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ORIGINAL ARTICLE

Catalase Enzyme Activity and Protein Estimation In Different Varieties if Sorghum (*Sorghum bicolor*)

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ABSTRACT
Protein estimation and catalase (Sorghum bicolor) was carried out in four different varieties of sorghum. Seeds of varieties viz., DSV 4, DSV 5, CSV 216R and M35 washed with pinch of mercuric chloride and repeatedly washed with distilled water were sown in sterile soil in pots. Estimation of protein is done through Folin-Cio-Calteau method. DSV-4 showed the highest concentration at 660 nm and CSV 216R showed least concentration in standard BSA graph. The blue coloured bands were observed immediately against bright background. The blue coloured bands indicated the presence of catalase enzyme which protect cells from toxic by product produced in cells . catalase isoforms were prominent in shoot samples and faint bands were observed in root samples.
Key Words : *Protein estimation , Sorghum,Mercuric chloride , Folin-Cio-Calteau method, Catalase enzyme , Isoforms*

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INTRODUCTION

Graminoids are a broader group which includes the grasses. They are monocotyledons, usually herbaceous plants with narrow leaves growing from the base. They include the "true grashehees", of the family Poaceae (also called Gramineae), Grasses are an important food for many animals including human being. Grasses range from tiny inconspicuous herbs less than an inch to the giant bamboos that grow up to 130 feet tall. Jowar(Sorghum bicolor) (L.)Moench) is one of the most important cereal crops in the world and is one of the major food grains of our country. Sorghum is an important crop of the dry land regions in our country. It is cultivated in *rabi* mainly for food purposes and in *kharif* for food, feed and fodder uses. it has immense potential as a high biomass and biofuel crop. Sorghum is well adapted to drought environments compared to other cereals [1-4], making it suitable for semi-arid tropical (SAT) agricultural production systems.

Sorghum is a self-pollinate diploid (2n=2x=20) C4 grass with a high photosynthetic efficiency. It has small genome size (730Mbp about 25% size of maize or sugar cane) is fully sequenced.

Catalase is a very common enzyme that is present in all organisms that are exposed to oxygen. Catalase protect the cell from toxic of by-products such as H_2O_2 generated as by product of cell metabolism the catalase enzyme helps getting rid of these compounds by breaking up hydrogen peroxide (H_2O_2) into harmless water and oxygen. However the complete mechanism of catalase is not currently known, the reaction is believed to occur in two stages:

$$H_2O_2 + Fe(III)\text{-}E \rightarrow H_2O + O{=}Fe(IV)\text{-}E(.+)$$

$$H_2O_2 + O{=}Fe(IV)\text{-}E(.+) \rightarrow H_2O + Fe(III)\text{-}E + O_2$$

Here Fe(III)-E represents the iron center of the heme group attached to the enzyme. Fe(IV)-E(.+) is a mesomeric form of Fe(V)-E, meaning the iron is not completely oxidized to +V, but receives some stabilising electron density from the heme ligand, which is then shown as a radical cation (.+)

With this concern, to investigate presence of catalase enzyme Activity in four varieties of sorghum was constructed.

MATERIAL AND METHODS

The Four Varieties of Sorghum Seed viz . DSV-4, DSV-5, CSV-216R, M-35 were procured from ARS Hitnalli , Vijaypur ,Karnataka.

Healthy and insect free seeds were washed them with pinch of mercuric chloride, and repeatedly washed with sterile distilled water. seed were sowed in pot with sterile soil & allowed to grow for 2-3 weeks . The roots and shoots were ground in a pre-chilled pestle and mortar in an ice-cold 50mM phosphate buffer pH (7.2). The extract was centrifuged at 4°C for 30min at 10,000 rpm. Supernatant obtained was used for Protein Estimation and also for determination of enzyme activity.

Fig1: The image is showing the length of shoot and root of each vareity of sorghum.

RESULTS AND DISSCUSION

Estimation of Protein done by FCR (Folin-Cio-Calteau Reagent) Using Standard Protein (BSA)solution And supernatant obtained from all four varieties DSV-4, DSV-5, CSV-216R, M-35. We quantified the protein by Lowry method. We compared with standard graph of protein estimation DSV-4 showed the highest concentration at 660 nm and it is more concentrated when Compared with other protein samples of sorghum plants. And CSV-216R showed the least concentration comparing with standard BSA graph.

Table 1: Quantification of protein in four different varieties of Sorghum

Sl no	standard protein(ml)	ml of diluents	Total volume	Alkaline copper reagent (ml)	FCR reagent	OD at 660nm	Conc. µg/ml
1	0.0	1.0	1.0	5.0	0.5	0.0	00
2	0.2	0.8	1.0	5.0	0.5	0.175	40
3	0.4	0.6	1.0	5.0	0.5	0.252	80
4	0.6	0.4	1.0	5.0	0.5	0.331	120
5	0.8	0.2	1.0	5.0	0.5	0.355	160
6	1.0	0.0	1.0	5.0	0.5	0.417	200
7	DSV- 4 1.0	0.0	1.0	5.0	0.5	0.492	210
8	DSV- 5 1.0	0.0	1.0	5.0	0.5	0.372	140
9	CSV-2161.0	0.0	1.0	5.0	0.5	0.345	130
10	M-351.0	0.0	1.0	5.0	0.5	0.437	180

Native Gel Was Run For All 4 Proteins Sample With Help of Electropheresis Unit At 110v For 2hours. Then Gel is Stained And Distained. Resolved Gel With Sample Was Taken For detecting catalase activity gel was incubated the gel in 25mM H_2O_2 solution (0.01%) for 10min. Then equal volume of warm 0.5% potassium fericyanide and 0.5% ferric chloride added. Blue Coloured band against Bright background was observed; the presence of catalase enzyme the band disappears within 1-2min. Hence the reaction was stopped by adding 7% acetic acid. The catalase isoforms were observed in all sorghum plant varieties. The shoot

showed the prominent bands than roots which showed faint bands. Thus Confirms The Presence of Catalase Enzyme in Sorghum Varieties [5-6].

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