

Comparison of changes in amylase activity in the cotyledons of radish and lablab

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Abstract

The food reserve of the seed may predominantly consist of fats, complex carbohydrates or storage proteins. To understand the chlorophyll content and various enzymatic activities taking place inside the seed i.e., in the cotyledons, it is very important and vital to analyze the degradation or synthesis of concerned products. However the work done so far with facts does open up some interesting possibilities of regulation of stored resources. The rate of degradation of starch is also slower than that of Radish. As we know that amylase activity is directly proportional to starch content. So this difference can be considered and justified. Thus it represents that the amylase is the major pathway of starch degradation in the axis and not that much significant in cotyledons of germinating seeds. The amylase activity is significant in cotyledons and is the best way to find out the parameters for the utilization of starch present.

Keywords: Amylase, germination, standard curve

INTRODUCTION

The major storage materials of the seeds are carbohydrates, mostly starch, protein and lipids. There occurs a great difference among the seeds in their reserve composition. Reserve materials are stored in the embryo or in the extra embryonic tissue. Usually, most of the stored carbohydrates and proteins reserve of cereals and graminaceous seeds is located in the endosperm. Fleshy cotyledons serve as the major storage organ in most non-endospermic legumes; belong to the former category, where the cotyledons serve as major storage organs with protein and carbohydrates as the major food reserves.

Soon after coming out of the soil the seedling turns green. In most plant showing epigeal germination, the cotyledons themselves function as the first leaves of the seedlings. In others the first leaf produced by the plumule. With the appearance of the green leaves, the seedlings become nutritionally independent.

The food reserve of the seed may predominantly consists of fats, complex carbohydrates or storage proteins. Seeds also possess some simple polysaccharides for functioning as intermediate respiratory substrate and for wall synthesis during early germination. Reserve food is broken down by the formation of various types of hydrolases (e.g., amylases, proteases, nucleases, lipases, etc.). Proteolytic enzymes are one of the first to be formed. They release some marked long lived RNA for controlling early metabolism. Due to the enzymatic activities DNA becomes active. New DNA synthesis and cell division occurs only after or near the emergence of radicle. Activation of DNA allows rapid synthesis of new RNA.

To understand the chlorophyll content and various enzymatic

activities taking place inside the seed i.e., in the cotyledons, it is very important and vital to analyze the degradation or synthesis of concerned products. Having proper idea and data about the mobilization or utilization of the nutritional content of the cotyledon is thus very necessary. Most of such work is done in cereal grains might be due to advantages inherent with cereals which ensure easy manipulation of the system. However the work done so far with facts do open up some interesting possibilities of regulation of stored resources. One such possibility is to extract, estimate, analyse and compare the amylase activity in two somehow different but economically important seeds from widely used plant species.

MATERIALS AND METHODS

Plant material – I

The first plant material is Radish, *Raphanus sativus*, Verna-muli. It is a small shrub type plant. It is cultivated throughout India. The root is consumed as vegetables widely. The green leaves are also edible. The taxonomic position of the plant is :

Division : Angiospermae
Class : Dicotyledonae
Order : Brassicales
Family : Brassicaceae
Genus : *Raphanus*
Species : *sativus*

Plant material – II

The first plant material is Lablab, *Dolichos lablab*, (*Lablab nigar*), Verna-sem. It is a twining herb. It is cultivated throughout India. The green pods and seeds are consumed as vegetables. The taxonomic position of the plant is :

Division : Angiospermae
Class : Dicotyledonae
Order : Leguminales
Family : Papilionaceae

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Genus : *Dolichos*
 Species : *lablab*

Process of germination

The seeds of high yielding variety of *Radish* and *Lablab* were taken for the investigation. Healthy seeds of uniform size and vigor were taken and sterilized in 1% sodium hypochlorite solution for 15min. 1% sodium hypochlorite solution acts as disinfectant. Then the imbibed seeds were germinated on moist vermiculite in a germinating chamber. The seedlings were exposed to light(4500Lx) set for 14hrs light and 10hrs dark daily cycle after cotyledonary emergence. The cotyledonary age was calculated from '0' hour at the beginning of imbibition till 120hrs. (5 days).

Then the extraction and estimation of starch was done at 24, 48, 72, 96 and 120hrs after the start of imbibitions.

Standard curve for reducing sugar for the estimation of amylase activity

In order to estimate the enzyme activity, first of all we need to prepare standard curve for a reducing sugar. 4ml of reducing sugar was added to 1ml of DNS(3,5,-dinitrosalicylic acid) reagent. It was followed by constant boiling in water bath for 7.5 mins. Then cooled and O.D. was taken at 540nm. Then the standard curve was drawn by taking the values.

Extraction and estimation of amylase

For the extraction and estimation of sugar, 1pair of cotyledon is taken in case of *Lablab*. Whereas 10 pairs of cotyledons were taken in case of *Radish* due to their extremely small size. The axis and seed coat is removed carefully before use.

Required numbers of cotyledons were taken both for *Radish* and *Lablab*. seeds were taken separately and homogenised with ice cold 0.05M citrate buffer at pH 5 in a prechilled mortar and pestle. Centrifuged the homogenate at 2000 r/m for 5 mins. at 4 degree C. Took the supernatant for enzyme assay. Kept the enzyme extract in ice box at 40 degree C. The enzyme was

assayed by adding DNS reagent to it. The O.D. was taken at 540 nm.

Both zero time and full time assay was done. For zero time assay, DNS reagent was used before adding enzyme extract. For full time, enzyme extract was added before DNS reagent.

An average of difference in O.D. was taken and respective amount of amylase present was shown in micro gram from the standard curve of reducing sugar. Now the amount of amylase activity can be calculated and it comes out in micro gram per pair of cotyledons.

RESULTS

Changes in the level of amylase activity in the cotyledons of germinating *Radish* seeds

The amylase activity in case of *Radish* cotyledons shows an interesting mode. First the activity increases steadily up to only 14% after 24hrs of imbibition. Again the activity increases rapidly by 38% between 48 to 72hrs. In this stage the enzymatic activity reaches to its peak and after this point it starts decreasing slowly in case of *Radish*. Again between 72 to 96hrs. after imbibition the activity decreases by 13% and between 96 to 120hrs. it becomes more less i.e. about 21% of the previous value.

Changes in the level of amylase activity in the cotyledons of germinating *Lablab* seeds

The amylase activity in case of *Lablab* cotyledons shows another interesting difference. First the activity increases steadily up to 18% between 24 to 48hrs. of imbibition. Again the activity increases rapidly by 29% between 48 to 72 hrs. of imbibition. After this increment, the enzymatic activity reaches its peak in between 72 to 96 hrs. of imbibition that is 35%. Proceeding from this point, the value starts decreasing very rapidly and becomes 19% less than the previous value. It shows an interesting difference at the duration between 72 to 96 hrs of imbibition. Here the rate increases the most whereas in case of *Radish* it starts decreasing from this stage of imbibition.

Table 1.Tabulation for the amylase activity in the cotyledons of *Radish* and *Lablab*

Hours after imbibitions	Amylase present in <i>Radish</i> Cotyledons (micro g / pair)	Amylase present in <i>Lablab</i> Cotyledons (micro g / pair)
24	9.6	93
48	10.9	110
72	15.0	142
96	13.1	191
120	10.2	154

DISCUSSION AND CONCLUSION

Starch is the major stored reserve in both type of seeds. The mechanism of starch was studied and it was found that there are two mechanisms of starch degradation, one is amylitic - involving amylase and the other is phosphorolytic - involving phosphorylase. The amylitic pathway is the major one for degradation of cotyledonary starch reserves in pea (Swain and Dekkar, 1966, Juliano and Varner, 1969, Abbot and Matheson, 1972) lentils (Tarrago and Nicolas, 1976) and cowpea (Mishra, 1983, Mishra et al, 1992).

There is a slight difference in amylase activity in the cotyledons

of *Radish* and *Lablab*. In case of *Radish* the maximum amylase activity occurs in between 48 to72 hrs. that is the time of hypocotyl expansion. After that the amylase activity slowly decreases. Whereas in case of *Lablab*, the amylase activity increases up to 96 hrs. after imbibitions. After this period it declines very slowly. It might be signifying that the amount of starch present per pair of cotyledons in case of *Lablab* is much more than that of *Radish*. The rate of degradation of starch is also slower than that of *Radish*. As we know that amylase activity is directly proportional to starch content. So this difference can be considered and justified. It encompassed within its span of rapid starch breakdown indicating its importance in the

mobilization of starch reserves in the cotyledons. Amylase activity is lower in cotyledons. Thus it represents that the amylase is the major pathway of starch degradation in the axis and not that much significant in cotyledons of germinating seeds.

The seeds of the Radish and Lablab were germinated and the pattern of their development was studied at every 24 hrs. interval. The starch degradation and the distribution of hydrolytic products were studied in this particular interval of time after imbibition.

The amylase activity is significant in cotyledons and is the best way to find out the parameters for the utilization of starch present. In cotyledons of Radish amylase activity increases up to 72 hrs. and then starts decreasing. but in case of cotyledons of Lablab, amylase activity increases up to 96 hrs. and after that it starts decreasing. The high amylase activity remains in the cotyledons even after most of the starch is degraded. But there is a general convention that amylase activity increases with a higher level of starch supply. From this investigation it can be concluded that

1. Amylase is the major pathway of starch hydrolysis.
2. The radicle emergence is independent of the reserve mobilization. The phase of hypocotyl extension and the epicotyl extension are highly dependent on it.
3. Very little amylase activity persists in the early stages of germination. So most of the amylase is synthesized de novo in the cotyledons.
4. The carbohydrate content shows a slightly more rapid changes in cotyledons of Radish than that of Lablab may be due to their comparatively very small size and due to their thinner seed coat. That is why Radish seeds show a rapid process of germination.

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