

SHORT COMMUNICATIONS

BIOCHEMICAL COMPOSITION OF STIGMA AND STYLE IN *CYAMOPSIS* SPP.

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SUMMARY

Guar [*Cyamopsis tetragonoloba* (L.) Taub.] syn. *C. psoraliodes* (Lamk; D. C. $2n=2x=14$) belongs to family Leguminaceae, is one of the most important **kharif** legume crops and is well adapted to arid and semi-arid regions of the world. The cultivated species of guar i. e. *C. tetragonoloba* takes about 90-150 days generally for maturation. The flowers are born in axillary raceme on long petioles. The crop is considered strictly self-pollinated. *C. serrata* Schinz ($2n=14$) is a wild, early maturing (40-50 days), while the other wild species i. e. *C. senegalensis* is also slow growing annual herb with narrow pentafoliate leaves and small pods and matures in 120-130 days. Protein content of stigma and style was nearly identical in all the three species, while total soluble carbohydrate content in *C. tetragonoloba* and *C. serrata* was nearly identical (5-6 mg/100 mg FW), while it was low in *C. senegalensis* (2.4 mg/100 mg FW).

Key words : Biochemical, composition, Stigma, style, *cyamopsis*

Among leguminous crops, clusterbean [*Cyamopsis tetragonoloba* (L.) Taub.] ($2n=2x=14$), commonly called as guar and belonging to family Fabaceae, is a drought tolerant commercial crop. It possesses deep tap root system and hence, well adapted to arid and semi-arid regions of the world. The origin of the crop has been suggested in India (Vavilov, 1951) and tropical Africa (Gillett, 1958).

C. tetragonoloba is an erect herb with indeterminate growth habit. It includes both branched and unbranched varieties with broad trifoliate leaves. The flowers are borne in axillary racemes on short pedicels and, it matures in about 90-120 days. However, one of its wild relatives i. e. *C. serrata* is an early maturing (40-50 days), slow growing and branched species with narrow trifoliate leaves, while the other species i. e. *C. senegalensis* is also slow growing annual herb with narrow pentafoliate leaves and small pods which mature in 120-130 days (Menon, 1973). Both these wild relatives possess some desirable attributes like drought resistance (Menon, 1973), photo- and thermo-insensitivity (Anonymous, 1982) and disease resistance (Orellana, 1966).

MATERIALS AND METHODS

Total Soluble Carbohydrates : The total soluble carbohydrate content (mg/g FW) was estimated by the method of Yemm and Willis (1954).

Extraction : Extraction of soluble carbohydrates was done according to Barnett and Naylor (1966) procedure. Fifty mg of fresh material of stigma+style was finely ground in 80 per cent alcohol by using pestle and mortar. Total soluble carbohydrates were extracted in 2 ml of 80 per cent ethanol (v/v) on a water bath at $50^{\circ}\pm 1^{\circ}\text{C}$ for 15 min. It was then cooled and centrifuged at $5000 \times g$ for 5 min. The supernatant (extract) was kept aside and the pellet re-extracted twice with 80 per cent ethanol. Total volume of extract was made to 5 ml with 80 per cent ethanol. This extract was used for the analysis of total soluble carbohydrates, while the pellet was used for extraction of the total soluble proteins.

Reagents

Anthrone reagent : 0.4% anthrone in concentrated sulphuric acid.

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Procedure : Aliquot (0.1 ml) of ethanol extract was evaporated to dryness in a test tube. After cooling, the residue was dissolved in 1 ml of distilled water and to it 4 ml of anthrone reagent was added. The mixture was then boiled in a water bath for 10 min. After cooling, absorbance was recorded at the wavelength of 620 nm against a reagent blank with the help of UV-Vis spectrophotometer. Standard curve was prepared using graded concentration of D-glucose (20-100 mg/ml).

Estimation of Soluble Proteins

Sample preparation : Pellet left after soluble carbohydrate extraction was extracted in 1.25 ml chilled Tris buffer (0.1 M, pH 8.0) containing 0.1 per cent polyvinyl pyrrolidone (PVP). It was centrifuged at 10000 rpm for 15 min. The supernatant containing the proteins was taken in a test tube and pellet was discarded and processed for the quantification of proteins by the method of Bradford (1976).

Reagents

Commassie brilliant blue G (CBBG)-250 reagent : One hundred mg of CBBG-250 reagent was dissolved in 50 ml of 95 per cent ethanol. To this solution 100 ml 85 per cent (w/v) phosphoric acid was added and final volume was made to 200 ml with double distilled water. The solution was filtered through Whatman No. 1 filter paper, and final volume was made to 1 litre and stored at 4°C in amber colour bottle.

Procedure : To 100 ml of the aliquot taken in test tube, 5 ml of the CBBG-250 reagent was added and mixed thoroughly either by inversion or vortexing. The optimal density (O. D.) was measured at 595 nm after 15 min and before 1 h against reagent blank. Standard curve was prepared using graded concentration of bovine serum albumin (20-100 mg/ml).

RESULTS AND DISCUSSION

Stigmatic exudate is an aqueous solution of sugars which stimulates pollen germination (Brewbaker and Kwack, 1964). However, an excessive amounts of free sugars in the exudate might interfere with the pollen grains functioning if the concentration of sugar contributed significantly to the viscosity of the exudates

(Konar and Linskens, 1966; Martin 1970, Martin and Telek, 1971). Fabaceae, in general, is characterized by wet stigma. The stigma was covered with surface cells that often lyse to release viscous secretion containing proteins, amino acids, lipids, polysaccharides and pigments. These secretions not only support retention and germination of pollen grains but protect stigma against desiccation. The role of proteins in "Wet" stigmas was not clear (Esau, 1977). Mattson *et al.* (1974) emphasized on the role of proteins in hydration of pollen grains. Water, nutrients and other small molecules are transported rapidly into pollen grains from the stigma exudates by mechanism that remain unclear. Certain proteins, amino acids may promote or inhibit the growth of pollen tubes *in vitro* (Copper 1939; Sawada, 1958; Sen and Verma, 1959, 1960, 1961; Brewbaker and Kwack, 1964).

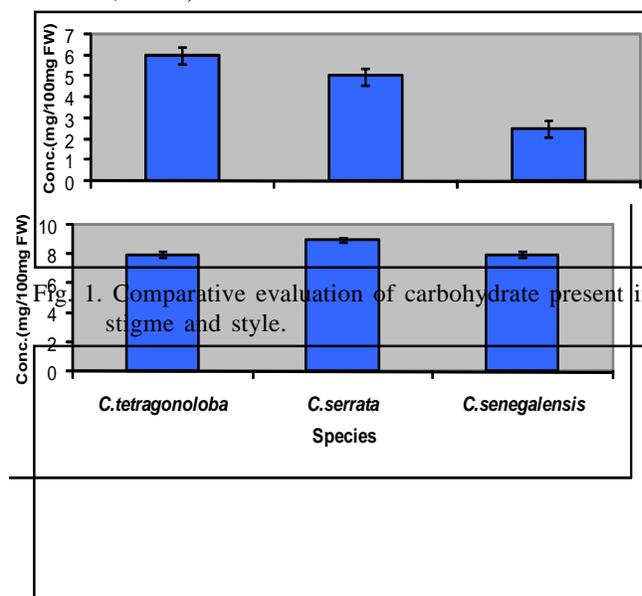


Fig. 1. Comparative evaluation of carbohydrate present in stigma and style.

Fig. 2. Comparative evaluation of protein content in stigma and style.

In present study, stigma and styles of *C. tetragonoloba* and *C. serrata* consisted of nearly identical (6 mg/100 mg FW) amount of total soluble carbohydrates (Fig. 1), while those of *C. senegalensis* consisted of 2.4 mg/100 mg FW (Fig. 2). On the other hand, protein content in all the three species was identical (8- 9 mg/100 mg FW). Lord and Webster (1979) reported 6.25 – 12.00 mg/ml proteins in the stigmatic exudate of *Phaseolus vulgaris*. However, none of the references could be found in the literature to corroborate the pressing slowly in guar.

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