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Comparison of changes in starch contents in the cotyledons of Radish and Lablab

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Abstract

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. 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. Since the sugar level is low in the quiescent seeds in the phase of radical emergence between 24 to 48 hrs the starch degradation is negligible. The rate of degradation is found to be 24% and 22% for *Radish* and *Lablab* respectively.

Keywords: Radish, Lablab, storage, carbohydrates

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 reseve 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.

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 becomes 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 analyse 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 starch 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.

Plant material – II

The second 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).

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

Standard curve for starch

In order to estimate the value of starch, first of all we need to prepare standard curve for starch. For this a working standard solution was prepared by taking 10mg of standard starch diluted to 10ml with 52% PCA(Perchloric acid). The standard solution were prepared by taking 0.2, 0.4, 0.6, 0.8 and 1ml of freshly prepared working standard starch solution in different test tubes making the volume 1ml in each test tube by adding water. 5ml of Anthrone reagent was added to each test tube followed by incubation for 5min of hot water bath. Then the standard solutions were cooled and the color developed was read at 630nm. Observations were tabulated and a standard curve was drawn.

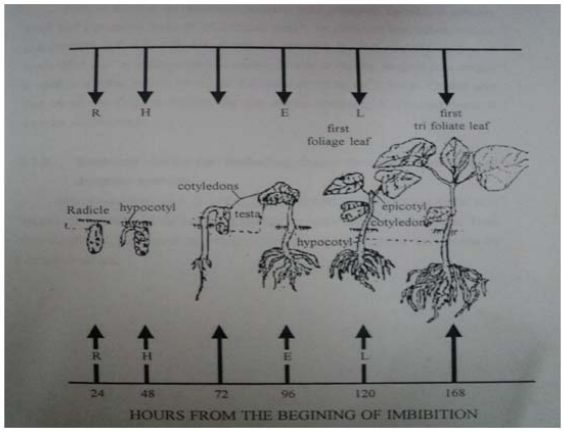


Diagram – 1

Extraction and estimation of starch

For the extraction and estimation of starch, 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; 1ml of 80% alcohol was added, grinded properly. Added 2ml alcohol and centrifuged for 10min and rejected the supernatant. Dried the residue left in the tube in an oven at 80 degree C. for starch extraction. Added 2ml of dist. Water to the centrifuge tube containing the dried residue. Put the tube in a boiling water bath for 15min and stirred occasionally. Allowed the tube to cool and added 2ml 9.2N HCLO4 while stirring constantly. Then stirred the solution for 15min. Suspension was made up to 10ml and centrifuged. Supernatants was collected and added 2ml 4.6N HCLO4 to the residue. The volume was made up to 10ml. Centrifuged, combined the supernatants and made the volume as per requirement. Starch was estimated by Anthrone reagent method and the O.D. was taken at 630nm. Then calculation was made as per the readings obtained. To get the starch content in mg/pair of cotyledon, calculation was done.

RESULTS

Changes in the starch level in the cotyledons of germinating Radish seeds :

There is a degradation of starch level in the cotyledons of germinating Radish seeds. Degradation level is low up to 48hrs. Thereafter the rate of degradation increases very rapidly up to 72hrs. but again the rate becomes low and remain almost similar up to 120hrs.

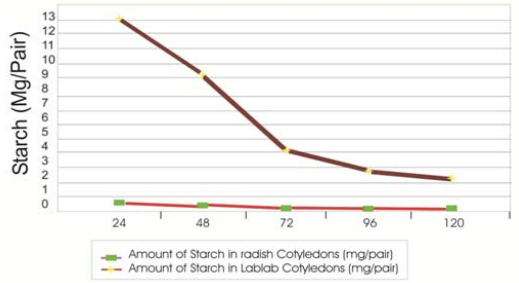
Comparing the rate of degradation between every 24hrs interval shows that about 24% loss occurs between 24 and 48 hrs and about 51% loss occurs between 48 and 72hrs which is the maximum one. The rate of starch loss is only 16% and 33% between 72 to 96 and 96 to 120hrs respectively. It corresponds with the morphological phases of hypocotyls extension leading to seedling emergence and crozier formation and epicotyls extension leading to unfolding of first leaves respectively.

Changes in the starch level in the cotyledons of germinating Lablab seeds :

As usual here also a degradation of starch level is found. The degradation level is low up to 48hrs. which is even lower than comparison to Radish. Thereafter the rate of degradation increases and becomes of highest at 72hrs. Then again the rate of degradation decreases but in a greater rate than before at 96hrs. But the rate becomes lowest as the germination proceeds that is at 120 hrs. Now comparing the rate of degradation between every 24hrs interval, it is found that about 22% loss occurs between 24 to 48hrs. and about 55% that is a drastic loss of starch between 48 to 72hrs. The rate of starch degradation is 31% in the seeds of *Lablab* in between 72 and 96 hrs. and about only 14% between 96 to 120 hrs. It corresponds similar with the morphological phases of hypocotyls extension leading to seedling emergence and crozier formation and epicotyls extension leading to unfolding of first leaves respectively.

Table -1: Tabulation for the starch content in the cotyledons of Radish and Lablab

Hours after Imbibition	Amount of starch in Radish Cotyledons (mg / pair)	Amount of Starch in Lablab Cotyledons (mg / pair)
24	0.67	12.1
48	0.51	9.4
72	0.25	4.2
96	0.21	2.9
120	0.14	2.5



GRAPH – 1: Graph for the starch content in the cotyledons of Radish and *Lablab*

Calculation of standard error

Table – 2: Tabulation for the calculation of standard error of the starch content of Radish

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*

Figure showing standard deviation for starch content of Radish

Table – 3: Tabulation for the calculation of standard error of the starch content of *Lablab*

Time after Imbibitions (Hrs.)	No. of observation	O.D. at 630nm	Mean ± Standard Deviation
24	1	0.922	0.917
	2	0.926	±
	3	0.911	0.007
	4	0.909	
48	1	0.721	0.716
	2	0.710	±
	3	0.726	0.008
	4	0.707	
72	1	0.329	0.323
	2	0.316	±
	3	0.311	0.011
	4	0.336	
96	1	0.233	0.225
	2	0.218	±
	3	0.236	0.01
	4	0.213	
120	1	0.104	0.109
	2	0.118	±
	3	0.101	0.007
	4	0.113	

Figure showing standard deviation for starch content of *Lablab*

DISCUSSION AND CONCLUSION

Starch is the major stored reserve in both Radish and Lablab cotyledons. Since the sugar level is low in the quiescent seeds in the phase of radical emergence between 24 to 48 hrs the starch degradation is negligible. The rate of degradation is found to be 24% and 22% for Radish and Lablab respectively. The starch degradation is comparatively slightly more in Radish cotyledon. Hence it shows a significant progress in germination at this interval of time than that of Lablab. Its seed coats become more loose and easily detachable at this time. Similar pattern of starch degradation are also observed in germinating lentils (Tarrago and Micolas,1976), pea (Juliano and Varner, 1969) cotyledons. The crozier formation occurs at the slow phase starch degradation. The epicotyls extension occurs at the second rapid phase of starch degradation between 48 to 72 hrs which is about 51% in Radish and 55% in Lablab. This is the stage of maximum starch degradation. Again between 96 and 120 hrs leading to unfolding of first leaves, there is a slower degradation of starch. The first phase of radicle formation is independent of mobilization of carbohydrate reserve in pea (Bain and Mercer, 1966) while the epicotyls and hypocotyls extension are highly dependent upon it with a period of rest between them. This regular decrease in the starch level of cotyledon shows that there must be an increase in starch

level in the axis of the seeds correspondingly. Because the starch thus degraded is used in the process of germination to provide required nutrition for the developing axis for its further independence.

The seeds of Radish and Lablab were germinated and the pattern of their development is studied at every 24 hrs interval after imbibitions and it is found that starch degradation in the cotyledons is rapid initially and slows down at the subsequent level. The degradation of starch starts after 48 hrs of imbibitions. It shows that the starch is to be converted into other organic compounds in order to provide nutrition to the axis of germinating seeds.

From all these investigations, it can be concluded that the starch degradation in the cotyledons is monophasic. More and more starch is degraded during the early phase of germination. Comparing the pattern of degradation in these two type of seeds, it is found that rate of utilization of starch is more rapid in case of *Lablab* in between 48 to 72 hrs. of imbibitions. Similarly maximum degradation occurs in between the same time interval in case of *Radish*. But the rate of degradation is much lower perhaps due to the small size of *Radish* seeds.

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