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Comparison of changes in sugar contents in the cotyledons of *Trigonella* and *Tamarindus*

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Abstract
Fleshy cotyledons serve as the major storage organ in most non-endospermic legumes. Cotyledons serve as major storage organs with protein and carbohydrates as the major food reserves. Reserve food is broken down by the formation of various types of hydrolases (e.g., amylases, proteases, nucleases, lipases, etc.). In germinating seeds, the starch degraded for the formation of sugar, it was found that at the level of starch degradation, the accumulation of sugar in cotyledons of both *Trigonella* and *Tamarindus* was quite prominent. It was marked that sugar was the hydrolytic product of starch. It accumulated in the cotyledons along with the subsequent degradation of starch. In most of the cases it showed a decline in its level due to its translocation into the axis which acted as a link.

Keywords: Amylases *, Trigonella* and *Tamarindus*

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.

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 become nutritionally independent.

The food reserve of the seed may predominantly consist 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 Fenugreek, *Trigonella foenum-graecum* L. Verna-methi.

It is a small shrub type plant. It is cultivated throughout India. The whole plant is consumed as vegetables widely. It has also a great medicinal value and the seeds are widely used for different purposes.

Plant material – II

The second plant material is Tamarind, *Tamarindus indica* L. Verna-imli.

It is a large tree and mostly wild. Though it is not cultivated but its products are widely consumed throughout India.

Process of germination

The seeds of high yielding variety of *Trigonella* and *Tamarindus* 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 24 hours of imbibitions at the beginning till 120hrs. (5 days) for the seeds of Trigonella and till 312hrs. (13 days) for Tamarindus. Because of a comparatively very longer time period taken for full germination in Tamarind seeds, some important intermediate stages during germination were taken under consideration for a comparative study.

The six most important stages were as follows:

1. Imbibition of 24hrs.("I") or 0hrs of germination .
2. Radicle expansion.("R").
3. Hypocotyl extension.("H").
4. Branching of radicle.("B").
5. Epicotyl extension.("E").
6. Unfolding of first pair of leaves.("L").

Then the extraction and estimation of sugar were done at these stages for both the types of seeds.

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 *Tamarindus*. Whereas 20pairs of cotyledons were taken in case of *Trigonella* due to their extremely small size. The axis and seed coat is removed carefully before use.

Required numbers of cotyledons were taken both for *Tamarindus and Trigonella*. Grinded separately and treated separately by the following method for extraction and estimation of their sugar content. The grinded samples were placed in a 15ml centrifuge tube and 10ml 80% ethanol was added. Kept in a water bath at 80 to 85 degree C. for 30mins. Centrifuged and decanted into a 50ml beaker. Repeated the process three more times.

Evaporated the alcohol extract on a water bath 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 *Trigonella* seeds

There was a gradual increase of sugar level in the cotyledons of germinating *Trigonella* seeds. The sugar level in the cotyledons of *Trigonella* increased sharply up to 72 hrs. of germination and again started decreasing further. In *Trigonella*, the rate of this increment or decrement was quite fluctuating. The sugar level increased to 10% in between "I" to "R" (that is from 24hrs. of imbibitions to 24hrs. of germination). This value increased to 16% in between the stages "R" and "H" (that is from 24hrs. to 48hrs. of germination). The increase in sugar content reached to its maximum that is up to 53% in between 48 to 72 hrs. of germination(that is from "H" to "B"). Sugar level became 51% less between 72 to 96 hrs. of germination means from the phase "B" to "E". The sugar level decreased to 19% from 96 to 120 hrs. of germination which refers to the value between phase "E" and "L". 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 epicotyl extension.

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

It was observed that the sugar level behaved almost similarly in the cotyledons of *Tamarindus*. The sugar level was maximum in between the period of 120hrs. to 192hrs. of germination (that is from the phase "H" to "B"). In *Tamarindus* the sugar level increased to about 8% from 24hrs. of imbibitions to 24hrs. of germination (between "I" and "R"). This increase in sugar level further continued to be about 18% from "R" to "H" (from 72hrs. to 120hrs. of germination) and the sugar level became maximum at the stage "B"(192hrs. of germination). The percentage of increment between "H" to "B" was recorded to be 52% (120hrs to 192hrs.). Now it was the time for decrement of the sugar level as usual. The sugar level decreased to 54% from stage "B" to "E" (192hrs. to 240hrs. of germination). The decrement still continued from "E" to "L" (240 to 312hrs. of germination) and the value was about 16%. The increase in sugar level corresponded with morphological phase of the hypocotyls extension and the rate of maximum decrease occurred prior to epicotyls extension.

Table 1. Tabulation for the sugar content in the cotyledons of *Trigonella* and *Tamarindus*

Stages of germination	*Amount of sugar in Trigonella Cotyledons (mg / pair)*	*Amount of Sugar in Tamarindus Cotyledons (mg / pair)*
I	*0.177*	*9.9*
R	*0.198*	*10.7*
H	*0.230*	*12.6*
B	*0.352`*	*18.9*
E	*0.183*	*8.7*
L	0.149	7.3

DISCUSSION AND CONCLUSION

Level of sugar started increasing just after the start of imbibitions of the seeds. Observations of sugar levels between the phases "I" and "R" were recorded to be increased by about 10%. Soaking of fenugreek seeds showed increase in sugar content in the cotyledons (Hooda & Jood, 2003). Germinated endosperms showed more sugar than that of ungerminated ones (Sathyanarayana et al, 2011). The sugar level increased rapidly up to the phase "B"(72hrs. and 192hrs. of germination in *Trigonella* and *Tamarindus* respectively). Sugar content in Tamarind increased up to near about 10 days of germination (Tawatchai et al, 2011). The maximum increase of sugar level in *Trigonella* occurs in between 48 to 72 hrs and the stages were from "H" to "B" that is about 53%. But in *Tamarindus* the same intermediate stages were came in to existence between the time periods of 120 to 192hrs. after the starting of germination and the increase was observed to be about 52%. 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 *Trigonella* seeds during 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 was no possibility of sugar accumulation due to photosynthesis because at this time the seedlings had not developed chlorophyll. The amino acid level in the cotyledons seemed to rise even after the peak sugar level started declining sharply. That's why the second position did not seem to be tenable. The former findings that the starch degradation by the cotyledonary respiration rose during germination and dropped after few days of germination(Beweley and Black, 1978) supported the formed hypothesis.

In germinating seeds, the starch degraded for the formation of sugar, it was found that at the level of starch degradation, the accumulation of sugar in cotyledons of both *Trigonella* and *Tamarindus* was quite prominent. It showed that the starch content decreased by the formation of sugar which acted as a source of nutrition and provided the required support for further germination of the seeds. By the percentage of formation of sugar, it can be concluded that the demand of carbohydrate during phase of epicotyl extension was more than the phases of radicle emergence and hypocotyl extension during the seedlings growth. But it was found that in *Trigonella,* the accumulation of sugar was more rapid than that of Lablab. Hence seeds of *Trigonella* were found to get their independent level of existence quite sooner before the *Tamarindus* seeds considered in this investigation.

From above investigation, it can be summarized that the level of sugar in cotyledons increased upon germination up to the phase "B", means till the proper development of hypocotyl. Then the level of sugar decreased towards the late phase due to unfolding of first leaves. The synthesis of sugar was more rapid in *Trigonella* than that of *Tamarindus*. Hence it showed a vigorous growth of its axis and soon attained full maturity. It showed that sugar was the hydrolytic product of starch. It accumulated in the cotyledons along with the subsequent degradation of starch. In most of the cases it showed a decline in its level due to its translocation into the axis which acted as a link.

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