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The Distribution of C_3 and C_4 Photosynthetic Species of the Centrospermeae Along an Altitudinal Gradient in Western Kenya

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Abstract: Two hundred and seventy eight species of the Centrospermeae were collected at different sites in Western Kenya representing gradients of altitude and aridity. Climate data were obtained from meteorological research stations. Species were examined for C_3 or C_4 photosynthesis using the anatomical Kranz syndrome, $\delta^{13}C$ values and carbon dioxide compensation points. C_4 photosynthesis is a feature of modern members of dicotyledoneae is of multiple evolutionary origins. It evolved independently in members of the same family and was found in one to several genera and then often only with two to three species. C_4 species are concentrated in lowland habitats subjected to high temperature, low precipitation and high evaporation. High $\delta^{13}C$ values is associated with low water availability which is a physiological syndrome and also a feature of saline habitats. The C_3 representatives of the Centrospermeae dominate in more moist and colder habitats, especially at higher altitudes. Only a few C_4 species occur at high altitudes (3000-4000 m) namely *Sagina gallica*, *Silene abyssinica* and *Melandrium nordiflorum*. The transition zone between C_3 and C_4-dicot is rather narrow between 1500 to 1700 m and thus much lower than that recorded for the monocots (2000-2200 m). The general pattern of $\delta^{13}C$ values distribution along the altitudinal gradient show that the values of -10.60 to -16.55, -17.75 to -18.87 and -18.89 to -32.42‰ that corresponds to altitudinal ranges, 0-1500, 1550-1700 and 1800-4200 m, respectively. The low altitudes are associated with drought and high temperatures. C_4 and C_3 dicot species can be intercropped to increase bioproductivity for the betterment of the flora and fauna in the semi-arid and arid ecosystem. C_4-species are potential candidates for exploitation in the agroforestry systems especially for long-term management programmes. The present study may also be relevant for better understanding of global change with respect to the diversity of photosynthetic pathways, herbivory and vegetation dynamics.

Key words: C_4 and C_3 photosynthetic species, climatic factors, altitude, transition zone, ecological significances

INTRODUCTION

The coexistence of diverse plant species with different photosynthetic pathways is now well established (Ehleringer *et al.*, 1997; Wang, 2004). The groups are characterized as CAM, C_4 and C_3 plants. There is consensus that the C_3 pathway evolved first and is wide spread in terrestrial and aquatic habitats. The C_4 syndrome is a phylogenetic younger achievement and apparently evolved independently in monocots and dicots perhaps as many as twenty times (Smith *et al.*, 1976). This interpretation is corroborated by the existence of different types of the C_4 syndrome, namely the NADP-malic type, the NAD-malic type and the PEP-Carboxykinase type (Hatch, 1987). C_4 photosynthesis: a unique blend of modified biochemistry, anatomy and ultrastructure. Nevertheless, 40% of the taxa in the Poaceae show the C_4 syndrome (Tieszen, 1978). A No. of genera contain both C_3 and C_4 species but only very few species in the Paniculoides and of the genus Flaveria are C_3-C_4 intermediates (Drincovich *et al.*, 1998; Edwards *et al.*, 2001). Thus, most species are clearly either C_3 or C_4 and are not interconvertible.

The C_3 and C_4 species possess different structural, physiological and biochemical features resulting in different water use efficiency and adaptation to climate (Ehleringer *et al.*, 1997; Wang, 2004) has been demonstrated in the Gramineae and Cyperaceae families. As C_4 plants are better adapted to higher temperatures and arid or saline conditions, knowledge of the C_4 syndrome and its occurrence in the plant kingdom is also required for the understanding and prediction of vegetation change during global climatic change and for the conservation and restoration of natural ecosystems (P'yankov *et al.*, 2000). Similar ecological studies of the dicots are too few for a sound assessment of a correlation

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between their occurrence and environmental variables. Centrospermeae group has been proposed to possess different photosynthetic types (Tieszen, 1981). But the proportion of the photosynthetic types has not been studied. Furthermore, ecological significances associated with C_3 and C_4 systems should be characterized in detail, especially in the dicot families. The objective of this study is to present data on the mesoscale distribution of the C_3 and C_4 syndrome in the Centrospermeae choosing gradients of altitude and aridity. Western Kenya was selected as sampling area because of its wide altitudinal range (up to 4200 m) broad range of air temperature, evaporation, precipitation in different rainfall regimes and even radiation, in habitats varying from open grassland to semi-arid/or arid deserts with intermittent saline habitats.

MATERIALS AND METHODS

This study was carried out in the 2000-2003 duration.

Geographical description of the gradient(s) and collection of the ecological data of the sampling sites: The studies were carried out in the semi-arid and saline areas. Sampling sites were randomly distributed along North West-North East belt transect. The transect about 30 km 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 365 km long rising from 670 m at Lodwar to 4200 m at Mt. Elgon through 250 m in Kerio Valley up to around 2,415 to 2293 m Mt. Kulal, 950 m Samburu Game reserve and 900 m at Kapedo-Nginyang. Sites were sampled in a broad range of vegetation types from semi-arid regions to near snowline. Attempt was made to sample at least some communities from each of the regions of available soil moisture as defined for Kenya by Woodland (1981). This annual index is based upon soil water storage, precipitation and Penman estimates of water surplus divided by the annual total evaporation. Climatic data were sourced from meteorological stations in the area of study. Ten-year climatic data were obtained from meteorological stations at the area of study - North West Kenya and their means calculated over the time period. Field climatic data was also measured. The stations included: Rohet, Lokori, Perkerra, Lodwar, Chewoyet and Kitale, Nginyang, Chemolingot, Lokichogio and Marigat areas. The mean annual values of the climatical variables collected included: maximum and minimum temperature (°C); monthly rainfall; relative humidity (%); daily radiation (J m^{-2}); monthly potential evaporation (mm^3 m^{-2}) and altitude (m), measured by a thermometer, maximum-minimum thermometer, rain gauge, thermopile pyranometer, litre pan water drying per meter area in a day and altimeter, respectively. Twenty-five Quadrats were

placed at 10 m interval random sites along the belt transect. Percentage of frequency of occurrence of each taxon of the sampled Quadrats was determined. Thus, the total number of each species divided by total species in the sampled quadrats times 100%.

Identification of the specimens: Identification was made in the field. Difficulty specimens were identified in the East African Herbarium or the Chiromo Campus herbarium, University of Nairobi, Kenya for verification. Nomenclature followed by Clayton (1974), Beentje (1994), Blundell (1992), Lotschert and Beese (1994) and Olembo *et al.* (1995), system of identification and documentation, respectively. Healthy leaves and desirable floral organ of the species were collected from different sites and enclosed in wet toweling paper in Jar(s). Enclosed containers for short transportation to the laboratory or brief storage were used. Leaves of species were dried in natural conditions between 25-30°C in the field.

Anatomical investigations: The classification of the dicot species into C_3 or C_4 or CAM groups was made on the basis of the structural Kranz anatomy (or, more specifically, the 0-3 cells or 5-15 cells or 4-5 cells for the maximum lateral cell count of the modified method of Hattersley and Watson (1975), $\delta^{13}C$ values and CO_2 compensation points.

Determination of the $\delta^{13}C$ data: Air oven at 25-30°C circulation dried leaf or vegetative organ until there no change in weight. Dried specimens were used for $^{13}C/^{12}C$ isotopic analysis of species at Bayreuth Universitat, Germany. 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). Standard gas of carbon dioxide was calibrated with respect to international standard (CO_2 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 $^{13}C/^{12}C$ isotopic ratios (denoted as δ values or $^{13}C/^{12}C$ values), were calculated according to the following equation:

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

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 $^{13}C/^{12}C$ ratios of the samples and standards, respectively (Gebauer and Schulze, 1987). The $\delta^{13}C$ values were recorded against each species studied.

Measurement of the carbon dioxide compensation points:
Six week old species in a pot enclosed in a gas-tight cover
using Vaseline oil just before the experiment to prevent
gas exchange with the soil, was put in an airtight, 19 L fan
stored glass chamber at 20°C controlled temperature. The
carbon dioxide concentrations (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 point measurements. A constant light
intensity of 350 μmol/m²/sec was used. The carbon
dioxide concentration in the chamber was arbitrarily set by
injecting small amount CO_2 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 1000 parts per million to
either 60-40 or 20-8 ppm CO_2. Sodium hydroxide pellets
were used to remove excessive transpired moisture from
the gas chamber. The carbon dioxide-sodium hydroxide
bind effect was nullified because it was used in each of
the experiment carried out.

RESULTS

Elements of climate for the ecological study area
temperature (°C), rainfall (mm), relative humidity (%),
radiation (cal cm^{-2} or J m^{-2}) and rates of potential
evaporation (mm m^{-2}) had their mean annual values
worked out. Climatic elements were correlated to the
abundance of either the C_3 or C_4 species along the
altitude.

The vegetation consists of drought resistant species.
High percentage of the region is bare especially in dry
spell. Annual grassland, with or without dwarf shrub
lands, mbuga grassland, scattered woody plant species
such as *Acacia reficiens* may exist. Perennial grassland
which is mostly ephemeral appears after a downpour
especially during the April long rains. Afro-Alpine belt
has groundsel, *Lobelia, Alchemilla*, dominated with
Podocarpus-Juniperus and large pasture. Sometimes
Podocarpus-Juniperus is mixed with *Arundinaria alpina*
and the subnival tussock grassland (*Festuca-Agrostis*).
At low end of the mountain ends of valleys are pastures
and partly replaced by *Dendrosenecio-FestucaAgrostis*.
Sometimes small forests of small ericaceous-leaved trees
(*Erica-Philippi*) occur.

Distribution of the species along the altitude: In total
more than two hundred species of the Centrospermeae
families were studied. Figure 1 shows the frequency of
Centrospermeae species along an altitudinal gradient from
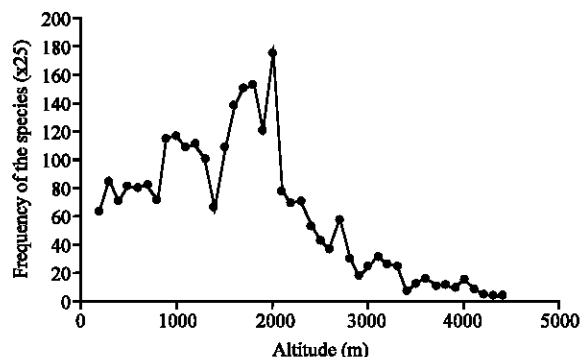200-4,200 m. The highest diversity of Centrospermeae

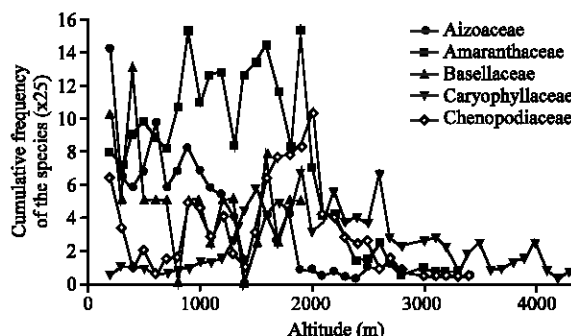Fig. 1: Distribution of the centrospermeae species along
the altitude

Fig. 2: Cumulative frequency of the species in the families
of the centrospermaea along the altitude

species was recorded between 900 and 2300 m. A dramatic
decrease in species richness was observed above 2000 m
and only very few species were recorded above 3500 m.
Nevertheless even *Sagina gallica, Silene abyssinica* and
Melandrium nordiflorum species were found on the top
of Mt. Elgon at an elevation of 4,200 m. The habitat exhibit
high degree of aridity. The habitat was characterized by
high diurnal temperature fluctuations, low moisture
availability, high relative humidity and high rates of
potential of evaporation.

Figure 2 and 3 show the representation of the
Centrospermeae families along the gradient.
Portulacaceae, Caryophyllaceae and Polygonaceae had
the widest range of distribution reaching from 200-4,200 m
and the species diversity of latter two families exhibited
several pronounced peaks between 1500 and 3000 m. The
highest species diversity was found with the
Amaranthaceae, the Phytolacaceae and the Polygonaceae.
The species in the families are typical lowland.
Biodiversity was an adaptation to environmental factors,
especially temperature.

The patterns of distribution of the species along
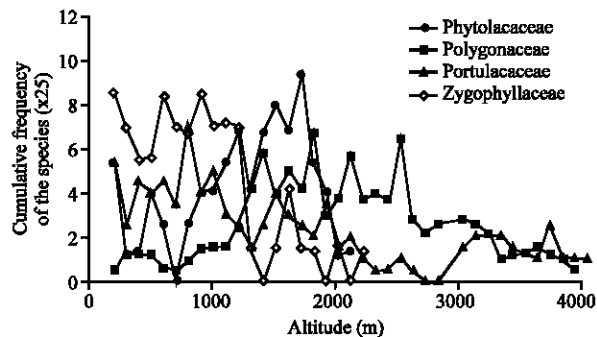the altitude in different families were very variable.

Fig. 3: Cumulative frequency of the species in the families of the centrospermaea along the altitude

Species of Amaranthaceae had broad range spatial distribution. Species of the Polygonaceae, Caryophyllaceae, Aizoaceae, Chenopodiaceae and Portulacaceae had average spatial distribution. Low species spatial distribution was associated with Nyctaginaceae, Phytolacaceae and Caryophyllaceae, Basellaceae.

The distribution of the species was predominantly genera specific. At the altitude less than 1300 m: *Gisekia*, *Mollugo* and *Trianthema* of Aizoaceae, *Aerva*, *Celosia*, *Digera* and *Amaranthus* of Amaranthaceae, *Polycarpon* and *Stellaria* of Caryophyllaceae, *Oxygonum* and *Polygonum* of Polygonaceae, *Calyptrotheca*, *Portulaca* and *Talinum* of Portulacaceae had high frequency compared to the species of Basellaceae and Phytolacaceae. At the altitude less than 1400 m: *Gisekia* and *Mollugo* were almost not available, *Delosperma* and *Limeum* showed low frequencies (with *Trianthema* at 1800 m) for the Aizoaceae, *Achyranthes*, *Aerva*, *Cyanthula*, *Amaranthus* of Amaranthaceae, *Cerastium*, *Comes*, *Drymaria*, *Pollichia* and *Polycarpon* had good ecological cross-over (GECO), *Silene* (Very-GECO), *Sagina* of Caryophyllaceae, *Chenopodium* and *Sueda* (GECO) and *Gyroptera* of Chenopodiaceae and most genera of Phytolacaceae (GECO). At altitude 1500-2300 m, individual species in the genera mentioned occurs in almost even frequency. Individual species in the genera: *Sagina* (at 3,500 m), *Stellaria* (at 1400 m and then towards 3300 m) and *Melandrium* of Caryophyllaceae (at 4,000 to 4,200 m mostly) and *Trianthema* of Aizoaceae (at 1800 m), are of ecological significance because deviate from the expected distribution trend. Too, they occur at almost snowline sites associated with high precipitation, very low temperatures, high radiation, increased moisture and relative humidity indices. Further, individual species of *Oxygonum*, *Polygonum* and *Rumex* of Polygonaceae does

well at high altitudes whereas that of *Tribulus* of Zygophyllaceae least occur or not at all above 1400 m of the sampled sites.

Carbon ($^{13}C/^{12}C$) isotope discrimination: The $\delta^{13}C$ values of the species show three distinct groups of the C_3, C_3-C_4 intermediate and C_4 photosynthetic types. The $\delta^{13}C$ values of the groups are summarized as follows: (i) 68 species fall between -10.60 and -16.55‰ with a mean of -13.65‰ and standard deviation of 1.8; (ii) 171 species between -21.16 to -30.93‰ with a mean of -26.40‰ and standard deviation of 1.94, respectively. An exception case of -32.42‰ value for *Chenopodium capitatum* was significant variation. Intermediate species, *Mollugo nudicaulis* (Aizoaceae) and CAM species: *Basella alba* and *Basella paniculata* (Basellaceae) had $\delta^{13}C$ values as -25.89, -18.75 and -17.79‰, respectively, with a mean of -20.81‰ and standard deviation of 4.4. The first cluster is characteristic of C_4 species, the second for the C_3 species, the third for the C_4-C_3 intermediate species and/or CAM species with $\delta^{13}C$ values which fall between -10.60 to -17.70‰, -21.16 to -30.93‰ and -17.79 to -18.75‰, respectively.

C_3-C_4 intermediate species occurred in the Aizoaceae (*Mollugo nudicaulis*) and CAM species in Basellaceae (*Basella alba* and *Basella paniculata*).

The C_4 species were conspicuously not recorded in two families - Basellaceae and Phytolacaceae. Basellaceae exhibit C_3-C_4 intermediate status but Phytolacaceae possess C_3 pathway. C_3-C_4 intermediate metabolism could be due to the transition evolution process in the Centrospermeae group. C_3-C_4 intermediate species included *Mollugo nudicaulis*, *Portulacaria afra* and *Portulaca* sp. Nov. The C_3-C_4 intermediate percent frequency of occurrence was approximately 1.25% of the Centrospermeae species. The C_4 type occur in the Amaranthaceae (16.7%), Caryophyllaceae (9.5%), Chenopodiaceae (70%), Nyctaginaceae (33.3%), Polygonaceae (11.1%), Portulacaceae (12.5%), Zygophyllaceae (50.00%) and Elatinaceae (25%) (Table 1). Amaranthaceae possess fewer C_4 species than Chenopodiaceae with almost similar growth habit and habitat. About 28.5% of the total species investigated were C_4 species, 70.3% C_3 species and 1.2% C_3-C_4 intermediate species. Concomitantly, in the C_4, a photosynthetic pathway whose distribution is both taxonomically rare and phylogenetically wide dispersed among the species occurs.

C_3 and C_4 species occurred in the genera- *Mollugo*, *Melandrium*, *Alternanthera*, *Trianthema* (Aizoaceae), *Aerva* (Amaranthaceae), *Silene* (Caryophyllaceae), *Gyroptera* (Chenopodiaceae) and *Dianthus*

Table 1 : Distribution of the C_3 pathway and C_4 pathway in the Centrospermeae group

Families	Genera (%)		Species (%)	
	C_4-species	C_3-species	C_4-species	C_3-species
Aizoaceae	63.6 (7)	26.4 (4)	50.0 (14)	50.0 (14)
Amaranthaceae	16.7 (3)	83.3 (15)	30.4 (14)	69.6 (32)
Caryophyllaceae	9.5 (3)	90.5 (19)	8.3 (4)	91.7 (44)
Chenopodiaceae	70.0 (7)	30.0 (3)	53.6 (15)	46.4 (13)
Nyctaginaceae	33.3 (1)	66.7 (2)	33.3 (2)	66.7 (4)
Phytolaccaceae	0.0 (0)	100.0 (2)	0.0 (0)	100.0 (7)
Polygonaceae	11.1 (1)	88.9 (8)	5.7 (2)	94.3 (33)
Portulaccaceae	12.5 (1)	87.5 (7)	53.6 (15)	46.4 (13)
Zygophyllaceae	50.0 (2)	50.0 (2)	50.0 (5)	50.0 (5)
Elatinaceae	25.0 (1)	75.0 (3)	16.7 (1)	83.3 (5)

Values in brackets are the total number species collected

(Caryophyllaceae), *Sesuvium sesuvioides* (-12.21‰) of Aizoaceae, *Silene abyssinica* (-10.9‰) of Caryophyllaceae, *Gyroptera gillettii* (-12.12‰), *Salsola pestifera* (-10.60‰) of Chenopodiaceae, *Tribulus terrestris* (-10.68‰) of Zygophyllaceae, *Boerhavia elegans* (-11.99‰) of Nyctaginaceae had their $\delta^{13}C$ values less negative than -12.22‰. These $\delta^{13}C$ values are occurred in the extreme dry habitats and could be an indicator of dryness of the habitat. The same is true for C_3 species with $\delta^{13}C$ values that are more positive just outside the $\delta^{13}C$ values range of C_3 species which is -22.40 to -30.34‰. These C_3 species were *Pandiaka lanunginosa* of Amaranthaceae (-21.42‰), *Lychnis viscaria* of Caryophyllaceae (-21.84‰), *Polygonum ampibium* of Polygonacaceae, *Talinum cuneifolium* and *P. paniculatum* of Portulacaceae. These six species have been found in the dry habitat and saline soils of Baringo and Turkana regions in Kenya. *Salsola pestifera* (-10.60‰) and *Tribulus terrestris* (-10.68‰) having a less negative $\delta^{13}C$ values are widely distributed mostly in extremely dry habitat ecotypes. *T. terrestris* is related to the more $\delta^{13}C$ negative value species of -12.56 and -13.40‰, for the species, *T. parvispinus* and *T. cistoides*, respectively. These regions show high degree of dryness. Most of the *Amaranthus* species were C_4 species showing wide distribution upto the transition zone for the C_3 species and C_4 dicot species along the altitude.

The distribution abundance of the C_3 and C_4 Centrospermeae species showed different relationships with the climatic variables: mean annual temperature (Fig. 4), mean annual rainfall (Fig. 5, 6), mean annual minimum temperature (Fig. 8) and mean annual evaporation (Fig. 7). The percentage of C_3 species and C_4 species were plotted against the climatic variables and their correlation coefficients, provided. The present studies showed correlation coefficients of r = 0.94, r = 0.91, r = 0.87, r^2 = 0.27 for the percentage of C_4 species against mean annual temperature (Fig. 4), mean annual rainfall (Fig. 5), mean annual evaporation (Fig. 7), relative humidity, respectively. The correlation coefficient,
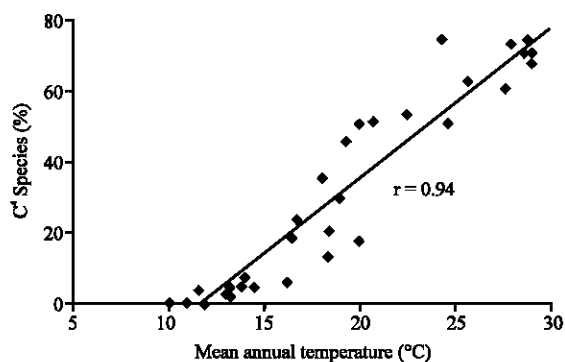

Fig. 4: Correlation distribution percent of C_4 species and mean annual temperature along the elevational transect
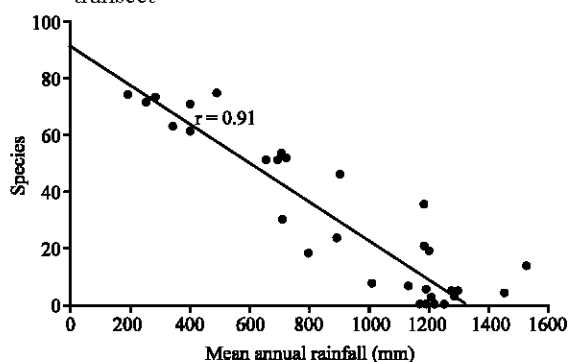

Fig. 5: Correlation distribution percent of C_4 species and mean annual rainfall along the elevation transect

r = 0.92, r = 0.91, r = 0.85 and r^2 = 0.097 for the percentage of C_3 against mean annual rainfall (Fig. 6), mean annual temperature, mean annual evaporation and mean annual relative humidity, respectively. The C_4 pathway was inversely correlated to increasing mean annual rainfall (Fig. 5) and vice versa for the C_3 pathway (Fig. 6). The mean annual temperature (°C) and mean annual rainfall (mm) were critical factors determining the abundance (%) of the C_3 and C_4 dicot species along the altitude (m) compared to the mean annual relative humidity and mean
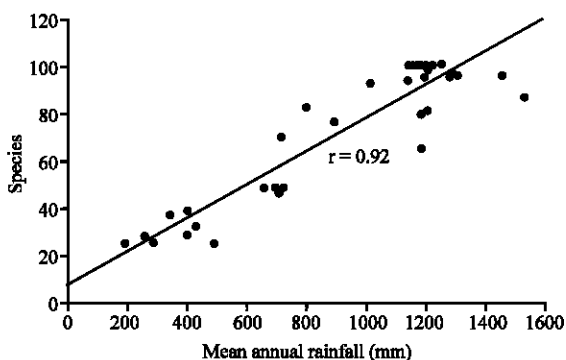
Fig. 6: Correlation distribution percent of C_3 species and mean annual rainfall along the elevation transect
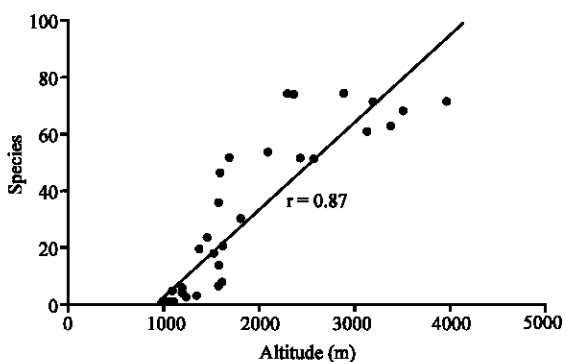

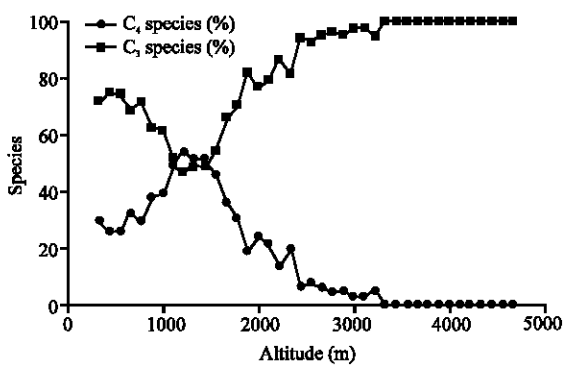Fig. 7: Correlation distribution percent of C_3 species and mean annual evaporation along the elevation transect

Fig. 8: Distribution percent curves of C_3 species and C_4 species of the centrospermeae along the altitudinal transect

annual evaporation. High temperature associated and low moisture (rainfall) favoured increased percentage of C_4 species (Fig. 4, 5) but low temperatures and high mean annual rainfall favoured increased number (or percentage) of the C_3 species along the increasing altitudinal gradient.

Mean annual evaporation was a promoter factor rather than the critical determinant factor for the abundance of the C_3 dicot species (Fig. 7) or C_4 dicot species. Mean annual relative humidity was neither a critical nor a promoter parameter. Therefore, mean annual temperature is the dominating determinant parameter correlated with the abundance of C_4 dicot species and its effects on the C_4 dicots species distribution increased with increasing aridity indices in the present study. Probably, relative humidity act as a synergistic factor with the major climatic determinant factors for example mean annual temperature and precipitation and evaporation.

The transition zone associated with the switch between C_3 dominance and C_4 dominance of abundance occurs between 1,500-1,700 m. Here, the transition zone was defined as the point at which C_4 abundance fell below 50%, based on the taxonomic basis and not necessarily on a percent C_4 biomass basis. This transition zone was defined by climatic variables as mean annual minimum temperature (°C), mean annual maximum temperature (°C, mean annual rainfall (mm), mean annual potential evaporation (mm^3 m^{-2}), mean annual radiation (J m^{-2}) and mean annual relative humidity (%) of 15.7, 25.6°C, 700 to 950 mm, 1575-1650 mm^3 m^{-2}, 450-500 J m^{-2} and 50-55%, respectively. Mean annual temperature range of 20.7 to 25.6°C is the temperature for the transition shift from the C_3 to C_4 dominance, regardless of elevation or latitudinal gradients. High temperatures, high evaporation and low moisture (rainfall or precipitation) are characteristic of aridity associated with the abundance of C_4 dicot species contrasting C_3 dicot species. The distribution and abundance (%) of the C_3 and C_4 species along the altitude show a transition phase as rather abrupt and sharp (Fig. 8).

DISCUSSION

Phylogenetic distribution: The taxonomic distribution of species exhibiting C_3 pathway and C_4 pathway attained 70.4 and 29.4% amongst the Centrospermeae group, respectively. Aizoaceae and Chenopodiaceae have high percentage of 63.6 and 70% of the genera exhibiting C_4 pathway. Similar phenomenon at the species level attained 50, 53.6 and 53.6% of the C_4 pathway in the Aizoaceae, Chenopodiaceae and Portulacaceae, respectively. Amaranthaceae, Basellaceae, Caryophyllaceae, Chenopodiaceae, Nyctaginaceae, Phytolacaceae, Polygonaceae, Portulacaceae, Zygophyllaceae and Elatinaceae recorded 30.4:69.6%, 0:0%, 8.3:91.7%, 53.6: 46.4%, 33.3:66.7%, 0:100%, 5.7:94.3%, 53.6:46.4%, 50:50% and 16.7:83.3% for C_4 species: C_3 species proportion percentages, respectively. C_3 and C_4 type (interspecific)

species occurred in the genera- *Sesuvium, Trianthema, Alternanthera, Melandrium, Silene, Gyroptera, Portulaca* and *Zygophyllum*. This suggests interesting and perhaps recent evolutionary origins in the C_4 photosynthesis restricted to advanced members of the Centrospermeae. Multiple evolutionary origins of the C_4 pathway were evidently recorded in 25 of the 98 genera of the Centrospermeae. This is calculated at 28.6% of the genera studied. C_3-C_4 intermediate pathway occurs in the *Mollugo nudicaulis, Portulacaria afra* species.

Monson *et al.* (1984) reported the occurrence of the super-orders of the dicotyledonae and monocotyledonae. Most of the species exhibiting the C_4 pathway are quite separated from each other and belong to different genera, The finding reinforces the notion that the C_4 pathway must have evolved independently multiple times. This view is held by Monson (1989), Ehleringer and Monson (1993) and Ehleringer *et al.* (1997). Within the monocots, C_4 photosynthesis is quite common among the Poaceae and Cyperaceae that are closely related taxonomic groups (Hattersley, 1987; Hattersley and Watson, 1992). Lack of occurrence of the C_4 syndrome in the Phytolacaceae suggests its old ancestral lineage and greater phylogenetic diversity among the Centrospermeae group. But, Caryophyllaceae and Polygonaceae could be the initial point of phylogenetic development based on the low percentage of the C_4 syndrome occurrence.

According to Ehleringer *et al.* (1997), the C_4 syndrome in the dicots occurs in only 16 of the approximately 400 families. The present study reveals that in each family, C_4 pathway occur in only one to several genera and then often only within two to three species. Exception to this trend occurred in the Aizoaceae, Amaranthaceae and Portulacaceae, where C_4 pathway is common among the genera. This is not the trend in Phytolacaceae. Furthermore, C_3-C_4 intermediate metabolism can exist in the genus that does not possess C_4 pathway, for example *Portulacaria afra*. The C_3 photosynthesis occurs in all the families of the Centrospermeae and shows high degree in the Caryophyllaceae (90.5%), Nyctaginaceae (66.7%), Phytolacaceae (100%), Polygonaceae (78.8%) and Portulacaceae (85.7%) families. This taxonomic distribution pattern is consistent with the fact that C_4 photosynthesis may has evolved recently in the tropical dicots (Ehleringer *et al.*, 1997).

Climatic variables and distribution of species: The photosynthetic species are influenced by climatic differences. Species at low altitudes are associated with high temperature, relative humidity, potential rates of evaporation, radiation and low degree of precipitation. The group consists of a higher percentage of the C_4

species. High temperature and low precipitation played a dominant and synergistic causal role, whereas the other factors modified the frequency percent distribution of the species. These variables associated with aridity reveal the C_4 syndrome is arid-adaptive. Similar studies in Poaceae of the Northern Saharan Desert (Winter *et al.*, 1976), in Kenya (Tieszen and Imbamba, 1976), in Java (Hofstra *et al.*, 1972), Ireland (Collins and Jones, 1985), Cyperaceae in Japan (Ueno and Takeda, 1992), Gramineae in North Argentina (Cavaganaro, 1988) and Poaceae in Australia (Hattersley, 1983), Israel (Vogel *et al.*, 1986) and salty areas of Hungary (Kalapos *et al.*, 1997), are also consistent with the present observation. In contrast, C_3 dicot species occur in high frequencies at high altitudes. The CAM species-*Basella alba* and *B. paniculata and* C_3-C_4 intermediate species-*Mollugo nudicaulis* and *Portulacaria afra* occur in the range between the extremes of the climatic factors.

The C_4 dicot species- *Melandrium nordiflorum* thrive in areas with the mean annual minimum temperature below 8°C. Similarly, Teeri and Stowe (1976) reported few or no C_4 species in habitats where July minimum temperatures were below 8°C.

Generally, the C_4 biochemical pathway concentrates carbon dioxide at the site of RUBP carboxylation (Hatch, 1976; Doliner and Jolliffe, 1979) that is of selective advantage whenever internal carbon dioxide concentration becomes low. These conditions occur under high light intensities, periods of water stress and stomatal closure. The sites at low altitudes are subjected to serious water stress, high temperatures, high evapotranspiration and high irradiances. The C_4 species are selectively adapted to the four climatic factors and therefore have competitive advantage against the C_3 species.

Ecological features of lowland habitats that allow the occurrence of C_3 species: There are few exceptions to the present general distribution pattern, which helps provide insight into the factors controlling distributions. The few C_3 dicot species at low altitudes were found mainly in the understorey of closed forests and their families often consisted of the C_4 species. Also, some of the C_3 dicot species exhibit short timing life cycle synchronized with the rainfall spell. Under these conditions irradiances would be quite low and leaf temperatures would only uncommonly exceed 32°C. Ehleringer (1978) and Ehleringer and Bjorkman (1977) showed that temperature below 32°C provide higher quantum yield for C_3 compared to the C_4 plants. Thus, all factors being equal, the C_3 species should have carbon balance advantage in the shaded environments and maximum leaf temperatures

below 32°C. The present studies support this view. Furthermore, most of the C_3 dicot annuals dominated some of the lowland or aquatic areas. Polygonaceae and *Caryophyllaceae* species formed a higher percentage of the understorey communities at low altitudes.

C_4 species growing at high altitudes: Few of the C_4 dicot species thrive at the high altitudes. The species include *Sagina gallica, Silene abyssinica* and *Melandrium nordiflorum* (4,000 m), *Calyptrotheca* species (3000 to 3300 m) species. The species have thick epidermal layer that would resist low temperatures and may partly account for the C_4 dicots at high altitude. The species occur in low frequency and disappear above 4,000 m. The species belong to the following genera: *Gisekia, Delosperma, Limeum, Mollugo,* (Aizoaceae), *Aerva, Amaranthus Gomphrena* (Amaranthaceae), Emex, *Polycarpea, Silene,* (Caryophyllaceae), *Gyroptera* (Chenopodiaceae), *Fagonia tribulus, Zygophyllum* (Zygophyllaceae), *Calyptrotheca, Portulaca, Talinum* (Portulacaceae) were common at very low arid altitudes. But, some of the C_4 genera: *Silene,* (Caryophyllaceae), *Sueda* (Chenopodiaceae), *Emex, Fagopyrum* (Polygonaceae) were less common in more arid areas. The present distribution show that species of the Caryophyllaceae, Chenopodiaceae and Polygonaceae thrive in hot moist habitats, often associated with saline conditions. They commonly occur near lakes in the arid, for example *L. baringo* and *L. turkana.*

The C_3 dicot species are found at the warmer ending regions of the C_3 monocot species distribution pattern and often coexist with increasing C_4 dicot species.. This frequency species-photosynthetic syndrome relationship, suggest high C_4 composition in the lowland understorey conditions compared to the C_3 composition at the high altitudes. There is monocot-dicot photosynthetic pathway relationship whereby at a given altitudinal range the frequency of the C_4 dicot syndrome overlaps C_4 monocot syndrome with decreased proportion and C_3 monocot syndrome overlaps C_3 dicot syndrome with increased proportion along the altitude. This is a good indicator system of the evolutionary process which is due to the synergistic effect of climatic factors in a given photosynthetic pathway at a given point. The net effect of the climatic factors influences the proportion of the photosynthetic species in the habitat along an elevation gradient.

The present studies shows that mean annual temperature and mean annual precipitation determines the distribution and percent frequency of the photosynthetic species in the semi arid and arid areas.

Current linear correlation coefficients between percent frequency of the species and altitude mean annual temperature, rainfall, potential rates of evaporation, radiation and relative humidity show good positive correlation, in this decreasing order. The C_3 dicot species show negative correlation coefficient with temperature, potential rates of evaporation parameters. The parameters are inversely related to the photosynthetic systems as altitude increases. This study confirms the observations of Tieszen *et al.* (1979) and Rundel (1980). At high altitudes (>4000 m) higher water availability also occur as a consequence of lower evapotranspiration and dewfall.

Tribulus terrestris, T. cistoides of Zygophyllaceae exhibited the shade tolerant phenomenon. The results indicate the potential for C_4 genotypes to adapt to shade conditions and acclimatize. The present data for relative distribution of C_4 and C_3 dicots demonstrate that patterns of the relative dominance of the photosynthetic systems along the climatic gradients is due to the synergistic degree of temperature, precipitation, shade and evapotranspiration. Further, the elevation transition zone between the C_3 and C_4 metabolic systems dominance is expected to be much sharper on the observed plant coverage compared to the floristic composition basis. The C_4 species susceptibility to low temperatures (Berry and Bjorkman, 1980) was mostly the NADP-ME type that may be related to Pyruvate Phosphate Dikinase cold liability (Sugiyama *et al.*, 1979). This cold acclimatization is known in various families (Caldwell *et al.*, 1977; Jones *et al.*, 1981; Winter, 1981; Hattersley, 1992). In general, C_4 dicot species like C_3 dicots increase in number with rainfall in their preferred temperature regime. The C_4 species are numerous where the area is hot and wet but the C_3 species in the cool and wet areas. The C_4 species decline with decreasing temperature and increasing seasonal rainfall whereas the C_3 species decline with increasing temperature and decreasing seasonal rainfall. Within the group of C_4 dicot the Malate-forming NADP-ME species favoured habitats where water stress was less severe.

Recently, some European C_4 plants have been observed extending towards northern latitudes, for example the *Spartina* species, *Salsola kali, Atriplex laciniata* (Long, 1983) and *Cyperus longus* (Jones *et al.*, 1981). Therefore, unique occurrence of *Melandrium nordiflorum* dicot species at 4,000 m raises significant ecological questions, in the present study. Evolution and adaptations in the C_4 species is a continuous process. While precipitation is obviously essential to initiate plant growth, temperature is such a dominant factor in regression analyses that it explains more than 90% of the total C_3/C_4 monocot distribution

variation across almost all geographic regions. Clearly, temperature has manifested itself as the critical determining factor in the distribution of the C_4 species.

The transition temperature was a critical switch between C_3 dominance and C_4 dominance in the present study. Here, transition temperature was defined as a point at which C_4 abundance fell below 50%, based on the taxonomic basis. Most of the studies converge on the daytime temperature range of 20-28°C as the temperature for the transition shift in C_3-C_4 dominance, either based on elevational or latitudinal gradients. The transition zone between C_3 and C_4 groups is rather abrupt and occurs at 1,500-1,700 m.a.s.l. along the altitude, less behind that recorded for the monocots in Java (Hotstra *et al.*, 1972) and Kenya (Tieszen *et al.*, 1979) at 2,000-2,300 m.a.s.l. altitude. This transition zone was defined by climatic variables as mean annual minimum temperature (°C), mean annual maximum temperature (°C, mean annual rainfall (mm), mean annual potential evaporation (mm m^{-2}), mean annual radiation (cal cm^{-2}) and mean annual relative humidity (%) of 15.7, 25.6°C, 700-950 mm, 1575 to 1650 mm m^{-2}, 450-500 J m^{-2} and 50-55%, respectively. Mean annual temperature range of 20.7-25.6°C is the temperature for the transition shift from the C_3 to C_4 dominance, regardless of elevation.

Earlier studies have shown that C_4 monocots might comprise well over half of the monocots taxa in a region. The present study has shown that the C_4 dicots constituted approximately 30% of the flora of the Centrospermeae. Studies in the Sonoran hot desert with summer precipitation reported 4.4% (Stowe and Teeri, 1978; Wentworth, 1983). The present studies showed that with decreasing degree of aridity index, the percent (or frequency) of C_4 dicot species decreased. In contrast, the C_3 dicot species increased. Similar observations have been reported in subtropical regions such as Florida (2.5%) and Texas (2.8%) (Ehleringer *et al.*, 1997) Europe (Collins and Jones, 1985), Japan (Okuda and Furukawa, 1990). The abundance of C_4 NAD-ME dicots increases with decreasing aridity unlike C_4 NADP-ME dicots.

The present data analysis show that the percent of C_4 dicots does not follow the same climatic factor as for C_4 monocots but are closely related to the aridity characters such as summertime pan evaporation rates and annual dryness ratio (ratio of annual net rates of radiation to heat required to evaporation mean annual precipitation). Instead, there was a stronger correlation coefficient of $r^2 = 0.89$, $r^2 = 0.83$ and $r^2 = 0.76$ between mean annual temperature, mean annual precipitation, mean annual rates of evaporation and the % C_4 dicot species, respectively. However, there was inverse relationship with the % C_3 dicot species. The conclusion is that the distribution and

abundances of C_4 monocots and C_4 dicots respond to the same climatic parameters, with varying intensity and behaviour in similar ecological regimes.

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