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FOLIAR PIGMENT ANALYSIS AND THEIR COREALTION WITH ADAPTATION IN GENUS *HABENARIA* WILLD.

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***Abstract*:**

Genus *Habenaria* is distributed throughout Western Ghats of peninsular India and Srilanka. This beautiful terrestrial orchid genus get escaped from the attention of researchers due to its short life span because of which many aspects such as cytology, anatomy, pollination biology and physiology remains unrevealed. Hence in present investigation an attempt was done to evaluate the foliar pigments in this terrestrial orchid genus. Foliar pigment analysis was done by following standard spectroscopic method. In present study eighteen species were analysed for their foliar content in leaves. Chlorophyll-a content (mg 100-^{1}g of fresh tissue) has been significantly less in four species having dorsiventrally flat leaves. A negative correlation between leaf thickness and pigment concentrations was observed during present studies while a positive correlation between leaf size and foliar pigment content is observed during present study.

***Index Terms*:** *Habenaria*, Chlorophyll, Pigments, Orchidaceae, Western Ghats.

I. INTRODUCTION:

Orchidaceae is one of the largest families among the flowering plants. Orchids exhibits incredible range of diversity in habit and habitat, shape, size, colour and fragrance of flower. Genus *Habenaria* Willd. belonging to family orchidaceae consists of mainly terrestrial or lithophytic, rarely epiphytic, tuberous and herbaceous species, represented by about 800 species distributed throughout the world especially tropical and temperate regions of the world [1] In India it is represented by 72 species including 38 endemic ones [2]. Very recently Choudhury *et al.* reported 59 species of *Habenaria* in India, holding 3rd position within the 5 dominant genera of India and it stands 5th position in the World [3]. For Western Ghats it is represented by 43 species out of which 28 are endemic. Over the years and decades layman and researchers get fascinated by the beauty and mystery of orchid flowers and their adaptations to different climatic conditions. In the number of work carried out on this genus, physiological aspect was very poorly represented because they escape from the attention of researchers due to their short life span. Probably due to the same reason the genus is poorly studied for its physiological aspects and adaptive strategies hence physiological marvels are remained unravel in this genus.

Physiological processes such as photosynthesis, respiration, photorespiration, water relations, mineral nutrition etc. plays important role in development of plant and subsequently its yield. A thorough understanding of all these processes is essential to improve the yield. A good and basic understanding of different parts of the orchids, their structure and function is essential for its large scale multiplication and maintenance. Leaves of orchids are variable in shapes, sizes and thickness. Information on anatomy and morphology of orchid leaves is important for both horticultural and scientific practices. Although orchid flowers present a relatively uniform structure, the organization of vegetative parts are notably variable among species [4]. Such features allow the family to thrive in different environments, and increase the number of its different growth forms [5]. Chlorophylls and carotenoids are required for photosynthesis, chlorophylls for the capture of light energy and as the primary electron donors and carotenoids as essential structural components of the photosynthetic apparatus, where they protect against photo-oxidation.

Most of the studies regarding pigment analysis have been carried out in many economically important crops as well as other plants. Orchids, are however, totally ignored for such studies. Hence, in present study, an attempt is made to study various plant pigments. Present study compares photosynthetic features of leaves of genus *Habenaria*, Foliar pigments i.e. Chl-a and Chl-b and carotenoids which were measured in 18 species of the genus *Habenaria* to find out distinction among the different species grown under same environmental conditions.

II. MATERIALS AND METHODS:

Plant specimens were collected from different localities of Western Ghats, identified with standard literature and maintained in botanical garden of Department of Botany, Shivaji University, Kolhapur, India. Foliar pigments were measured and analyzed for 18 species of genus *Habenaria* viz *Habenaria brachyphylla* (Lindl.) Aitch., *H. commelinifolia* (Roxb.) Wall. ex Lindl., *H. crinifera* Lindl., *H. digitata* Lindl., *H. diphylla* (Nimmo) Dalzell, *H. foetida* Blatt. and McCann, *H. furcifera* Lindl., *H. gibsonii* Hook. f., *H. grandifloriformis* Blatt. & McCann, *H. heyneana* Lindl., *H.longicorniculata* J. Graham, *H. longicornu* Lindl., *H. marginata* Colebr., *H. ovalifolia* Wight, *H. plantaginea* Lindl., *H. rariflora* A. Rich., *H. roxburghii* Nicolson and *H. suaveolens* Dalzell to find out the content of chlorophyll a, that of chlorophyll b, total chlorophyll content, the ratio of chlorophylls a and b, Carotenoids and flavonoids to find out variation among species grown under same environmental conditions. Chlorophyll contents were determined by using method of Arnon [6], Carotenoids by Kirk and Allen [7] and Flavonoids by Luximon - Ramma *et al.* [8].

III. RESULTS AND DISCUSSION:

3.1. Chlorophyll (Chl. a, Chl. b, Chl. a+b and Chl. a/b ratio): (Table 1)

During present analysis it has been found that four species viz. *H. brachyphylla*, *H. diphylla*, *H. grandifloriformis* and *H. roxburghii* grows with dorsiventrally flat leaves appressed to the ground, while remaining species have aerial leaves or not like as mentioned above. Chlorophyll-a content (mg 100-1g of fresh tissue) has been significantly less in all these four species while remaining species showed higher content of Chl. a. These four species showed a range of Chl. a from 20.00 ± 0.07 to 44.47 ± 0.17 $mg100^{-1}g$ while other 14 species show Chl. a range between 21.16 ± 0.39 – 85.15 ± 0.11 $mg100^{-1}g$ of fresh tissue. Least amount of Chl. a has been observed in *H. diphylla* (20.00 ± 0.07) while maximum amount of Chl. a has been observed in *H. foetida* (85.15 ± 0.11), followed by *H. furcifera* (79.36 ± 0.24).

Chl. b content is found maximum in *H. ovalifolia* (35.75 ± 0.13) with a minimum in *H. diphylla* (6.21 ± 0.02). Chl. a + b (mg $100^{-1}g$ of fresh tissue) was found to be higher in *H. foetida* (114.21 ± 0.44) followed by *H. furcifera* (109.23 ± 0.31) with a minimum in *H. diphylla* (26.21 ± 0.04). Chakrabarti and Sarkar [9] estimated foliar pigments and its relationship with leaf thickness in 16 species of genus *Eria* and concluded a negative correlation between leaf thickness and pigment concentrations. Similar types of results were obtained in present analysis; species such as *H. brachyphylla*, *H. diphylla*, *H. grandifloriformis* and *H. roxburghii* have thick leaves as compared to other species studied and showed minimum amount of chlorophylls.

Chl. a/b ratio varies from 2.0 – 2.8 for shade-adapted plants to 3.5 – 4.9 for plants adapted to fully exposed conditions [10], while Anderson concluded that shade-adapted plants tend to have lower Chl. a/b ratios than sun adapted plants [11]. In present analysis all species showed Chl. a/b ratio less than 3.5 i.e. all species are shade loving with a maximum ratio in *H. diphylla* (3.22) while minimum in *H. ovalifolia* (1.74). It is also presumed that plants showing Chl. a/b ratio >3 are C4 plants, while < 3 are C3 plants.

Lovegrove [12] stated that prostrate leaves act as water-trapping umbrellas, reducing the rate of water loss, and creating favourable microclimates for growth. A CO_2 enriched environment might increase photosynthetic CO_2 uptake. CO_2 enrichment generally causes plants to develop more extensive root systems to exploit additional pockets of water and nutrients and to enhance the activity of bacteria and other organisms that break nutrients out of the soil, which the plants can then exploit [13]. During present investigation similar type of observations were observed, as above discussed 4 species has prostrate leaves they act as a water trapping umbrellas which in turn provide favourable CO2 enriched environment for growth of these species. Gaikwad, *et al.*, [14] proposed grass like leaves provide camouflage among grasses, and are therefore more difficult for herbivores to recognize; thus protecting plants against herbivory. Such kinds of observations were observed in all 4 species but the ideal was represented by *Habenaria grandifloriformis*. This species was a perfect example against herbivory, most of the times population of this species was very vast, more than 500 individuals profusely grows in grasses. As leaves were adpressed to ground in this species it becomes very difficult for animals to detect these species among grasses, exhibiting perfect camouflage among grasses. Present results clearly show that species growing in grasses and having dorsiventrally flat leaves which are appressed to ground receive very less or poor light. Due to this it may concluded that the content of Chlorophyll in these species significantly drops down, while species having aerial leaves get exposed to much more light compared to these 04 species hence show more amount of Chl. a. In the present study, *H. crinifera* which grows on mango trees (partially in epiphytic habitat) receives less light thus resulting in minimum amount of both Chlorophylls i.e. Chl. a and b (21.16 ± 0.39 and 8.77 ± 0.17 respectively). Result shows significant variation in chlorophylls among the different sp. maximum content of chlorophyll-a and chlorophyll-b pigments were recorded in *Habenaria foetida* Blatt. and McCann and *Habenaria ovalifolia* Wight i.e. 21.29 ± 0.03 mg $100g^{-1}$ and 8.94 ± 0.03 mg $100g^{-1}$ respectively while minimum pigment content was seen in *H. diphylla* (Nimmo) Dalzell as compared to other species of *Habenaria*. . The chlorophyll a/b ratio varies from 1.74 to 3.22 ± 0.02, maximum in *H. diphylla* with minimum in *H. ovalifolia*. From these observation it may concluded that Chlorophyll content was directly proportional to exposure of light intensity, as light reduces Chlorophyll content reduces and vice versa. From all these studies it was concluded that all species belong to C3 types of plants showing Chl. a/b ratio < 3, while thick leaves among *Habenaria* species may have significant role in relation to drought tolerance, desiccation and fungal diseases.

3.2 Carotenoids: (Table 1)

In case of Carotenoids, minimum content was observed in *H. diphylla* (6.76 ± 0.16 mg 100^{-1}g) with a maximum in *H. furcifera* (30.52 mg 100^{-1}g). According to [10] Carotenoid pigments impart distinctive red, orange and yellow colours and a number of Carotenoids-derived aromas to many fruits and flowers. Thus, Carotenoids make fruit and flowers commercially important in agriculture, food manufacturing and cosmetic industry. Specifically, Carotenoids are ubiquitous components of all photosynthetic organisms, as they are required for assembly and function of the photosynthetic apparatus. Carotenoids are also vital part of our diet as antioxidants and precursors to vitamin A. In the present investigation, maximum species have white to pure white coloured flowers with a few greenish coloured and one species has yellow coloured flower viz. *H. marginata*. From the analysis of Carotenoids it is very difficult to predict exact role of Carotenoids in genus *Habenaria*. However, according to Davies (*loc. cit.*) the Carotenoids plays a role in photosynthesis and nutrition, which account for the absolute requirement of Carotenoids in the survival of plants and mammals alike. The highest Carotenoids content was observed in *H. furcifera* Lindl. i. e. 30.52 mg 100 g^{-1} with a minimum in the *H. diphylla* i.e. 6.76 ± 0.16 mg 100 g^{-1}. In case of flavonoids highest was observed in *Habenaria foetida* i.e. 229 ± 0.15 mg 100 g^{-1} with a minimum 54.98 ± 0.77 mg 100 g^{-1} in *H. marginata* Colebr.

3.3 Flavonoids: (Table 1)

In case of Flavonoids maximum content was observed in *Habenaria foetida* (229.58 ± 0.15 mg 100-1g) and a minimum in *H. marginata* (54.99 ± 0.77 mg 100-1g), with a range of 60 – 200 mg 100-1g in rest of the species. Most of the earlier workers reported frequently occurring flavonoid classes in leaves of orchidaceae are flavone C-glycosides, flavonols, flavones, 6-hydroxyflavones, proanthocyanidins and xanthones. [10] suggested that flavonoids have most obvious and best characterized role to provide floral visual cues for insect and animal pollinators. They are the most important floral pigments, occurring throughout the angiosperms and providing most colours in the visible spectrum. In present analysis only flavonoids content has been observed and measured. It is assumed that they may be playing a role in providing floral visual cues for insect and animal pollinators.

IV. CONCLUSION:

According to current research, leaf thickness affects chlorophyll concentration; thin leaves have a far greater chlorophyll content than thick leaves. This can be because of the species' habitat. The pigment concentration of the thick-leaved species under study varies significantly since all of the basal species have leaves that are barely touching the ground and grow alongside grasses, which blocks direct sunlight from reaching the leaves. This is in contrast to the aerial leaves of other species. In the current study, a positive association was found between foliar pigment content and leaf size, while a negative correlation was found between pigment concentrations and leaf thickness.

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Table 1. Foliar pigment content of *Habenaria* species.

Sr. No.	Name of the species	Chl. a mg 100^{-1}g	Chl. b mg 100^{-1}g	Chl. a + b mg 100^{-1}g	Chl. a /b ratio	Carotenoids mg 100^{-1}g	Flavonoids mg 100^{-1}g
1	*H. brachyphylla*	44.47 ± 0.17	17.28 ± 0.29	61.73 ± 0.20	2.57	21.21 ± 0.02	125.32 ± 0.00
2	*H. commelinifolia*	57.59 ± 0.12	21.79 ± 0.14	79.35 ± 0.14	2.64	21.41 ± 0.10	135.55 ± 0.26
3	*H. crinifera*	21.16 ± 0.39	8.77 ± 0.17	29.93 ± 0.29	2.41	8.48 ± 0.08	79.45 ± 0.15
4	*H. digitata*	66.83 ± 0.40	24.44 ± 0.78	91.25 ± 1.06	2.73	24.56 ± 0.16	193.86 ± 0.51
5	*H. diphylla*	20.00 ± 0.07	6.21 ± 0.02	26.21 ± 0.04	3.22	6.76 ± 0.16	61.55 ± 0.15
6	*H. foetida*	85.15 ± 0.11	29.08 ± 0.43	114.21 ± 0.44	2.92	29.24 ± 0.12	229.58 ± 0.15
7	*H. furcifera*	79.36 ± 0.24	29.91 ± 0.19	109.23 ± 0.31	2.65	30.52 ± 0.00	209.21 ± 0.89
8	*H. gibsonii*	56.92 ± 0.19	22.91 ± 0.48	79.81 ± 0.67	2.48	25.51 ± 0.10	175.70 ± 0.92
9	*H. grandifloriformis*	29.76 ± 0.04	15.99 ± 0.43	45.72 ± 0.44	1.86	11.83 ± 0.08	93.61 ± 0.77
10	*H.heyneana*	60.64 ± 0.20	20.88 ± 1.39	81.51 ± 1.25	2.90	21.84 ± 0.08	93.35 ± 0.77
11	*H.longicorniculata*	55.67 ± 0.37	26.08 ± 0.18	81.72 ± 0.44	2.13	20.35 ± 0.02	153.45 ± 0.00
12	*H. longicornu*	63.17 ± 0.07	21.12 ± 0.12	84.27 ± 0.14	2.99	21.19 ± 0.05	194.54 ± 0.15
13	*H. marginata*	42.17 ± 0.11	16.44 ± 0.43	58.60 ± 0.44	2.56	16.43 ± 0.05	54.99 ± 0.77
14	*H. ovalifolia*	62.50 ± 0.01	35.75 ± 0.13	98.23 ± 0.11	1.74	23.76 ± 0.08	166.41 ± 1.03
15	*H. plantaginea*	71.32 ± 0.50	30.31 ± 0.11	101.61 ± 0.42	2.35	27.24 ± 0.08	171.53 ± 0.53
16	*H. rariflora*	60.63 ± 0.17	26.14 ± 0.06	86.76 ± 0.23	2.31	25.56 ± 0.12	158.57 ± 0.51
17	*H. roxburghii*	39.46 ± 0.14	17.07 ± 0.11	56.53 ± 0.18	2.31	15.16 ± 0.07	104.86 ± 0.77
18	*H. suaveolens*	53.40 ± 0.40	25.96 ± 0.25	79.34 ± 0.17	2.05	21.84 ± 0.12	132.99 ± 0.51

(± SD, n = 10)