



Phylogenetic Distribution of the C₃ and C₄ Syndrome in the Centrospermeae (Dicot) Species

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ABSTRACT: *Centrospermeae* species were collected at different sites along gradient of altitude and aridity in the semi-arid, saline and arid habitats in western region of Kenya. $\delta^{13}\text{C}$ values, Kranz leaf anatomy and carbon dioxide compensation points were used to examine for C₃, C₄, C₃-C₄ intermediate and Crassulacean metabolism (CAM) species photosynthesis. The C₄, C₃, C₃-C₄ and CAM species were confirmed to be present in the *Centrospermeae* group in different proportionate percentage. Interspecific species occur in the group. The percent frequency of the photosynthetic type show that the C₄ syndrome exhibit recent phylogenetic development and might have evolved independently several times in the dicot species. Further, the distribution of C₄ pathway is both taxonomically uncommon and phylogenetically wide spread among the species of the *Centrospermeae*. Results show C₄ photosynthesis is a trait of modern dicotyledonae species and is of multiple evolutionary origins. Thus, the C₄ syndrome is polyphyletic in origin. C₄ subtypes exist in the *Centrospermeae* group. The data on the distribution and occurrence of the C₄, C₃, C₃-C₄ and CAM syndrome types can be used to understand the phylogenetic affinity in a given taxonomic unit of the angiosperm. More data on the distribution of the C₄, C₃-C₄ in the angiosperm is required to understand the phylogenetic affinity and their biological functions and consequences during the evolutionary processes at different development phases of the plant species.

Keywords: *Centrospermeae*, Dicot species, C₄, C₃, C₃-C₄ and CAM species, Phylogenetic affinity, Polyphyletic origin

I. INTRODUCTION

There is consensus that C₃ pathway evolved first and wide spread in terrestrial and aquatic species and habitats (Sikolia, Onyango, Beck and Kinyamario, 2009[1]). The C₄ syndrome is phylogenetic younger achievement and apparently evolved independently in monocots and dicot perhaps as many as twenty times (Quade and Cerling, 1995[2]; Edwards, Franceschi and Vozsenenskaya, 2004[3]; Edward, Furbank, Hatch and Osmond, 2001[4] [1] Sikolia, 2016[5]). This interpretation is corroborated by the existence of different types of the C₄ syndrome, namely, the NADP-Malic type, the NAD-malic type and the PEP-Carboxykinase [1]. Phylogenetic distribution of the C₄ syndrome has been extensively investigated in the Gramineae and Cyperaceae families but few dicot species. Studies in the dicot do not provide comprehensive data for analysis of the taxonomic distribution of the plants possessing C₃ and C₄ photosynthetic pathway. Furthermore, the few studies on the dicots have rarely been carried in the semi-arid and or saline tropical ecosystems. Exhaustive studies are therefore warranted. Downton (1975) [6], Imbamba and Papa (1979) [7], Mateus-Andre's (1993) [8] and AKhana, Trimborn and Ziegler (1997)[9] have listed some of the investigated C₄ perennial species. However, in the Euphorbiaceae some of the C₄ tree forms do occur (Percy and Troughton, 1975) [10].

An exhaustive study in the Middle East and USSR (Winter, 1981) [11] has shown most of the flora are C₄ species. They include trees or tall shrubs of high biomass with economic value, in that they are fast growing, sand binders, for fuel, honey, improved biodiversity and source of alkaloids. Clearly, there is need for more information on the occurrence of C₄ species, especially from semi-arid and arid regions outside North America [11]. This missing gap and/or situation on the C₄ dicot studies and data availability have not changed much [5]. Similarly, there is little information about the relationships within the Sedges, Cyperaceae, and *Centrospermeae* and more so about their quantitative occurrence. The relative causal factors that influence C₄ species partitioning

in their ecosystem, whether monocots or dicots have recently been discussed in detail (Ehleringer *et al*, 1997[12] [5]). The current studies will attempt to correct the imbalance in research and investigation bias against the dicot studies. Thus, in C₄ photosynthesis, we have C₄ pathway whose quantitative data is both taxonomically unknown and phylogenetically not completely described [1]. Exhaustive studies should enable an evolutionary assessment of the hierarchical dichotomous tree of the angiospermous species and across the families (Raghavendra, 1980[13]; Das and Raghavendra, 1973[14]). Further studies of the occurrence of interspecific differences in the operation of the C₄ pathway would provide biological lead to examine the close relationship between C₃ and C₄ species [13] and therefore the evolutionary selective forces for photosynthetic pathway development. Taxonomically, species misplacement in a family can be explained, especially from semi-arid ecosystems.

Takeda, Ueno and Agata (1980) [15] reported C₄ pathway in three of the seven tribes of Cyperaceae: Cyperdeae, Fimbristyldeae and Rhynchosporae, and Brown (1977) [16] reported in the Poaceae. Further, C₄ syndrome is reported in the Poaceae (Hattersley and Watson, 1976[17]; Hattersley, 1984[18]; Prendergast, Hattersley and Stone, 1987) [19], *Sueda monoica* (Shomer-Ilan, 1975) [20], *Arudinella hirta* (Crookston and Moss, 1973) [21], Asteraceae (Smith and Turner, 1975) [22], *Eleocharis*, a sedge (Ueno, Samejima and Koyama, 1989) [23], *Scaevola* and *Euphorbia* (Robichaux and Percy, 1984) [24], *Flaveria* (Monson and Charles, 1991) [25], Chenopodiaceae [9], intermediate *Flaveria* (Ku, Monson, Littlejohn, Nakumaoto, Fischer and Edwards, 1983) [26]. The study is informed that there is little information on the ecology or ecophysiology of C₃-C₄ intermediates [26]. Further, no information is available on the characteristics of C₃-C₄ intermediates in their natural habitats. This lack of information has hindered the understanding of the adaptive and evolutionary characteristics of the C₃-C₄ intermediates [26]. Furthermore, these studies are useful in the postulation of phylogenetic affinities within a given family or families. This paper provides data on the phylogenetic distribution and affinity amongst the dicot species of the Centrospermeae families. The study involved Aizoaceae, Amaranthaceae, Basellaceae, Caryophyllaceae, Chenopodiaceae, Nyctaginaceae, Phytolacaceae, Polygonaceae, Portulacaceae, Zygophyllaceae and Elatinaceae families of the Centrospermeae. Further, the study provides the much required quantitative data on the C₃, C₄ and C₃-C₄ intermediate dicot plant species in their semi-arid, arid and saline habitats.

II. MATERIALS AND METHODS

Sampling Sites

Sampling sites were randomly selected along North West-North East belts transect. The belt transect was about 30km wide between 36° 30'E and 34° 30'E, ran from south-west of Mt. Elgon to Mt. Kulal near Lake Turkana. It was about 365km long rising from 670m at Lodwar to 4200m at Mt. Elgon through 250m in Kerio valley up to around 2,415m to 2293m Mt. Kulal, 950m Samburu game reserve and Kapedo-Nginyang. Twenty-five quadrats were placed at 10m interval random sites along the belt transect. The dicot species of the Centrospermeae were collected for identification from each quadrat, counted and recorded. Percentage frequency of occurrence of each species sampled in the quadrats was determined. Thus, the total number of each species divided by the total species in the sampled in the quadrats times 100% was worked out.

Identification of the Plant Species

Identification was done both in the field and laboratory. Unknown species were sent to the East African Herbarium or Chiromo Campus Herbarium, University of Nairobi, Kenya for verification. Healthy floral organs and leaves organs were collected for plant identification and Kranz leaf anatomy studies, respectively. Leaves were dried in natural conditions between 25 °C -30 °C in the field.

Determination of the C₃ and C₄ species

Kranz leaf anatomy, δ¹³C values and carbon dioxide compensation points were used to distinguish between the C₃ and C₄ dicot species [1] [5].

Anatomical Studies

Microtome sections for Kranz leaf anatomy (presence/absence) were prepared and studied. C₄ and C₃ species exhibited Kranz leaf anatomy and non-Kranz leaf anatomy, respectively. Modified anatomical description of 0-3 cells or 5-15 cells or 4-5 cells for the lateral cell count was adopted in the studies [17].

Measurement of the $\delta^{13}\text{C}$ Data

Leaves were dried with circulating air in the oven at 25 °C -30 °C until there was no change in weight. Dried leaves of each species were milled into minute grains used for the determination of ¹³C/¹²C isotopic analysis of the species. Dried leafy-milled grains of each species were analyzed using an elemental analyzer (HERAEUS CHN.O RAPID) for Dumas combustion of the samples, a FINNIGAN MAT Delta (δ) gas isotope

mass spectrometer with a dual inlet system, a method of Gebauer and Schulze (1987) [27]. Standard gas carbon dioxide was calibrated with respect to international standard (CO in Pee Dee Belemnite) by use of the reference substance NBS 16 to 20 for carbon isotopic ratio provided by the International Atomic Energy Agency (IAEA), Vienna. The ¹³C/¹²C isotopic ratios (denoted as δ values or ¹³C/¹²C values), were calculated using the formula equation below:

$$\delta_x = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000(\text{‰})$$

where, δ_x is the isotope ratio of carbon in delta units relative to the international standards and R_{sample} and R_{standard} are the ¹³C/¹²C ratios of the samples and standards, respectively [27]. The δ¹³C values were assigned against each species investigated.

Measurement of the Carbon Dioxide Compensation Points

Six week old plant species in enclosed in gas-airtight cover using vaseline oil just before the experiment to prevent gas exchange with the soil, was put in an airtight 19litre fan stored glass chamber at 20 °C controlled temperature. The carbon dioxide concentration(ppm) were continuously measured by pumping a stream of air through a closed, oxygen tight, circuit with an Infra-Red Gas Analyzer (Biros 1.1, Leybold, Hanau, Germany) for carbon dioxide compensation points measurements [5]. A constant light intensity of 350 μmol/m²/sec was used. The carbon dioxide concentration in the chamber was arbitrary set by injecting small amount carbon dioxide with a calibrated syringe through a small rubber plug in the jar lid, at a time to avoid fluctuations on the scale recorder. Experiments were run in atmospheres ranging from 1000ppm to 60-40ppm or 20-8ppm carbon dioxide. Sodium hydroxide pellets were used to remove excessive transpired moisture from the gas chamber. The carbon dioxide bind effect was nullified because it was used in each of the experiment carried out.

III. RESULTS

The families of Centrospermeae studied were as follows: Aizoaceae, Amaranthaceae, Basellaceae, Caryophyllaceae, Chenopodiaceae, Nyctaginaceae, Phytolacaceae, Polygonaceae, Portulacaceae, Zygophyllaceae and Elatinaceae. Two hundred and fifty-eight species were collected and analyzed for the photosynthetic pathway types. About seventy-two plant species possess the C₄ photosynthetic pathway whereas one hundred and eighty possess the C₃ photosynthetic type. Basellaceae was represented with *Basella Alba* and *B. paniculata* and investigation indicates the presence of CAM pathway as shown by carbon dioxide compensation points. Studies indicated *Mollugo nudicaulis* (Aizoaceae) is a C₃-C₄ intermediate dicot species. *Portulacaria afra* species require further investigations to ascertain the status of its photosynthetic type. The syndrome shows C₃-C₄ intermediate syndrome to C₃ syndrome manifest at different stages of development. About 28.5% of the total species investigated were C₄ species, 70.3 % were C₃ species and 1.2% C₃-C₄ intermediate/CAM species. Concomitantly, the C₄ photosynthetic pathway occurs in the dicot species of the Centrospermeae group in the angiosperm. Interspecific photosynthetic species occurred in the genera of *Mollugo* and *Trianthema* in the Aizoaceae, *Aerva* and *Alternanthera* in the Amaranthaceae, *Melandrium* and *Silene* in the Caryophyllaceae, *Gyroptera* in the Chenopodiaceae and *Dianthus* in the Elatinaceae.

The C₃ and C₄ syndrome in the Aizoaceae, Amaranthaceae, Caryophyllaceae, Chenopodiaceae, Nyctaginaceae, Phytolacaceae, Polygonaceae, Portulacaceae, Zygophyllaceae and Elatinaceae were represented by 63.6%:36.4%, 21.1%:79.9%, 9.5%:90.5, 72.7%:27.3%, 33.3%:66.7%, 0%:100%, 22.2%:78.2%, 14.3%:85.7%, 50%:50% and 25%:75% percent frequency at the generic level, respectively as shown in Figure 1. In the figure 1, legend 1 represents the C₄ genera and legend 2 represents C₃ genera whereas the families: Aizoaceae, Amaranthaceae, Caryophyllaceae, Chenopodiaceae, Nyctaginaceae, Phytolacaceae, Polygonaceae, Portulacaceae, Zygophyllaceae and Elatinaceae are represented on -axis by 1,2,3,4,5,6,7,8,9 and 10, respectively. It is significant to observe that there is equal probability that C₄ and C₃ syndrome exist in the Zygophyllaceae family whereas no possibility of the C₄ syndrome in the Phytolacaceae family. Caryophyllaceae shows low degree of C₄ syndrome occurrence but Phytolacaceae shows the C₃ syndrome only. Further, Portulacaceae shows low frequency of the C₄ syndrome

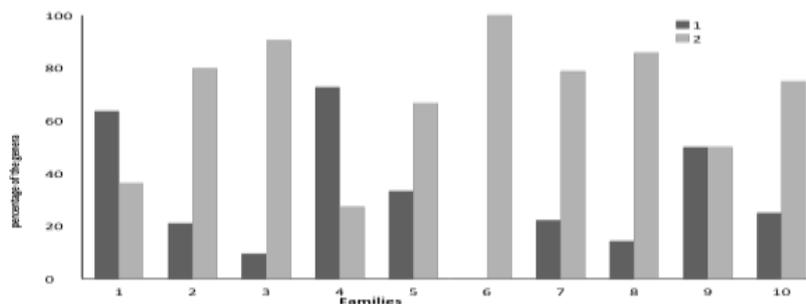


Figure 1. The percent distribution and occurrence of the C₃ and C₄ genera in the dicot families of the Centrospermeae

compared to the C₃ syndrome. Thus, the progressive and sequence of the C₄ syndrome assumes the following trend: Chenopodiaceae (72.7%) >> Aizoaceae (63.6%) >> Zygophyllaceae (50%) >> Nyctaginaceae (33.3%) >> Elatinaceae (25%) >> Polygonaceae (22.2%) >> Amaranthaceae (21.1%) >> Portulacaceae (14.3%) >> Caryophyllaceae (9.5%) >> Phytolacaceae (0%), in the Centrospermeae group. This reflects also the possible degree of phylogenetic affinity amongst the parameters that determine the syndrome at the generic level. This option can be interpreted differently depending on the observation of further studies of the C₄ syndrome between and within the genera and genus, respectively.

The C₃ and C₄ syndrome in the Aizoaceae, Amaranthaceae, Caryophyllaceae, Chenopodiaceae, Nyctaginaceae, Phytolacaceae, Polygonaceae, Portulacaceae, Zygophyllaceae and Elatinaceae were represented by 53.9%:46.1%, 28.3%:71.7%, 8.2%:91.8%, 50.0%:50.0%, 33.3%:66.7%, 0%:100%, 9.1%:98.9%, 53.9%:46.1%, 50.0%:50.0%, 14.3%:85.7% percent frequency at specific epithet level, respectively, as shown in Figure 2. In the figure 2, legend 1 represents the C₄ species and legend 2 represents C₃ species whereas the families: Aizoaceae, Amaranthaceae, Caryophyllaceae, Chenopodiaceae, Nyctaginaceae, Phytolacaceae, Polygonaceae, Portulacaceae, Zygophyllaceae and Elatinaceae are represented on x-axis by 1,2,3,4,5,6,7,8,9 and 10, respectively.

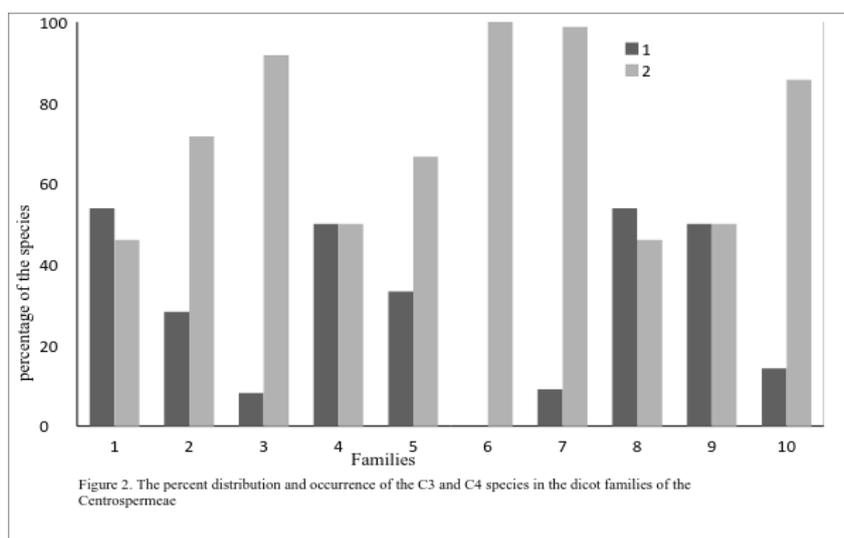


Figure 2. The percent distribution and occurrence of the C₃ and C₄ species in the dicot families of the Centrospermeae

It is worth to observe the percent frequency difference in the distribution and occurrence of the C₄ syndrome in the Aizoaceae, Chenopodiaceae and Portulacaceae at the specific epithet level and generic level. Further, there is minimal percent frequency difference in the distribution of the C₄ syndrome in the Amaranthaceae, Caryophyllaceae, Polygonaceae and Elatinaceae but no difference is recorded in Nyctaginaceae, Phytolacaceae and Zygophyllaceae families.

IV. DISCUSSION

Three principal mechanism of carbon dioxide reduction occur in the Centrospermeae families, the dicot group of angiosperm. The C₃ pathway is certainly the most common followed by C₄ pathway, C₃-C₄ intermediate, and Crassulacean metabolism in the investigated dicot species. Studies have shown that C₄ pathway evolved recently from the C₃ syndrome [1][12]. The distribution of C₄ pathway is both taxonomically uncommon and phylogenetically wide spread among the species of the Centrospermeae. The C₄ syndrome exhibit recent phylogenetic development and might have evolved independently several times in monocots and

dicots [1]. The low composition and distribution index of the C₄ mechanism amongst the Centrospermeae indicates its relatively recent description on the evolutionary mesoscale and has been reported in a small percentage (< 1%) of extant species (Ehleringer, Sage, Flanagan and Pearcy, 1991[2] [3]) and is highly polyphyletic in the angiospermous families [1]. This is further exemplified with the transition in the Kranz leaf anatomy paradigm in different species of angiosperms in different terrestrial and aquatic species and habitats (Monson, 1989[29]; Monson, Edwards, and Ku, 1984[30][12]; Drincovich, Casati, Andreo, Chessin, Franceschi, Edwards and Ku, 1998[31]; Ueno, Takeda, Samejima and Koyama, 1986 [32]; Vozsenenskaya, Franceschi, Kiirats, Freitag and Edwards, 2001[33]; P'yankov, Vozsenenskaya, Franceschi, Kiirats, Freitag and Edwards, 1997[34]; Crookston and Moss, 1973[21]; Brown, 1975[35], 1977[16]). Similarly, the CAM lends further evidence to the progressive evolutionary phenomenon in the nature of photosynthesis development. Regimes observed or recorded in the environmental factors played a disproportionate role in the evolutionary trends of the biological reactions and structural ramifications in the Centrospermeae dynamics.

The percentage frequency of the genera or species showing a particular photosynthetic mechanism showed varied occurrence and distribution with respect to the two levels of taxonomic determination. The C₄ photosynthetic pathway was genera specific with the exception of the Portulacaceae. The percent frequency of C₄ composition in the genera, 12.5%, could not be used to deduce an estimated percent frequency of 53.6% for the species level. The C₄ syndrome occurrence in the genera should be reference point of divergence from C₃ syndrome but define or show high degree of phylogenetic affinity in that genera or species epithet level. Deviation from this paradigm is an indication of disparity in the taxonomic identification and determination process of the genera or specific epithet of the plant species along the hierarchy of species, genera and family lineage and placement. The parameters used in the determination should be re-evaluated if there are doubts about taxonomic placement. However, the distribution of the syndrome is noticeable in different taxonomic units.

The highest degree of occurrence and distribution of C₄ pathway was recorded in Aizoaceae but least in Caryophyllaceae and non-existent in the Phytolacaceae and Basellaceae. Similarly, C₃ pathway was widely distributed in the genera of the Phytolacaceae, Caryophyllaceae, Polygonaceae, Portulacaceae, Amaranthaceae and Elatinaceae with over 80% in the sampled species. The distribution had lower index in the Zygophyllaceae and Chenopodiaceae but lowest in the Aizoaceae. An observation that Portulacaceae recorded high percent of C₃ mechanism occurrence and distribution at the genera level is significant. This is because the habit of succulence offers morphological advantage of the C₄-like activity in their habitat. The general trend is a high percentage of C₄ species occurrences with corresponding reduced proportion at the genera level, for example, in the Portulacaceae and Amaranthaceae. This phylogenetic percent shift, for example in the Amaranthaceae, may be exhibited by their advanced refining adjustment responses towards variable climatic ecotypes and therefore its ecotypes superiority against the species in the other families, between 200m a.s.l. to 2000m a.s.l. Similar trend of occurrence does not exist for the C₃ mechanism in a given family.

The percent distribution of the C₃ and C₄ mechanisms and the associated species in the families of the Centrospermeae are characterized by specific interrelated attributes of the syndrome which function well in their habitats. These attributes are anatomical, biochemical, physiological and ecological in nature. The attributes and climatic variables [1] [12] [31] [32] [33], P'yankov, Vozsenenskaya, Kondratchuk and Black, (1997) [35] synergistically plays role in the partitioning of the C₃ and C₄ photosynthetic types, abundance and occurrence of the species in their habitats [1] [12] [32]. This process of partitioning must have impacted on the evolution and expansion of the C₃ photosynthetic pathway variants, the C₄, C₃-C₄ and Crassulacean acid metabolism (CAM) during the early period of angiosperm development and became common in families present around 60M BP [3].

The percent C₃ species and C₄ species distribution in the Centrospermeae families reveals varying degree of development expansion of the photosynthetic mechanism that enables taxa to spread and occupy different ecological habitats with varied conditions. Similar observations have been recorded in different habitats in Namibia (Ellis, Vogel and Fuls, 1980 [36]; Vogel, Fuls and Ellis, 1986 [37]) [1]. These evidences are indicator indices for the partitioning of the photosynthetic mechanisms caused by earlier (2.5 to 3.3 billion years BP) and prevailing varying climatic and environmental conditions[12], Sage (2004) [38]. An oxygenic atmosphere, for example, did not develop until 18 to 2.3 billion years BP [3] [4] [33] and relatively recent (60M BP) climatic conditions became more seasonal and certainly in some geographical areas, more and likely saline. This must have influenced and modified the structural apparatus, physiological and biochemical processes to adapt to the emerging conditions and sustain the primary bioproductivity of the dicot species [1], Hatch and Slack (1998) [39]. The changing status in the carbon dioxide metabolic organization must have provided selective force for the highly polyphyletic nature existing in the Centrospermeae families today.

Further subdivision of the C₄ photosynthetic mechanism reveal three subgroups based on differences in the mode of carboxylation: NADP-Malic enzyme type, NAD-Malic enzyme type and PEP Carboxykinase type. Analysis of the three subtypes of the C₄ species suggests that the malate –forming NADP-me species thrive in

less water stress habitats while the aspartate-forming NAD-me species are successful under xeric conditions [37], Schulze, Ellis, Schulze, Trimbom and Ziegler (1996) [40]. The C₄ subtypes, NAD-me, for example, *Portulaca oleracea* and NADP-me, for example *Portulaca grandiflora*, *P. quadrifida* and *P. pilosa* showed decreasing relative abundance gradation along the altitude as temperature increased, respectively [1]. Other examples of NADP-me type, NAD-me type and PEP Ck-type species included *Amaranthus hybridus*, *Portulaca quadrifida* and *Zygophyllum simplex* species, respectively (Sikolia, Ph.D. Thesis) [41].

Recent studies has revealed unique and novel anatomies observed in dicot species including some Chenopodiaceae species (Freitag and Stichler, 2000) [42]; Asteraceae species (Guadalupe and Katinas, 2003) [43] and *Eleocharis vivipara* (Minorsky, 2002) [44]. Thus, there is need to investigate the angiosperm species to provide concise and elaborate structural and cellular organization of the different photosynthetic types and subtypes to understand the unique blending with the physiological and biochemical specialization in enhancing bioproductivity in different environmental conditions, worldwide. The data derived will also be useful in understanding phylogenetic relationships of the C₃, C₄ and C₄ variants, C₃-C₄ intermediate syndromes between species and genera and within the genera in different habitats and different families of the angiosperm. Further, data on the C₃-C₄ and C₄ species is significant for the evaluation and prediction of vegetation change during global climatic and geographic change and for the restoration and conservation of natural ecosystems (2004) [45]. The studies of C₃-C₄ intermediate are encouraged and can provide knowledge and evidences to evaluate the extent of the evolutionary process of C₄ photosynthesis.

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