Research Article

COMPARATIVE CARBOHYDRATES STATUS IN LEAF DEVELOPMENTAL STAGES OF *CLEOME* SPECIES

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ABSTRACT

Carbohydrates are the major micronutrients containing carbon, hydrogen and oxygen atoms in their structures. The carbohydrates are divided roughly into three groups the monosaccharides, the oligosaccharides and the polysaccharides. There are marked variations in the amounts of carbohydrates in various parts of plants and also in kinds of carbohydrates present, which will help to understand taxonomic relationship within different species. In *Cleome*, carbohydrate content is highest in *Cleome viscosa* mature leaves, while lowest in *Cleome simplicifolia* senescent leaves. It is evident from results that total carbohydrate content stored in such pattern that *Cleome viscosa> Cleome gynandra> Cleome chelidonii> Cleome simplicifolia> Cleome speciosa.*

Keywords: *Cleome,* soluble sugar, starch, carbohydrate, leaf developmental stages.

INTRODUCTION

As carbohydrates are the direct products of photosynthetic carbon fixation and act as precursors for the respiratory process and serve as a main source of energy essential for growth and development of plants. There are marked variations in the amounts of carbohydrates in various parts of plants and also in kinds of carbohydrates present. Carbohydrates are accumulated largely in parenchyma cells and death of a cell is accompanied by incorporation or withdrawal of these reserves[1]. There are several cases in which sugar plays a role as a signaling molecule that regulates a variety of genes[2].

MATERIALS AND METHODS

Carbohydrates were estimated from different leaf developmental stages within five *Cleome* species according to the method described by Nelson[3]. 0.5 g oven dried plant material was homogenized in mortar with pestle and extracted with 80% alcohol. It was filtered through Bucher's funnel using Whatman No.1 filter paper. The residue on filter paper was washed with 80% alcohol repeatedly. All the washings and filtrate were mixed together. This filtrate was used for estimation of soluble sugars while the residue was saved for estimation of starch.

a) Reducing sugars:

The filtrate was condensed on the water bath to about 2-3 ml and to it, were added Lead acetate and Potassium oxalate (1 g each) to decolourise. It was mixed together with the help of glass rod with the addition of some water. It was again filtered and washed with distilled water 2-3 times, colleting the washings in the same filtrate. The final volume of filtrate was made 50 ml with distilled water. This filtrate was used for estimation of reducing sugars (A).

b) Total sugars:

From the above filtrate, 20 ml were taken into a conical flask and hydrolyzed with 2-3 ml conc. HCl in an autoclave at 15 lbs pressure for half an hour. The contents were cooled, neutralized with Na_2CO_3 and filtered. This filtrate was used for the estimation of total (reducing + non-reducing) sugars (B). The volume of the filtrate was noted down.

c) Starch:

The residue on the filter paper saved for starch estimation was transferred to a conical flask with 50 ml of distilled water and 3-5 ml of conc. HCl. This was hydrolyzed, neutralized and filtered as stated above. This filtrate contains reducing sugars produced as a result of hydrolysis of starch. The sugars so available were estimated to determine the starch present in the tissue (C). The volume of the filtrate was also noted down.

The requisite quantity, 2ml of the above filtrates A and B and 0.1 ml of filtrate C were taken separately in 10 ml marked test tubes. 1 ml of alkaline copper tartarate reagent- (4 g $CuSO_4.5H_2O$, 24 g anhydrous Na_2CO_3, 16 g Na-K- tartarate and 180 g anhydrous Na_2SO_4 were dissolved in distilled water and volume was made to 1000 ml) was added to each test tube.

All the test tubes containing the reaction mixtures were subjected to boiling water bath for about 10 min and then cooled to room temperature. 1ml of arsenomolybdate reagent (25 g ammonium molybdate in 450 ml distilled water and to this were added 21 ml of conc. H_2SO_4. This was mixed with solution containing 3 g sodium arsenate dissolved in 25 ml distilled water. The mixture of the solutions was placed in an incubator at 37°C for 48 hours) was added to each test tube and shaken vigorously. The volume of the reaction mixture in each test tube was made 10 ml with distilled water. A blank was prepared in the same way but without sugar solution. After 10

minutes, the absorbance was read at 560 nm on double beam spectrophotometer (Shimadzu, UV-VIS 190). A standard curve of glucose (0.1mg.ml^{-1}) was prepared and the sugar content was calculated.

Table 1: Carbohydrate Content* of the Leaves of *Cleome viscosa* L.

Plant part	Statistical parameter	Reducing Sugars	Total Sugars	Starch	Total Carbohydrates
YL	Mean	0.09	0.28	26.32	26.60
	S. D.	0.02	0.02	0.10	0.08
	S. E. of Mean	0.01	0.01	0.06	0.05
ML	Mean	0.23	0.56	28.08	28.64
	S. D.	0.02	0.02	0.82	0.33
	S. E. of Mean	0.01	0.10	0.18	0.19
SL	Mean	0.30	0.47	22.77	23.24
	S. D.	0.04	0.03	0.22	0.20
	S. E. of Mean	0.02	0.02	0.13	0.11

Table 2: Carbohydrate Content* of the Leaves of *Cleome simplicifolia* (Camb.) Hook f. & Thoms

Plant part	Statistical parameter	Reducing Sugars	Total Sugars	Starch	Total Carbohydrates
YL	Mean	0.24	0.99	11.55	12.53
	S. D.	0.03	0.01	0.11	0.13
	S. E. of Mean	0.02	0.01	0.07	0.07
ML	Mean	0.13	1.36	12.36	13.72
	S. D.	0.01	0.02	0.21	0.19
	S. E. of Mean	0.01	0.01	0.12	0.11
SL	Mean	0.31	0.65	7.51	8.16
	S. D.	0.02	0.04	0.10	0.14
	S. E. of Mean	0.01	0.02	0.06	0.08

Table 3: Carbohydrate Content* of the Leaves of *Cleome chelidonii* L.f.

Plant part	Statistical parameter	Reducing sugars	Total Sugars	Starch	Total Carbohydrates
YL	Mean	0.070	0.55	17.75	18.30
	S. D.	0.008	0.04	0.13	0.17
	S. E. of Mean	0.004	0.02	0.07	0.10
ML	Mean	0.093	0.75	14.50	15.25
	S. D.	0.009	0.02	0.35	0.37
	S. E. of Mean	0.005	0.01	0.20	0.21
SL	Mean	0.061	0.47	12.52	12.99
	S. D.	0.012	0.03	0.10	0.13
	S. E. of Mean	0.007	0.02	0.06	0.07

Table 4: Carbohydrate Content* of the Leaves of *Cleome gynandra* L.

Plant part	Statistical parameter	Reducing Sugars	Total Sugars	Starch	Total Carbohydrates
YL	Mean	0.08	0.12	16.64	16.76
	S. D.	0.02	0.02	0.18	0.20
	S. E. of Mean	0.01	0.01	0.11	0.12
ML	Mean	0.11	0.36	19.82	20.18
	S. D.	0.01	0.02	0.13	0.15
	S. E. of Mean	0.01	0.01	0.08	0.09
SL	Mean	0.07	0.18	17.88	18.07
	S. D.	0.01	0.02	0.21	0.22
	S. E. of Mean	0.01	0.01	0.12	0.13

Table 5: Carbohydrate Content* of the Leaves of *Cleome speciosa* Raf.

Plant part	Statistical parameter	Reducing Sugars	Total Sugars	Starch	Total Carbohydrates
YL	Mean	0.11	0.61	10.96	11.55
	S. D.	0.01	0.07	0.19	0.14
	S. E. of Mean	0.01	0.04	0.11	0.07
ML	Mean	0.22	1.03	7.75	8.76
	S. D.	0.02	0.05	0.23	0.19
	S. E. of Mean	0.01	0.03	0.13	0.10
SL	Mean	0.17	0.36	8.05	8.41
	S. D.	0.02	0.03	0.07	0.09
	S. E. of Mean	0.01	0.01	0.04	0.05

* Values in % dry weight; S. D. = Standard Deviation; S. E. of Mean = Standard Error of mean; YL= Young Leaves; ML= Mature Leaves; SL =Senescent Leaves

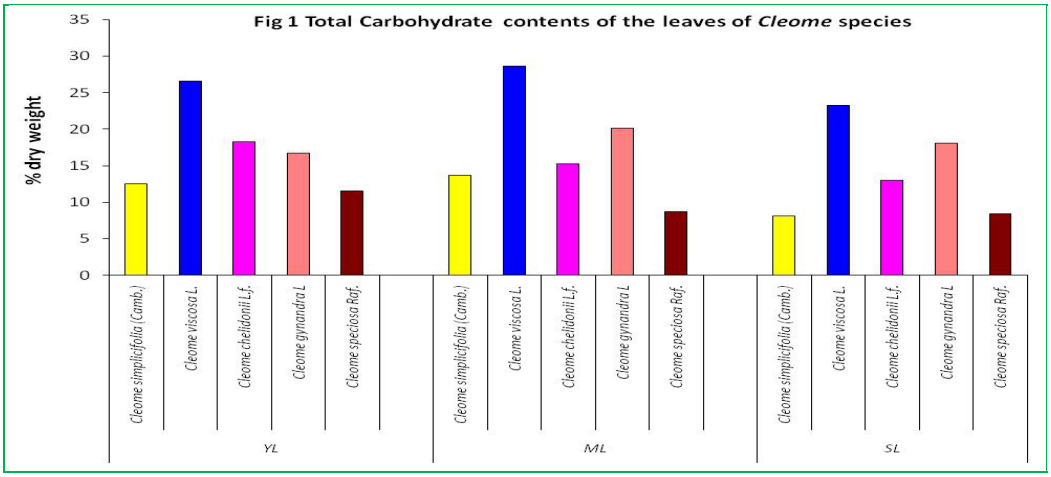
Fig 1 Total Carbohydrate contents of the leaves of Cleome species
% dry weight
35
30
25
20
15
10
5
0
Cleome simplicifolia (Camb.)
Cleome viscosa L.
Cleome chelidonii L.f.
Cleome gynandra L
Cleome speciosa Raf.
YL
ML
SL

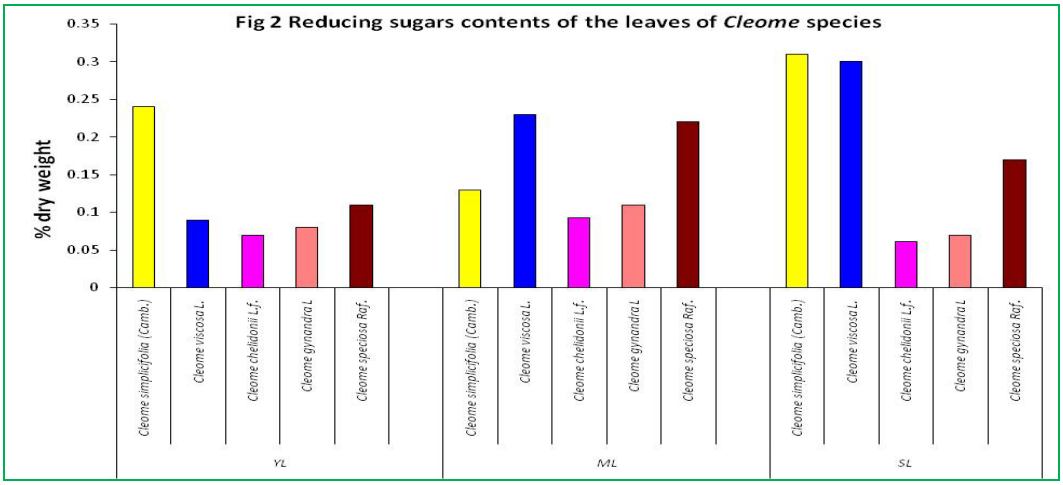
Fig 2 Reducing sugars contents of the leaves of Cleome species
% dry weight
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Cleome simplicifolia (Camb.)
Cleome viscosa L.
Cleome chelidonii L.f.
Cleome gynandra L
Cleome speciosa Raf.
YL
ML
SL

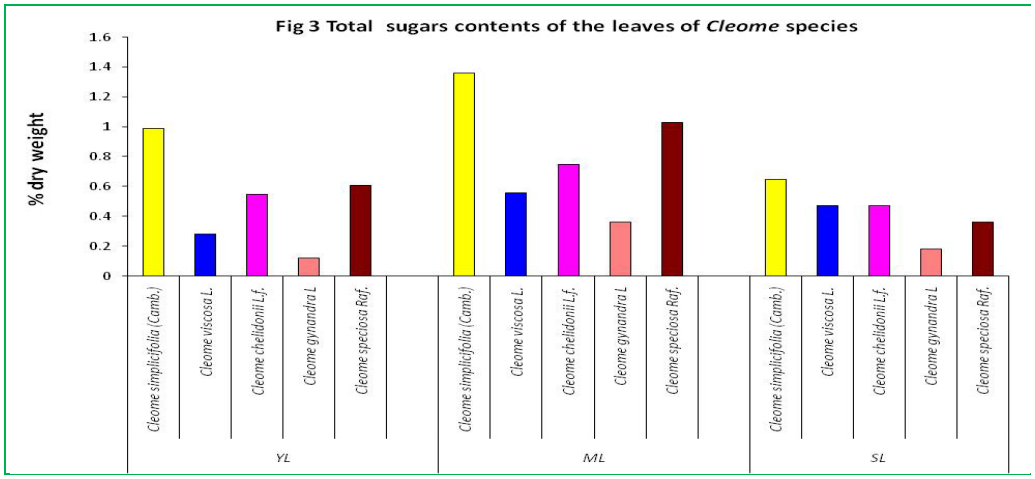
Fig 3 Total sugars contents of the leaves of Cleome species
% dry weight
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Cleome simplicifolia (Camb.)
Cleome viscosa L.
Cleome chelidonii L.f.
Cleome gynandra L
Cleome speciosa Raf.
YL
ML
SL

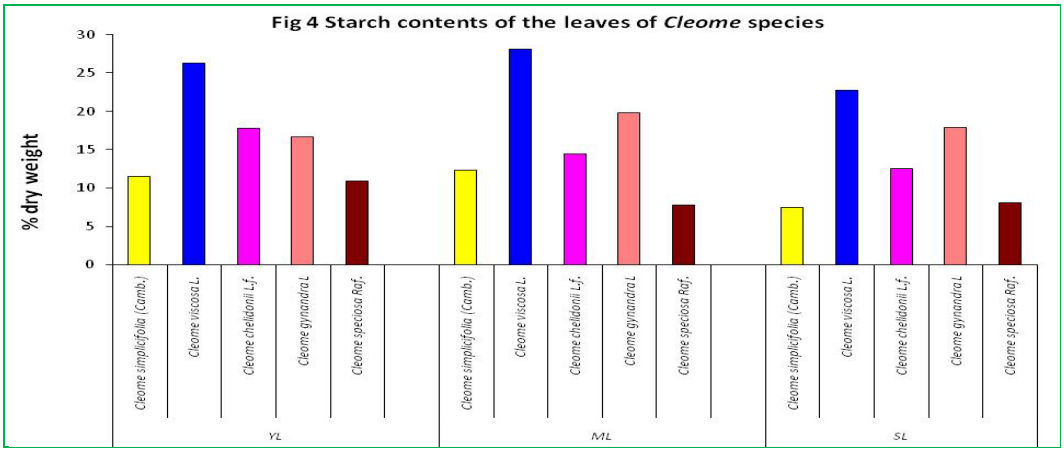
Fig 4 Starch contents of the leaves of Cleome species
% dry weight
30
25
20
15
10
5
0
Cleome simplicifolia (Camb.)
Cleome viscosa L.
Cleome chelidonii L.f.
Cleome gynandra L
Cleome speciosa Raf.
YL
ML
SL

RESULTS AND DISCUSSION

The total soluble sugars and starch (total carbohydrates) contents of the young, mature and senescing leaves of five different *Cleome* species have been recorded in table 1-5. It is evident from the results that the values of starch are much higher than those of soluble sugars in all species.

Total Carbohydrate Contents

In *Cleome*, carbohydrate content (fig 1) is the highest in *Cleome viscosa* mature leaves, while that lowest in *Cleome simplicifolia* senescent leaves. Young leaves of *Cleome chellidonii* and *C. speciosa* have more amounts of total carbohydrates than that in mature leaves. Similar types of results have been recorded in *Cassia* species by Patil[4]. However, in species like *Cleome viscosa, C. gynandra* and *C. simplicifolia* an opposite trend is observed as mature leaves have shown higher level of total carbohydrates than that in young leaves.

In *Cleome gynandra*, *C. viscosa* and *C. simplicifolia*, the carbohydrates are stored maximally in the mature leaves. In *Cleome chelidonii* and *C. speciosa* the young leaves are very rich in total carbohydrates with their low level in the mature and senescing leaves. It may help to understand relationship or evolutionary criteria of these species. The *Cleome* species in the present investigation can be arranged according to their leaf carbohydrate level in the following order, *C. viscosa> C. gynandra> C. chelidonii> C. simplicifolia> C. speciosa.*

Reducing Sugars Content

Reducing sugars content (fig 2) is the highest in the senescing leaves of *Cleome simplicifolia*, while it is the lowest in the senescing leaves of *C. chellidonii*. In *C. gynandra* and *C. chelidonii* the reducing sugars are stored more in the mature leaves. The pattern of reducing sugars content ML > YL >SL is exactly opposite to that observed by Thombare[5] in *Portulaca quadrifida* and *Aptenia cordifolia*. However, in *C. simplicifolia* the reducing sugars appear to be stored more in senescing and young leaves than that in mature leaves. Similar observations were made by Jamale and Joshi[6], Karadge[7] and Deshpande[8] respectively in *mangroves* (*Sonneratia acida* Linn. *Excoecaria agallocha* Linn. and *Lumnitzera racemosa* Willd.), *Portulaca oleracea* and *Cajanus cajan*. Kataoka *et al.*[9] studied change in sugar content of *Phalaenopsis* leaves.

In *Cleome viscosa* also the reducing sugars content is maximum in the senescing leaves. In *C. speciosa* these are maximum in the mature leaves and minimum in the young leaves. The pattern of reducing sugars level in the leaves of *Cleome* species can be given as *C. simplicifolia > C. viscosa> C. speciosa > C. gynandra> C. chelidonii.*

Total Sugars Content

The total sugars content is the highest in *the mature leaves of Cleome simplicifolia*, while it is the lowest in the young leaves of *C. gynandra* (table 4 and figure 3). The total sugars content seems to be the highest in the mature leaves of all *Cleome* species studied.

In *C. gynandra* and *C. viscosa* the level of total sugars is maximum in the mature leaves. However, in *C. simplicifolia, C. speciosa* and *C. chelidonii* the total sugar content is lower in senescing leaves than that in young leaves.

Starch Contents (Fig. 4)

Starch is a carbohydrate composed a large number of glucose units joined together by glycosidic bonds. This polysaccharide is produced by all green plants as an energy store. It is the most common carbohydrate in the human diet and is present in large amounts in potatoes, wheat, maize and rice. Depending on the plant, starch is generally composed of 20 to 25% amylose and 75 to 80% amylopectin[10].

Starch content is highest in the mature leaves of *C. simplicifolia, C. speciosa, C. viscosa* and *C. gynandra*, while in *C. chelidonii* it is the highest in the young leaves. In *Cleome simplicifolia* and *C. viscosa* the pattern of starch level in the leaves is almost identical with its higher level in the mature leaves.

In *C. speciosa* the pattern of starch level is YL>SL>ML and that in *C. gynandra* it is ML>SL>YL. However, in *C. chelidonii* pattern it is YL> ML>SL. The *Cleome* species investigated here can be arranged as *C. viscosa > C. gynandra > C. chelidonii > C. simplicifolia > C. speciosa* with respect to the starch content of leaves.

CONCLUSION

Higher level of total carbohydrates in *C. viscosa* indicates the highest photosynthetic rate. While that the lowest in *C. speciosa.* In all *Cleome* species CO_2 fixation is higher at young and mature stages of leaf development.

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