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Biochemical changes during imbibition stages of seed priming in Tomato

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Abstract
Tomato (*Solanum lycopersicum* L.) is one of the major consuming vegetable all over the world. We are in a position to improve or enhance the productivity of tomato seeds. Now a days, the seed enhancement techniques *i.e.,* seed priming is widely use to improve uniform germination and yield. Against this background, this research was undertaken with the imbibition stages of seed priming in tomato seeds. Tomato seed priming involves 48 h of seed imbibition and subsequent dehydration (hydropriming) (Venkatasubramanium and Umarani, 2007) [10]. Tomato seeds were subjected to 12, 24, 36 and 48 h of imbibition. Seed samples from each of the seed imbibition duration along with control was subjected to various analysis *viz.,* moisture content of the seeds, α-amylase content, starch content and total sugar content without subjecting to dehydration. The moisture content, α-amylase content and total sugar content of the seeds during various stages of imbibition, goes on increasing from 12 h of imbibition to 48 h of imbibition than control. The starch content was gradually decreased among various stages of imbibition than control.

Keywords: Imbibition**,** seed priming, hydrolytic enzymes, starch and total sugar content

1. Introduction
Tomato (*Solanum lycopersicum* L.) is a self-pollinated vegetable crop. According to Indian Minimum Seed Certification Standards (IMSCS), the germination of tomato seed is only 70%. Balance between embryo growth potential and the mechanical resistance of the endosperm, determined the emergence of radicle during germination of seeds. The embryo is surrounded by the rigid endosperm in tomato seed. While the endosperm plays an important role in supplying nutrition for growing embryo. After germination, the rigid tissue is an obstacle to radicle emergence. The micropylar region of the endosperm *viz.,* endosperm cap which is adjacent to the radicle tip provides a mechanical resistance. The endosperm cap must be weakened for germination of seeds to occur.

During seed germination, absorbed water causes cells of an embryo to release gibberellin, which diffuses through the seed into the aleurone layer of the endosperm to produce hydrolytic enzymes *viz.,* amylase. This enzyme diffuses into the starch-packed endosperm, where it breaks down stored starch molecules. The embryo then begins to use the released glucose for aerobic respiration, which fuels rapid cell divisions of meristem cells in the embryo and helps for seed germination. Synthesis or activity of hydrolytic enzymes that break down endosperm cell walls prior to completion of tomato seed germination. Hydrolysis of endosperm cell walls is a prerequisite for tomato seed germination because it decreases the mechanical restraint imposed on the embryo (Groot *et al.,* 1988) [3].

The uptake of water by the dry seed, involves absorption of water by cell wall and protoplasmic macromolecules, is called as imbibition. The movement of water into the seed is due to diffusion and capillary action with water moving from a region of higher to lower water potential. Among the three components of seed water potential, *i.e.,* osmotic potential, matric potential and turgor pressure, the matric potential of cell walls and their contents are primarily responsible for imbibition. Permeability of the testa, or seed coat, is a major factor controlling the rate of water uptake. Low vigour legume seeds with permeable seed coats are especially susceptible to imbibitional injury. The imbibition period offers opportunity as well as hazard. Seeds may be primed for increased vigour by imbibing and then drying back. High membrane permeability during early imbibition may facilitate insertion of germination promoting and anti-pathogen chemicals into seed tissues (Woodstock, 1988) [11].

Against this background, an experiment was conducted to determine the moisture content of the seeds, α-amylase content, starch content and total sugars during seed germination.

Materials and Methods

Tomato variety, Arka Vikas with the initial germination and moisture content of 67% and 8%, respectively was obtained from Coimbatore for conducting seed priming experiments in tomato. Laboratory experiments were conducted at the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore. Hydropriming has been standardized as an effective method of seed priming for tomato. The methodology involves 48 h of seed imbibition in water and subsequent seed drying (Venkatasubramanium and Umarani, 2007) [10]. Based on these duration for seed priming, the imbibition stages are taken and various analysis were undertaken.

Tomato seeds were subjected to 12, 24, 36 and 48 h of imbibitions. Seed samples from each of the seed imbibitions duration was subjected to various analysis without subjecting to dehydration.

Moisture content (%)

Two replications of 5 g seeds each were taken in moisture bottle, weighed to record the initial weight and dried at 103 ± 2°C in a hot air oven for 17 h. After cooling in desiccators for 30 min. the dry weight of the seeds were recorded. The moisture content was calculated and the mean expressed in percentage adopting the following formula (ISTA 1999) [5].

$$\text{Moisture content (\%)} = \frac{M2-M3}{M2-M1} \times 100$$

Where,

M1 = Weight of the container.

M2 = Weight of the container + Initial weight of the sample.

M3 = Weight of the container + weight of the sample after drying.

Starch content

The starch content of the seeds was determined by the method of Hodge and Hofreiter, 1962 [4]. Homogenize the seed sample with hot 80% ethanol to extract sugars. Starch content was extracted using anthrone reagent and read the intensity of green dark green colour at 630nm by an Optima UV-VIS spectrophotometer (Model SP-3000). The starch content was expressed as mg g^{-1} of sample.

α-Amylase activity (Paul *et al.*, 1970) [7]

Two replicates of 500mg of pre-germinated seed samples were homogenised in 1.8ml of cold 0.02M sodium phosphate buffer (pH 6.0) and centrifuged at 20,000 rpm for 20 min. to extract enzymes. To 0.1ml of enzyme extract, one ml 0.067 per cent starch solution was added. The reaction was stopped after 10 min. of incubation at 25 ºC by the addition of one ml of iodine HCl solution (60mg KI and 6mg I_2 in 100ml of 0.05N HCl). Change in colour was measured at 620nm. The activity was calculated and expressed as mg maltose min^{-1}

$$\text{α- Amylase activity} = \frac{\text{OD value}}{\text{Volume of sample pipetted out}} \times \frac{1000}{500}$$

Total Sugar content

Weigh 100mg of the sample into a boiling tube. Hydrolyze by keeping it in a boiling water bath for 3 hours with 5ml of 2.5N HCL and cool to room temperature. Neutralize it with solid sodium carbonate until the effervescence ceases. Make up the volume to 100ml and centrifuge. Collect the supernatant and take 0.5 and 1ml aliquots for analysis. Prepare the standards by taking 0, 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard. „0" serves as blank. Make up the volume to 1ml in all the tubes including the sample tubes by adding distilled water. Then add 4ml of anthrone reagent. Heat for 8 minutes in a boiling water bath. Cool rapidly and read the green to dark green colour at 630 nm. Draw a standard graph by ploting concentration of the standard on the X-axis versus absorbance on the Y-axis. From the graph calculate the amount of carbohydrates present in the sample tube and expressed in% mg.

Data were analysed using an analysis of variance (ANOVA) as a factorial combination of treatments. Means were separated on the basis of least significant difference (LSD) only if F test of ANOVA for treatments was significant at the 0.05 or 0.01 probability level. Percentage data were arcsine transformed before analysis. Percentage data were arcsine transformed before analysis

Results and discussion

The moisture content of the seeds of imbibition in various stages were presented in Table 1. The moisture content (%) of the seeds was found to be significantly affected due to stages of imbibition. As the duration of imbibition progressed, there was an increase in moisture content (%) of the seeds. The increase of the moisture content (%) was from 8.9% (12 h imbibition) to 10.00% (48 h imbibition), while the dry seed recorded the lowest moisture content of 7.8% (Fig. 1).

The starch content (mg/g of glucose) of the seeds was found to be significantly differed due to various stages of imbibition. As the duration of imbibition progressed, there was a decrease in starch content (mg/g of glucose) of the seeds. The results revealed that, the dry seed (70.2 mg/g of glucose) registered highest starch content (mg/g of glucose) and it is on par with 12 h of imbibition (70.2 mg/g of glucose). The starch content (mg/g of glucose) of the seeds was decreased to 41.4, 39.6 and 30.6 during 24, 36 and 48 h of imbibition (Table 1) (Fig. 1). Stored starch plays an important role in the development of embryo during germination of seeds. The increase in metabolic activity in germinating seeds is due to the induction of some of the hydrolytic enzymes. Amylase and invertase are two important hydrolytic enzymes that increase sugars in germinating seeds of rice. Starch is hydrolysed by the amylolytic enzymes to provide substrate and energy sources for the embryo during germination. The simultaneous increase in soluble sugars and amylase activity and decrease in starch in *vicia faba* could be due to faster breakdown of starch (Kashem *et al.*, 1995) [6]. 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 (Shweta Tiwari and Sharma, 2011) [8].

The α-Amylase content (mg of maltose/min) of the seeds was found to be significantly increased due to stages of imbibition. The increase of the α-Amylase content (mg of maltose/min)

was from 6.24 mg of maltose/min (12 h imbibition) to 9.78 mg of maltose/min (48 h imbibition), while the dry seed recorded the lowest value of 5.44 mg of maltose/min (Table 1) (Fig. 1). Das and Sem-Mandi (1992) [1], working with wheat seeds observed a considerable increase in α-amylase activity during imbibition and an association between the start of germination with the increase in α-amylase activity. Amylase activity was low at initial stages and increased steeply upto 120 h of germination in cotton seeds (Kashem *et al.*, 1995) [6], this results are in agreement with the finding of Thimmiah (1989) [9] who found that amylase activity was variable among the wheat varieties and activity was low at initial stages and increased steeply and further decreased at later stages of germination.

Total sugar content (mg) of the seeds was found to be significantly increased among different stages of imbibition. As the duration of imbibition progressed, there was an increase in total sugar content (mg) of the seeds. The increase of the total sugar content (mg) was from 4.46 mg (12 h imbibition) to 7.79 mg (48 h imbibition), while the dry seed recorded the lowest value of 1.88 mg (Table 1) (Fig. 1). The sugar content was significantly increased with the period of germination. The rate of accumulation of sugars was higher in wheat varieties *i.e.*, Balaka and Barkat at 96 h and it was 5 times greater than the ungerminated seeds (Kashem *et al.*, 1995) [6]. These results are agreement with those observed by Dhanunjayanath *et al.* (1993) [2], who found 74% depletion of starch and 4 times increase of sugar at 96 h over the zero period of germination in horse gram.

Table 1: Effect of imbibition stages of seed priming on biochemical characteristics (mg/g of glucose) of tomato

Imbibition (h)	Moisture content (%)	Starch content (mg/g of glucose)	Total sugars (mg)	α-Amylase content (mg of maltose min^{-1})
Dry seed	7.8 e (16.43)	70.2 a	1.88 e	5.44 e
12 h (I)	8.9 d (17.46)	70.2 a	4.46 d	6.24 d
24 h (I)	9.6 c (18.44)	41.4 b	5.60 c	7.66 c
36 h (I)	9.9 b (18.44)	39.6 c	7.76 b	8.52 b
48 h (I)	10.00 a (18.44)	30.6 d	7.79 a	9.78 a
Mean	9.24 (17.46)	50.4	5.50	7.53
SEd	0.24	1.34	0.12	0.20
CD (0.05)	0.53	2.98	0.26	0.44

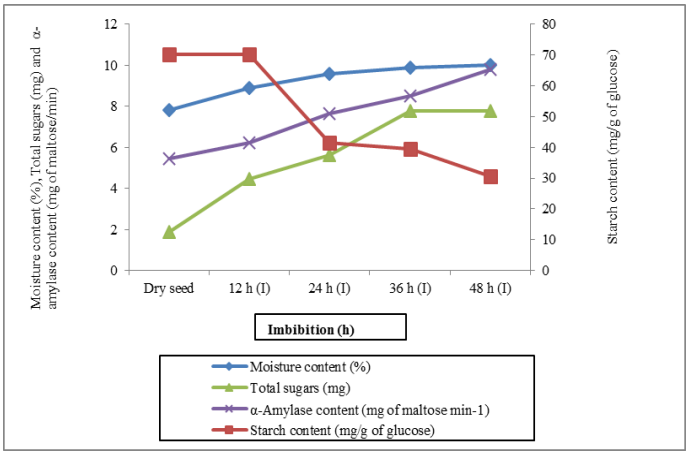

Fig 1: Effect of imbibition stages of seed priming on biochemical characteristics (mg/g of glucose) of tomato

Conclusions

The results obtained in the present study allow the following conclusions to be drawn:

- Moisture content, α-amylase content and total sugar content of the seeds were drastically increased during various stages of imbibition in tomato seeds.
- At the same time the starch content was drastically reduced, among different stages of imbibition of seeds than control in tomato.

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