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Leaf Morphology and Stomatal Anatomy Indicates Inter-Varietal Variability in Water Chestnut (*Trapa natans* var. *bispinosa* Roxb.)

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ABSTRACT

Water chestnut (Trapa natans var. bispinosa Roxb.) is a commercially important yet underutilized aquatic, annual herb grown for its valuable fruit but it has not been documented for germplasm variability in India. Thus, 20 morphotypes of Trapa, collected from various blocks of district Lucknow (Uttar Pradesh) and maintained in artificial pit created as experimental pond at the Vocational Research Farm of our department. Significant differences were recorded in Selection – 6 among the collected genotypes for leaf characters with highest number of leaves per plant (29.67), maximum length of leaves (19.78cm), length (5.65cm) and width of lamina (8.78cm), petiole length (13.34cm) and stomatal pore width (5.16ìm) while, maximum pulvinus diameter (15.94 mm) and fruit yield (255.07 g plant^{-1}) were recorded for Selection- 12 and similarly pulvinus: petiole ratio (0.29), stomatal width (8.05ìm) and stomatal density (50ìm^{-2}) were observed maximum in Selection-14 and maximum pulvinus length (3.17cm) recorded in Selection- 10. Minimal environmental influence on the morphological expression of the characters under study is indicated by similar genotypic coefficient of variance (GCV) andphenotypic coefficient of variance (PCV) values. The highest PCV (35.44), GCV (35.07), h^2% (97.90) and genetic advance as percent of mean (147.42%) was observed for width of stomatal pore, while highest genetic advance (26.01) was observed for stomatal density. Thus, phenotypic expression of these characters was governed at the genetic level since they show high heritability contributing to genetic advance over mean and thus, these selected characters could be useful for selection traits for future crop improvement programs.

Key words: Phenotypic, Stomata, *Trapa natans* var. *bispinosa*, Variability, Water chestnut

INTRODUCTION

Water chestnut (*Trapa natans* var. *bispinosa* Roxb.) is a commercially important aquatic, angiospermic, annual, warm season herb grown for its valuable fruit but variation in its germplasm has not been documented in India. *Trapa natans* var. *bispinosa* Roxb has two forms, one is red (leaf, petiole and fruit) and the other is green (leaf, petiole and fruit) each bearing fruit large in size having two dull spines (Faruk *et al*., 2012). It is basically a tropical crop, cultivated commercially in tropical, subtropical and temperate zones of the world (Arima *et al.,* 1999). In India, it is cultivated in waterlogged areas in shallow ponds and catchment areas of irrigation canals etc. in most north Indian states (Chakor, 1974). The water chestnut fruits are nutritive and possess medicinal importance and are used in the traditional ayurvedic system of medicine, yet it is highly neglected for study of its inter variability in Indian agro-climatic situation (Babu *et al.,* 2011). This research has focussed on diversity in water chestnut on the basis of leaf morphological and stomatal characters since success of any breeding program depends upon the quantum of genetic variability available for exploitation and the extent to which the desirable characters are heritable (Tiwari *et al*., 2011) contributing to genetic advance.

MATERIALS AND METHODS

Twenty germplasm of water chestnut from the various blocks of district Lucknow of Uttar Pradesh, were collected during end of June to July, 2016 and established in ponds $2\times1\times1m^3$ in dimension at Vocational Research Farm, Department of Horticulture, Babasaheb Bhimrao Ambedkar University, Lucknow following standard management practices (Chattopadhyay, 1996).

Leaf Morphology Studies

Leaves of uniform age and physiological maturity at 6-8 internode from the apex of rosette were collected as per leaf sampling technique (Wolf, 1982). Length of leaves and lamina, width of lamina, petiole and pulvinus length (cm) was measured using measuring scale while pulvinus diameter was measured using digital vernier callipers (Mitutoyo, Japan). The experiment was laid out in randomized block design. Observed data was analyzed using Windows-based computer software ICAR-SPAR (Statistical Package for Agricultural Research) as per procedure for analysis of variance at 5% level of significance, PCV and GCV was estimated as per procedure by Burton and De Vane (1953) whereas, heritability in broad sense (h^2) and genetic advance were calculated following the method of Allard (1960).

Stomatal Anatomy Study through Scanning Electron Microscopy (SEM)

Variations in the anatomical characters of stomata were studied by scanning electron microscope (JSM-6490LV, JEOL, Japan) at University Scientific Instrumentation Centre of the university. Leaf samples were prepared as per procedure given by Fischer *et al.* (2013) with slight modifications (Fig.1).Stomatal density (μm^{-2}) was measured at 500X magnificence while, all stomatal dimensions were measured at 5000X magnificence.

RESULTS AND DISCUSSION

Variability Study by Leaf Morphology Analysis

A statistical significant variability was observed in leaf morphological characteristics of water chestnut (Table 1). The maximum values were observed for leaf number (29.67), leaf length (19.78cm), lamina length (5.65cm), lamina width (8.78cm) and petiole length (13.34cm) in Selection-6, while, maximum pulvinus diameter (15.94mm) and fruit yield (255.07g) were recorded for Selection-12 and pulvinus length (3.17cm) and pulvinus: petiole ratio (0.29) were observed in Selection-10 and 14, respectively. These leaf morphological characters helped to identify primarily the inter-varietal variability of water chestnut as also described by other scientific workers who narrated morphological characterisation is one of the simple, rapid and inexpensive methods which was conventionally applied in mango (Begum *et al*., 2014; Kishor *et al.,* 2019), in jamun (Swamy *et al*., 2017), in banana (Kundu *et al.,* 2018 and in other horticultural crops Salparni (Manivel *et al.,* 2019) and in *Gingko biloba* (Klimko *et al.,* 2015) which even facilitating the identification and classification of fragments of *Gingko* fossil leaves. But, number of these traits is limited, unstable and unable to establish variations in closely related accessions (Konarev, 2000). These may however, be controlled by epistatic and pleiotropic gene effects and face heritability problems (Begum *et al*., 2014). Thus, prime advantages of genotyping on the basis of morphological traits are simplicity and rapid, inexpensive assays, even from herbarium specimens and other dead tissues (Begum *et al.,* 2014). However, it is limited and lack decisiveness because environmental variations also affect expression of these characteristics. Thus, these morphological characters may not adequately represent the genetic variability among accessions of a single cultivar. Hence, characterization of inter-varietal variability based on morphological traits was complemented with stomatal studies through scanning electron microscopy (SEM) since stomatal initiation is controlled by both environmental and genetic factors (Casson and Hetherington, 2010) and is indicative of clonal variability.

Study on Stomatal Characters

The maximum stomatal density ($50\mu m^{-2}$) and stomatal width (8.05 µm) were recorded in Selection- 14. However, the highest stomatal length (17.43 µm) and stomatal pore length (13.52 µm) were recorded in Selection-19 and maximum stomatal pore width (5.16 µm) was recorded for Selection-6 (Table 1). Stomatal density is reported to vary within leaves, plants and individuals even of a single species (Afas, 2006) which may be due to environmental factors (Woodward and Kelly, 1995) but, is also genetically controlled by additive genes (Gailing *et al*., 2008) which indicates that selection of these stomatal characters would be more effective for crop improvement.

Table. 1 Vegetative performance and scanning electron microscopy of leaves of water chestnut (*Trapa natans* var. *bispinosa* Roxb.)

Morpho types	Number of leaves	Length of leaves (cm)	Length of lamina (cm)	Widths of lamina (cm)	Petiole length (cm)	Pulvinus length (cm)	Pulvinus diameter (mm)	Pulvinus: petiole ratio	Stomatal Density (μm^{-2})	Stomatal length (µm)	Stomatal widths (µm)	Stomatal pore size (µm)		Yield g/plant
												Length	Width	
Sel-1	22.50	16.40	5.62	7.50	11.60	2.77	6.58	0.25	25	13.73	7.36	9.12	2.48	201.60
Sel-2	24.34	12.87	4.55	5.09	7.65	1.80	5.34	0.25	38	13.88	6.04	9.91	3.44	101.44
Sel-3	29.67	13.49	4.55	5.45	9.47	2.29	7.34	0.26	47	12.95	4.68	8.68	1.40	76.67
Sel-4	27.17	12.50	4.20	4.22	7.02	1.60	3.84	0.19	31	15.24	6.56	8.68	2.20	97.67
Sel-5	23.50	16.90	4.74	5.82	12.29	2.32	8.30	0.24	29	17.00	6.28	12.81	3.44	119.54
Sel-6	29.67	19.78	6.44	8.78	13.34	2.88	7.80	0.22	39	16.00	6.68	12.78	5.16	139.77
Sel-7	25.34	18.44	5.14	6.87	13.18	3.05	7.86	0.23	46	12.55	6.85	6.94	1.84	105.80
Sel-8	24.84	17.04	5.12	6.80	12.95	2.97	7.39	0.23	44	11.53	6.4	8.08	3.72	103.67
Sel-9	26.00	17.22	5.55	5.65	12.35	2.74	11.13	0.24	40	14.88	6.76	8.42	3.04	139.00
Sel-10	21.67	17.30	5.29	7.69	12.67	3.17	8.49	0.27	39	14.28	5.29	8.54	1.92	129.04
Sel-11	22.00	16.20	5.15	5.77	10.28	2.42	10.97	0.24	38	14.44	4.76	10.78	1.76	104.37
Sel-12	22.67	16.79	5.53	8.53	12.53	2.92	15.94	0.27	35	15.44	6.76	11.27	1.60	255.07
Sel-13	24.50	13.30	4.22	5.25	9.29	2.20	9.27	0.24	48	11.80	5.44	8.24	2.72	80.67
Sel-14	27.67	13.79	4.99	5.67	9.23	2.53	10.58	0.29	50	13.33	8.05	8.22	3.44	113.40
Sel-15	27.17	13.14	4.69	5.83	9.35	2.40	9.18	0.28	42	15.73	5.48	10.61	2.56	133.80
Sel-16	26.67	11.93	4.30	5.43	7.47	1.68	5.33	0.23	42	13.20	6.52	8.59	3.64	82.60
Sel-17	24.50	14.87	4.55	5.45	9.33	2.07	7.92	0.22	44	13.40	6.56	9.36	6.56	93.04
Sel-18	23.84	16.17	4.70	5.82	10.67	2.37	8.75	0.23	44	14.16	7.52	10.28	3.83	145.22
Sel-19	23.34	17.09	4.78	6.38	12.10	2.53	9.80	0.22	42	17.43	5.53	13.52	1.84	191.43
Sel-20	23.67	17.40	4.52	6.10	12.85	2.35	8.47	0.20	46	11.48	5.92	6.64	2.36	246.60
SEM	1.461	1.050	0.333	0.342	0.464	0.143	0.478	0.013	1.559	0.756	0.363	0.763	1.559	5.260
CD	6.399	4.599	1.458	1.497	2.032	0.626	2.093	0.060	2.067	1.353	0.649	1.365	2.790	23.038

Table 2 Estimates of PCV, GCV, heritability, genetic advance and genetic advance as percent of mean in water chestnut (*Trapa natans* var. *bispinosa* Roxb.)

Characters		Grand Means	Minimum	Maximum	PCV	GCV	h^2 (%)	GA	GAM (%)
No. of leaves		25.03	21.67	29.67	10.11	0.13	0.00*	0.00*	0.00*
Length of leaves (cm)		15.62	11.93	19.78	17.70	13.34	56.80	6.67	42.70
Length of lamina (cm)		4.92	4.20	5.62	15.37	9.96	42.00	1.33	27.03
Widths of lamina (cm)		6.20	4.22	8.78	20.70	18.35	78.60	4.28	69.03
Petiole length (cm)		10.78	7.02	13.34	19.17	17.65	84.80	7.43	68.92
Pulvinus length (cm)		2.45	3.84	15.94	20.01	17.26	74.40	1.54	62.85
Pulvinus: Petiole ratio		0.23	1.60	3.17	10.65	3.22	9.10	0.00*	0.00*
Pulvinus diameter (mm)		8.51	0.19	0.29	21.02	18.64	78.60	5.97	70.15
Stomatal density (μm^{-2})		40.43	25.00	50.00	16.51	15.82	91.8	26.01	64.33
Stomatal length (µm)		14.12	11.48	17.43	13.10	11.34	74.9	5.89	41.71
Stomatal widths (µm)		6.25	4.68	8.05	15.23	13.47	78.2	3.15	50.40
Stomatal pore size (µm)	**Length**	9.55	6.64	13.52	21.55	19.20	79.4	6.94	72.67
	Width	2.72	1.40	5.16	35.44	35.07	97.9	4.01	147.42
Yield g/plant		133.01	76.67	255.07	6.86	0.34	0.30	0.10	0.077

*Very negligible

The extent of variability among genotypes was further determined in terms of PCV and GCV estimates which were found very close (Table 2) and reveal that most of the stomatal characters expressed appear to be independent of the environmental effect in their phenotypic expression (Majumder *et al*., 2012). The characters having high genotypic coefficient of variation, phenotypic coefficient of variation, heritability and genetic advance indicate predominance of additive gene action for these characters and these characters would have possibilities of selection towards desired direction (Ogunniyan and Olakojo, 2014) for further breeding programme. In the present experiment it was observed that the highest PCV (35.44), GCV (35.07) and heritability (97.9) were recorded for stomatal pore size (width). While, the highest genetic advance (26.01) was observed for stomatal density. So, stomatal pore size had very good distinguished parameter to specify and characterise the inter variability of water chestnut.

Genetic variability was recorded based on morphological studies which was further elucidated with the help of stomatal studies through SEM. Similar values for PCV and GCV for morphological as well as stomatal characters indicated genetic control of their phenotypic expression since the influence of the environment was minimal in case of water chestnut. Significantly high values for PCV, GCV heritability and genetic advance were recorded for stomatal characters as well as some morphological traits *viz*., stomatal density, petiole length indicating that these traits would have possibilities of selection in desired direction. Present study has significance since development of superior varieties in this hitherto, underutilised crop like water chestnut could have far reaching impact on developing an alternative crop which has the capacity to grow in waterlogged, degraded lands.

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