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Comparative cytological and fruit biochemical studies in two populations of *Solanum torvum* Sw. - an ethnobotanical species of Northeast India

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ABSTRACT: Comparative cytological and fruit biochemical characteristics were studied between two wild populations of *Solanum torvum* Sw. to assess variability in fruit nutritional values. Constant somatic chromosome number 2n=24 was recorded in both the populations with distinct n=12 bivalents without any irregularities. Fruits of the two populations were morphometrically characterized and compared with their fruit size and average seed distribution per fruit at three developmental stages of their maturation and revealed no significant differences in average diameter of mature fruits and number of seeds per fruit. Soluble sugar content in mature fruits of population I was significantly high compared to population II. On the contrary, the level of flavonoid was found higher in population II. In addition, significant variation was also recorded in soluble protein content in different stages of fruits maturation in both the populations. Distinct variability in different biochemical attributes between the populations was recorded with 2n=24 somatic chromosome numbers. The present study highlights significance of fruit nutritional value of *S. torvum* used as non-conventional vegetable by the people of this region.

KEY WORDS: *Solanum torvum,* Somatic chromosome number, Fruit maturation, Biochemical attributes, Population.

INTRODUCTION

Tripura is a hilly landlocked state of North East India enriched with many ethonobotanicaly important plants species used by the different communities of the state. *Solanum torvum*, a wild ethnobotanical species used as a source of fruit vegetables by local tribal people of this region (Choudhury *et al*., 2010). The fruit of the species is used in the treatment of fever, cough, cold, tuberculosis, diabetes, high blood pressure, asthma, tooth decay, liver and spleen enlargement (Yuanyuan *et al.,* 2009; Jaiswal, 2012; Kannan *et al.*, 2012; Zubaida *et al.,* 2013; Priyanaka *et al.,* 2014). The ripened fruits are also used in the preparation of tonic and haemopoietic agents and also for the treatment of pain (Kala, 2005). It grows as a weed in the open field, road side and other wastelands of Tripura. The species contains alkaloid like Solasodine, torvogenin (Dopke *et al.,* 1975) and flavonoids such as torvanol A and torvoside H (Arthan *et al.,* 2002) and reported to have antimicrobial, antioxidant, sedative, digestive, haemostatics and diuretic activities (Agarwal *et al.,* 2010). The leaves of the species contain rich source of essential vital minerals, iron, magnesium, zinc, sodium, potassium, vitamins and proteins (Dickson *et al.,* 2014). The species is reported to have wide morphological variations in their wild populations indicating the possibility of existence of intraspecific variation (Kumar *et al.,* 2013) which may play significant role in species diversity of the taxon. In spite of ethnomedicinal importance there has been no significant research records in the field of cytological and biochemical studies of different populations of *S. torvum* along with its diversity and the nutritional potential. On the basis of above background, the present study was undertaken to determine the somatic chromosome number and biochemical characteristics during developmental stages of maturing fruits in two populations of S*olanum torvum*.

MATERIALS AND METHODS

Plants were collected from two different populations, *viz.* Dharmanagar (population-I) and Agartala (population-II) and their geographical coordinates were recorded (Table.1). The species was identified with the help of Flora of Tripura (Deb, 1983) and finally confirmed in consultation with Prof. B. K. Datta, Plant Taxonomy and Biodiversity laboratory, Department of Botany, Tripura University and the herbarium was submitted at the herbarium centre of Tripura University along with definite collection numbers. In addition, fruits at three developmental stages (immature, young and mature) were also collected from the respective plants of population-I and population-II for further experimental studies.

Morphometric study: Different fruit morphometric characters like fresh fruit weight, fruit diameter, number of seeds per fruit and number of seeds per 100 mg were

Table.1: Geographical coordinates of ***Solanum torvum*** Sw. at collection sites with representative accession numbers.

Place of collection	Accession number	Latitude	Longitude	Altitude (m)
Dharmanagar (Population-I)	TUH-463	N24°19'31.4"	E92°09'26.6"	34
Agartala (Population-II)	TUH-464	N23°46'14.7"	E91°15'47.3"	30

measured and presented in tabular form.

Cytological study: Mitotic metaphase chromosome of both the population were carried out following modified method of Sharma and Sharma (1980) and meiotic metaphase bivalent chromosomes was studied following the method of 2% acetocarmine staining technique. At least 50 mitotic and meiotic metaphase cells were scanned for determination of somatic chromosome number and meiotic bivalent status.

Biochemical study: Three different stages of maturing fruits such as immature 10 days old measuring 0.5-0.6 g, young 20 days old measuring 0.9-1.2 g and mature 30 days old measuring 1.5-1.8 g of *S. torvum* from two different populations were used for estimation of different biochemical parameters. Extraction of total soluble protein was carried out from the 500mg tissue sample crushed in 0.1M Tris HCl buffer (PH 6.8) and centrifuged at 14000rpm for 45 minutes at 4°C. The supernatant was collected and used for quantitative estimation of protein following the modified method of Lowery *et al*., 1951 and the calibration of optical density was done at 690 nm. Estimation of ascorbic acid as vitamin-C was done with fresh fruit crushed in 4 % oxalic acid solution and centrifuged at 5000rpm for 5 minutes. 10ml of supernatant was collected in a conical flask and 10-15 drops of bromine water was added drop by drop to remove enolic hydrogen atoms. After thorough mixing, the sample was used for quantitative estimation at 540 nm in UV Vis spectrophotometer following the method of Sadasivam and Manickam (1992).

Extraction and estimation of total soluble sugar was done following the method of Hodge and Hofreita (1962). Soluble sugar was obtained from 500mg tissue sample crushed in 50% chilled ethanol and centrifuged at 5000rpm for 20 minutes. Total soluble phenol of the fruit sample was obtained by crushing with chilled 80% ethanol and centrifuged at 5000rpm for 20 minutes. The supernatant was evaporated in boiling water and the residue thus obtained was dissolved in distilled water and used for quantitative estimation of phenol (Swain and Hillis, 1959).

The flavonoid of fresh tissue sample was determine following the method of Eom *et al.,* 2007. The supernatant was obtained by crushing the tissue sample with 80% methanol and centrifuged at 10000 rpm for 5 minutes. Optical density of the reaction mixture was measured at 415 nm in UV vis spectrophotometer. Total free amino acids sample was obtained from the fruit tissue crushed in 10 ml of 50% aqueous ethanol. The slurry thus obtained was centrifuged at 1000 rpm for 10 minutes. The clear supernatant was used for quantitative estimation of total free amino acids following the method of Yemm and Cocking (1955).

Data generated in the present study were analysed using one way and two way analysis of variance (ANOVA) and Fisher's least significant difference (LSD). A probability value of $p < 0.05$ was considered to denote a statistically significant difference.

RESULTS AND DISCUSSION

Fruit sample collected from two different geographical coordinates (Table.1) revealed no such significant variation in terms of fresh immature and mature fruit weight, fruit diameter and number of seeds per mature fruit. Significant variation was recorded in young fruit weight and number of dry seeds per 100mg unit. Fresh mature fruit weight of two populations was found to be 1.67 ± 0.17 mg (Pop-I) and 1.56 ± 0.19 mg (Pop-II) respectively. The average diameter of fruits of population-I was found to be 1.30±0.06 cm with 256.80±14.60 number of seeds and that of population-II revealed 1.27±0.04 cm with 259.40±21.00 number of seeds (Table.2). Cytological study of mitotic metaphase plate of both populations of *S. torvum* shows 2n= 24 chromosome with n=12 bivalents (Fig.1) in meiotic metaphases. This observation is in corroboration with previous records (Obute *et al.,* 2006; Das *et al.,* 2015). Our present findings clearly validate that the somatic chromosome number in the two populations are constant and also there is as such no variation in meiotic bivalent chromosomes. Biochemical estimates of fruits of *S. torvum* revealed variations in terms of contents mg/g fresh weight between the populations. Amount of protein was found higher in young fruits of both the populations with 11.1±0.70 mg/g fresh weight (pop-I) and 12.58±0.45 mg /g fresh weight (pop-II) respectively. However, a decrease in protein content was observed in mature fruits of both the populations with 9.24±0.34 mg/g fresh weight (pop-I) and 9.82±0.16 mg/g fresh weight (pop-II) respectively. The decreasing amount of protein recorded during maturation of fruits may be attributed due to degradation of pigments and phospholipids by free radicals as the trend of radical scavenging system declines (Prochazkova *et al.,* 2001) as well as the rising activities of hydrolytic and proteolytic enzymes like proteases (Rastegar *et al.*, 2012). In spite of cytological homogeneity in mitotic and meiotic cells, a significant difference in the biochemical parameters among the stages of fruits was observed in both the populations. Amount of phenol in both the species revealed characteristic increase in mature fruits with 6.10±0.32 (pop-I) and 3.40±0.19 mg/g fresh weight (pop-II) respectively. Mature fruit of population-I has

Table. 2: Comparative fruit morphometric study of ***S. torvum*** (Mean ± SD).

Populations	Fresh fruit weight in g			Mature fruit diameter in cm	No. of seeds per mature fruit	No. of dry seeds per 100 mg
	Immature	Young	Mature			
Population-I	0.57 ± 0.16	1.11 ± 0.09	1.67 ± 0.17	1.30 ± 0.06	256.80 ± 14.60	112 ± 3.31
Population-II	0.54 ± 0.05	0.97 ± 0.03	1.56 ± 0.19	1.27 ± 0.04	259.40 ± 21.00	104.4 ± 3.72
P value	0.6244	0.0003*	0.2009	0.0520	0.7922	0.0258*

Note: Mean ± SD of 10 replicates; *significant at P <0.05

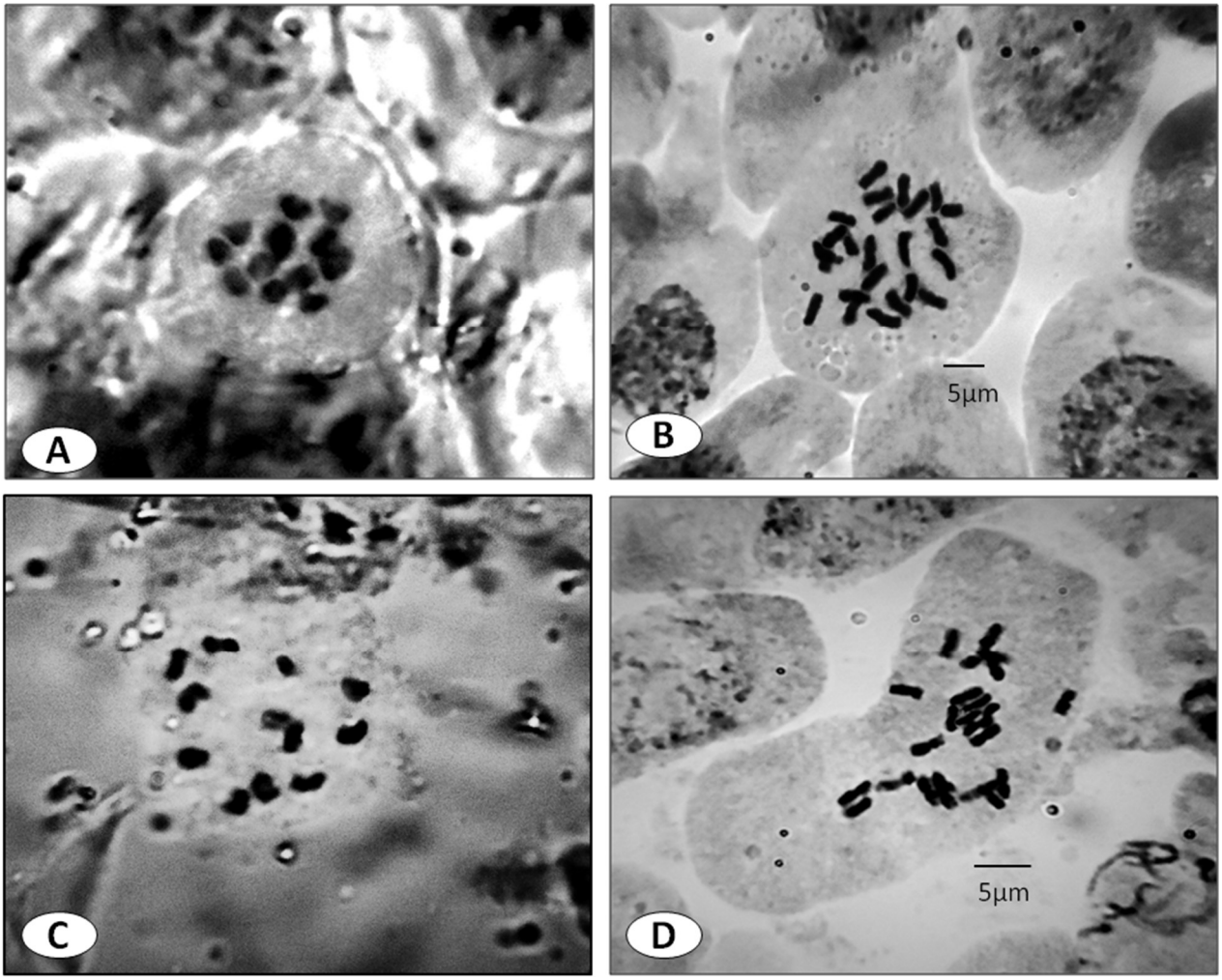

Fig. 1: Cytological study of ***S. torvum*** **A**. Meiotic metaphase of Population-I showing n=12; **B**. Mitotic metaphase of population-I showing 2n=24; **C**. Meiotic metaphase of population-II showing n=12; **D**. Mitotic metaphase of population-II showing 2n=24 (x1400)

the maximum phenol content with 6.10±0.32 mg/g fresh weight. Phenols are the secondary metabolites of plants, which have numerous health benefit effects due to its antioxidant potential (Antolovich *et al.*, 2000; Servili and Montedoro 2002). Increase in sugar level was also observed in mature fruits of both the populations whereas the highest value of total soluble sugar was obtained in mature fruits of population-I with 12.39±0.65 mg/g fresh weight. An appreciable increase in flavonoid content was noticed in all the fruits on maturation. The maximum flavonoid content was noticed in mature fruits of both the populations with 0.28±0.015 (pop-I) and 0.53±0.012 (pop-II) mg/g fresh weight respectively. However, in comparison to other biochemical content, the flavonoid levels were very low in both the populations. Vitamin-C (Ascorbic acid) was found higher in young fruits in population-I with 11.62±1.49 mg/g fresh weight as compared to the population-II. Considerable level of vitamin-C was recorded in young fruits in both the populations as compared to their immature and mature stages. The diminishing amount of vitamin- C content during maturation of fruits of *S. torvum* is in agreement with data recorded in other species (Muhammad *et al.*, 2014). Higher level of total free amino acid contents was found in young fruits with 1.48±0.05 fresh weight in

Table. 3: Comparative biochemical estimates at three developmental stages of fruits of ***S. torvum*** collected from two different populations.

Parameters	Population-I			Population-II			LSD
	Immature mg/g fr. Wt. (mean±SD)	Young mg/g fr. Wt. (mean±SD)	Mature mg/g fr. Wt. (mean±SD)	Immature mg/g fr. Wt. (mean±SD)	Young mg/g fr. Wt. (mean±SD)	Mature mg/g fr. Wt. (mean±SD)	
Soluble protein	11±0.38	11.1±0.70	9.24±0.34	12.08±0.56	12.58±0.45	9.82±0.16	0.93[a]
Soluble phenol	5.57±0.22	3.36±0.07	6.10±0.32	3.31±0.16	2.77±0.28	3.40±0.19	-
Soluble sugar	10.44±1.35	9.59±0.39	12.39±0.65	8.70±0.97	7.19±0.41	9.13±0.97	1.28[b]
Flavonoid	0.16±0.024	0.19±0.01	0.28±0.015	0.35±0.015	0.35±0.012	0.53±0.012	0.076[b]
Ascorbic acid	5.97±0.79	11.62±1.49	10.87±0.75	3.96±0.23	5.68±0.11	3.58±0.10	-
Total free amino acids	0.83±0.06	1.05±0.08	0.80±0.031	0.59±0.01	1.36±0.04	1.48±0.05	-

Note: Mean of five replicates; a. Significant difference between the stages of fruits; b. Significant difference between the populations

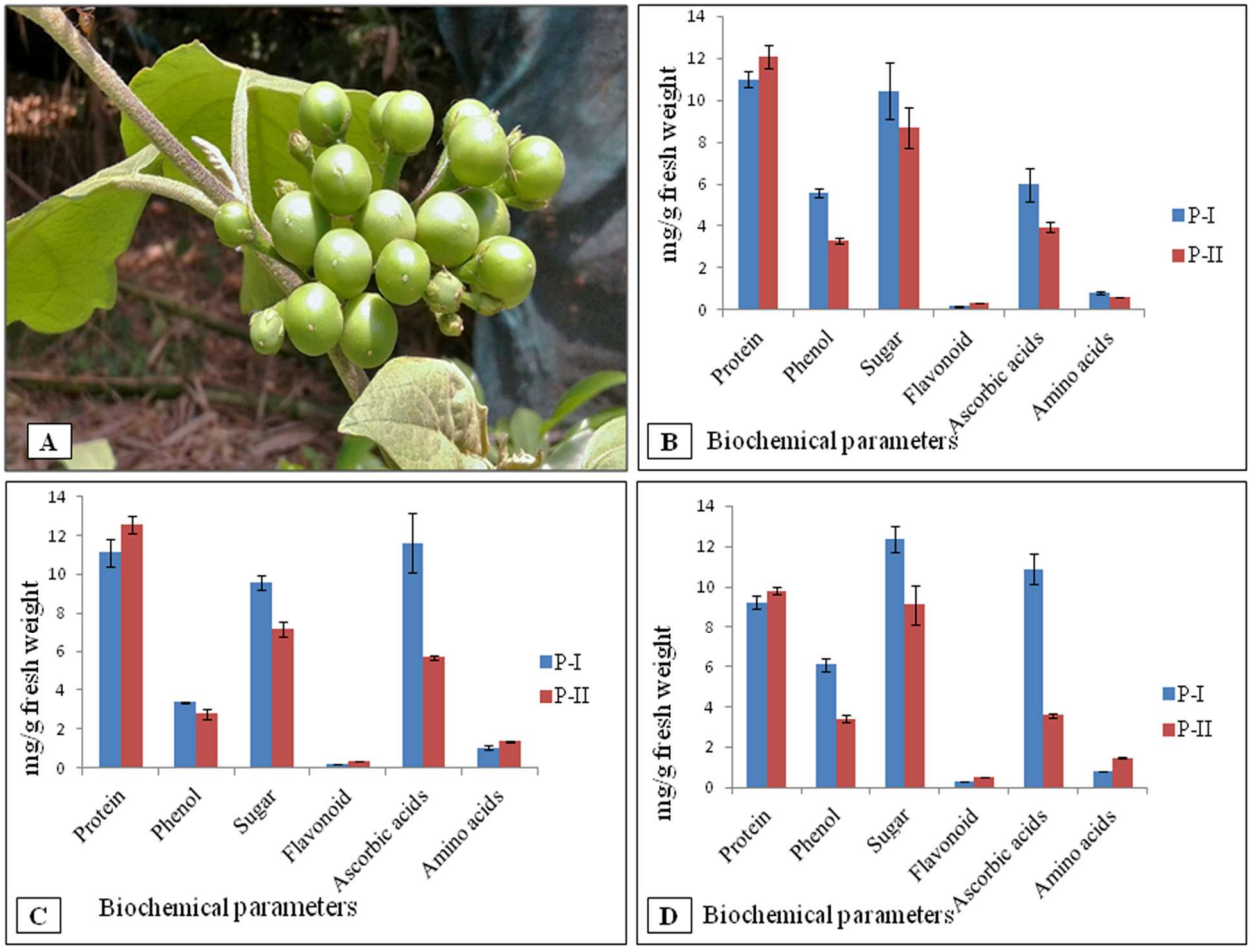

Fig.2: **A**. Fruiting twig of the ***S. torvum***. **B-D**. Graphical representaion of different biochemical attributes in three developmental stages of maturing fruits in two populations (P-I and P-II) of ***S. torvum*** **B**. Immature fruits; **C**. Young fruits; **D**. Mature fruits

population-II. The present study clearly highlights the variability in certain biochemical parameters between the two populations of *S. torvum* in spite of similar somatic chromosome number (2n=24) of the species. This variation in different biochemical contents may be attributed due to variation in fruit morphology and different eco climatic factors and the effect of soil type on fruit characteristics, which has been reported in the other fruits (Sharma *et al.,* 2010; Ugese *et al.,* 2010; Gurashi *et al*., 2016). Different biochemical characteritsics recorded in the present study was also significant in terms of sugar and flavonoid content between the two populations (Table.3 and Fig.2). Overall young fruits are nutritionally rich in terms of different biochemical contents as compared to immature and mature fruits and found to be useful as a good vegetable supplement to the local people of this region.

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