SciMedCentral

International Journal of Plant Biology & Research

Research Note

Real-Time Leaf Morphometrics Field Recognition Characters for Five Species of Vernonia in Nigeria

Ebigwai JK*

Department of Botany, University of Calabar, Nigeria

***Corresponding author**

Ebigwai JK, Department of Botany, University of Calabar, Calabar, Nigeria, Email: ebijoe4@gmail.com

Submitted: 20 July 2017

Accepted: 25 September 2017

Published: 26 September 2017

ISSN: 2333-6668

Keywords

- *Vernonia*
- Identification
- Morphology
- Leaf and taxonomy

Abstract

The need for rapid, reliable and accurate species identification is one if the goal of taxonomy. This is often achieved by the use of morphological characters. Regrettably, it is often fraught with unreliability. The application of numerical taxonomy has contributed to solving this challenge. Field identification of the five *Vernonia* species in Nigeria is very challenging. This study applied quantitative measurements of the leaf length, leaf width, petiole length, inter nodal length, twig length, length of mid rib, length of lateral nerves, and width of intra marginal nerves on thirty replicates per each of the five species to confirm significant differences. The species were grown in controlled environment and use of line thread and hand lens was used. The result showed that significant difference among all the species using leaf length existed with a minimum of 102.4 leaves from an individual plant was measured. In the same vein, the study showed that Leaf width was able to separate *V. cinerea*, length of twig was able to differentiate *V. thompsoniana*, length of mid rib was able to separate *V. amygdalina* while petiole length was able to differentiate *V. conferta* from others. With the exception of leaf length, no other character was able to discriminate *V. colorata* from others.

INTRODUCTION

The bedrock on which taxonomic practice is anchored is species identification. Several taxonomic lines of evidence exist in order to aid species identification. These are anatomical, palynological, cytological, molecular, phytochemistry, and serological [1]. These markers are dependent on skilled experts, good laboratory and functional equipment. In Nigeria as in most developing countries with paucity of research funds availability, the cost outlay is sometimes huge for individual researchers. More importantly, generation of data from these sources and ultimately species identification cannot be achieved in real time and hence cannot be used for field recognition. Prompt, efficient and accurate field recognition of species is key to several sectors of the Nigerian economy. For instance, Environmental Impact Assesment (EIA) is a critical requirement for developmental projects for which flora studies is an integral component (EIA Act 2004). The ever increasing incidences of alien and invasive species proliferation need accurate and prompt field recognition. In the tropics with high susceptibility for speciation, development of field recognition characters is crucial. A critical observation on the IUCN database for Red Listed species showed that most flora species in comparison with fauna species are data deficient or has not been evaluated against the criteria (IUCN redList 2017). This is owed to largely to dearth of field identification taxonomist. The challenge of environmental factors causing mimicry among species has raised the decibel on the importance of field recognition. Worst still, there are no field guides or reference texts on any of the protected forest in Nigeria. Apart from funding, studies in literatures devoted to field species recognition in Nigeria are limited at best. To further buttress the need for field recognition studies Nigeria Trees, the standard reference texts in Nigeria which regrettably is in dire need of revision was compiled by Keay, an English man.

Application of accurate field recognition techniques is required in several fields. Researchers on medicinal plant often conduct their experiment on species whose identity is questionable. Often extraction of active ingredients is conducted on species with self authentication. In few instances of authentication by a certified taxonomist, a documented field characteristic to aid the expert is lacking. Worst still, in institutions where herbarium exists, there is the need for trainings for the curators on species identification. Often, the identity of the species is wrong. This puts a huge question on the results and validity of the research findings. More worrisome is that most of the researchers do not deposit voucher specimens in the herbarium from which experiments could be repeated.

Vernonia is a genus of Asteraceae. It is characterized by Farumbi and Owoeye 2011. There are five species of *Vernonia* in Nigeria (*Vernonia thomsoniana* Oliv.& Hiern; *Vernonia amygdalina* Del.; *Vernonia colarata* (Willd.) Drake; *Vernonia cinerea* (Linn) Less. and *Vernonia conferta* Benth). for which *V. amygdalina* is the most known. Apart from few studies (IUCN Red List 2017) on the anatomy and taxonomy of *Vernonia* species, several others on medicinal, nutritional compositional and other ecosystem

services mention *Vernonia amygdalina* only. To most researchers, only *V. amygdalina* exists in the country and it is the only species called bitter leaf. They treat, name and ascribe any species with resemblance of *Vernonia* as *amygdalina*. It is improbable that all such studies were conducted on *V. amygdalina* alone. Some would certainly have been on others.

Although some published works on anatomy [2,3] succeeded in differentiating these species, it is unimaginable for such experiments to be conducted prior to authentication. Therefore there is the need or quick, reliable and documented morphological process that could be repeated and validated.

It is in light of these challenges that this study aims to determine reliable and ever present leaf characters that could discriminate among these species in real time.

METHODOLOGY

Stem cuttings for the study were obtained from National Centre for Genetic Resources and Biotechnology. The identity of the obtained voucher specimens were confirmed and authenticated by Prof BL Nyananyo of the University of Port Harcourt. Detail of the planting materials is shown in (Table 1).

Thirty replicates of each cutting were sown in a screen house of the Department of Botany, University of Calabar, Calabar, Nigeria using a spacing of 1.0 × 1.0 m and a depth of 3.0 cm. Poultry manure was applied to the plots after two weeks to enhance speedy regeneration. Modified methods of [4,5] was adopted. For example, on foliar development after an average of 7 days, measurements were done on the leaves and stem. The parameters measured are length of midrib, length of twigs, inter nodal length, leaf length, leaf width, length of lateral nerves, width of intra marginal nerves and length of petiole. Measurements were done weekly either with thread or line ruler over a three months period.

The morphological readings were compiled on recording sheets for each operational taxonomic unit (OTU). Mean figures were entered into an Excel spreadsheet and the raw data coded to allow analysis using Unistat 4.0 for Windows. For analysis the ratios of these figures were calculated. Analysis of variance (ANOVA) was carried out for the eight leafy quantitative measurements. The level of significance was recorded for each measurement. Cluster Analysis was performed for these parameters. The quantitative characters were subjected to PCA ordination.

The study was conducted from May to July 2016.

RESULT

Leaf morphological variations among the thirty individuals of each species were studied using the nine aforementioned quantitative characters. Means and standard deviations are shown as (Table 1.1).

Analysis of variance (ANOVA) was performed to test the differences between taxa.

DISCUSSION

The use of morphological characters in species identification though fraught with challenges of environmental mimicry would undoubtedly remain pivotal in nomenclatural practice.

Length of mid rib

Literature is scanty on the application of midrib as character for taxon diagnosis [6]. Its taxonomic significance as a delimitation tool was exposed by [5] on seventeen members of Asteraceae family. In the present study, the mean length of the mid rib ranged from 9.22 + 0.24m in *V. amygdalina* to 9.61 + 0.54 cm in *V. colorata*. The mean mid rib length for *V. amygdalina* individuals were 9.22 + 0.87cm.A statistical significance value ($p < 0.001$) exist only in *V. amygdalina.* This implied that *amygdalina* could be separated on the basis of mid rib length from the other species. The length of the mid ribs seems to be controlled in part

Table 1: Source and Accession number for Stem Cutting.

Serial No	Accession number	Species
1	NG/07/JAN/12/023	Vernonia thomsoniana Oliv.& Hiern
2	NG/07/JAN/12/024	Vernonia amygdalina Del.
3	NG/07/JAN/12/025	Vernonia colarata (Willd.) Drake
4	NG/07/JAN/12 026	Veronia cinerea (Linn) Less.
5	NG/07/JAN/12/027	Vernonia conferta Benth.

Table 1.1: Mean ± Standard Deviation of the Quantitative Characters of the Three Studies Species.

Species	Length of Mid rib	Length of twigs	inter nodal length	leaf length	leaf width	Length of lateral nerves	width of intra marginal nerves	length of petiole
V. thomsoniani	9.54 ± 0.2	7.48±1.0	1.93 ± 0.6	11.2 ± 1.1	6.48 ± 1.4	2.70 ± 1.4	0.78 ± 0.1	1.79 ± 0.4
V. amygdalina	9.28 ± 0.8	7.39 ± 0.9	1.93 ± 0.5	11.7 ± 1.5	6.45 ± 2.0	2.71 ± 1.2	0.79 ± 0.1	1.88 ± 0.3
V. colarata	9.61 ± 0.5	7.31 ± 1.1	1.96 ±1.0	12.9 ± 3.3	6.46 ± 2.3	2.66 ±1.2	0.84 ± 0.1	1.85 ± 0.7
V. cinerea	9.56 ±1.3	7.32 ± 1.5	1.94 ± 2.0	12.1± 2.1	6.54 ± 2.3	2.69 ± 1.2	0.75 ± 0.1	1.74 ± 0.9
V. conferta	9.40 ± 0.9	7.29 ±1.2	1.88 ± 1.8	10.9 ± 1.7	6.49 ± 1.7	2.67 ± 1.7	0.71± 0.1	1.56 ± 1.2
Unit of measurements =Cm								

Vernonia conferta Benth

Vernonia cinerea (Linn) Less

Vernonia colorata (Willd) Drake

Vernonia amygdalina De

Vernonia thomsoniana Oliv & Hiern

Plate 1.1 Species of *Vernonia*.

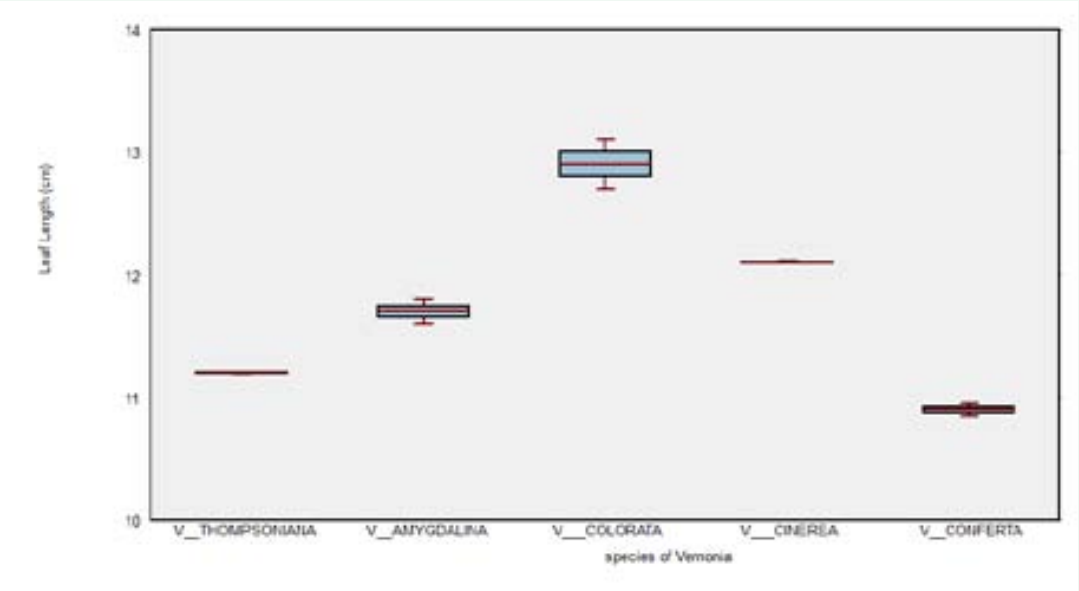

Figure 1 Box and whiskers plot discriminating among five *Vernonia* species using Leaf Length.

by its anatomical outlines. The concave, convex, arc, v- shaped and U- shaped of *V. amygdalina, V. cinerea, V. conferta,V. colarata,* and *V. thompsoniana* as showed by [2,5,7] would have resulted in the observed mid rib length differentials.

Identification Key for the five *Vernonia* members using length of mid rib

1a Length of mid rib below 9.25cm - *V. amygdalina*

1b Length of mid rib above 9.25cm - *V. Thompsoniana, conferta, cinerea* and *colorata*

Length of Twigs: [8] demonstrated variations in twig leaf patterns among some species and suggested that it could play a defining role in taxon diagnosis. The average twig length among the species under investigation showed a range of 7.29 cm in *V. conferta* to 7.48 cm in *V. thompsoniana*. When the result was subjected to Newmans-Kuels Multiple Comparison Test at the 0.05% confidence interval, a significant difference was observed for *V. Thompsoniana* against all others. [9] reported that length of twigs is both maturity dependent and species specific. In a study involving 234 species, [10] related positive correlation of large twig size to higher leaf area ratio. A further re examination of the twig length of the species under investigation showed a higher Leaf area ratio for *V. thompsoniana* than others. Similarly, [11] attributed twig size to shade and light tolerant species. The temperature requirements for optimum growth of *Vernonia* is less than 30^0C [12] and the species could be light or shade tolerant (Native Plant data base 2010).

Identification Key for the five *Vernonia* members using Length of twigs

1a Length of twigs above 7.45cm - *V. thompsoniana*

1b Length of twigs below 7.45cm - *V. amygdalina, colarata, cinerea* and *conferta*

Inter nodal length

Nodal morphology is of great taxonomic importance [13]. [14] used the number of nodes in the stem of sesame for delimitation. The mean length of the five species under study was 1.93cm, 1.93cm, 1.96cm, 1.94cm and 1.88cm for *thompsoniana, amygdalina, colarata, cinerea* and *conferta* respectively. The result was not significant for any of the species hence none could be delimited based on this character. This result made the construction of an identication key irrelevant.

Leaf length

[15] conducted a model digital morphometrics on leaf characters. Leaf length has been shown to relate closely to leaf shape and leaf size [16]. The leaf length for the *Vernonia* species was measured as 11.2cm, 11.7cm, 12.9cm, 12.1cm and 10.9 cm for *V. thompsoniana,V. amygdalina, V. colarata, V. cinerea* and *V.conferta* respectively. A statistical significant result ($p < 0.001$) for all the species was obtained at the 0.05% confidence limit. On further re examination, a minimum measurement of the leaf length of 116.4 leaves from a single individual yielded a mean that was sufficient to discriminate the five taxa under study with a standard error of 0.1cm.

Leaf width

Several studies utilizing leaf width as discriminatory character exist [4,17,18,]. Statistically, this parameter clearly discriminates *V. Cinerea* from others. Other members under study showed no statistical significant difference.

Identification Key or the five *Vernonia* members using Leaf Width

1a Leaf width above 6.50cm- *V. cinerea*

1b Leaf width below 6.50cm - *V. thompsoniana, conferta, amygdalina* and *colorata*

Length of lateral nerves

The length of lateral nerves seems to be a function of the leaf width, right angle to margin (parallel) or if looping away from margin. In all the species investigated in this study, the lateral nerves loops away from the margin though at various distances. The mean length for lateral nerves in the five species were 2.70 cm, 2.71cm, 2.66cm, 2.69cm and 2.67cm for *V. thompsoniana, V. amygdalina, V. colarata, V. cinerea* and *V. conferta* respectively. There was no significant difference among the species.

Identification Key of the five *Vernonia* members using Length of lateral nerves

1a Length of lateral nerves below 2.69cm – *V. colarata, cinerea* and *conferta*

1b Length of lateral nerves above 2.69cm – *V. thompsoniana* and *amygdalina*

Width of intra marginal nerves

Taxonomic significance of intra marginal nerves is owed partly to its various patterns, structure and orientation. These variations are expressed somewhat differently in most species and to a large extent, they exhibit generic uniformity. Several studies utilizing this character are found in [19,20]. [21] applied the diagnostic features of intra marginal nerves in characterizing members within the *Buxus* genus in just as [22] applied same to the occurrence and distribution of seas grasses in Malaysia archipelago. These intra marginal nerves are closely packed or widely spaced apart [23] and these intra marginal spaces serve taxonomic purposes [24]. The genera *Tabernaemontana* and *Voacanga* are morphologically delimited amongst other features by the number and space of intra marginal nerves in the abaxial surface. In this study, the mean width of intra marginal nerves are 0.78cm, 0.79cm, 0.84cm, 0.75cm and 0.71cm for *V. thompsoniana, amygdalina, colorata, cinerea* and *conferta* respectively. Statistically, the NKMCT was able to discriminate *V. thompsoniana, amygdalina, colorata* from *conferta* and *cinerea.*

Identification Key for the five *Vernonia* species using width of Intra marginal Nerves

1a Width of intra marginal nerves above 0.75cm – *V. thompsoniana, amygdalina, colorata*

1b Width of intra marginal nerves below or at 0.75cm – *V. cinerea* and *conferta*

Length of petiole

Several discontinuous character states found in leaf petiole are discriminatory in nature and hence excellent taxonomic

markers. The discriminatory role played by the presences or absences of Petiole and external features (stellate hairs, glandular dots), nature and shape (winged/marginate, elliptic, angulate, canaliculated, ovoid, semi circular) and thickness are well documented [23-25]. The mean length of petiole in the five species of *Vernonia* were 1.79cm, 1.88cm, 1.85cm, 1.74cm, and 1.56cm for *V. thompsoniana, amygdalina, colorata, cinerea* and *conferta.* Statistical analysis using NKMCT was able to clearly separate *V. conferta.* The test also separated *V. amygdalina* and *colorata* from *V. thompsoniana* and *cinerea*.

Identification Key for the five *Vernonia* members using leaf petiole

1a Petiole length below 1. 70cm - *V. conferta*

1b Petiole length above 1.70cm …….. .. 2

2a Petiole length between 1.71 to 1.80cm - *thompsoniana* and *cinerea*

2b Petiole length between 1.81 to 1.88cm - *amygdalina* and *colorata*

As evident in the results of the study, not all the leaf morphological characters were able to discriminate among the various *Vernonia* species. Generally, the use of morphology in generating taxonomic distinctness among taxa had its limitations. Reports of species mimicry by environmental influences resulting in false taxon identity are well documented [26]. Unfortunately, no one of the taxonomic evidences is error - proof. Interestingly real time species identification offered by morphological evidences is totally absent in others. For instance, the extensive requirements of laboratory techniques and protocols needed by all others taxonomic markers makes species identification open not only to a skilled expert, but laborious, expensive, time consuming, equipment dependent. All other published reports on *Vernonia* species identification utilizing other taxonomic markers cannot be achieve in real time on the field [2]. Notably, other taxonomic lines of evidence are applied only when the use of vegetative morphological evidence has failed. The need for extensive identity protocols for *Capparis tomemtosa* (8cm long), *Capparis fascicularis* (2-4cm long) and *Capparis deciduas* (12cm long) does not arise since leaf length was sufficient to separate them [23]. In the same vein, the need for other markers to differentiate *Cadaba farinose* (with 5-6 pairs of lateral nerves) and *Cadaba glandulosa* (2-3 pairs of lateral nerves) species can not arise since variations in intra marginal nerves was sufficient for their delimitation [23]. It seems plausible to suggest that limitations on the use of leaf morphological characters for plant species identity are borne on limited number of replicates. For example, the study re-examined the minimum number of leaves from fully blown reproductive individuals for each character that was able to discriminate among the species. The use of 102.4 *Vernonia* leaves was the minimum number required to measure leaf length that could separate all species of *Vernonia* studied. Similar measurements were conducted on the number of leaves measurement required to differentiate among *Rhizophora* species in the Niger Delta (Ebigwai *et al*., in press). Application of this novel technique would reduce the challenge associated with false identity of species using morphological while enhancing cost effective, no laboratory and real time field identification process.

CONCLUSION

The identification of the various species of *Vernonia* in Nigeria has been very challenging. The study experimented on the use of nine quantitative leaf morphological characters to ascertain if any could discriminate among them. The study proved that measurement of the length of 102.4 leaves was the minimum number required to separate all the species. Leaf width was able to separate *V. cinerea*, length of twig was able to differentiate *V. thompsoniana*, length of mid rib was able to separate *V. amygdalina* while petiole length was able to differentiate *V. conferta* from others. With the exception of leaf length, no other character was able to discriminate *V. colorata* from others.

RECOMMENDATION

The use of leaf morphometrics could help in real time field recognition of species and hence should be encouraged.

ACKNOWLEDGEMENT

The author registers his appreciation to his post graduate studies.

REFERENCES

1. Garcia-Martinez J, Delgado-Ramos L, Pelechano V, Medina DA, Carrasco F, Gonzalez R, et al. The cellular growth rate controls overall mRNA turnover, and modulates ether transcription or degradation rates of particular gene regulations. Nucleic Acids Res. 2016; 44: 3643-3658.
2. Mabel FA, Johnson AA, Temitope OO. Petiole anatomy of some species of Asteraceae in southwest Nigeria. Afr J Plant Sci. 2013; 7: 608-612.
3. Adekanmbi OH, Ogundipe OT. Pollen grains of some cultivated plants in Nigeria. J Sci Res Dev. 2006; 10: 101-110.
4. Conesa M, Mus M, Rosselló J. Leaf shape variation and taxonomic boundaries in two sympatric rupicolous species of Helichrysum (Asteraceae: Gnaphalieae), assessed by linear measurements and geometric morphometry. Biol J the Linnean Soc. 2012; 106: 498-513.
5. Ekeke C, Mensah SI. Comparative anatomy of midrib and its significance in the taxonomy of the family Asteraceae from Nigeria. J Plant Sci. 2015; 10: 200-205.
6. Mantovani A, Pereira TE. Comparative anatomy of leaf and spathe of nine species of Anthurium (section Urospadix; subsection Flavescentiviridia) (Araceae) and their diagnostic potential for taxonomy. Rodriguesia. 2015; 56: 146-160.
7. Eltahir AS, Abu BI. Microscopical studies on the leaf and petiole of *Vernonia amygadlina* Del. Adv Appl Sci Res. 2011; 2: 398-406.
8. Meng F, Cao R, Yang D, Niklas K, Sun S. Within-twig leaf distribution patterns differ among plant life-forms in a subtropical Chinese forest. Tree physiology. 2013.
9. Fralish JS, Franklin SB. Taxonomy and Ecology of Woody Plants in North American Forests: (Excluding Mexico and Subtropical Florida). New York. John Wiley & Sons. 612pp. $100 cloth. 2002.
10. Yang D, Niklas KJ, Xiang S, Sun S. Size-dependent leaf area ratio in plant twigs: implication for leaf size optimization. Ann Bot. 2010; 105: 71-77.
11. Milla R, Reich PB. The scaling of leaf area and mass: The cost of light interception increases with leaf size. Proceedings of the Royal Society. Biological Sciences. 2007; 274: 2109-2114.
12. Sharma KC. The stem-node-leaf-continuumin Pithecellobium dulce

(Roxb.) Benth. J Indian Bot Soc. 2007; 5: 37-40.

13. Mbinglo SB. "Survey on the production of bitter leaf Vernonia spp. in Bamenda, N.W. Cameroon" Student Project Report for Natural Resource Institute, Chatham, United Kingdom/Dschang University, Cameroon. 1998.

14. Alege GO, Akinyele BO, Ayodele SM, Ogbode AV. Taxonomic importance of the vegetative and pod characteristics in three Nigerian species of sesame. Afr J Plant Sci. 2011; 5: 213-217.

15. Cope JS, Corney D, Clark JY, Remagnino P, Wilkin P. Plant species identification using digital morphometrics: A review. Expert Syst Appl. 2012; 39: 7562-7573.

16. Tsuyuzaki S. Characteristics of "number of veins" to estimate leaf maturity in Pteris mutilate (Pteridaceae). J Plant Res. 2000; 113: 415-418.

17. Iroka CF, Clement UO, Alex II, Nkumah CO, Bio LN, Ekwealor KU. Taxonomic significance of morphological characters in the species of Stachytarpheta found in Awka, Nigeria. Int J Plant & Soil Sci. 2015; 8: 1-6.

18. Simpson M G. Plant Systematics. 2d edn. New York: Academic Press. 2010.

19. Ramasubbu R, Divya C, Anjana S. A note on the taxonomy, field status and threats to three endemic species of Syzygium (myrtaceae) from the southern western Ghats. Indian Journal of Threatened Taxa. 2016; 8: 9384-9390.

20. Maslin BR, Murphy DJ. The international plant names index. London Journal of Botany. 2009; 1: 1842.

21. KohIer E, Bruckner P. The genus Bltxilr (Buxaceae): aspects of its differentiation in space and time. Plant Syst Evol.1989; 162: 267-283.

22. Muta Harah B, Sidik J. Seagrasses: A threatened coastal resource. *Fishmail.* 2013; 12: 6-14.

23. Arbonnier M. Trees, Shrubs and Lianas of West African Dry Zones. Weikersheim, Cirad-Margraf. 2004; 573.

24. Keay RWJ. Trees of Nigeria. Clarendon press Oxford. 1991; 11: 322.

25. Coutinho MF, Santos JI, Alves S. Less Is More: Substrate Reduction Therapy for Lysosomal Storage Disorder. Int J Mol Sci. 2016; 18: 178.

26. Auerbach JE, Bongard JC. Environmental influence on the evolution of morphological complexity in machines. PLoS Comput Biol. 2014; 10: e1003399.

Cite this article

Ebigwai JK (2017) Real-Time Leaf Morphometrics Field Recognition Characters for Five Species of Vernonia in Nigeria. Int J Plant Biol Res 5(4): 1074.