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Research article

Comparative studies on some biochemical parameters of *Cajanus scarabaeoides* (L.) Thouars and *Cajanus cajan* (L.) Millsp.

Joyeeta Dey*, Puja Rani Saha, Priyanki Debnath and R. K. Sinha

Plant Physiology and Biochemistry Lab., Department of Botany, Tripura University, Tripura, India

*****Corresponding Author: joyeeta.dey10@gmail.com

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Abstract: Studies of some biochemical parameters in wild *Cajanus scarabaeoides* was carried out and compared with *Cajanus cajan* as its cultivated relative. *C. scarabaeoides* is considered to have a potent source of genetic variation carrying genes for resistance to various biotic and abiotic stresses and other morphological traits. Comparative analysis of biochemical parameters revealed distinct genomic diversity between the two species. Electrophoretic study of leaf isoperoxidases in two species of *Cajanus* indicated the presence of species specific variability in terms of relative mobility values of isozymes. Higher values of total amino acids, sugar and protein content in *C. scarabaeoides* also indicates ethnobotanical significance of the species as compared to widely cultivated *C. cajan*.

Keywords: *Cajanus* species - Biochemical attributes - Isoperoxidase - Genomic diversity.

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INTRODUCTION

Cajanus scarabaeoides (L.) Thouars belongs to the family Fabaceae, locally known as Bonkalai. This species is the closest wild relative to *Cajanus cajan* (L.) Millsp. [Pigeon pea]. *C. scarabaeoides* is reported to be used as ethnomedicine by the tribal healers around different states of India. Tribal people of Madhya Pradesh use the plant decoction as a tonic after delivery; the fresh leaf paste is applied on swellings of leg. The pods are also eaten for this purpose. In Bihar tribal people use the root paste and use it with coconut oil to check falling hairs to cure baldness (Sharma & Kumar 2013). Native people of Andhra Pradesh use this plant to cure piles (Murty & Rao 2010), skin diseases (Rao *et al*. 2006). This plant is also used as fodder and effective in reducing diarrhea in cattle. Besides, the species is an important weed legume with various ethnomedicinal properties. It also possesses wound healing, anti-diabetic, anti-inflammatory, hepatoprotective, anti-diarrheal, anti-bacterial activities (Pattanayak *et al.* 2009, 2011). Significance of *C. scarabaeoides* as a source of ethnobotanicals in terms of carotenoid content was recorded (Dey & Sinha 2015). *C. scarabaeoides* was also reported to have higher levels of draught tolerance, and resistance to insect pests compared to cultivated types (Tikka *et al.* 1997). The genetic traits of drought tolerance and the higher levels of resistance to insect pests of *Cajanus scarabaeoides* can be utilized to improve the crop's productivity of *Cajanus cajan* (Sharma *et al.* 1987, Upadhyaya *et al*. 2011). In view of the above context, the present study is aimed to study certain biochemical attributes of wild *C. scarabaeoides* and its comparision with cultivated *C. cajan*.

MATERIALS AND METHODS

The two species, *Cajanus cajan* (L.) Millsp. and *Cajanus scarabaeoides* (L.) Thouars were collected in an around Suryamaninagar area (Fig. 1) of West Tripura district; having a geographical location of N 23º45′40.6″ and E 091º16′04.3″. The healthy plant materials were collected during the month of March, 2016. Herbarium specimen of respected species were prepared and identified with the help of floristic literature (Deb 1983, Hooker 1885). The herbarium specimen with accession number TU/BOT/471 and 472 were assigned for *C. cajan* and *C. scarabaeoides* respectively and submitted to the herbarium of the Department of Botany, Tripura University for reference (Fig. 2).

Healthy fresh leaves were used as a source of ethnobotanicals purposes and thus biochemical investigation

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of respective species were performed to estimate the total soluble sugar with Anthrone Reagent by the method of Yemm & Willis (1954). In this, 500 mg freshly harvested leaves of plants were homogenized in 10 ml of 50% aqueous ethanol with a pinch of activated charcoal. The slurry was centrifuged at 5000 rpm for 10 min and free amino acids were extracted in the form of a clear supernatant. The volume of supernatant was raised to 10 ml with aqueous 50% ethanol. To 1 ml of the supernatant 2 ml of 2% freshly prepared anthrone reagent was added. The absorbance of the green coloured complex was taken at 620nm. Glucose was used as standard.

Figure 1. Map showing the study area (Suryamaninagar) of the West Tripura district, Tripura.

Figure 2. A, Herbarium specimen of *Cajanus cajan*; **B,** Herbarium specimen of *Cajanus scarabaeoides*.

The amount of total free amino acid was estimated following the method of Yemm & Cocking (1955). Freshly harvested leaves (500 mg) of plants were homogenized in 10 ml of 50% aqueous ethanol with a pinch of activated charcoal. The slurry was centrifuged at 5000 rpm for 10 min and free amino acids were extracted in the form of a clear supernatant. The volume of supernatant was raised to 10 ml with aqueous 50% ethanol. To 1 ml of the supernatant 2 ml of 2% Ninhydrin (w/v in dehydrated alcohol) was added. The mixture was kept on water bath at 75±2 ºC for 10 minutes and after cooling, aqueous alcohol (1:1) was added to make up the volume to 3 ml. The absorbance of the violet complex was measured at 570 nm on a spectrophotometer. The amount of total free amino-acids was calculated with the help of a standard curve prepared from glycine and was expressed as mg of amino acids per gram fresh weight of the sample.

The phosphate buffer soluble protein was estimated following the procedure of Lowry *et al.* (1951). Fresh leaves (500 mg) were crushed in 5 ml cold potassium phosphate buffer solution (pH 7.5; 0.1 M) on ice-bath using pestle and mortar. The homogenate was filtered through cheese cloth and the filtrate was centrifuged at 12000 rpm for 25 min in a refrigerated centrifuge. The supernatant was taken in a 10 ml measuring cylinder and

its volume was raised to 10 ml with same buffer. The pellet was used for insoluble protein. This supernatant contains soluble protein. In 1 ml of supernatant 1 ml of tri-chloro acetic acid (TCA) solution (10%, w/v) was added in a centrifuge tube. Immediately a whitish or cream coloured precipitate appeared. The precipitate was centrifuged at 5000 rpm for 5 min and after centrifugation the supernatant was discarded and the pellet contained precipitate of soluble protein. To this pellet 2 ml of ethyl alcohol (95%) was added and stirred the precipitate so as to remove the TCA sticking on the surface of the protein as well as to remove pigments, if any. The tube containing the precipitate was re-centrifuged (1000 rpm for 5 min) and the supernatant was discarded. The pellet was used for estimation of soluble protein using reagents prescribed in the Lowry method and the absorbance of blue colour complex was read at 690 nm using Spectrophotomter. BSA was used as standard protein.

The amount of total soluble phenol were determined by the following the methods Swain & Hillis (1959). For this 500 mg fresh leaves were taken and crushed with 80% chilled ethanol. Then the slurry was centrifuged at 5000 rpm for 20 mins. The supernatant was collected and evaporated to dryness. After drying the pellet was dissolved in water and this was used as aliquot. From this aliquot 0.05ml was taken in a test tube and 0.5 ml Folin reagent, 2.5 ml distilled water and 2 ml saturated sodium carbonate solution were gradually added. The OD of the blue coloured complex was taken at 560 nm . Tannic acid was used as a standard.

Assay of *in vivo* Nitrate Reductase was measured in fresh leaves by the method of Hageman & Hucklesby (1971). Fresh 500 mg leaves were cut into thin strips (2 mm × 3 mm size) from respective plant species and incubated in the assay mixture containing 4 ml of potassium-phosphate buffer (0.1 M; pH 7.4), 0.5 ml KNO_3 (100 mM) and 0.5 ml of 5% aqueous propanol. After 30 mins of incubation period at 32±2 ºC 1 ml of incubated assay was transferred to a test tube contain 1 ml of sulphanilamide and 1 ml of NED mixture. The pink coloured complex was used for determination of nitrite spectrophotometrically at 540 nm. Sodium nitrate was used as standard. Antioxidant activity in the leaves was measured by following the method of Patel & Patel (2011).

For isozyme study slab vertical polyacrylamide (without SDS) gel electrophoresis (Laemmli 1970) was adopted. In this process, 500 mg freshly harvested leaves were taken and homogenized in 3 ml extraction buffer in cold; and the homogenate was centrifuged at 12,000 rpm for 45 mins at 4ºC for 7–8 times. The supernatant was collected and used as the material source for isozyme study. For the isozyme study, the vertical PAGE was performed without using SDS. Respective mean values of the biochemical attributes were compared following the simple students's 't' test between the means.

RESULTS AND DISCUSSION

Total free amino acid and soluble sugar was found to be higher in the leaves of *C. scarabeoides* (Table 1) whereas the amount of total soluble phenol was significantly higher in *C. cajan*. Efficiency of nitrate reductase activity was much higher and significant in *C. cajan* though soluble protein content was almost similar in both the species. The antioxidant activity measured in the leaves of *C. scarabaeoides* was 45.54% inhibition and much less against the reported data of *C. cajan* (Mahitha *et al*. 2015).

Table 1. Comparison of some biochemical parameters of *Cajanus cajan* and *Cajanus scarabaeoides*.

Parameter	***Cajanus cajan***	***Cajanus scarabaeoides***	**t-value**
Total free amino acid ($mg.g^{-1}$ fresh wt.)	5.59 ± 0.50	7.18 ± 0.95	3.31*
Soluble sugar ($mg.g^{-1}$ fresh wt.)	6.67 ±1.20	8.34 ±1.44	1.99(NS)
Soluble phenol ($mg.g^{-1}$ fresh wt.)	1.29±0.12	0.97±0.08	4.96*
Soluble protein ($mg.g^{-1}$ fresh wt.)	28.21±1.74	28.26±1.22	0.06(NS)
Antioxidant activity	†52.12% (inhibition)	45.54% (inhibition)	-
Nitrate reductase activity (µ moles NO_2 produced $h^{-1}g^{-1}$leaf fr. wt.)	3.4 ± 0.29	1.59±0.05	7.45*

Note: *Significant at 5% level; NS= Not Significant; **Mean of five replicates (†source: Mahitha *et al.* 2015).

Cajanus scarabaeoides is having high ethnomedicinal values as it is used in tonic after delivery, fresh leaf paste or pod used to cure swelling of leg, root paste also used in night fever, dropsy, anaemia, burns and wounds by the indigenous people of Tripura (Majumdar & Datta 2013). Since leaves are also used as vegetables by

certain communities (Choudhury *et al.* 2015) , the present study highlights significant differences in terms of high soluble sugar and free amino acid content as compared to *C. cajan*. The antioxidant property and phenolic contents of the leaves are however superior in *C. cajan.* Soluble protein content in the leaves is very close between the two taxa in spite of high Nitrate Reductase activity recorded in *C. cajan.* Iso-enzyme pattern reveal remarked genetic differences between the two taxa. As many as seven isozymes were recorded in *C. cajan* with different relative mobility (Rm) values ranging from 0.08 - 0.61 compared to five isozymes ranging from 0.08–0.59 in *C. scarabaeoides* (Fig. 3). Only three isozymes were found common in both the species. In spite of differences in many biochemical traits and isozyme patterns between the two taxa, *C. scarabaeoides* is reported to be compatible with *C. cajan*. Successful F_1 hybrid production between the two taxa was already reported by other workers (Mishra *et al*. 2012). This result also suggests that genetic distance between cultivated *C. cajan* and its wild relative is not related to their hybridization barrier as also reported by Mudaraddi *et al*. (2013).

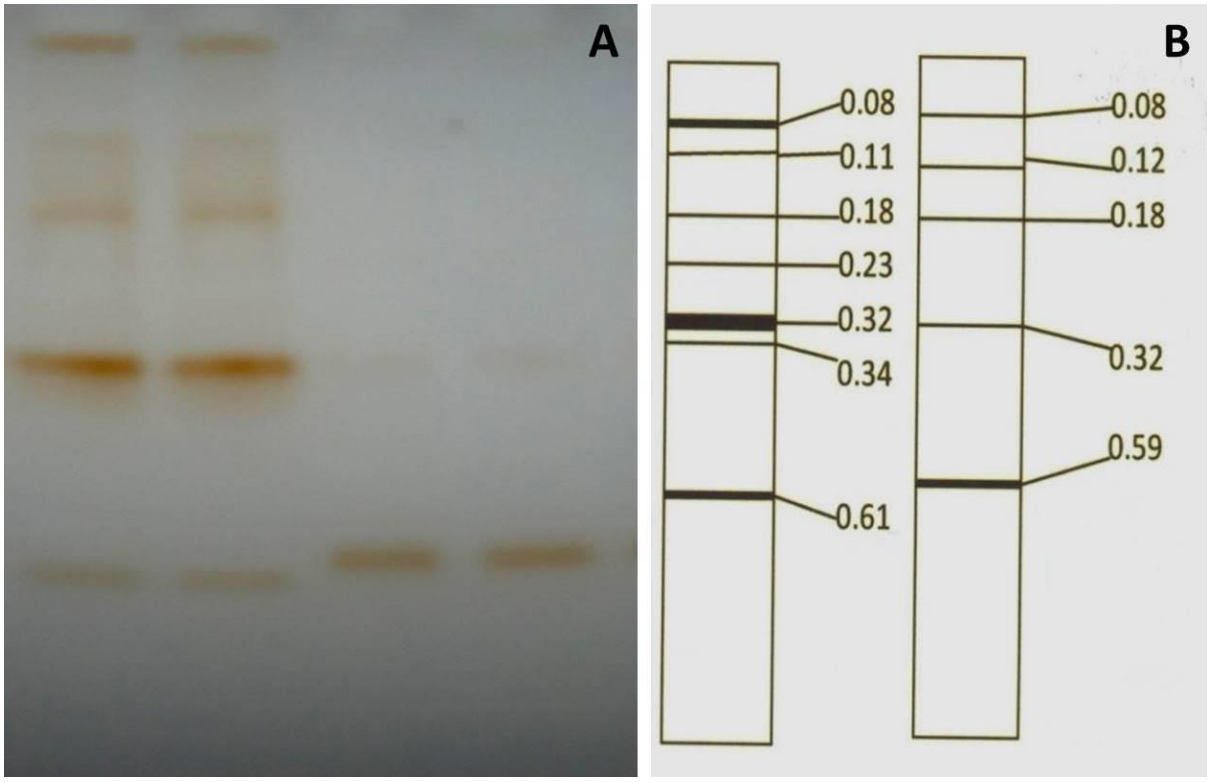

Figure 3. Zymogram pattern of leaf isoperoxidases: **A,** *Cajanus cajan*; **B,** *Cajanus scarabaeoide*s.

CONCLUSION

The present study highlights the genetic diversity of wild *C. scarabaeoides* in terms of certain biochemical attributes and its relative significant difference in free amino acids, phenol contents and nitrate reductase activities between the two species. Relatively high value of soluble sugar and protein contents is also attributing characteristic of nutritional value of *C. scarabaeoides* leaves.

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