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Comparison of changes in sugar contents in the cotyledons of radish and lablab

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Abstract
Seed germination is one of the most important phases in the life cycle of plants. Starch is the major stored reserve in both Radish and Lablab cotyledons. 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 does open up some interesting possibilities of regulation of stored resources. The sugar level decreases to 29% from 96 to 120 hrs after imbibitions in case of Radish. In case of Lablab The sugar level decreases to 60% from 72 to 96 hrs after imbibitions. The decrement still continues from 96 to 120 hrs and the value is about 24%.

Keywords: Radish, Lablab, storage, carbohydrates

INTRODUCTION

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. The seed germination involving the emergence of cotyledons above the soil is known as epigeal germination. When the cotyledons remain inside the soil, seed germination is said to be hypogeal germination.

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 utilization of the vital reserves like sugar in two somehow different but economically important seeds from widely used plant species.

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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.

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.

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 inhibition 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 sugar

In order to estimate the value of sugar, first of all we need to prepare standard curve for sugar. Glucose standard solution was prepared by adding certain fixed and calculated amount of distilled water. In different test tubes except one contains 0.1, 0.2, 0.3, 0.4, 0.5 ml of standard glucose and the volume is made up to 1ml by

adding distilled water. 2ml of Anthrone reagent was added in each test tube. Boiled them for 7.5 mins at 70 to 80 degree C temp. Cooled and then taken O.D. at 620 nm. By the values obtained a standard curve is done for sugar graph.

EXTRACTION AND ESTIMATION OF SUGAR

For the extraction and estimation of sugar, 1pair of cotyledon is taken in case of Lablab. Whereas 10pairs 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*. Grinded separately and treated separately by the following method for extraction centrifuge tube and 10ml 80% ethanol was added. Kept in a waterbath at 80 to 85 degree C. for 30mins. Centrifuged and decanted into a 50ml beaker. The process Repeated three more times. Evaporated the alcohol extract on a waterbath at 80 to85 degree C., until most of the alcohol is removed and the volume becomes 3ml. Diluted the sample extract as per requirement. The sugar level is measured by using Anthrone reagent and the color thus produced is evaluated by taking O.D. at 620nm. Then the sugar content can be calculated further to analyze the amount of sugar in mg/pair of cotyledons.

RESULTS

Changes in the sugar level in the cotyledons of germinating *Radish* seeds

There is a gradual increase of sugar level in the cotyledons of germinating *Radish* seeds. The sugar level in the cotyledons of Radish increases sharply up to 72 hrs. after imbibitions and again starts decreasing further. In Radish, the rate of this increment or decrement is quite steady. The sugar level increases to 25% in between 24 to 48 hrs. after imbibitions. This value increases to its maximum that is up to 65% in between 48 to 72 hrs. after imbibitions. Sugar level becomes 54% less between 72 to 96 hrs. The sugar level decreases to 29% from 96 to 120 hrs. after imbibitions. The increase in sugar level in the cotyledons corresponds with morphological phase of the hypocotyls extension and the rate of maximum decrease occurs prior to epicotyls extension.

Changes in the sugar level in the cotyledons of germinating *Lablab* seeds

It is observed that the sugar level behaves almost similarly in the cotyledons of Lablab. The sugar level is maximum in between the period of 48 to 72 hrs. after imbibitions. In Lablab the sugar level increases to30% from 24 to 48 hrs. of imbibitions. The sugar level becomes maximum at 72 hrs. after imbibitions. The percentage of increment between 48 to 72 hrs after imbibitions is 73%. Now it is the time for decrement of the sugar level as usual. The sugar level decreases to 60% from 72 to 96 hrs after imbibitions. The decrement still continues from 96 to 120 hrs and the value is about 24%. The increase in sugar level corresponds with morphological phase of the hypocotyls extension and the rate of maximum decrease occurs prior to epicotyls extension.

Table 1.Tabulation for the sugar content in the cotyledons of *Radish* and *Lablab*

Hours after imbibitions	Amount of sugar in Radish Cotyledons (mg / pair)	Amount of Sugar in Lablab Cotyledons (mg / pair)
24	0.165	2.3
48	0.205	3.0
72	0.330	5.2
96	0.154	2.1
120	0.110	1.6

DISCUSSION AND CONCLUSION

The sugar level increases rapidly up to 72 hrs of imbibitions. The maximum increase of sugar level in *Radish* occurs in between 48 to 72 hrs that is about 65% and in Lablab at the same interval of time that is about 73%. After this period the sugar level declined. There was accumulation of sugar in chick pea following germination (Tarrago et al, 1978) whereas no accumulation was observed in pea cotyledon (Juliano and Varner, 1969, Abbott and Matheson, 1972).

The accumulation of sugar up to 72 hrs observed in the present investigation might be due to the translocation of starch degradation products or due to the synthesis of sugars from amino acid pool. There is no possibility of sugar accumulation due to photosynthesis because at this time the seedlings have not developed chlorophyll. The amino acid level in the cotyledons seems to rise even after the peak sugar level starts declining sharply that's why the second position does not seem to be tenable. The former findings that the starch degradation by the cotyledonary respiration rises during germination and drops after few days of germination(Beweley and Black, 1978) supports the formed hypothesis.

In germinating seeds, the starch degrades for the formation of sugar, it is found that at the level of starch degradation, the accumulation of sugar in cotyledons of both *Radish* and *Lablab* is quite prominent. It shows that the starch content decreases by the formation of sugar which acts as a source of nutrition and provide the required support for further germination of the seeds. By the percentage of formation of sugar, we can conclude that the demand of carbohydrate during phase of epicotyls extension is more than the phases of radicle emergence and hypocotyls extension during the *Radish* and *Lablab* seedlings growth. But it was found that in Radish the accumulation of sugar is more rapid than that of Lablab. Hence Radish seed is found to get its independent level of occurrence quite soon before the *Lablab* seeds considered in this investigation.

From above investigation, it can be summarized that the level of sugar in cotyledons increases upon germination up to 96 hrs. Then the level of sugar decreases towards the late phase due to unfolding of first leaves. The synthesis of sugar is more rapid in

Radish than that of Lablab. Hence it shows a vigorous growth of its axis and soon attains full maturity. It shows that sugar is the hydrolytic product of starch. It accumulates in the cotyledons along with the subsequent degradation of starch. In most of the cases it shows a decline in its level due to its translocation into the axis which acts as a link.

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