparative Study of Three Different Cell Surfaces of the Rat." *J. Biol. Chem.*, 246 (1971), 6339-6346.

[18] Brewbaker, J.L.; Upadnya, M.D.; Makinen, Y., and Macdonald, T. "Isoenzyme Polymorphism in Flowering Plants. III. Gel Electrophoretic Methods and Applications." *Physiol. Pl.*, 21 (1968), 930-940.

[19] Horikoshi, M.; Kobayashi, H.; Yamazoe, Y.; Mikami, B. and Morita, Y. "Purification and Completed Amino Acid Sequence of Major Prolamin of Rice Endosperm." *J. Cer. Sci.*, 14 (1991), 1-14.

[20] Went, T.N and Luthe, D.S. "Biochemical Characterization of Rice Glutelin." *Pl. Physiol.*, 78 (1985), 172-177.

[21] Thevenot, C.; Lauriere, C.; Mayer, C.; Simod-Cote, E. and Daussant, J. "α-Amylase Changes During Development and Germination of Maize Kernels." *J. Pl. Physiol.*, 140 (1992), 61-65.

[22] Tanaka, Y.; Ito, T. and Akazawa, T. "Enzymic Mechanism of Starch Breakdown in Germinating Rice Seeds. III α-Amylase Isoenzymes." *Pl. Physiol.*, 45 (1970), 650-654.

[23] Sticher, L. and Jones, R.L. "α-Amylase Isoforms are Postransitionally Modified in the Endosperm of the Barley Aleurone Layer." *Pl. Physiol.*, 98 (1992), 1080-1086.

[24] Al-Helal, A.A. "Degradation of Storage Protein of *Acaccia Laeta* L. Seeds." *Qyton*, 50 (1989), 103-107.

[25] Wilson, K.A.B.R.; Chen, J.C. and Tan-Wilson, A.L. "Differential Proteolysis of Glycinin and β-Conglycinin Polypeptides During Soyabean Germination and Seedling Growth." *Pl. Physiol.*, 82 (1986), 71-76.

هدم النشا والبروتين في حبوب الأرز النابتة

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(سُلِّم في ٤ جمادى الآخرة ١٤١٥هـ؛ وقُبل للنشر في ١٨ جمادى الآخرة ١٤١٦هـ)

ملخص البحث. تمَّ فصل البروتينات المختلفة (الألبمين، والجلوبين، والبرولين والجلوتين) المستخلصة من حبوب الأرز (صنف أحسائي) كهربائياً باستخدام طريقة SDS-PAGE ، أوضحت النتائج أنه لا توجد حزم بروتينية في الألبمين والبرولين في حين الجلوبين يحتوي على عدة حزم بروتينية والجلوتين يحتوي على حزمتين رئيسيتين مع عدد من الحزم ذات المحتوى البروتيني المنخفض وكذلك يحتوي الجلوتين على حزمتين من البروتين الكربوهيدراتي.

كذلك تمَّ دراسة هدم: النشا، والبروتين، ونشاط أنزيمي الأنفرتيز والأمليز أثناء مراحل مختلفة من الإنبات ولمدة ١١ يومًا. **يتضح** من النتائج المتحصل عليها أن الوزن الجاف للبذور النابتة ينقص بشكل خطي مع الزمن، ومحتوى البذور من النشا ينقص بمعدّل خطي في الأيام التسعة الأولى من الإنبات ثم يحدث النقص بشكل أسرع. البذور الجافة تحتوي على نسبة كبيرة من النشا والذي يمثّل ٥٠٪ من الوزن الجاف في حين نسبة البروتين الكلي والمستخلص بواسطة SDS تمثّل ٢٪ من الوزن الجاف.

عند فصل البروتين الكلي المستخلص من البذور في مراحل مختلفة من الإنبات بواسطة SDS-PAGE أوضحت النتائج أن الحزم البروتينية المختلفة تختلف في معدّل هدمها.

البذور الجافة لا تحتوي على نشاط لأنزيم **الأنفرتيز**. ومحتواها من نشاط **الأمليز** الكلي منخفض. يزداد نشاط أنزيم **الأمليز** بشكل كبير أثناء الإنبات ويصل النشاط إلى حدُّه **الأقصى** (٣٦ ضعفًا) في اليوم الحادي عشر من الإنبات في حين نشاط أنزيم **الأنفرتيز** يزداد بشكل بطيء حتى اليوم التاسع من الإنبات.

أوضحت نتائج التحليل الكهربائي لنشاط **الأمليز** بواسطة PAGE وجود حزمتين لنشاط هذا الأنزيم في البذور الجافة والبذور النابتة بالإضافة إلى وجود حزمة ثالثة ابتداءً من اليوم الثالث للإنبات.