Accumulation of photosynthetic pigments in *Cenchrus* species under different light intensities

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**ABSTRACT**

The highest accumulation of chl a was recorded under minimum light intensity (I₂5). *C. pennisetiformis* showed higher accumulation of chl a under minimum light intensities (I₂5) and a significantly less accumulation of chl a was observed in the same species under I₁₀₀ in comparison to I₁₀₀. *C. glaucus* showed minimum accumulation of chl a under I₁₀₀. The pigment chl b showed an increasing trend in all the species of *Cenchrus* with respect to the decreasing light intensity. *C. ciliaris* showed a maximum accumulation of chl b under I₂₅ and the minimum was accumulated under I₁₀₀. There was a significant difference in the accumulation of photosynthetic pigment among the treatments. *C. pennisetiformis* showed maximum accumulation under I₂₅ followed I₁₀₀ and I₅₀. In *C. glaucus* the scenario of the total photosynthetic pigment is something different. In this species the highest accumulation of total photosynthetic pigment was observed under I₁₀₀ followed by I₂₅ and I₅₀. All the species of *Cenchrus* showed a decreasing trend in the chlorophyll a: b ratio when the light intensity decreases from 100% to 25%. In *C. setigerus* the accumulation of carotene was observed in an increasing order when the light intensity decreases from 100 to 25% In *C. pennisetiformis* the highest accumulation was recorded under I₁₀₀ followed by I₂₅ which is having no significant difference among each other. *C. glaucus* showed a maximum accumulation of carotene under minimum light intensity I₂₅ Variation in the accumulation of Photosynthetic pigment over the period of shading. The highest accumulation of photosynthetic pigment was recorded in *C. glaucus* under open (100% light intensity) followed by I₁₀₀ and I₅₀, however *C. setigerus* showed accumulation of maximum amount of photosynthetic pigment under minimum light intensity (I₂₅). Under 75% light intensity *C. glaucus* showed a maximum value of chl a:b at 4th week stage and the value of *C. ciliaris* and *C. setigerus* showed an increase from 1st week to 4th week stage of the crop and then declined. Maximum accumulation of carotene was recorded under minimum light intensities in *C. setigerus* followed by *C. pennisetiformis* and *C. glaucus*.

**Key words:** *Cenchrus*, light intensity, photosynthetic pigments, perennial, flowering stage, carotene.

**INTRODUCTION**

With current production figure of 70 million tones, India has emerged as the largest milk producer in the world. Again, the productivity and profitability is too low to be sustainable. A greater concern is now emerging fast to improve the productivity of livestock by enhancing the availability of quality feeds and fodders. Due to constraint of land resources attention has now been diverted towards the utilization of degraded waste lands. The growth patterns of a plant may be determined by ecological factors, which fluctuate in the environment due to inter and intra specific relations. These ecological factors like, temperature, photoperiod and the light energy etc; influence the plant growth either through change of photosynthetic activity of leaf or change in ratio of leaf surface to the total plant weight. The adaptability of plant is related not only to its structural and functional
responsiveness but also to the speed of readjustment to fluctuation which frequently encountered under natural conditions. The plant productivity is determined by the net photosynthesis, which is directly related to the solar radiation. Lot of work has been done on grain crops under low light situation. The adaptation of grasses to light stress condition has received little attention and thus requires extensive studies. The shade-type chloroplasts of shade leaves, low-irradiance leaves, and shade plants are characterized by much larger grana stacks and higher stacking degree (Anderson et al. 1973, Guillot-Salomon et al. 1978, Lichtenthaler et al. 1981) than the sun-type chloroplasts, which have much less chloroplast lamellae. High-irradiance chloroplasts are represented by higher ratio of chlorophyll (Chl) a/b and lower ratios of xanthophylls/carotene (x/c) and Chl a/prenylquinones (Lichtenthaler 1979, Lichtenthaler et al. 1981).

The characteristics of photosynthetic reactions differ between shade tolerant species grown in shade and shade-intolerant species acclimated to higher irradiance (Björkman 1981). Photon-saturated net photosynthetic rate (P_{\text{max}}) and Chl a/b ratios were higher in foliage of canopy positions exposed to higher irradiance as compared to shaded crown layers. The shade tolerant species showed relative shade-type characteristics at a given radiation environment, both P_{\text{max}} and Chl a/b ratio lower in needles of the shade tolerant species. In high plants, the amount of incident solar radiation available during growth produces distinct differences in the composition, function, and structure of chloroplasts (Leong and Anderson 1984, Lechowicz et al. 1986). At light saturation, the leaves and chloroplasts of high light plants possess a higher photosynthetic CO_{2}-fixation capacity on a chlorophyll, chloroplast and leaf area basis than the low light and shade leaves with low light chloroplasts (Boardman et al. 1974, Wild 1979, Lichtenthaler et al. 1981 and 1982). In this study efforts were made to define typical differences in the accumulation of photosynthetic pigments between sun-type and shade-type leaves of four Cenchrus species.

MATERIALS AND METHODS

Four perennial Cenchrus species viz Cenchrus ciliaris (IGFRI-3108), Cenchrus setigerus (EC-397331), Cenchrus pennisetiformis (EC-397528) and Cenchrus glaucus (EC-397614) were grown in the seedbeds. One-month-old seedlings were transplanted in the field with a 2 x 2m plot size with a 50 by 50 cm distance from plant to plant and row-to-row. All plots were in full sunlight for one season for the proper establishment of the tussocks. After one season low light treatment was given by putting agro shade nets with different light transmission efficiency at Indian Grassland and Fodder Research Institute Jhansi (25° 27’ N, 78° 35’ E and 271m.a.s.l.) during 2003-05. The soil was clay loam in texture, neutral in reaction (pH 6.54) and non-saline in (EC 0.29 dsm^{-1}) salt content. The content of organic carbon (0.48%) and available nitrogen (23.52g m^{-2}) in the surface soil were low. The available phosphorus and potassium contents were in the medium range (1.27 g (P) m^{-2} and 27.88 g (K_{2}O))². Four irradiance, i.e. 100\left(1_{100 \text{ control}}\right), 75\left(1_{75}\right), 50\left(1_{50}\right) and 25\left(1_{25}\right) % of full sunlight were applied for each sub plot. Solar radiation in \left(1_{100}\right) was 1200-1400\mu\text{mol} m^{-2} s^{-1}. The agro shade nets were mounted on wooden frames 10 x 5 m set 2 m above the ground. At the eastern and western ends of each screen the shade was extended an extra 1 m and angled at 45° to exclude most of the early morning and late evening sun from plots. The shading treatment was imposed before the onset of monsoon (June-July) as the growing season of these tropical grass species ranges from the onset of monsoon (June-July) to the beginning of the winter (November-December).

Photosynthetic Pigments

Chlorophyll a, b, carotene and total chlorophyll contents were determined by extraction in dimethylsulfoxide (DMSO), following non – maceration technique of Hiscox and Israelstam (1979). Leaf slices weighing 25 mg were transferred to a test tube containing 5ml of DMSO. The tubes were then incubated in a hot air oven at 65°C for 2 hrs. Then tubes were cooled to room temperature and absorbance was recorded at 4 wavelength 480, 510, 645 and 663 nm using a Pc based spectrophotometer (IUCAM make) by taking DMSO as blank pigment content were calculated using the formula:

\[
\text{Chl a} = \frac{(12.7 \times O.D_{663})-(2.69 \times O.D_{645})}{[\text{Volume}]} \\
\text{Chl b} = \frac{(22.9 \times O.D_{645})-(4.68 \times O.D_{663})}{[\text{Volume}]} \\
\text{Carotene} = \frac{(7x O.D_{480})-(1.47 \times O.D_{510})}{[\text{Volume}]} \\
\text{Total Chlorophyll} = \frac{(20.2 \times O.D_{645})+(8.02xO.D_{663})}{[\text{Volume}]} \\
\text{Where} \ V = \text{Volume of the solvent (Here dimethyl sulphoxide)} \\
\text{W = Weight of the leaf tissue taken}
\]

The same out of leaf sample (25mg) was kept for drying and its dry weight was recorded and the pigment content was expressed as mg g^{-1} dw.

RESULTS AND DISCUSSION

Accumulation of Photosynthetic Pigments viz. chlorophyll a (chl a), chlorophyll b (chl b), total chlorophyll (chl a+b), ratio of chlorophyll a and b (chl a:b) and the carotene of the fully expanded 2nd leaf was recorded on dry weight basis four times in week interval at the peak of growth stage (50% flowering stage) through out the season the data represented as the mean of four observations.
Chlorophyll a

All the four species of *Cenchrus* showed a change in the accumulation of chlorophyll *a* in respect to the decreasing light intensity. In *C. ciliaris* there was not much variation in the accumulation of chlorophyll *a* among the light intensity, however the highest accumulation of chlorophyll *a* was recorded under minimum light intensity (*I*<sub>50</sub>) (5.926 mg g<sup>-1</sup> dw<sup>-1</sup>). There was no significant difference in the accumulation of chlorophyll *a* between *I*<sub>100</sub> and *I*<sub>50</sub>. In *C. setigerus* there was no significant difference in the accumulation of chlorophyll *a* under different light intensities. *C. pennisetiformis* showed higher accumulation of chlorophyll *a* under minimum light intensities (*I*<sub>25</sub>) (6.624 mg g<sup>-1</sup> dw<sup>-1</sup>) and a significantly less accumulation of chlorophyll *a* was observed in the same species under *I*<sub>50</sub> (5.464 mg g<sup>-1</sup> dw<sup>-1</sup>) in comparison to *I*<sub>100</sub>. However, there was no significant difference in the accumulation of chlorophyll *a* between *I*<sub>100</sub> and *I*<sub>50</sub>. *C. glaucus* showed no significant difference in the accumulation of chlorophyll *a* under *I*<sub>50</sub> (5.779) which is not significantly different over *I*<sub>100</sub>. However, this species showed a maximum amount of chlorophyll *a* accumulation under *I*<sub>75</sub> and *I*<sub>25</sub>, both of having similar value of chlorophyll *a* (6.08 mg g<sup>-1</sup> dw) (Figure 1A).

Chlorophyll b

The pigment chlorophyll *b* which is considered as maximum light harvesting efficiency showed an increasing trend in all the species of *Cenchrus* with respect to the decreasing light intensity. *C. ciliaris* showed a maximum accumulation of chlorophyll *b* (1.232 mg g<sup>-1</sup> dw<sup>-1</sup>) under *I*<sub>25</sub> and the minimum was accumulated under *I*<sub>100</sub>. *C. setigerus* did not show a specific trend in the accumulation of chlorophyll *b*, however the maximum accumulation was observed under *I*<sub>25</sub> (1.473 mg g<sup>-1</sup> dw<sup>-1</sup>) and the minimum was observed under *I*<sub>50</sub>. Of course there was no significant difference in the accumulation of chlorophyll *b* was observed under *I*<sub>75</sub> and *I*<sub>50</sub>. The same trend was observed in *C. pennisetiformis* with the maximum accumulation of (1.182 mg g<sup>-1</sup> dw<sup>-1</sup>) under *I*<sub>25</sub>. *C. glaucus* behaved in the same way as *C. ciliaris* which showed an increasing trend in the accumulation of chlorophyll *b* with respect to the decreasing light intensity with the maximum accumulation under *I*<sub>25</sub> (1.632 mg g<sup>-1</sup> dw<sup>-1</sup>) and minimum accumulation of chlorophyll *b* was recorded under *I*<sub>100</sub>. (Figure 1B).

Total Chlorophyll (*a+b*)

The Total Chlorophyll of the leaf varies when the light intensity varies from open field with 100% sunlight to minimum light intensity of 25%. In *C. ciliaris* the maximum accumulation of pigments was recorded under *I*<sub>25</sub> while the minimum accumulation was in open field grown grasses under 100% light intensity. No significant difference in the accumulation of total pigment was recorded in the grass grown under 50% and 75% light intensity. *C. setigerus* also showed in the same trend as *C. ciliaris* with the maximum accumulation of total photosynthetic pigment under *I*<sub>25</sub> (7.71 mg g<sup>-1</sup> dw<sup>-1</sup> and the minimum was recorded under open grown crop. The highest accumulation of total photosynthetic pigment was recorded under *I*<sub>25</sub>. There was a significant difference in the accumulation of photosynthetic pigment among the treatments.

The highest accumulation of photosynthetic total pigment in *C. pennisetiformis* was also influenced by the light regimes, with a maximum accumulation under *I*<sub>25</sub> followed by *I*<sub>100</sub> and *I*<sub>50</sub>. In *C. glaucus* the scenario of the total photosynthetic pigment is something different. In this species the highest accumulation of total photosynthetic pigment was observed under *I*<sub>25</sub> followed by *I*<sub>50</sub> and *I*<sub>25</sub>. There was no significant difference observed in the accumulation of total photosynthetic pigment when the crop was grown under *I*<sub>50</sub> and *I*<sub>25</sub>. The minimum accumulation of total photosynthetic pigment was recorded when the crop was subjected to open field condition with 100% solar radiation throughout the growing season (Table. 1).

Chlorophyll *a*: *b* ratio

All the species of *Cenchrus* showed a decreasing trend in the chlorophyll *a*: *b* ratio when the light intensity decreases from 100% to 25%. In *C. ciliaris* the maximum value of chlorophyll *a*: *b* ratio was recorded under *I*<sub>100</sub> (8.398) and the minimum at *I*<sub>25</sub> (5.06). Other three species showed maximum value of chlorophyll *a*: *b* ratio under *I*<sub>25</sub> and the minimum was under *I*<sub>50</sub>. The minimum value was recorded in all the species under minimum light intensity due to the higher accumulation of chlorophyll *b* which is showing the maximum light harvesting efficiency under shading environment. (Table 1).

Carotene

The carotene, which includes the violaxanthin, antheraxanthin and zeaxanthin showed the quality of the fodder and was accumulating in an increasing order when the light intensity decreases from 100% to 25%. In *C. ciliaris* the maximum accumulation of carotene was recorded under *I*<sub>50</sub> (2.219 mg g<sup>-1</sup> dw<sup>-1</sup>) followed by *I*<sub>25</sub> (2.0233 mg g<sup>-1</sup> dw<sup>-1</sup>). There was no significant difference in the accumulation of carotene was observed under *I*<sub>100</sub> and *I*<sub>25</sub> in *C. setigerus* the accumulation of carotene was observed in an increasing order when the light intensity decreases from 100% to 25% with the maximum accumulation under *I*<sub>25</sub> (2.81 mg g<sup>-1</sup> dw<sup>-1</sup>) and the minimum was recorded under open field grown crop of the *C. setigerus*. In *C. pennisetiformis* the highest accumulation was recorded under *I*<sub>100</sub> (2.233 mg g<sup>-1</sup> dw) followed by *I*<sub>25</sub> (2.128 mg g<sup>-1</sup> dw) which is having no
Figure 1. Photosynthetic pigment accumulation (A: chl a, B: chl b and C: carotene) in Cenchrus species as influenced by irradiance level.
Table 1. Total photosynthetic pigment (chl a+b) and the ratio of chl a/b in Cenchrus species as influenced by irradiance level

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Chlorophyll a+b (mg g⁻¹ dw⁻¹)</th>
<th>Chlorophyll a/b</th>
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<tbody>
<tr>
<td></td>
<td>$I_{100}$</td>
<td>$I_{75}$</td>
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</table>

CD at 0.05 p Irradiance, I: 0.7840
Plant species, S: 0.4982

CD at 0.05 p Irradiance, I: 0.7659
Plant species, S: 1.1810
S x I: 0.9965
S x I: 2.3620

significant difference among each other. No significant difference in the accumulation of carotene was recorded when the crop was grown under $I_{75}$ and $I_{50}$. C. glaucus showed a maximum accumulation of carotene under minimum light intensity $I_{25}$ (2.64 mg g⁻¹ dw⁻¹) and the minimum recorded under open field grown crop (1.639 mg g⁻¹ dw⁻¹). In the same species carotene accumulation under $I_{75}$ and $I_{50}$ showed similar value showing no significant difference among these two treatments. Overall the four species of Cenchrus showed in the improvement of quality of the fodder by accumulating higher amount of carotene under shading environment. (Figure 1C).

Variation in the accumulation of Photosynthetic pigment over the period of shading periodic accumulation of photosynthetic pigments was recorded at weekly intervals from the date of imposing shade to $6^{th}$ week growth stage of the crop. The chlorophyll a accumulation in the leaves of Cenchrus species over the period under different irradiances level showed species variation. In the shading environment the accumulation of chlorophyll a remained steady from $2^{nd}$ week to $4^{th}$ week stage from the date of initiation of shading and then declined. It seems towards the maturity of the leaf the accumulation decreases may be due to the degradation of chlorophyll synthesis. In our experimental result higher accumulation of chl a was recorded in C. glaucus under all the irradiance level followed by C. setigerus. (Figure 2). The accumulation of the chl b pigment considered to be the light harvesting pigments varied under different light intensities in different Cenchrus species in the successive growth week. Under open and 50% light intensity the maximum accumulation of chl b was recorded in C.glaucus, however under $I_{75}$ and $I_{25}$, C.setigerus showed maximum accumulation of chl b pigments. Under all the irradiance level the accumulation was in a steady rate from $2^{nd}$ to $4^{th}$ week stage of the crop and then declined. (Figure 3). The accumulation of carotene which showed the improvement of quality of the crop showed variation in the Cenchrus species under different light intensities maximum accumulation of carotene was recorded under minimum light intensities in C. glaucus followed by C. pennisetiformis and C. setigerus. Under all the irradiance level the accumulation remained steady up to $4^{th}$ week stage of the crop and then declined. (Figure 4).

The total chlorophyll, the sum of chl a and chl b was recorded from the $1^{st}$ week of the stress imposed up to $6^{th}$ week stage. Variation in the total pigment accumulation in different Cenchrus species under different light intensity was recorded. The highest accumulation of photosynthetic pigment was recorded in C. glaucus under open (100% light intensity) followed by $I_{75}$ and $I_{50}$, however C. setigerus showed accumulation of maximum amount of photosynthetic pigment under minimum light intensity ($I_{25}$) (Table 2). Chlorophyll a: b ratio which indicate the shade tolerant efficiency of a particular crop showed maximum accumulation in C. ciliaris under open field followed by C.setigerus. Under 75% light intensity C.glaucus showed a maximum value of chl a:b at $4^{th}$ week stage and the value of C. ciliaris and C. setigerus showed an increase from $1^{st}$ week to $4^{th}$ week stage of the crop and then declined. The ratio in C.pennisetiformis showed a steady rate from $1^{st}$ week to $4^{th}$ week stage and then declined. (Table 2).

The increased pigment content in shaded leaves is attributed to the increase in number and size of chloroplasts, the amount of chl per chloroplast, and better grana development (Boardman 1977). Under the shade, the accumulation of chl a:b was higher than in plants grown under $I_{100}$. Shaded plants have higher relative content of chl b than chl a (Singh 1994). However, under different irradiances chl b behaved variably. C.glaucus accumulated more chl-b than other species of Cenchrus indicating its potential for shade adaptation. Lichtenhaler (1981) found in radish seedlings a significantly higher chl a/b in plants grown under high (HI) than low (LI) irradiance, but chl content per dry mass and the height and width of grana stacks were in turn always greater in the LI leaf. Higher chl a/b in HI-leaves indicates lower amount of light harvesting chl-protein, which was shown for HI-leaves of Raphanus and barley (Armond and Arntzen 1977, Buke et al. 1979). Chl a/b ratio are
Figure 2. Variation in the accumulation of Photosynthetic pigment (chlorophyll a) over the period of shading.
Figure 3. Variation in the accumulation of Photosynthetic pigment (chlorophyll b) over the period of shading.
Figure 4. Variation in the accumulation of Photosynthetic pigment (carotene) over the period of shading.
typically higher in sun leaves than shade leaves (Boardman 1977, Anderson et al. 1988); (a) sun leaves possess higher capacities for photosynthesis and (b) Place less emphasis on light-harvesting, chl \(a/b\) can rise a result of different ratios of photo system (PS-II) cores to peripheral light-harvesting complexes (Anderson and Osmond 1987). Nevertheless, higher chl \(a/b\) ratios in sun than shade are sometimes found e.g. in Juglans regia (Atanasora et al. 2003). This means a lesser emphasis in photon collection versus PS-II photochemistry. In addition, chl \(a/b\) ratios in the open field condition are higher in chlorophyll complexes of PS-I. Compared to those of PS-II (Thayer and Björkman 1992). Thus the higher Chl \(a/b\) ratios in the open condition indicate differences in either the ratio of PS-II core to light-harvesting complexes or the ratio of Chl complexes associated with PS-I versus PS-II or both. Adjustments of the leaf pigment composition in response to irradiance have been described in numerous studies. Chl based contents of photo-protective Cars and Chl \(a/b\) ratios are higher in sun versus shade leaves of individual plants (Thayer and Björkman 1990, García-Plazaola and Becerril 2000, Lichtenthaler et al. 2000, Hansen et al. 2002) and in sun-grown versus shade-grown plants (Logan et al. 1998). Chl \(b\) is enriched in outer antennae, relative to the core complexes (CC) of PS-I and PS-II and in PS-II versus PS-I. A higher contribution of outer antennae Chl to the total Chl, leading to lower Chl \(a/b\) ratios, enhances the efficiency of photon capture under limited photon supply.

Baig et al. (2005) experimented by taking three tropical range grasses (C.ciliaris, D.annulatum, Panicum antidotale) and two legumes [Macroptilium atropurpureum (pirato) and Stylosanthes hamata (stylo)] by growing under four irradiance i.e. 100 \(I_{100}\), control) 75 \(I_{75}\), 50 \(I_{50}\) and 25 \(I_{25}\) % of full sunlight and they supported our present finding by concluding that accumulation of chl \(b\) increased but that of chl \(a\) decreased under low irradiance. The greater accumulation of chl \((a+b)\) in grasses (particularly in D. annulatum and Panicum antidotale) under shade predicted their shade adaptability. Among legumes Stylosanthes hamata was more adaptive to the shade than Macroptilium atropurpureum due to its higher accumulation of carotenoids under \(I_{25}\) over \(I_{100}\) was observed in all the species, which shows the increase in quality of the fodder under limited irradiance.

Corresponding results were obtained by Hansen et al. (2002) on deciduous tree species, Fagus sylvatica and Quercus petrea, in a mixed beech/oak forest: shade-type pigment characteristics for shade leaves relative to sun leaves. At a given irradiance, leaves of the more shade-tolerant beech showed lower Chl \(a/b\) ratios than those of the less shade tolerant oak. The average Chl amount per leaf fresh mass was higher under shade compared to full

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<tr>
<th>Total chlorophyll ((a+b))[mg g(^{-1}) dw(^{-1})]</th>
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<th>C.setigerus</th>
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<table>
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<th>(C.pennesetiformis)</th>
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sunlight, suggesting a higher investment in pigment-protein complexes. The variability in Chl content among the species was much profound and possibly arising from content of water and amount of non-photosynthesising tissues. There was substantial variation between species in the extent and nature of alteration in photosynthetic characteristics.

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REFERENCE


