

$\delta^{13}\text{C}$ Values of the *Centrospermeae* Species and Their Ecological Implications in the Semi Arid Conditions

¹S. Sikolia, ²E. Beck, ³J.I. Kinyamario, ¹J.C. Onyango and ¹G. Ouma

¹Maseno University, P.O. Box 333, Maseno, Kenya

²Lehrstuhl Fur Pflanzenphysiologie, University of Bayreuth, 95440, Germany

³School of Biological Sciences, University of Nairobi, P.O. Box 30197-00100, Kenya

Abstract: $\delta^{13}\text{C}$ values of the *Centrospermeae* species are presented. 69.5, 28.45, 1.25 and 0.8% of the total species are C_3 , C_4 , $\text{C}_3\text{-C}_4$ and CAM photosynthetic species. The $\delta^{13}\text{C}$ values are species dependent. $\delta^{13}\text{C}$ values for the C_3 range from -21.16 to -30.28‰ while the C_4 species vary from -10.60 to -16.55‰. An exceptional $\delta^{13}\text{C}$ value of -32.28 is reported for *Chenopodium capitatum*. $\delta^{13}\text{C}$ value for the CAM species vary from -16.00 to -18.50‰. $\text{C}_3\text{-C}_4$ species includes *Mollugo nudicaulis*, *Portulacaria afra* and *Portulaca* sp. nov. with $\delta^{13}\text{C}$ values -25.89, -20.93 and -15.66‰, respectively. Temperature and precipitation are the dominant causal climatic factors that influence the distribution of the C_3 and C_4 species inversely and by extension the $\delta^{13}\text{C}$ values along the altitude. Other climatic factors act synergistically. A difference in the $\delta^{13}\text{C}$ values is a biochemical dual function of the Rubisco and suberized lamella anatomical structural organization. The occurrence of some C_4 species in the unusual high altitude includes *Melandrium nordiflorum* and *Silene abyssinica* and may be due to the Pyruvate Phosphate Dikinase (PPDK) enzyme functional activity. Ecological significances of the $\delta^{13}\text{C}$ values are discussed.

Key words: $\delta^{13}\text{C}$ values, photosynthetic path ways, C_3 and C_4 transition altitudinal zone, centrospermeal species, ecology

INTRODUCTION

The C_3 and C_4 species are distinguished using $\delta^{13}\text{C}$ values (Bender, 1971; Epstein *et al.*, 1997). Plants promote isotopic fractionation of carbon dioxide atmospheric source during photosynthesis, such that lighter isotope (^{12}C) is favoured than heavier isotope (^{13}C). This lead to less ^{13}C isotope compared to higher atmospheric carbon dioxide in plants. The degree of discrimination varies between C_3 and C_4 plants but also amongst the species of a given photosynthetic metabolism. The C_4 species category has less negative values compared to the C_3 species category. Knowledge on the $\delta^{13}\text{C}$ value is mainly accrued from studies of the monocots. However, the degree of isotopic fractionation in the dicot species requires more $\delta^{13}\text{C}$ value data from diverse climatic conditions for ecological comparison.

Differences in the isotopic ratio values are used to solve scientific problems, for instance, to trace the flow the flow of carbon as differential dietary inputs (Hobson, 1999), reconstruct paleodiets (Schoeninger *et al.*, 1983; Macko *et al.*, 1999), follow the transport of carbon across ecosystems (Conte and Weber, 2002), establish the authenticity of liquids, determine climatic patterns as strong geographic signature (Craig, 1961), address forensic problems such as determining the point of origin of illicit drugs (Ehleringer *et al.*, 2000; Carter *et al.*,

2002), trace origin of infectious microbial source (Kreuzer-Martin *et al.*, 2003, 2004), fingerprints of biological agents as forensic tool (Horita and Vass, 2003). Recently, isotopic ratio value analysis has been used to study the distribution of photosynthetic species along the altitude and climatic factors that influence their distribution (Sikolia *et al.*, 2008). Further, knowledge of the occurrence C_3 and C_4 species in the plant kingdom is required to understand and predict vegetation change in the present global climatic scenario to conserve and restore our natural ecosystems (P'yankov *et al.*, 2000). Similarly, ecological studies in the dicots are too few for sound correlation between their occurrence and environmental variables compared to studies in the monocots.

Centrospermeae group has been proposed to possess different photosynthetic types (Ehleringer *et al.*, 1997), whose proportion has not been studied. Furthermore, ecological significance associated with C_3 and C_4 photosynthetic systems should be characterized in detail, especially in the dicot families. It is main objective in this study, to present data on the mesoscale proportion of the C_3 and C_4 photosynthetic species in the Centrospermeae using the isotopic ratio analysis method. Western Kenya was selected as study area because of its broad altitudinal range (up to 4200 m), wide 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

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. 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. Difficult specimens were identified in the East African Herbarium, Kenya, for verification. Nomenclature followed by Agnew and Shirley (1994), Blundell (1992), Lotschert and Beese (1994) and Olemba *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.

Determination of the δ¹³C data: Air oven at 25-30°C circulation dried the leaf or vegetative organ until there was no change in weight. Dried specimens were used for ¹³C/¹²C isotopic analysis 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 (1991). Standard gas of carbon dioxide was calibrated with respect to international standard (CO₂ 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 according to the following equation:

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

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 (Gebauer and Schulze, 1991). The δ¹³C values were recorded against each species studied.

RESULTS

CARBON (¹³C/¹²C) isotope discrimination: The δ¹³C values of the species show four distinct groups of the C₃, C₃-C₄ intermediate, C₄ and CAM photosynthetic types. The δ¹³C value of the C₄ and C₃ 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) 165 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), *Portulacaria afra* (Portulacaceae) and CAM species: *Basella alba* and *Basella paniculata* (Basellaceae) had δ¹³C values as -25.89, 20.93, -18.75 and -17.79‰, respectively, with a mean of -18.44‰ and standard deviation of 4.4. The first cluster is characteristic of C₄ species, the second for the C₃ species, the third for the C₄-C₃ intermediate species and/or CAM species with δ¹³C values which fall between -10.60 to -17.70‰, -21.16 to -30.93‰ and -17.79 to -18.75‰, respectively.

The C₄ species were not recorded in two families-Basellaceae and Phytolacaceae. Phytolacaceae possess C₃ species only. The C₃-C₄ intermediate percent frequency of occurrence was approximately 1.25% of the Centrospermeae species. The percentages of the C₄ and C₃ type occurrence in the families of Centrospermeae are shown in Table 1. Amaranthaceae possess fewer C₄ species than Chenopodiaceae yet have almost similar growth habit and habitat. About 28.45, 69.5, 1.25 and 0.8% of the total species investigated were C₄, C₃ species, C₃-C₄ intermediate and CAM species, respectively.

Table 1: Distribution of the C₃ pathway and C₄ pathway in the Centrospermeae group

Families	Genera (%)		Percentage of species	
	C ₄ -species (%)	C ₃ -species (%)	C ₄ -species (%)	C ₃ -species (%)
Aizoaceae	63.6 (7)	26.4 (4)	50 (14)	50 (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 (7)	30 (3)	53.6 (15)	46.4 (13)
Nyctaginaceae	33.3 (1)	66.7 (2)	33.3 (2)	66.7 (4)
Phytolacaceae	0 (0)	100 (2)	0 (0)	100 (7)
Polygonaceae	11.1 (1)	88.9 (8)	5.7 (2)	94.3 (33)
Portulacaceae	12.5 (1)	87.5 (7)	53.6 (15)	46.4 (13)
Zygophyllaceae	50 (2)	50 (2)	50 (5)	50 (5)
Elatinaceae	25 (1)	75 (3)	16.7 (1)	83.3 (5)

Values in brackets are the total No. of species collected

Table 2: $\delta^{13}\text{C}$ values of the centrospermeae species

Family/species	$\delta^{13}\text{C}$ values
Aizoaceae	
<i>Carbichonia decumbens</i>	-12.50
<i>Delosperma abyssinica</i>	-13.73
<i>Delosperma nakurense</i>	-13.45
<i>Gisekia africana</i>	-11.77
<i>Gisekia pharnacoides</i>	-11.99
<i>Glinus lotooides</i>	-26.79
<i>Glinus oppositifolius</i>	-25.04
<i>Glinus seliflorus</i>	-25.37
<i>Hypertelis bowkeriana</i>	-23.32
<i>Limeum fruticosum</i>	-26.82
<i>Limeum indicum</i>	-24.51
<i>Limeum praetermissum</i>	-23.75
<i>Limeum viscosum</i>	-24.40
<i>Mollugo nudicaulis</i>	-25.89
<i>Mollugo cerviana</i>	-15.36
<i>Sesuvium portulacastrum</i>	-24.88
<i>Sesuvium sesuvioides</i>	-12.21
<i>Tetragonia acanthocarpa</i>	-22.90
<i>Tetragonia tetragonoides</i>	-23.84
<i>Tetragonia expansa</i>	-23.81
<i>Trianthema ceratosepala</i>	-23.66
<i>Trianthema portulacastrum</i>	-12.48
<i>Trianthema triquetra</i>	-13.25
<i>Trianthema salsoides</i>	-13.86
<i>Trianthema sedifolia</i>	-13.86
<i>Zelaya pentandra</i>	-12.26
Amaranthaceae	
<i>Achyranthes aspera</i> L. var. <i>pubescens</i> (Moq.) C.C. Townsend	-30.89
<i>Achyranthes aspera</i> L. var. <i>Sicula</i> L.	-30.93
<i>Achyroopsis fruticola</i>	-25.13
<i>Achyroopsis greenwayi</i>	-25.18
<i>Aerva javanica</i>	-14.41
<i>Aerva lanata</i>	-28.12
<i>Aerva leucura</i>	-26.53
<i>Alternanthera pungens</i>	-14.75
<i>Alternanthera sessilis</i>	-25.51
<i>Amaranthus caudatus</i>	-16.55
<i>Amaranthus dubius</i>	-15.14
<i>Amaranthus graecizans</i>	-15.83
<i>Amaranthus hybridus</i>	-14.70
<i>Amaranthus lividus</i>	-12.96
<i>Amaranthus patulus</i>	-13.12
<i>Amaranthus retroflexus</i>	-13.48
<i>Amaranthus spinosus</i>	-13.98
<i>Amaranthus thunbergii</i>	-13.45
<i>Amaranthus species</i>	-13.48
<i>Celosia anthehmintica</i>	-25.66
<i>Celosia argentea</i>	-25.42
<i>Celosia hastata</i>	-28.22
<i>Celosia polystachya</i>	-27.35
<i>Celosia sweinfurthiana</i>	-27.97
<i>Celosia trygina</i>	-23.55
<i>Centemopsis gracilentia</i>	-26.73
<i>Centemopsis kirkii</i>	-27.97
<i>Celosia rubra</i>	-25.34
<i>Centrostachya aquatica</i>	-23.15
<i>Centrostachya coriacea</i>	-28.35
<i>Centrostachya cylindrica</i>	-27.42
<i>Centrostachya orthacantha</i>	-27.99
<i>Centrostachya polycephala</i>	-28.24
<i>Centrostachya uncinulata</i>	-26.91
<i>Digera muricata</i>	-25.67
<i>Gomphrena celosioides</i>	-13.08
<i>Hemstaedia gregorii</i>	-29.59
<i>Pandaka lanuginosa</i>	-21.42
<i>Psilotrichum elliotii</i>	-23.30
<i>Puppalia grandiflora</i>	-24.36
<i>Puppalia lappacea</i>	-24.17
<i>Puppalia micrantha</i>	-25.67

Table 2: Continued

Family/species	$\delta^{13}\text{C}$ values
<i>Sericomopsis hilderbrandtii</i>	-23.41
<i>Sericomopsis pallida</i>	-25.56
<i>Volkensinia prostrata</i>	-25.26
Basellaceae	
<i>Basella alba</i>	-18.75
<i>Basella paniculata</i>	-17.79
Caryophyllaceae	
<i>Arenaria foliacea</i>	-24.83
<i>Arenaria montana</i>	-25.66
<i>Cerastium adnivale</i>	-27.53
<i>Cerastium afromontanum</i>	-25.68
<i>Cerastium corymbosa</i>	-27.67
<i>Cerastium indicum</i>	-26.26
<i>Cerastium octandrum</i>	-25.13
<i>Corrigola capensis</i>	-25.78
<i>Corrigola litoralis</i>	-27.47
<i>Comes abyssinica</i>	-26.76
<i>Drymaria cordata</i>	28.69
<i>Gypsophila elegans</i>	-26.82
<i>Gypsophila oldhamiana</i>	-25.67
<i>Gypsophila gillettii</i>	-25.64
<i>Lychnis alba</i>	-30.28
<i>Lychnis githago</i>	-22.41
<i>Lychnis cororiana</i>	-25.58
<i>Lychnis viscaria</i>	-21.84
<i>Melandrium nordiflorum</i>	-13.57
<i>Melandrium rubrum</i>	-13.11
<i>Melandrium persicum</i>	-26.75
<i>Mimuartia elenbeckii</i>	-27.57
<i>Mimuartia juniperiana</i>	-30.24
<i>Polichia campestris</i>	-25.75
<i>Polycarpea corymbosa</i>	-13.12
<i>Polycarpon prostratum</i>	-27.23
<i>Polycarpon tetraphyllum</i>	-28.00
<i>Sagina abyssinica</i>	-27.09
<i>Sagina apetala</i>	-26.65
<i>Sagina afroalpina</i>	-25.36
<i>Silene abyssinica</i>	-10.91
<i>Silene burchelli</i>	-26.76
<i>Silene dioica</i>	-25.70
<i>Silene gallica</i>	-25.72
<i>Silene longitubulosa</i>	-25.79
<i>Silene nocteolens</i>	-29.52
<i>Silene macrosolen</i>	-26.75
<i>Silene species</i>	-26.85
<i>Silene vulgaris</i>	-27.27
<i>Spercula arvensis</i>	-25.94
<i>Stellaria marnii</i>	-26.72
<i>Stellaria media</i>	-27.69
<i>Stellaria sennii</i>	-28.26
<i>Uebelinia abyssinica</i>	-27.45
<i>Uebelinia cf. rotundifolia</i>	-25.01
<i>Uebelinia crassifolia</i>	-24.75
<i>Vaccaria pyramidata</i>	-28.75
<i>Sponaria vascaria</i>	-26.65
<i>S. depressus</i>	-26.56
Chenopodiaceae	
<i>Arthrocnemum indicum</i>	-13.70
<i>Atriplex coriacea</i>	-14.35
<i>Atriplex farinosa</i>	-16.33
<i>Atriplex halimus</i>	-12.39
<i>Atriplex semibaccata</i>	-15.18
<i>Atriplex muelleri</i>	-14.68
<i>Beta vulgaris</i>	-13.25
<i>Chenopodium album</i>	-26.70
<i>Chenopodium ambrosioides</i>	-27.45
<i>Chenopodium botryoides</i>	-26.80
<i>Chenopodium capitatum</i>	-32.42
<i>Chenopodium carinatum</i>	-25.03
<i>Chenopodium fasciculosum</i>	-27.44
<i>Chenopodium murale</i>	-25.64
<i>Chenopodium opolifolium</i>	-25.72

Table 2: Continued

Family/species	$\delta^{13}\text{C}$ values
<i>Chenopodium procerum</i>	-25.47
<i>Chenopodium pumilio</i>	-27.70
<i>Chenopodium rubrum</i>	-26.70
<i>Chenopodium schwaderanum</i>	-25.44
<i>Fadenia zygophylloides</i>	-13.50
<i>Gyroptera gillettii</i>	-12.12
<i>Gyroptera somalensis</i>	-25.54
<i>Kochia indica</i>	-12.54
<i>Kochia scoparia</i>	-12.94
<i>Salicornia pachystachya</i>	-26.70
<i>Salsola dendroidea</i>	-13.62
<i>Salsola pestifera</i>	-10.60
<i>Suaeda monoica</i>	-14.92
Phytolacaceae	
<i>Gallesia gorarema</i>	-25.65
<i>Hillarie latifolia</i>	-29.01
<i>Phytolaca americana</i>	-28.37
<i>Phytolaca chilensis</i>	-27.53
<i>Phytolaca dioica</i>	-23.79
<i>Phytolaca dodecandria</i>	-23.49
<i>Phytolaca octandria</i>	-23.32
Polygonaceae	
<i>Antipogon leptopus</i>	-28.62
<i>Coccoloba uvifera</i>	-30.08
<i>Emex australis</i>	-12.79
<i>Emex spinosus</i>	-12.36
<i>Fagopyrum esculentum</i>	-13.53
<i>Harpagocarpus snowdenii</i>	-28.18
<i>Homocladium platycladium</i>	-26.72
<i>Oxygonum atriplicifolium</i>	-24.67
<i>Oxygonum sinuatum</i>	-26.65
<i>Polygonum afromontanum</i>	-26.11
<i>Polygonum ampibium</i>	-21.46
<i>Polygonum aviculare</i>	-25.37
<i>Polygonum capitatum</i>	-29.84
<i>Polygonum chinense</i>	-26.19
<i>Polygonum convolvulus</i>	-29.76
<i>Polygonum equisetiforme</i>	-29.91
<i>Polygonum nepalense</i>	-27.32
<i>Polygonum pulchrum</i>	-27.94
<i>Polygonum salicifolium</i>	-26.67
<i>Polygonum senegalense</i>	-27.04
<i>Polygonum setulosum</i>	-27.18
<i>Polygonum strigosum</i>	-23.99
<i>Polygonum tomentosum</i>	-27.51
<i>Rumex abyssinica</i>	-28.35
<i>Rumex acetosa</i>	-30.15
<i>Rumex bequaertii</i>	-27.30
<i>Rumex crispus</i>	-27.66
<i>Rumex lunaria</i>	-29.85
<i>Rumex nervosus</i>	-27.04
<i>Rumex trianguivalvis</i>	-27.76
<i>Rumex rugosus</i>	-27.96
<i>Rumex ruwenzoriensis</i>	-26.55
<i>Rumex usambarensis</i>	-28.01
Portulacaceae	
<i>Cayptrotheca somalensis</i>	-24.86
<i>Cayptrotheca taitensis</i>	-23.34
<i>Montia fontana</i>	-28.53
<i>Portulacaria afra</i>	-20.93
<i>Portulacacorticans</i>	-12.36
<i>Portulaca fascicularis</i>	-12.37
<i>Portulaca foliosa</i>	-12.29
<i>Portulaca grandiflora</i>	-12.33
<i>Portulaca kermesina</i>	-12.43
<i>Portulaca oblonga</i>	-13.11
<i>Portulaca oleracea</i>	-14.98
<i>Portulaca parensis</i>	-12.40
<i>Portulaca petersii</i>	-13.69
<i>Portulaca pilosa</i>	-13.68

Table 2: Continued

Family/species	$\delta^{13}\text{C}$ values
<i>Portulaca quadrifida</i>	-15.66
<i>Portulaca</i> species (K)	-14.50
<i>Portulaca</i> species (T)	-11.48
<i>Portulaca wightiana</i>	-12.98
<i>Ravinatinctoria</i>	-30.11
<i>Talinum cafferum</i>	-24.34
<i>Talinum crispatulum</i>	-23.41
<i>Talinum cuneifolium</i>	-21.16
<i>Talinum paniculatum</i>	-21.32
<i>Talinum patens</i>	-28.18
<i>Talinum portulacifolium</i>	-23.72
<i>Vallamila peruwiana</i>	-29.52
Zygophyllaceae	
<i>Fagonia indica</i>	-25.25
<i>Fagonia isotricha</i>	-24.82
<i>Fagonia paulayana</i>	-23.20
<i>Fagonia schweinfurthii</i>	-23.75
<i>Tribulus cistoides</i>	-13.40
<i>Tribulus</i> cf <i>T. cistoides</i>	-14.68
<i>Tribulus parvispinus</i>	-12.56
<i>Tribulus terrestris</i>	-10.68
<i>Zygophyllum cordifolium</i>	-24.57
<i>Zygophyllum simplex</i>	-13.77
Nyctaginaceae	
<i>Boerhavia diffusa</i>	-12.20
<i>Boerhavia elegans</i>	-11.99
<i>Commicarpus grandiflora</i>	-25.10
<i>Commicarpus pendunculatus</i>	-25.03
<i>Commicarpus plumbagineus</i>	-27.70
<i>Mirabilis jalapa</i>	-30.34
Elatinaceae	
<i>Bergia ammanioides</i>	-24.42
<i>Bergia decumbens</i>	-25.17
<i>Bergia suffruticosa</i>	-23.99
<i>Dianthus barbatus</i>	-15.73
<i>Dianthus plumarium</i>	-30.92
<i>Dianthus</i> species	-25.86
<i>Elatinum triandria</i>	-23.76

Both the C₃ and C₄ species can occur in the same genus; for example in *Sesuvium*, *Trianthema* (Aizoaceae), *Aerva*, *Alternanthera* (Amaranthaceae), *Melandrium*, *Silene* (Caryophyllaceae), *Gyroptera* (Chenopodiaceae), *Zygophyllum* (Zygophyllaceae) and *Dianthus* (Elatinaceae)(Table 2).

The frequencies of the carbon discrimination values were analyzed at different altitudes. $\delta^{13}\text{C}$ values of -10.60 to -17.78‰ occurs in high frequencies between 500 m to 1,000 m, decreasing at 2,000 to 2,500 m, almost rare at 3,000 m and did not occur at 3,500 m (Fig. 1). Carbon discrimination values of -17.79 to -18.75‰ occurs at 500 m altitude only while -20.0 to -21.0 to -30.5‰ show an upward trend from 500 to 3,500 m (Fig. 1). $\delta^{13}\text{C}$ values between -23.0 to -27.0‰ show an upward frequency composition trend up to 3,000 m whereas $\delta^{13}\text{C}$ values -28.0 to -30.1‰ show constant frequency composition trend from 500 to 3,500 m (Fig. 1).

Low altitude has high temperature, high rate of evaporation and low moisture and high relative humidity factors. These climatic indices define the degree of aridity at low altitudes where high relative occurrences of low $\delta^{13}\text{C}$ values are recorded. Furthermore, some $\delta^{13}\text{C}$ values

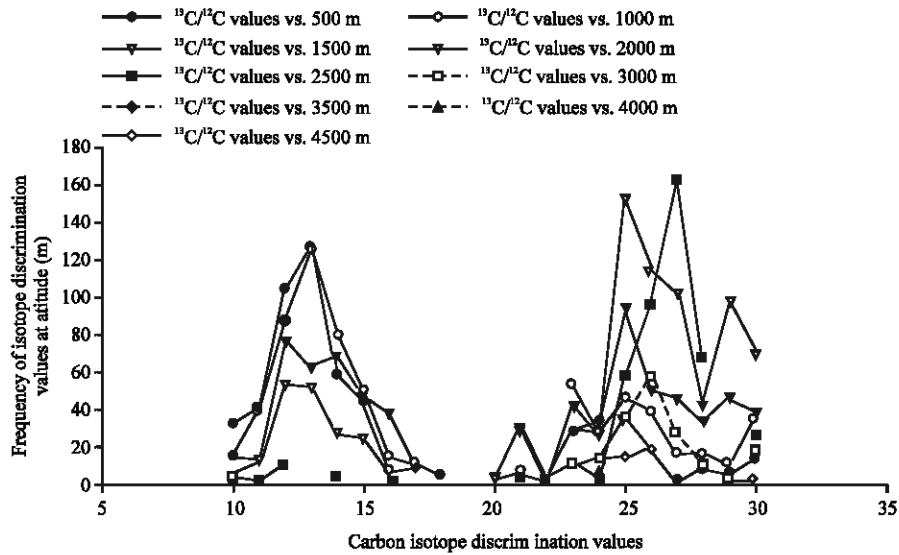


Fig. 1: Distribution curves of carbon isotope discrimination values for different dicots species of the centrospermeae along the elevation. Measurements from the centrospermeae species subdivided into C_3 species (170), C_4 species (67), CAM species (2) and C_3 - C_4 intermediate (3) species

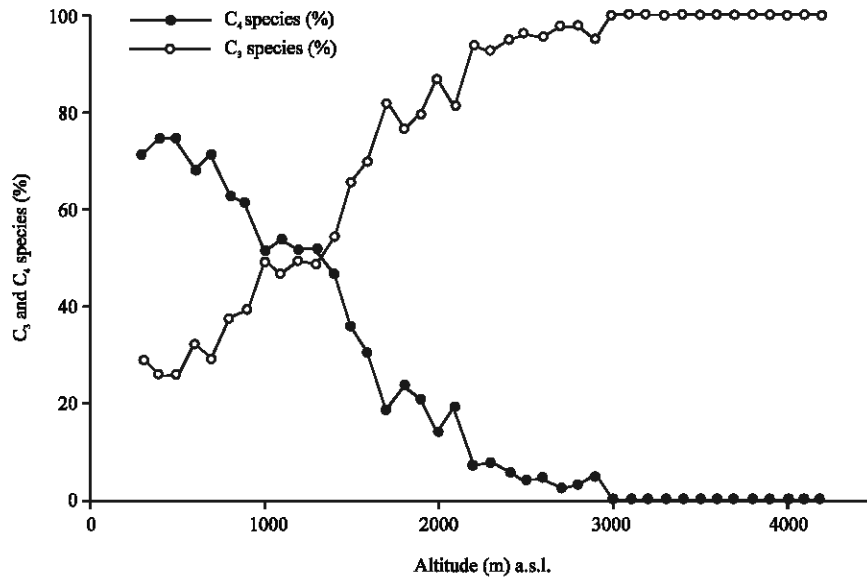


Fig. 2: Distribution percent curves C_3 and C_4 species of the centrospermeae along the altitudinal transect. Note the sharp abrupt transition zone between the photosynthetic systems represented by respective C_3 and C_4 species

such as -20.0 to -21.0‰ that occurs in low aridity habitats corresponds to their unique occurrence of the C_3 dicot species. These habitats show high percentage of the C_4 species, with $\delta^{13}C$ values range between -15.00 and -16.55‰. These $\delta^{13}C$ values describe the transition zone of the C_3 and C_4 types, a proportion influenced by climatic attributes at a given altitude (Fig. 1). High relative frequency of the C_3 species as a function of increase in

the $\delta^{13}C$ value negativity occurs at high altitudes (Fig. 1) compared to that of the C_4 species. Two main frequency peaks of $\delta^{13}C$ values occur separately at -12.50 and -27.0‰ (Fig. 1). This reflects high proportion of the C_4 dicot species and C_3 dicot species that show mean $\delta^{13}C$ values closer to -13.0 and -27.0‰ values (Fig. 1), respectively.

The tendency for the species to achieve the mean frequency peaks of the $\delta^{13}\text{C}$ values is representative of the cumulative nature of the different frequencies of carbon isotope discrimination value-peaks at different altitudes (Fig. 1). The cumulative intensity in the stratification (an index of occurrence of the species) is more pronounced in the C_3 dicot species for $\delta^{13}\text{C}$ values tending towards -27.0‰ as shown by two main peaks at -25.0 and -27.0‰ (Fig. 1). The relative distribution of the C_4 species and C_3 species percentage composition along the altitude is shown (Fig. 2). There is a high percentage of the C_4 species at the low altitude (below 1,400 m) compared to the C_3 species which has a high occurrence at the high altitudes (Fig. 2).

The transition zone occurs between 1,400 to 1,900 m.

DISCUSSION

The C_3 species constitute approximately 69.5% of the total species. The species has $\delta^{13}\text{C}$ values between -21.16 and -30.28‰ while, an exceptional high $\delta^{13}\text{C}$ value -32.42‰ is recorded in *Chenopodium capitatum*. The plant, *C. capitatum*, grows in moist regions of Baringo and Mt. Elgon. The C_4 species constitute approximately 28.45% of the total species and has $\delta^{13}\text{C}$ values in the range of -10.6 to -16.55‰ , with a high cluster around -12.0 to -13.0‰ . The CAM species had $\delta^{13}\text{C}$ values of between -16.0 and -18.5‰ . Bender (1971) reported -10 to -20‰ $\delta^{13}\text{C}$ values for C_4 species and -22 to -33‰ $\delta^{13}\text{C}$ values for C_3 species. Less negative $\delta^{13}\text{C}$ value is associated with low moisture index, high temperatures, high radiation and low relative humidity areas, especially in Turkana and Baringo (Sikolia *et al.*, 2008). Similar trend is occurs in the warm, moist and saline areas near Lake Turkana and Perkerra irrigation scheme (Baringo).

High composition of the $\delta^{13}\text{C}$ negativity correspond to increase in the occurrence of the C_4 plants along the low altitudes which is associated with low temperatures, high potential rates of evaporation, high precipitation and high relative humidity factors. Concomitantly, the degree of $\delta^{13}\text{C}$ negativity increase along the altitudinal gradient function. This change in the $\delta^{13}\text{C}$ values reflects percent change in the C_4 species dominance tendency relative to the C_3 species along the altitude.

The $\delta^{13}\text{C}$ values of the C_3 trees leaves have been found to vary from -25.0 to -35‰ (Edwards and Walker, 1983; Buchmann *et al.*, 1997b; Martinelli *et al.*, 1998). The difference is primarily due to variations in the net stomatal conductance during photosynthesis. This affects the ci/ca ratio and to a lesser degree because off the variations of the atmospheric carbon dioxide. The $\delta^{13}\text{C}$ values of C_3 plants in the tropics have been reported to vary from -29.0 to -34.0‰ (Medina and Minchin, 1980; Medina *et al.*, 1991; Ducatti *et al.*, 1991; Kapos *et al.*, 1993; Martinelli *et al.*, 1994; Fischer and Tieszen, 1995;

Buchmann *et al.*, 1997a; Martinelli *et al.*, 1998), while in the temperate areas typical $\delta^{13}\text{C}$ values vary from -25 and -29‰ (Schleser, 1992; Flanagan *et al.*, 1996; Hanba *et al.*, 1997; Buchmann *et al.*, 1997b). These differences in the $\delta^{13}\text{C}$ value in the wetter tropical conditions result in higher ratios of stomatal conductance. This increase the ci/ca ratio results in lower $\delta^{13}\text{C}$ values in the wet areas (Farguhar *et al.*, 1989).

The differences in isotopic fractionation are either C_3 or C_4 species dependent. But, small change in the $\delta^{13}\text{C}$ value (isotopic fraction) is due to fluctuation in environmental factors. The most differential climatic factors are temperature and precipitation (Sikolia *et al.*, 2008). The influence of temperature as dominant determinant factor in the photosynthetic types partitioning has been reported in the monocot species (Ehleringer *et al.*, 1997). Thus, the most limiting climatic factor in the ecosystem determines the trend of negativity or positivity of the $\delta^{13}\text{C}$ values while the other factors act synergistically.

The difference in the $\delta^{13}\text{C}$ values for the C_3 , C_4 and CAM species has both biochemical and anatomical function explanations. Carbon dioxide availability through the external and internal structural organization to reach Rubisco is species dependent. Rubisco reacts with $^{12}\text{CO}_2$ more easily than $^{13}\text{CO}_2$ due to a kinetic isotope effect. PEP Carboxylase is less discriminating against $^{13}\text{CO}_2$ than Rubisco. Both C_3 and C_4 species prefix atmospheric CO_2 through the PEP Carboxylase activity in mesophyll cells. Then, C_4 species fix all these CO_2 through the Rubisco activity in the bundle sheath cell compartment (gas tight). The C_4 species discriminates against $^{13}\text{CO}_2$ more than C_3 species. Thus, C_4 species ends up with greater $\delta^{13}\text{C}$ value in the range of -14.0‰ than the C_3 species $\delta^{13}\text{C}$ value in the range of -28.0‰ . This means that carbon isotope composition of C_4 dicot species has the potential to be used as an indicator for variations in the isotopic composition and concentration in the atmospheric CO_2 , especially for the past climate construction. The assumption is that, C_4 species photosynthetic discrimination range against ^{13}C remains constant in a wide range of environmental conditions, including the past and present climates (Lloyd and Farguhar, 1994; White *et al.*, 1994).

Farguhar *et al.* (1982) suggest that the causes of variation in the C_3 species may be associated with variations in the ratio of intercellular and atmospheric partial pressures of carbon dioxide that give rise to change in water use efficiency. This quantitative function in the ratio to the proportion of bundle sheath cells which leaks back into the mesophyll cells and the ratio of intercellular and atmospheric partial pressure of carbon dioxide for the C_4 species $\delta^{13}\text{C}$ value variation was explained by Farguhar (1983). The results support suggestions that leakage may be significant (Berry and

Farguhar, 1978) and differences in the $\delta^{13}\text{C}$ values in C_4 species may reflect variations in the amount of leakage (Hattersley and Browning, 1981).

Within the conventional C_3 , C_4 , $\text{C}_3\text{-C}_4$ intermediate and Crassulacean acid metabolism pathways, variations in the $\delta^{13}\text{C}$ values are observed in the present study. The general pattern of $\delta^{13}\text{C}$ values distribution along altitude gradient as a climatic function shows that $\delta^{13}\text{C}$ values of -10.60 to -16.55‰, -17.15 to -18.87‰ and -21.16 to -32.42‰ occur at low altitudes (0-1500 m), intermediate altitude (1550-1700 m) and high altitude (1800-4200 m) (Sikolia *et al.*, 2008), respectively. The altitudinal $\delta^{13}\text{C}$ values distributions for the dicot species is within the range reported in the C_3 and C_4 monocot species (Smith and Brown, 1973; Tieszen *et al.*, 1979). Within the intermediate altitudinal range (1550-1700 m), species with $\delta^{13}\text{C}$ values in the range between -10.6 to -16.55‰ and -21.16 to -32.42‰ occur in low diversity index. These $\delta^{13}\text{C}$ value distributions correspond to the floristic distribution pattern of the C_3 and C_4 dicot species along the altitude (Sikolia *et al.*, 2008). The transition shift between C_3 dicot and C_4 dicot species is a rather sharp and abrupt change along the altitude. Less $\delta^{13}\text{C}$ value negativity is a potential aridity indicator and C_4 syndrome marker. Even $\delta^{13}\text{C}$ values of the C_4 species in low altitudes tend to be very less negative and show a photosynthetic paradigm tendency towards C_4 -like physiological/biochemical significance in the understorey of closed forests, for example in the Amaranthaceae.

The more the dicot species are stressed by low light intensity, high temperature and limited water supply, the lower their $\delta^{13}\text{C}$ value and the higher their carbon isotope discrimination. This differentiation of the $\delta^{13}\text{C}$ value, as a result of CO_2 leakage could be related to suberized middle lamella. The anatomical function is compensated by the centripetal chloroplasts arrangement and position in the bundle sheath. This increases CO_2 diffusion efficiency in the mesophyll cell and a higher bundle sheath surface to volume ratio (Hattersley and Browning, 1981; Henderson *et al.*, 1992). Increasing stomatal closure as a result of increase in the aridity causes decrease in carbon isotope discrimination in leaves (Ehleringer and Cerling, 1995; Liu *et al.*, 1996). The decline in carbon isotope discrimination in the more arid regions can also occur as a function of both low species diversity like the *Basellaceae* species (Sikolia *et al.*, 2008), high seasonal and unpredictable rainfall regime (Schulze *et al.*, 1996), decreased humidity (Williams and Ehleringer, 1996) and soil water availability (Ehleringer and Cooper, 1988), that vary along the altitude.

The plants show differential distribution along soil moisture gradient. This is because species vary in their ability to tolerate low soil moisture availability coupled with stomatal resistance to gaseous exchange or compete for the limiting stomatal resistance. Thus, the differential

$\delta^{13}\text{C}$ values depend on the ability of the individual species to compete for soil moisture and carbon dioxide availability. This leads to observed $\delta^{13}\text{C}$ values along the climatic gradient. Furthermore, a decrease in $\delta^{13}\text{C}$ value indicates stomatal limitation to photosynthesis and also an increase in leaf water use efficiency as exemplified by greenhouse experimental $\delta^{13}\text{C}$ values.

The C_3 and C_4 $\delta^{13}\text{C}$ values differences are also species dependent water-use efficiency and habitat conditions. Water-use efficiency contributes directly in a predictable manner to the long term growth and survival of high species index. This relationship occurs under the unpredictable and fluctuating drought conditions that characterize the semi-arid or arid. The conditions are punctuated with salinity that affects water diffusion into the plant root. Finally, the influence of climatic factors on the composition species results in competition between species in their habitat. This affects the association, vegetation zonation, longevity, diversity of the species and their $\delta^{13}\text{C}$ values. The $\delta^{13}\text{C}$ value becomes a predictor of spatial diversity and shift of the species along the altitudinal gradient of climatic factors.

Some C_4 species record unusual lower $\delta^{13}\text{C}$ values than expected at high altitudes, for example *Melandrium nordiflorum* and *Silene abyssinica*. This contradicts availability of high carbon dioxide concentration that would favour C_4 species unlike C_3 species at high altitude regions. This is attributed to the Pyruvate Phosphate dikinase (PPDK) enzyme activity which requires ADP and not ATP. This is subject to dark/light regulation and optimal temperatures instead of the norm activity at low temperatures at the high altitudes. The mesophyll enzyme PP dikinase which exhibit different states of activation becomes non-functional and attain standard photosynthetic reaction in the C_4 species. This leads to reduced or increased biochemical non-functional requirements of the PP dikinase enzyme for continuous metabolic pathway. But, PP dikinase enzyme is not a biochemical functional requirement in the C_3 species which utilizes malate dehydrogenase (or malic enzyme) in the PEP case of the Calvin cycle. Therefore, the C_3 species survive well in cold temperatures compared to the warm conditions of the C_4 species (occur up to 3,000 m). The present results imply that CO_2 assimilation would decrease with increasing altitude for lack of normal biochemical functions of the metabolic mechanisms in the C_4 species for their growth and survival in high altitudinal habitats controlled by low temperatures (Sakai and Larcher, 1987; Rada, 1992; Cabrera *et al.*, 1998). Therefore, more negative $\delta^{13}\text{C}$ values (C_3 species) occur at high altitudes compared to the less $\delta^{13}\text{C}$ values of the C_4 species dominated low altitudes.

Two main peaks representing the frequency of carbon isotope discrimination values represents high relative occurrence of the species that reflects the

cumulative $\delta^{13}\text{C}$ values of the species. There is increased species diversity at specific altitudes that translates to the two $\delta^{13}\text{C}$ value peaks. The first peak that occurs at about -12.50‰ of $\delta^{13}\text{C}$ value is a major attribute of the C_4 species. The second peak between -25.0 to -27.0‰ of $\delta^{13}\text{C}$ value is mainly due to the contribution of the C_3 species.

The $\delta^{13}\text{C}$ value has been used to determine climatic factors that influence the distribution of the C_3 and C_4 species along the altitude gradient, in Western region in Kenya. Further, $\delta^{13}\text{C}$ value can be applied to determine the taxonomic hierarchical level of plant species. For example, the C_4 species shown by -11.77 and -11.99‰ $\delta^{13}\text{C}$ values of *Gisekia* species would place the genus in a different family like Portulacaceae instead of Aizoaceae. It is expected that C_4 species in a given taxonomic group should reflect almost similar degree of carbon isotope discrimination index. The degree of carbon discrimination should reflect evolutionary hierarchy in the taxonomic significance such that Rubisco in a given taxonomic group perform with similar efficiency with regards to the carbon isotope discrimination index.

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