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A Comparative Study of β-galactosidase and Alanine Amino Transferase Enzymes during the Germination of Rice, Wheat, Ragi and Bajra

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Biomolecules are converted by the action of enzymes during the germination process and these are translocated to the growing seedlings serving as an enzyme source and for the synthesis of new biomolecules. The objective in the present study was to quantify the enzymatic activity of β-galactosidase and alanine amino transferase in rice, wheat, ragi and bajra on different days of germination. Germination was carried out for a period of 8 days. The enzymes activity increased differentially to an extent on the different days on germination in rice, wheat, ragi and bajra. This indicates the breakdown of molecules differs and the mechanism which is operating during the germination varies from species to species, depending on the requirement for the developing seedlings.

Keywords: Germinating seedlings; alanine amino transferase; β-galactosidase; rice; wheat; ragi and bajra.

1. INTRODUCTION

The mobilization of reserve biomolecules from the storage tissue is an essential feature during germination. The complexity of metabolic changes and its regulation is not clearly

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understood. The major metabolic events occurring during germination are not the same in seeds of different species because of genetically determined diversity in seed morphology, physiological maturity, developmental pattern, chemical reserves and the signal transduction occurring during the germination.

Hydrolytic enzymes play a set of role in the biochemical mechanism of germination. A special attention was focused on the enzymatic activity of the β-galactosidase and alanine amino transferase activity. Changes mainly responsible for the breaking of glucans residues and on interconversion of keto acids to amino acids.

β-galactosidases (EC: 3.2.1.23) are the hydrolytic enzymes occurring in different organs of several plant species. It has been suggested that they are responsible for the removal of galactose from cytosolic or membrane bound glycoproteins and from cell wall pectins and hemicelluloses [1]. Inspite of much effort their physiological role is still not well understood [2]. These enzymes have been associated with depletion of oligo and polysaccharides during seed reserve mobilization [3] as well as during the process of cell wall loosening for cell elongation [4]. Furthermore, they have been related to fruit ripening processes [5].

Mobilization of nitrogen is a crucial process that controls germination as nitrogen serves as source of energy and nutrients to the growing new tissues. Several works based on ^{14}C labeling have shown that amino acids during germination provide a large quantity of carbon, which is essential for respiration and synthesis of monosaccharides. This event is further enhanced by the interconversion of amino acid and keto acids as a result a complete pool of amino acids is available for the protein synthesis and hence this plays a regulatory role in the seedling germination control [6]. Alanine amino transferase (L-alanine; 5-oxoglutarate amino transferase; EC: 2.6.1.2) converts pyruvate and glutamate to alanine. In doing so, it is involved in both carbon and nitrogen metabolism in plants which have been reported in many plant tissues [7]. It has been proposed that this enzyme plays a major role in the synthesis of the alanine which occurs in cotyledons of many seeds during germination. This alanine is then transported from cotyledons to the axis of the germinating seeds. Alanine could be synthesized from pyruvate and glutamate by alanine amino transferase. The activity of the Alanine amino transferase enzyme is also known to increase during the greening of leaves of plants having the C_4-photosynthetic pathway [8]. This enzyme shows a requirement for pyridoxal phosphate, similar to most amino transferases.

The development of aspartate transaminase synthesis during seed germination has been studied in a variety of plants. In an early investigation, a marked increase in activity of the aspartate and alanine transaminase were found in the embryo during the first few days of germination in Cucurbita [9] seeds and also in the entire seedlings of *Phaseolous vulgaris* and *Triticum vulgare*. In pisum cotyledonary tissue, on the other hand, the specific activity of apartate amino transferase was found to reach a peak by the 4th day of germination, after which it steadily decreased [10]. In Cucurbita seedlings grown on light or darkness, the total activity of amino transferase activity was also found to show a similar pattern during development [7].

High activities of amino transferases in the imbibed seeds with subsequent decline during germination of Indian bean resemble the sequence of events in pea nuts, beans and horse gram [11] which has high levels of aspartate amino transferase in the dry seeds but declined during the first few days of germination and enhanced later. Formation of keto acids is important for respiration pathways and thus a very early metabolic event during imbibition appears to be the formation of keto acids from amino acids by deamination and transamination reactions.

Metabolism of carbohydrates, proteins and lipids are very well understood during germination in different plants. In contrast very little information is available on amino transferases and β-galactosidases, which are the key enzymes for the interconversion of amino acid and keto acid the breakdown of galactosidic linkage during the germination. Hence, the present work is an attempt of furthering our knowledge towards better understanding of the above concept in germinating cereals and millets. In the present investigation a comparative study was done on the activity of alanine amino transferase and beta-galactosidase in rice, wheat, ragi and bajra.

2. MATERIALS AND METHODS

2.1 Plant Materials

Seeds of Rice cultivar (MTU-1001), Ragi (GPU-28), Wheat (Improved variety) and Bajra were obtained from Dharwad Agricultural University, Dharwad and Karnataka.

2.2 Preparation of Enzyme Extraction

Uniformly germinated seedlings (1gm) were picked from day 0 to 8 days, cut into small pieces and homogenized in a pestle and mortar with ice cold saline containing 2% (w/v) insoluble PVPP (Polyvinyl polypyrrolidone). The volume was made up to 5ml. This extract was filtered through four layers of muslin cloth and the filtrate was centrifuged at 15000xg for 20 min. at 4ºC. The supernatant was used for enzyme assay.

β-galactosidase activity was determined by a modified Dey and Pridham [12] method. Alanine amino transferase activity was determined according to the procedure of Tonhazy et al. [13]. The protein was estimated by the Lowry et al. method [14].

Experiments are performed in triplicates. Each time three sets were taken and data was subjected to statistical analysis. The values are expressed as mean ± SD.

3. RESULTS AND DISCUSSION

Seeds are rich in reserve biomolecules like carbohydrates, proteins and lipids which make the seeds self-sufficient with respect to their food and energy supply. Several biochemical changes occur during germination which has received considerable attention. During the germination reactivation of enzymes occurs leading to the breakdown of the reserve materials, which results in the production of energy. The energy is utilized for protein synthesis, cell division, maturation etc., where there is a perfect co-ordination between different metabolic reaction and initiating the seed germination. In our present investigation we have checked the activity of β-galactosidase and Alanine amino transferase from 0 to 8th day of germination of rice, wheat, ragi and bajra seeds. The total protein content of the seeds on different germination days as shown in the Fig. 1.

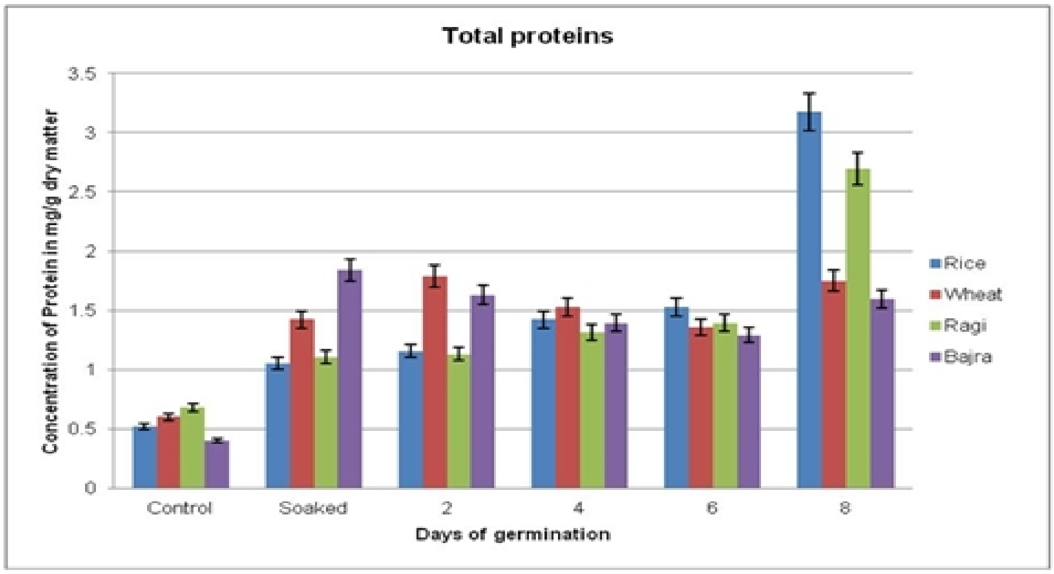

Fig.1. Total protein content of the seeds on different germination days

3.1 β--galactosidase Activity during Germination of Rice, wheat, Ragi and Bajra

Changes in the specific activity of β-galactosidase enzyme in the seedling of rice, wheat, ragi and bajra on different days of germination have been studied and the results are presented in Table 1.

The specific activity was maximum on 6^{th} day in rice, wheat, and bajra (14, 30.91 and 24.02 nmoles/mg/min) respectively and on 4^{th} day in ragi (9.69 nmoles/mg/min). There were 2, 4, 6 and 2.5 fold increase in 6^{th}, 6^{th}, 4^{th} and 6^{th} of germination in rice, wheat, ragi and bajra respectively. The enzyme activity further decreased on 8^{th} day of germination in all the seedlings.

Table 1. Variation of β-galactosidase specific activity during seed germination of rice, wheat, ragi and bajra

Days of germination	Rice	Wheat	Ragi	Bajra
0	6.9±0.3	7.95±0.6	1.69±0.8	9.0±0.2
2	7.1±0.7	5.07±0.7	5.01±0.5	10.2±0.8
4	10.0±1.1	15.07±0.5	9.6 ±1.2	11.29±0.3
6	14.0±0.8	14.00±0.8	5.08±0.5	24.02±1.4
8	9.0±1.6	16.84±1.0	3.15±0.8	16.44±0.4

Both the β-galactosidase enzyme as its sustrate (β-galactosyl linkages), are found in a wide variety of sources [15]. A large number of reports on the occurrence of β--galactosidases in plant tissues, especially in seeds have appeared since 1950. Leguminous seeds have both the β--galactosidases enzymes and their natural substrates, the carbohydrates with β--galactossyl linkages. The reserve carbohydrate levels decrease during seed germination and the forms of β--galactosidases are said to be involved in the breakdown process [15].

Several fold increases in their activity during seed germinating has been reported [16]. Galactose containing oligosaccharides present in seeds serves as a source of energy during the early phases of seed germination. Both the β-galactosidase enzyme as its sustrate (β-galactosyl linkages), are found in a wide variety of sources [17]. The β--galactosidases activity increases in cotyledons of *Vigna sinunsis* and *Anacardium occidentale* [17]. In the present study the β--galactosidase activity in rice, wheat, ragi and bjra increased during germination. The increase in activities may be due to the *de novo* synthesis of enzyme protein [18].

3.2 Alanine Amino Transferase Activity during Germination of Rice, Wheat, Ragi and Bajra

The activity of the alanine amino transferase enzyme was not detected in ungerminated seeds (0 days of germination). However there was an increase the transaminase activity on the onset of germination till the 4th day where the maximum activity could be seen and further decreased and no detectable activity were observed on the 8th day of germination. In the case of wheat, ragi and bajra a basal activity was present and bajra had a maximum (14.67nmoles/mg/min). This activity further increased by 5 folds on 2nd day germination of wheat and further the activity declined till the 6th day of germination and a slight increase in the activity was observed later on the 8th day of germination. A similar activity pattern was seen in the ragi seeds, in which the maximum activity of the alanine amino transferase was found at the 4th day of germination In the case of bajra seeds it was observed a slight increase in the activity of this enzyme in the second and fourth day of germination, with a higher increase on the sixth day and finally a decrease on the eighth day of germination time. In the case of bajra seeds it was observed a slight increase in the activity of this enzyme in the second and fourth day of germination, with a higher increase on the sixth day and finally a decrease on the eighth day of germination time (Table 2). Form the above studies, it may be concluded that the activity of different amino transferases may vary quantitatively from one species to another during seed germination and seedling growth.

Table 2. Variation of alanine amino transferase specific activity during seed germination of rice, wheat, ragi and bajra

Days of germination	Rice	Wheat	Ragi	Bajra
0	0.0±0.0	1.9±0.2	4.89±0.5	14.67±0.5
2	7.0±0.4	10.56±1.1	7.16±0.7	8.28±0.7
4	22.8±1.4	6.5±0.6	12.32±1.3	1.94±0.6
6	1.77±0.7	5.3±0.7	1.96±0.6	4.18±0.4
8	0.0±0.0	10.34 ± 0.8	3.0±0.8	7.70±0.8

In the present study it was observed that a great variation of alanine amino transferase activity in due course of germination. There was an increase on a particular day with further decrease and there were increase and decrease and increase pattern in the seeds during germination. The increase in the alanine transaminase may be associated with increase in gluconeogenesis. Due to high level of protease activity as reported in our earlier studies [19]. The liberated amino acid may undergo amino transamination by this enzyme and become keto acids. These keto acids may undergo oxidation to provide energy for the growing axis. The initial high level of the activities of this enzyme appears to decline steadily which may be due to a low declining rate of protein synthesis. However, in the case of bajra, a decline of amino transferase was observed till 6th day and further a slight increase was observed. Difference in the onset and relative magnitude of alanine transaminase activity has been observed in rice, wheat, ragi and bajra during germination, In conclusion, the result obtained from this study indicate developmental pattern of β-galactosidases and alanine amino transferase are different. Further work is in progress in investigating the isoenzyme during germination and its regulation which may help in understanding the physiology of seed germination in cereals and millets.

The comparison of amino transferase and β--galactosidase on different days of germination in rice, wheat, ragi and bajra shows the activity and day of the activity is different which may be due to the difference in the activation of enzyme production which differs from the cereals and millet. Further investigation on its isoenzyme pattern and their activity correlation on different days of germination may reveal the mechanism and regulation of the above enzymes in cereals and millets.

4. CONCLUSION

The amino transferase and β--galactosidase enzymes activity increased differentially to an extent on the different days on germination in rice, wheat, ragi and bajra. This indicates the developmental pattern of β-galactosidases and alanine amino transferases are different in different species depending on the requirement for the developing seedlings and their physiology.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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