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Capsule growth and calyx protein content in *Hibiscus sabdariffa* L. var. sabdariffa

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Abstract: Protein rich calyx of sorrel (*Hibiscus sabdariffa L.* var. sabdariffa) is used in herbal tea, drink, jam and jelly preparations. Capsule growth and calyx protein content were determined at 7, 15, 30, 45 and 60 days after anthesis (DAA) to find out appropriate time of calyx harvest and to determine physiological maturity (PM) of seeds. Results revealed that maximum dry weight of seeds or PM was observed at 45 DAA with good protein contents (9.09%). Results further revealed that calyx size and fresh weight also reached maximum at PM identified by deep red colour with capsule beginning to shatter. This is the first report on capsule maturity and calyx protein content in Bangladesh that capsule may be harvested at PM for higher calyx yield with good protein content in sorrel.
Key words: Sorrel, PM, growth, DM, calyx yield and protein.

Introduction

Sorrel or roselle (*Hibiscus sabdariffa* L. var. sabdariffa) is a short-day and multipurpose shrub of agroforestry species in Malvaceae in the tropics and subtropics. Sorrel can be grown as a monocrop or in the different agroforestry systems in fallow highland, roadside, backyard and other unutilised public places (Islam *et al*., 2008). Hence, this species would not compete with rice land. The most important uses of sorrel worldwide include: fiber crop, leaf vegetable, and refreshing beverage from calyx (Purseglove, 1988). The main edible part is persistent and accrescent fresh and dry calyx. The fresh calyxes are rich in protein and carbohydrates and traces of fiber, calcium, iron, carotene and vitamin C (Teske and Trentini, 1995). According to Babalola (2000), calyxes of the red and dark red-coloured type are extracted and sweetened to produce refreshing drink while calyxes and leaves of the green type are used for making vegetable stew.

The fruit is an oval dehiscent capsule, 2.0 cm long, where seeds are inserted and are covered by the fleshy calyx developing after fecundation, which in addition to persistent; it develops and surrounds the fruit (Vidal and Vidal, 1990).The harvest is timed according to the ripeness of the seed. Maximum dry mass (DM) accumulation in seed is considered as physiological maturity (PM) (Copeland and McDonald, 1995). The right stage of PM is essential to preserve seed and to obtain high percentage of seed germination. Usually attainment of seed PM is followed by maximum size and weight of the fruits and other floral parts (Fakir and Abdullah, 2007; Fakir and Hossain, 2010; Rahman *et al.* 2010). Harvesting capsule at PM, therefore, would provide higher calyx yield in sorrel (Islam *et al.* 2008). So the best time to obtain higher calyx yield, good nutrient and seed, maturity depends upon the fruit growth stages after pollination and fertilization. The wet red fleshy calyxes are harvested after the petals have dropped but before the seeds have completely dried and shattered. The longer time the capsule remains on the plant after the seeds begin to ripen, the more susceptible the calyx is to sores, sun cracking, and general deterioration in quality. Harvesting of fleshy calyx and dry seeds for maximum yield, almost simultaneously, would be required. McClaleb (1998) highlighted the importance of harvest before capsules dry and open, for in addition to making handling difficult, drying does not become uniform, undervaluing the quality of calyxes. According to Castro *et al*. (2004) the period of the start of flowering till complete maturation of calyxes ranged in sorrel from 30 to 40 days. Although information on the capsule growth and yield in sorrel is available in the world literature there is only one report on capsule growth in Bangladesh (Islam *et al*., 2008). Therefore, it is necessary to carry out research on this aspect in Bangladesh. The present research was aimed to investigate the growth of calyx and capsule and calyx protein content for assessment of optimum harvesting time for calyxes and capsules.

Materials and Methods

The experiment was conducted at the Experimental field of the Department of Crop Botany, Bangladesh Agricultural University (24°75′ latitude and 90°50′ longitude, elevation 18 m), during April 2006 to April 2007. The soil was silt loam, poorly drained. Standard fertilization was practised. Seeds were sown in well prepared plots using 1m x 1m spacing in triplicates. There were 16 plants in $4m^{-2}$ plots. Randomised complete block design was followed. Six seeds were sown each pit at 2-3 cm depth on 15 April, 2006. Finally, one healthy plant pit^{-1} was kept at 15 days after sowing (DAS). Weeding and mulching were done whenever necessary. During seedling establishment stage, watering was done but later no irrigation was required due to rainfall in April. For yield, maturity of the crop was ascertained when 70-80% of capsules were dry and turned brown. Plants were harvested between first and second week of April, 2007. Capsule yield per plant was estimated from separately tagged plants but seed yield/plant was obtained by harvesting all the plants in a plot.

Four guarded plants per replicate were selected for capsule growth study, and flowers were tagged at the beginning of anthesis so that data on different ages of capsule i.e. 7, 15, 30, 45 and 60 days after anthesis (DAA) were obtained. At each stage, 60 capsules, twenty from each replicate, were randomly harvested and were enclosed in polythene bag to avoid loss of water. Length and diameter of calyx, epicalyx and capsule were recorded. Freshly harvested capsules were separated into pedicel, epicalyx, calyx, pericarp and seeds. Fresh and dry (80 ± 2^0C for 48h) weights of different plant parts were measured at each stage. The crude protein content of calyx was determined by AOAC (1990). The collected data on various characteristics were complied and analysed statistically. The mean differences were evaluated by

Duncun's Multiple Range Test (Gomez and Gomez, 1984).

Results and Discussion

Capsule dry matter and calyx protein: Dry matter content of different parts of capsule generally increased with increasing ages (days after anthesis, DAA) reaching highest between 45 and 60 DAA (Table 1). Seed dry mass was the highest at 45 DAA and did not increase significantly thereafter indicating physiological maturity (PM). Dry calyx and seed yields also reached peak at PM indicating highest calyx yield could be obtained at PM. Protein content also increased from 7.09% at 30 DAA to 9.09% 45 DAA and 11.02% at 60 DAA (Table 1). The dry yield of calyx and seed plant^{-1} at 45 DAA (48.89 and 84.12%) did not increase much thereafter at 60 DAA (49.79 and 84.24%) (Table 1). Results indicated that maximum seed yield and capsule maturity was concurrently accompanied by highest calyx yield.

Table 1. Dry matter and protein (dry wt basis) contents of capsule and seed at different days after anthesis (DAA) in sorrel (*Hibiscus sabdariffa* L. var. sabdariffa)

Age (DAA)	Dry matter content (%)					Calyx protein (%)
	Thalamus plus pedicel	Epicalyx	Calyx	Pericarp	Seed	
7	25.26 c	12.91 d	15.36 b	34.96 d	45.33 c	-
15	34.96 b	26.15 c	16.67 b	46.2 c	35.26 d	-
30	42.06 a	37.30 b	18.26 a	74.66 b	60.42 b	7.09 c
45	43.58 a	42.93 ab	19.50 a (48.89)	87.79 a	92.86 a (84.12)	9.09 b
60	43.71 a	46.87 a	19.34a (49.79)	88.29 a	92.86 a (84.24)	10.11 a

In a vertical column values having same letter(s) do not differ at 5% level. Each figure is the mean of 60 capsules. **- :** data not available, Figures within parenthesis are dry wt plant^{-1} (g).

Capsule growth and maturity: Generally capsule length increased linearly and significantly up to 45 DAA (Fig. 1A). Ovary length followed a quadratic trend which was also similar to that of capsule length. Capsule diameter followed a sigmoid pattern of growth (Fig. 1B). In contrast, epicalyx length was significantly greater at 60 DAA (.88 cm) than at 7 and 15 DAA (Fig. 1C). Calyx length linearly increased up to 45 DAA (Fig. 1 D). Ovary diameters became greater at 45 DAA (1.80 cm) than at the other ages and it followed a quadratic pattern of growth (Fig. 1F). The fresh weight of different parts of capsule was significantly different at different DAA (Fig. 2). Thalamus plus pedicel fresh weight became maximum at 30 DAA followed by a decline (Fig. 2A). Seed fresh weight followed a trend that was similar to growth of thalamus plus pedicel fresh weight (Fig. 2F). Epicalyx fresh weight was significantly greater at 30, 45 and 60 DAA than at 7 and 15 DAA. Generally, calyx grew larger with aging. In contrast, calyx fresh weight increased linearly and significantly up to 45 DAA (Fig. 2C). Pericarp fresh weight increased up to 30 DAA followed by a decline in later stages (Fig. 2D). Pericarp generally grew larger with ages. Fresh weight of calyx plus pericarp increased linearly up to 40 DAA followed by little increase (Fig. 1G-E). Dry weight of thalamus plus pedicel became greater at 30 DAA than at other ages (Fig. 2G). Epicalyx dry weight increased linearly and significantly up to 45 DAA (Fig. 2H). Dry weight of calyx and seed

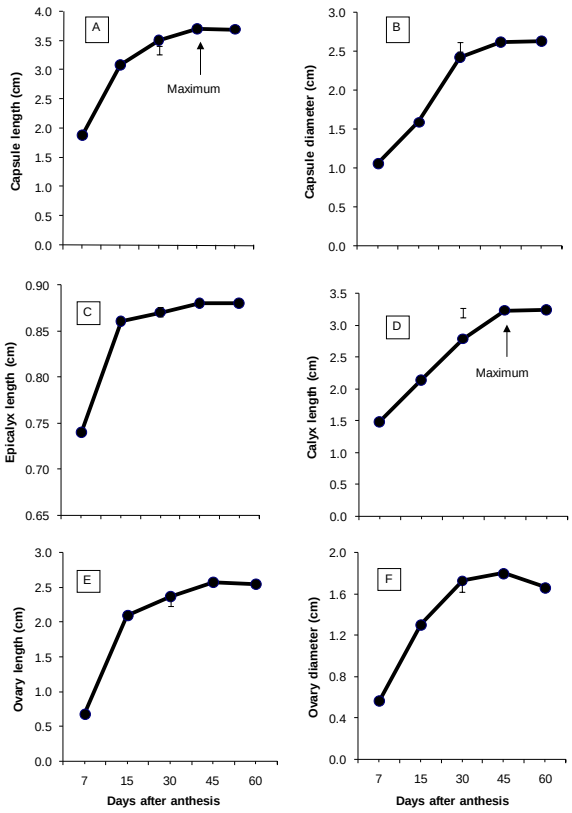
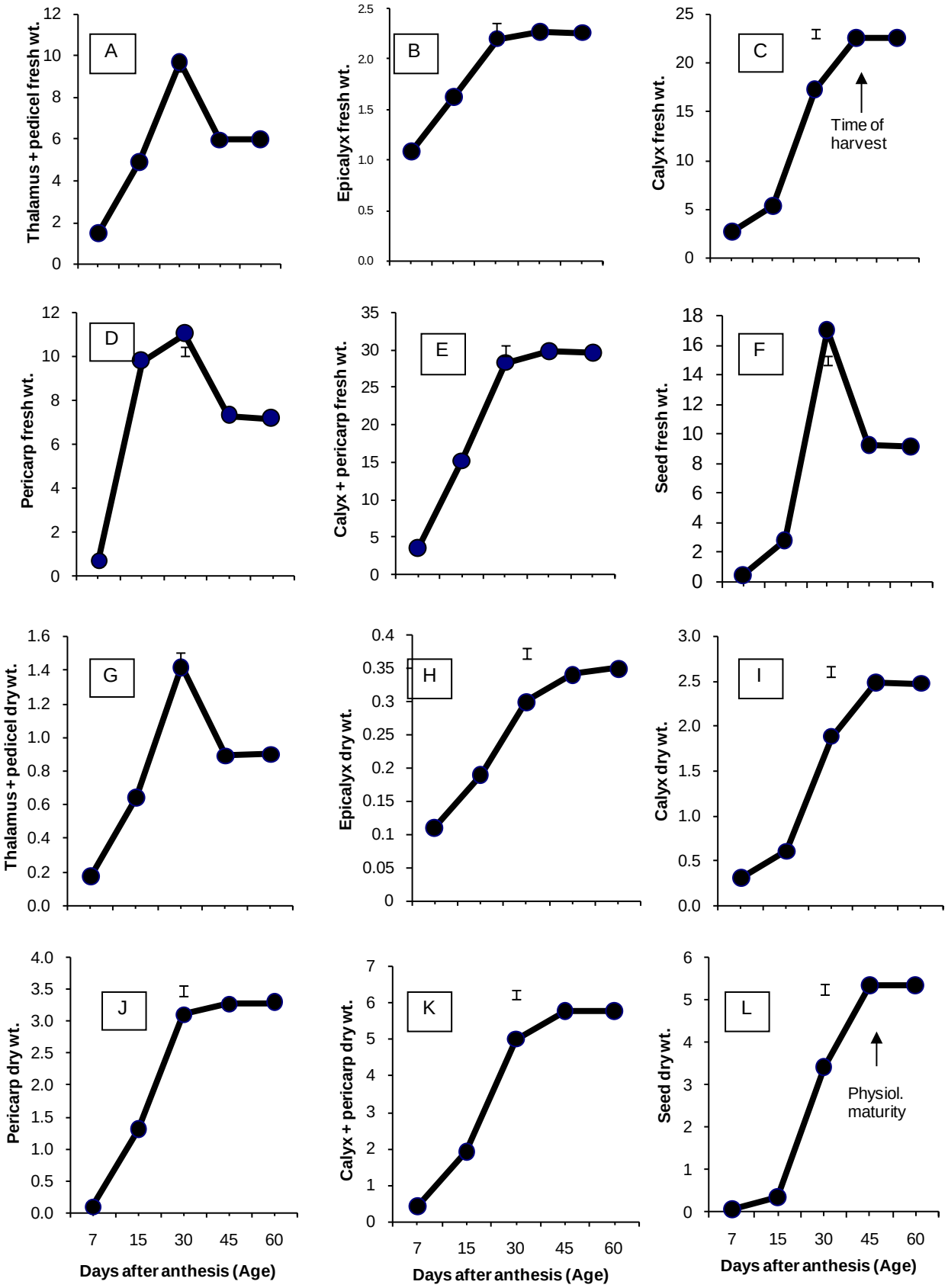


Fig. 1. Change in capsule length (A), capsule diameter (B), epicalyx length (C), calyx length (D), ovary length (E) and ovary diameter (F) at different days after anthesis in sorrel (*Hibiscus sabdariffa* var. sabdariffa)



F8.5ig. 2. Change in fresh weight of thalamus + pedicel (A), epicalyx (B), calyx (C), pericarp (D), calyx + pericarp (E), seed (F); dry weight of thalamus + pedicel (G), epicalyx (H), calyx (I), pericarp (J), calyx + pericarp (K), and seed (L) (g/10 capsules) at different days after anthesis in sorrel (*Hibiscus sabdariffa* var. sabdariffa). Vertical bars are $Lsd_{0.05}$.

followed a trend also similar to that of epicalyx dry weight. Change in dry weight was similar between pericarp and seed. In both cases, growth was very slow at earlier stage, rapid and linear at intermediate and reached a plateau at maturity. Determination of optimum time of calyx harvest is an important consideration for high calyx yield and quality in roselle (Castro *et al.*, 2004). In the present investigation size of capsule, epicalyx, calyx and ovary became maximum around 45 DAA (Figs. 1 and 2) and fresh and dry weight of calyx also became maximum by 45 DAA (Fig. 2). These data indicate that optimum time for calyx harvest is around 45 DAA and capsule at this stage is characterised by deep red coloured calyx. This is similar to the report by Kalavathi *et al.* (2001) who also identified the stage by changing capsule colour from green to brown, and concluded that beyond this stage shattering of seeds occurred.

Physiological maturity (PM) is characterised by maximum dry weight accumulation in seed. In this study, maximum dry weight in seed was achieved around 45 DAA (Fig. 2, Table 1) indicating PM; hence, capsule may safely be harvested for seed at PM in roselle. The current result agrees with the report of Raifa *et al.*, (2005) who also found that the most suitable harvest age for roselle sepals is between 45 and 55 days after the appearance of flowers. Optimum time of fresh calyx and capsule harvest was around 45 DAA since size and weights of capsule, epicalyx, calyx and ovary were the highest around that time.

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