

Changes of the Enzymes Activity During Germination of Different Mungbean Varieties

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Abstract

The changes in the contents of enzymes activity of the seed of three varieties of mungbean were analysed at different hour of germination. Amylase and invertase activity were tremendously increased 200-220 % and 165-175 % respectively at 24 hour of germination and decreased gradually from 48-96 hour of germination. Protease activity was remarkably increased at 24 hour then further increased upto 48 hour (131-161%) and then decreased from 72-96 hour of germination. BARIMung-3 variety showed the best result i.e the highest amount of enzymes activity among the three varieties of mungbean at 24 hour of germination.

Introduction

Seed germination was regarded as a series of steps which normally occur prior to the emergence of the radicle from the seed coat (Mayer and Shain, 1974). During germination of seeds, a massive breakdown of the reserve substances begin with the help of amylolytic, proteolytic and lipolytic enzymes and the products are transported to the growing seedlings for their development. The remaining small amount of proteins represents enzymes concerned in metabolic processes during seed development and germination (Miller and Thomson, 1975).

Different proteins, enzymes and minerals play significant roles in various ways at different times of seed germination. Germinated

seed and grain showed an increase in proteins, minerals, vitamins and enzymes from 25 to 4,000 percent (Azulay, 1997). Protease activity during germination was studied in mungbean (Chrispeels and Boutler, 1981) and legume seeds (Lichenfeld *et al.* 1981). Amylase and invertases are the important hydrolytic enzymes which are found in plants. The activity of amylase and invertase were studied on legume seeds (Koshiba and Minamikawa, 1983 and Morohashi, 1982).

Mungbean (*Vigna radiata*) is one of the major pulses grown in Bangladesh. BARIMung-2, BARIMung-3, and local variety of mungbean have been selected to study the enzyme activity viz. amylase, invertase

and protease during different germination period.

Materials and Methods

Different varieties of mungbeans were collected from Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur. There were three treatments viz. Mungbean (Local), BARIMung-2 and BARIMung-3. For each treatment 20 seed of any one kind were placed on water soaked filter paper in each sterilized petridish. Distilled water was applied to the experimental seed at an interval of 24 hours. Completely Randomised Design (CRD) were followed for the placement of petridishes on the laboratory bench with three replication petridishe for each treatment. Then enzyme activities were carried on at 0 (without germination), 24, 48, 72 and 96 hour of germination.

Preparation of crude enzyme extract

The mungbean (2g) was grinded in a mortar with cold 0.1M phosphate buffer of respective P^H (for amylase- P^H 6.7, for invertase and protease- P^H 7.0) and finally crushed into paste using a homogenizer. The temperature was maintained at 4°C by putting ice in the outer chamber of the homogenizer. The suspension was then filtered through few layers of cheese cloth in the cold room. The filtrate was collected and clarified further by centrifugation in a refrigerated centrifuge at 10,000 rpm for 15 min at 4°C.

Amylase activity was assayed by the method as described (Jayaraman, 1981). One percent starch solution was used as substrate (1 g in 100 ml of 0.1M phosphate buffer, P^H 6.7). The amylase activity was measured by estimating the release of maltose calculated from the standard curve prepared with maltose. One unit of amylase activity was defined as the amount required for liberating 1 mg of maltose in 15 min at 37°C.

Invertase activity was assayed by the method as described (Mahadevan and Sridhar, 1982) using sucrose as substrate. The invertase activity was measured by estimating the release of glucose calculated from the standard curve prepared with glucose. One unit of invertase activity was defined as the amount required for liberating 1 mg of glucose in 15 min at 37°C.

Protease activity was assayed by the method as described (Reimerdes and Meyer, 1976) using milk protein casein as substrate. The protease activity was measured by estimating the release of tyrosine calculated from the standard curve prepared with tyrosine. One unit of protease activity was defined as the amount required for liberating 1 mg of tyrosine in 30 min at 45°C.

Least Significant Difference (LSD) is used for comparing the treatment means (Steel and Torrie, 1960).

Results and Discussion

The changes of enzyme activity of the three varieties of mungbean under different germination condition are presented in the Table I. During the different germination period, the highest amylase activity was found at 24 hour in BARI Mung-3 variety (5.05 unit/g) followed by BARI Mung-2 (4.85 unit/g) and local variety (4.65 unit/g), while the amylase activity was decreased drastically from 48 to 96 hours due to germination. The present findings indicated that amylase activity increased tremendously i.e from 200 to 220 % at 24 hours of germination and thereafter decreased. The maximum decrease was 50 to 55 % at 96 hours of germination.

The maximum invertase activity was observed in BARI Mung-3 (1.10 unit/g) at 24

hours of germination followed by BARI Mung-2 (1.04 unit/g) and local variety (0.96 unit/g). However, the invertase activity decreased gradually from 48 to 96 hours. Results also showed that the highest increase was 165 to 175 % and maximum decrease was 40 to 50 % at 24 and 96 hours respectively.

BARI Mung-3, showed the highest protease activity (8.16 unit/g) at 48 hours followed by local variety (7.78 unit/g) and BARI Mung-2 (7.14 unit/g). At 24 hours, the protease activity was also found to increase but it was decreased gradually from 72 to 96 hours among the three varieties of mungbean. The present observation indicated that protease was remarkably increased from 131 to 161 % and then decreased but the decrease was maximum 30 to 35 % at 96 hours.

Table I. Activities of amylase, invertase and protease of mungbean during the germination

Activity	Variety	Hour of germination				
		0	24	48	72	96
Amylase (Unit/g)	Mungbean (Local)	1.53	4.65	4.10	2.45	1.1
	BARI Mung-2	1.56	4.85	4.34	2.52	1.22
	BARI Mung-3	1.58	5.05	4.38	2.54	1.25
LSD (0.05)		NS	NS	NS	NS	NS
Invertase (Unit/g)	Mungbean (Local)	0.96	2.62	2.30	1.40	0.75
	BARI Mung-2	1.04	2.76	2.45	1.50	0.85
	BARI Mung-3	1.10	2.95	2.56	1.58	0.92
LSD (0.05)		NS	NS	NS	NS	NS
Protease (Unit/g)	Mungbean (Local)	3.05	6.32	7.78	5.58	3.70
	BARI Mung-2	3.08	6.50	7.14	5.70	3.76
	BARI Mung-3	3.12	6.65	8.16	5.82	3.85
LSD (0.05)		NS	NS	NS	NS	NS

Amylase and invertase activities were found to be increased tremendously at 24 hours and then declined gradually which are in agreement with the results of previous studies (Koshiba and Minamikawa, 1983 and Morohashi, 1982). Similar result for the changes of enzyme activity was also reported (Azulay, 1997).

Conclusion

The enzymes activity of three varieties of mungbeans were analysed at different hours of germination. The present investigation clearly revealed that amylase and invertase activities increased at 24 hours and then decreased drastically at 96 hours of germination. But the protease activity increased at 48 hours of germination and then decreased.

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