



Profiling and Isoenzyme Analysis of Green Gram Leaves by Using Different Organic Manures

Anbuselvi S*, Jeyanthi Rebecca

¹Industrial Bio Tech, Bharath University, Chennai, India.

*Corresponding author's E-mail: anbuselvichennai@yahoo.com

Accepted on: 23-02-2016; Finalized on: 31-03-2016.

ABSTRACT

Molecular study of plants gives the better idea for the cultivation of plants which having importance commercially. The protein content of green gram is very high and commercially, it having more in demand. By the help of better manure if yielding will be high, then the economical condition will be increased. Now-a-days green manuring is gaining popularity as a method that successfully improved soil productivity. Green manuring with legumes, adds organic residues conserves, recycles plant nutrients and protects the soil from erosion. In this study the researcher have done the comparison of protein profiling and isoenzyme analysis in green gram leaves by three different organic manures.

Keywords: Isoenzyme analysis, Ammonium sulphate Precipitation, Peroxidase analysis, Native PAGE.

INTRODUCTION

Organic manures are derived from decaying material of plant or animal origin. Inorganic manures, also know as fertilizer, are derived from chemical and nutrients, such as nitrogen that is trapped by bacteria in the soil¹. Legumes form a symbiotic association with certain soil bacteria such as rhizobia. This bacteria colonize the root hairs of legumes and multiply swellings which become nodules. The bacteria benefit from the legumes which convert nitrogen into ammonium salts in the soil². Soil productivity is an important concern for farmers. Green manuring is gaining popularity as a method that successfully improves soil productivity. Now a days use different organic manures such as seaweed manure, cowdung, vermicompost, coir waste and peat mass have been used to increase the fertility of the soil³. The organic manure helps to grow the plants faster when compared with chemical fertilizer. Isoenzyme analysis is based on the existence of enzymes with similar or identical specificity, but different molecular structure eg, Peroxidase. Peroxidases are those enzymes which are involved in the transfer of oxygen from peroxides to substance to be oxidized. This enzyme catalyses a host of reactions in which hydrogen peroxide is a specific oxidizing agent and a wide range of substrates act as electron donors⁴.

MATERIALS AND METHODS

Protein Extraction from Leaves of Green Gram (*Vigna radiata*)

Leaves were collected from different pots which were grown by using three different manures (Table 1). Then the leaves were grinded with phosphate buffer (P^H 7.5) and it was centrifuged for 15 minutes. After centrifugation, the supernatants were collected and protein concentration was estimated by Lowry's method⁵.

Desalting columns are pre-packed, ready to use columns for group separation between high and low molecular weight substances. The desalting columns are prepared by packing size exclusion matrix. The matrix is beds of cross linked dextran with epichlorohydrin. The matrix allows excellent resolution with good flow rates. The fractionation range for globular proteins is between 1000-5000Da.

Sample Preparation

500 mg of leaves from three different plants were weighed. The weighed samples were grinded properly followed by the addition of 1.5 ml of Phosphate buffer (pH7). This was then centrifuged for 15 minutes and the supernatant was collected. 150 µl of the supernatant was used in finding the enzyme activity, protein content and the specific enzyme activity of the crude enzyme and the remaining volume was subjected to gel filtration and subsequent measurement of the enzyme activity of the filtered enzyme.

Ammonium Sulphate Precipitation and Sample Loading

The 150 µl supernatant was used for determining the specific activity of the crude enzyme by the method of spectrophotometrically at 620 nm.

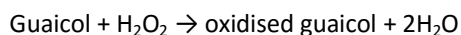
Isolation of Peroxidase by Native Page

The supernatant was electrophoresed in starch or polyacrylamide buffered (non denaturing) stack gel at a low temperature (4-8)°C. Each lanes should be loaded with equal amount of proteins after normalizing the proteins content in exact in small volume as possible (25-50 µl) later electrophoresis the gel is incubated in a solution containing all the necessary components for enzyme reaction. The colored reaction product stains the gel where the enzymes were located. The sample leaves were collected from the plants which were grown by



three different manures such as seaweed, coir waste and peat mass. 1 gm of sample was homogenized with phosphatase buffer, centrifuged and the supernatant were collected. 30 μ l of supernatant was mixed with 5 μ l of native sample buffer. The sample was loaded carefully and gel was running using a power pack. The entire setup was maintained in a refrigerator at 4°C because the protein does not get denatured. Then the gel was removed from the glass plates and incubated in staining solution (7% acetic acid). It was kept on a rocker for 10 min and the zymogram obtained was photographed⁶.

Estimation of Peroxidase Activity



The resulting oxidase (dehydrogenated) guaicol was probably more than one compound and depends on the reaction condition. The rate of formation of guaicol dehydrogenation product is a measure of the POD activity and was assayed spectrophotometrically at 436nm.⁷

RESULTS

Extraction of protein and isolation of isoenzyme was done successfully with the purification from the leaves of green gram (*Vigna radiata*). The growth of plants was faster in case of plants having manures in comparison to the control. The germination of seeds appeared on second day of plantation. The flowering and fruiting were occurred on the age of 51st day of plants. The extraction of crude protein and its concentration were mentioned in table 2 and isolation of peroxidase have been carried out on 48th day. The table 3 shows the partial purification of protein by desalting column. Organic manures enhance the growth of the plants. Among the four different samples the protein concentration and peroxidase activity were found to be higher in plant with coir waste than other plants (Table 4). Plants with seaweed manure showed somewhat better in protein concentration than peat mass.

DISCUSSION

Comparing with crude extract, the recovery of purified enzyme was not very high, but purity, as was shown by SDS-PAGE analysis and RZ, was very high. Therefore this method may be useful for purification of peroxidase from sources with high protein content. It has been reported

that peroxidase isoenzymes from various sources have different molecular weights ranging from 30,000-60,000 Dalton, e.g. four isoenzymes of turnip peroxidase have molecular weights between 37,000 and 57,000 Dalton and 30,000- 54,000 for Japanese radish isoperoxidases. The coir waste manure contain high amount of C: N ratio, cellulose and lignin. So it acts as a good fertilizer containing NPK having greater effect than other manures. Seaweed manure shows the better yield than the peat mass. The activity of peroxidase enzyme in crude extract is lesser than the partially purified sample. Hence it concluded that the partial purification of enzymes successfully done by the followed process. The peroxidase isoenzyme having a significant effect on the green gram plant (*Vigna radiata*).

Table 1: Types of Manures Used

Sample No.	Manures Used
1	Control without manure
2	Seaweed+aminoacid
3	Peatmass
4	Coirwaste + BGA

Table 2: Crude Protein concentrations of leaves

Sample	Amount of Sample Taken (mg)	Protein Concentration (mg/ml)
1	500	2880
2	500	3441
3	500	3360
4	500	3680

Table 3: Partial purification of protein using desalting and its concentration

Sample	Amount of Sample Taken (mg)	Protein Concentration (mg/ml)
1	100	400
2	100	548
3	100	520
4	100	660

Table 4: Partial purification of peroxidase

S. No.	1		2		3		4	
	C	PP	C	PP	C	PP	C	PP
Sample	C	PP	C	PP	C	PP	C	PP
Volume (ml)	3	3	3	3	3	3	3	3
Protein Content	2880	390	3441	527	3360	480	3680	448
Total Activity	0.06	0.091	0.093	0.124	0.12	0.135	0.16	0.163
Specific Activity	0.02	0.233	0.027	0.235	0.035	0.281	0.043	0.357



The NATIVE PAGE bands for peroxidase after its partial purification

Figure 1 observed the native PAGE band for enzyme specifically the peroxidase. The appearance of band explained the presence of peroxidase isoenzyme.

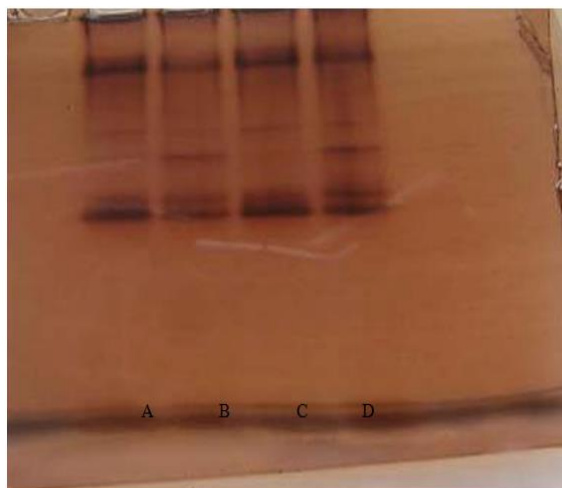


Figure 1: Native page (A-well contain sample 1, B- well contain sample 2, C- well contain sample 3 and D- well contain sample 4)

CONCLUSION

Protein profiling and isoenzyme analysis has done successfully. The protein content of green gram (*Vigna*

radiata) in sample no. 4 is higher than the other plants. The coirwaste manure shows the better yield than the other manures. This is due to symbiotic nitrogen fixation in legumes and nutrient holding capacity of coir waste. The activity of peroxidase enzyme is high of green gram (*Vigna radiata*) plants. Mostly from this work we concluded that the enzymatic activity of coir waste manure treated leaves were found to be higher than the other pots.

REFERENCES

1. Boller, E. Hani. Manures and Soil Amendments. Ideal book on functional biodiversity at the Farm level: 2004, 78.
2. Wagner, Georg. Legume Green Manuring. Ecology. 4th Edition, TataMcGraw Hill: 2002, 45-54.
3. Azam F., Ashaf M., Mahmood T., Qureshi .M. Effect of Manures on Legumes, J. Biology and Fertility, 4(2), 2004, 2-8.
4. Mazza G., Job C. and Bouchet M. Chemical composition and hydrodynamic characteristics of turnip peroxidases. J. of Env. Science. 14(2), 1998, 218-223.
5. Lowry O.H., Roserbrough N.J., Fail A.L. 'Protein measurements with foilin phenol reagent', J. of Biology and Chemistry. 19(3), 1951, 65-75.
6. Laemmli U.K. Manual techniques in molecular biology. 13(3), 2000, 23-30.
7. Putter, J. Methods of enzymatic analysis, 14th edition, Academic press Newyork: 1998, 685.

Source of Support: Nil, Conflict of Interest: None.