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# Exploring Statistical Tools in Measuring Genetic Diversity for Crop Improvement

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## 1. Introduction

Increase in global numerical population especially in developing nations has gradually led to food shortage and hence increase in poverty. Addressing and tackling the issue and causes of poverty in the developing nations is one major challenge to breeders (Fu and Somers 2009). The different theories of econometircs have identified the human and material resources traceable to poverty, but fail to identify the crop improvement techniques in addressing world food shortage (Baudoin and Mergeai 2001). Crop improvement techniques therefore remains a major concern to plant breeders (Akbar and Kamran, 2006; Aremu *et al,* 2007a). Several factors affect crop improvement for specific or general environment performance. Such factors include climate, weather, soil, edaphic and biological and more importantly crop genotype (Aremu, et al, 2007b). Crop genotypes are composed of different crop forms including inbred or pure lines hybrids, landraces, wildraces germplasm accessions, cultivars or varieties. These crop genotypes have wide and diverse origin and genetic background known as genetic diversity. Genetic diversity study is a major breakthrough in understanding intraspecie crop performance leading to crop improvement (Aremu, 2005). Knowledge of crop performance in genetic diverse population reveals the differences in the nature of genetic materials used.

Genetic diversity studies therefore, is a step wise process through which existing variations in the nature of individual or group of individual crop genotypes are identified using specific statistical method or combination of methods (Christini *et al.* 2009; Warburton and Crossa 2000; Aremu, 2005; Weir 1996). It is expected that the identified variations would form a pattern of genetic relationship useable in grouping genotypes.

Several researchers including breeders have employed different data source and type from diverse crops in their methods to study genetic diversity. Such data source include morphological and agronomic, pedigree, proximate or biochemical and molecular data (Aremu, *et al.,* 2007a in cowpea; Liu *et al*., 2000 in cotton; Mostafa *et al.,* 2011 in wheat; Adewale *et al*., 2010 in African Yam bean; Christine *et al*., 2009 in bentgrass.

The choice of statistical method to be used is dependent on the achievable objectives laid out in the studies. This chapter reveals the underlying importance of genetic diversity and

reviews useable statistical techniques for identifying and grouping genotypes for intraspecies crop improvement.

## 2. Need for germplasm resource in genetic diversity preservation

Crop genotypes sourced as germplasm accessions, landraces, breeding lines, wild species, have rich and variable genetic integrity explorable for breeding programmes. The first step of any meaningful breeding programme is to identify crop plants that exhibit exploitable variation for the trait(s) of interest. However, these genetic diverse crops are under threat. Continuous hybridization and crossing systems have reduced the genetic variations in cropping programmes and leave a dearth in harvesting and utilization of novel crop types with exploitable traits. Also, the continuous threat or loss of genetic diversity as a result of replacement of landraces, wild species and other primitive term of crop species by exotic high- yielding varieties remains an insurmountable problem to plant breeders. Another major source of loss of genetic diversity is by changes and or increase in population size, resulting in land use acts promoting deforestation, wars, industrialization, urbanization and other factors. According to Brown (1989), preservation of genetic diversity is possible when genetic or germplasm resource is realized as the most precious asset in conserving genetic diversity. Germplasm therefore is an essential resource for successful plant breeding. Certain areas of the world exhibit high level of genetic variability for crops (Vavilov, 1950). Falconer and Mackay (1996); Eivazi *et al;* (2007); reported that such areas are considered as regions or center of genetic diversity. Therefore genetic diversity in crop may be associated with the origin of the crop. This is supported by Christine *et al.* (2009), who reported genetic diversity to be associated with origin. Potter and Doyle, (1992) reported Tropical Africa to be the centre of diversity for African yam bean. Van Bueningen and Busch (1997), reported genetic diversity of wheat to be centered in North America. Ariyo and Odulaja (1991), found correlation between genetic diversity and eco-geographic background in okro. Some grouping methods in genetic diversity studies identified origin and geographical diversity not important in measuring genetic diversity. Nair *et al*. (1998) discovered diversity in sugarcane not to be associated with origin. Aremu *et al*. (2007a), discovered that center of origin is not a measure of genetic diversity in cowpea. If crop origin is somewhat not important in the measure of genetic diversity a resource centre is therefore needed to preserve and maintain the wide genetic sources exploitable in breeding programmes. Genetic relationship and diversity are useful for developing germplasm conservation strategies and utilization of crop genetic resources. The use of genetic diversity resource centre cannot be under estimated as earlier discussed.

## 3. Importance of genetic diversity studies

Study on genetic diversity is critical to success in plant breeding. It provides information about the quantum of genetic divergence and serves a platform for specific breeding objectives (Thompson et al, 1998). It identifies parental combinations exploitable to create segregating progenies with maximum genetic potential for further selection, as proven by Akoroda (1987), Weir, (1996), Liu *et al*.( 2000); Dje *et al*.(2000), (Aremu *et al*, 2007b). Genetic diversity exposes the genetic variability in diverse populations and provides justification for introgression and ideotype breeding programmes to enhance crop performance. Mostafa *al et.* (2011), postulated that genetic diversity studies provides the understanding of genetic relationships among populations and hence directs assigning lines to specific heterogeneous

groups useable in identification of parents and hence choice selection for hybridization. Choice of parent has been identified to be the first basic step in meaningful breeding programme (Akoroda 1987); (Aremu et al. 2007a); (Islam 2004), (Rahim *et al,* 2010). Furthermore, the choice of parent selection in diversity studies is valuable because it is a means of creating useful variations in subsequent progenies. ); Dje *et al.* (2000), discovered that the higher the genetic distance between parents, the higher the heterosis in the developed progenies. Hence the heterotic progenies can be further hybridized and selections based on transgressive segregation. Akbar and Kamran, (2006). exploited this parental selection technique in wheat breeding program through hybridization. Mostafa *et al*. (2011), investigated genetic distance among 36 winter wheat genotypes cultivated in different regions of Iran using principal component analysis and discovered five major groups in the genotypes to distantly related. Comprehensive and significant emphasis are made by researchers especially plant breeders on the analysis of genetic diversity in a number of field crops white and yellow yam, (Akoroda, 1987); cowpea, (Adewale and Aremu, 2010); African yam bean, (Baudoin and Mergeai 2001); Flax, (Mohammadi *et al.* 2010); wheat, (Mostafa *et al.* 2011) and several other crops.

The diversity studies on these crops at their respective primitive levels (Landrace, wildtype, accessions, lines *etc*) led to the development of their widely distributed cultivars and varieties with proven characteristics based on stability and adaptability of performance with consistent tolerance to adverse weather conditions and resistant to diseases around the world. Fu and Somers (2009) supported that the use of identified wheat parents resistant to environmental stress under different growing conditions has led to increased world wheat production. The early report of Mohammadi and Prasna (2003) revealed that appropriate parent selection for hybridization in maize using a definite diversity study technique, Bohn *et al* (1999), identified six groups of wheat land races in the Western Iran that can be grown in different geographical locations for improved yield. Martin *et al.,* (2008) discovered 42 cultivars of bentgrass in the mancet city and that only diversity studies would identify reliable and definite cultivar(s) with varietal purity and ensure protection of breeder and consumer rights. Understanding the inter and intra specie genetic relationships as provided by diversity studies has proven to increases hybrid vigor and reduce or avoid re-selection within existing germsplasm. It is worthy of note that existing cultivar populations have narrow genetic bases, hence need for creating variability within and among cultivars using genetic diversity methods.

## 4. Genetic diversity measurement tools

Genetic diverse populations arising from pure lines, accessions, landraces, wild or weed races are analyzed using a number of methods. Such method can be single or in combination of two or more methods. Franco *et al.* (2001) stressed the need for careful considerations to be made when measuring genetic diversity within and between crop populations in research. Such considerations include:

1. Use of multivariate data collected from morphological or agronomic traits. Such data may effectively display discrete, continuous, binomial ordinal etc. variables.
2. Use of multiple data sets arising from morphological, biochemical and DNA-based collections. The use of such multiple data sets in diversity study helps to reveal the adequacy in terms of strength and constraints in the choice of each of the data sets. The use of multiple data pose some puzzles including can analysis and result interpretation

be based on individual or combined data sets? And more worrisome is the puzzle on how to effectively combine the different data sets and still achieve meaningful result. To provide answers to these puzzles, Wrigley *et al.* (1982), studied phylogenetic relationships among triticeae species using individual and combined analysis of data sets consisting of morphological and DNA-based traits and discovered divergent results in the analysed individual and combined data. The discrepancies in the results may be attributed to the discrete nature of DNA-based data and the continuous variable nature of the morphological data. No wonder Hillis 1987; Chippindale and Wein (1994) suggested the assignment of specific numbers to both quantitative and qualitiative traits in morphological, biochemical and molecular data set. In view of this, Pedersen and Seberg (1998) advised that both individual and combined data sets can be analyzed in many possible and meaningful ways to draw conclusions on genetic divergence. In 1999 and 2001, Taba *et al.* and Franco et al., respectively utilized the modified Location Model (MLM) which combines all variables into one multinomial variable called "W" to classify maize accessions from the genetic resource centres of Latin America. Better still, this MLM can combine molecular and morphological data to classify data better than when individual data set is employed. Individual data from morphological, biochemical or molecular data set can be analyzed using one or a combination of techniques. These techniques shall be discussed.

3. Expected objective to be achieved. This dictates choice of statistical tool in measuring genetic distance and the level of clustering of the intragenic factors in use. Such objective(s) include to determine the quantum of variation and grouping such genotype based on genetic distance, identify action following parental selection. In essence, breeding focus determines applicable method in explaining the nature of genetic divergence.

Variations are recorded in the measurement of genetic diversity in genotype relationships based on genetic distances and grouping populations from individual genotypes such as accessions, lines, wild races etc. The recorded variations are primarily because of the differences in the nature of genetic materials. Therefore, the basis or genetic variance theories which identifies genotype relationships based on genetic distance estimating genetic diversity depends largely on statistical genetic variance theories which identifies genotype relationships based on genetic distance / variance.

## 5. The use of morphological data to measure genetic distance

Nei, (1973), first defined Genetic distance as the difference between two entities that can be described by allelic variation. This definition was later in 1987, modified to "extent of gene differences among populations that are measured using numerical values. Betterstill, in 1998, Beaumont *et al.,* provided a more comprehensive definition of genetic distance as any quantitative measure of genetic difference at either sequence or allele frequency level calculated between genotype individuals or populations.

The first early work of Anderson (1957), proposed the use of metrogliph and index-score to study the pattern of morphological variations in individual data set. In the early seventies (Singh and Chaudhary 1985) used this method to study morphological variation in green gram. This method uses a range of variations arising from trait such that extent of trait variation is determined by the length of rays on the glyph. The performance of a genotype is

adjudged by the value of the index score of that genotype. The score value determine the length of ray which may be small, medium or long Akoroda (1987); Ariyo and Odulaja (1991) and Van Bueningen and Busch (1997), extensively explored the use of metroglyph and index-score to morphological variations in yellow yam, Okro and wild rye accessions respectively.

Similar to metroglyph and the score index is Euclidian Distance (ED) measurement. According to Nei (1987), Euclidian distance measures similarity between two genotypes, populations or individuals using using statistical measures where two individuals *i* and *j,* having observations on morphological traits (*p)* denoted by $x_1, x_2, x_3,\ldots\ldots x_n$ and $y_1, y_2,\ldots\ldots y_n$ for *i* and *j* individuals respectively.

Metroglyph and index-score methods measures genetic distance by use of morphological traits. Euclidian distance measurements utilize both morphological and molecular based marker data sets. Smith *et al.* (1991), applied the following statistic to measure ED.

$$dij = \varepsilon[(T_{1(i)} - T_{2(i)}^2)/\sigma^2 T_{(i)}]^{1/2}.$$

Where $T_1$ and $T_2$ are the values of the ith trait for 1 lines and 2 and $\sigma^2$ T(i) is the variance for the ith trait over all the lines used. Much later, Weir (1996) developed a formula for calculating genetic distance to be.

$$d(I,j) = [(x_1 - y_1)^2 + (x_2 - y_2)^2 + \ldots..(x_p - y_p)^2]^{1/2}$$

where i and j is the ED between two individuals lines having morphological traits (p)

$x_1, x_2\ldots\ldots x_p$ is the traits for i individuals and
$y_1, y_2\ldots\ldots x_p$ is the traits for j individuals

from here, the individual character distances are summed and then divided by the total number of characters scored in both individuals. ED measurement allows the use of both qualitative and quantitative data several workers identified genotype distances using ED. Van Bueningen and Busch (1997) in wheat, smith *et al*, 1987 in sorghum and Ajmone – Marsan (1998) in maize.

## 6. The use of molecular data in measuring genetic distances

The advent and explorations in molecular genetics led to a better definition of Euclidean distance by Beaumont *et al;* (1998) to mean a quantitative measure of genetic difference calculated between individuals, populations or species at DNA sequence level or allele frequency level.

Various genetic distance measurements are proposed for analyzing DNA-based data for the purpose of genetic diversity studies. Powel *et al.* (1996), identified different DNA-based marker techniques to include Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Restriction Fragment Length Polymorphic (RFLPs) and the most recent Simple Sequence Repeats (SSR) and Microsatellite (MT) of single nucleotide polymorphism (SNPs). The above nucleotide differences can be used effectively to run individual or combined data sets of morphological, biochemical or DNA based data. For DNA based data, where the amplification products are equated to alleles, the allele

frequencies can be calculated and the genetic distance between i and j individuals estimated as follows.

$$d(ij) = 1 < \left[ \sum^{n} (X_{ai} - X_{aj}) \right]^{1/r}$$

Where $X_{ai}$ is frequency of the allele a for individual I, and n is the number of alleles per loci; r is the constant based on the coefficient used. In its simple form, i.e *r* = 1, genetic distance can be calculated as:

$$d\int (ij) = 1/2 \left[ \sum^{n} (X_{ai} - X_{aj}) \right]$$

Where r = 2, d(*i,j)* is referred to as Rogers (1972) measure of distance (RD), where

$$RD_{ij} = I/2[\ \Sigma(\ x_{ai}\text{-}x_{aj})\ ^2]^{1/2}$$

Where allele frequencies are to be calculated for some of the molecular markers, the data must first generate a binary matrix for statistical analysis. Binary data has been long and widely used before the advent of molecular marker data to measure genetic distance by Rogers (1972); Nei and Chesser (1983) coefficient and known as $GD_{MR}$ and $GD_{NL}$ respectively.

In the use of any given statistical formula to determine genetic diversity in molecular based data, one specific problem usually encountered is the failure of some genotypes to show amplification for some primer pairs. Robinson and Harris (1999) noted that lack of amplification may be due to "null alleles". Most often, it is difficult to ascribe lack of amplification to "null allele". It is therefore the reposed confidence of the researcher, that a "null allele" status of a genotype will not be considered as missing data during computation of genetic similarity- distance matrix so as to avoid gross error during result interpretation.

DNA based marker data have been successfully used to measure genetic distance in some crops (Pritchard *et al.* (2000) in pigeon pea; Beaumont *et al.* (1998) in wheat; Franco *et al.*, (2001) in maize; Dje *et al.* (2000) in Sorghum.

## 7. Grouping techniques in measuring genetic diversity

Genetic relationship among and with breeding materials can be identified and classified using multivariate grouping methods. The use of established multivariate statistical algorithms is important in classifying breeding materials from germplasm, accessions, lines, and other races into distinct and variable groups depending on genotype performance. Such groups can be resistant to diseases, earliness in maturity, reduced canopy drought resistant etc. The widely used techniques irrespective of the data source (morphological, biochemical and molecular marker data) are cluster analysis, Principal Component Analysis (PCA), Principal Coordinate Analysis (PCOA) Canonical Correlation and Multidimensional Scaling (MDS).

Cluster analysis presents patterns of relationships between genotypes and hierarchical mutually exclusive grouping such that similar descriptions are mathematically gathered

into same cluster (Hair *et al*. 1995); (Aremu 2005). Cluster analysis have five methods namely unweighted paired group method using centroids (UPGMA and UPGMC), Single Linkages (SLCA), Complete Linkage (CLCA) and Median Linkage (MLCA). UPGMA and UPAMC provide more accurate grouping information on breeding materials used in accordance with pedigrees and calculated results found most consistent with known heterotic groups than the other clusters (Aremu *et al.,* (2007a).

Principal components, canonical and multidimentional analyses are used to derive a 2-or 3-dimensonal scatter plot of individuals such that the geometrical distances among individual genotypes reflect the genetic distances among them. Wiley (1981), defined principal component as a reduced data form which clarify the relationship between breeding materials into interpretable fewer dimensions to form new variables. These new variables are visualized as different non correlating groups.

Principal components analysis first determines Eigen values which explain the amount of total variation displayed on the component axes. It is expected that the first 3 axes will explain a large sum of the variations captured by the genotypes. Cluster and principal component analysis can be jointly used to explain the variations in breeding materials in genetic diversity studies.

## 8. Conclusion

Genetic diversity studies is in no measure the first basic step in meaningful breeding programme and therefore require accurate and reliable means for estimation. Data sets sourced can morphological biochemical several workers successfully utilized various statistical tools in analysis diverse data sets and identified two major framework to really explain divergence in genotype performance. Genetic distance among and within individual data sets can be conveniently determined using specific tools while classificatory and cluster analysis require principal component and polymorphic sequence tools. Since each data set provide different molecular type of information, based marker data set is visualized to provide more reliable differentiate information on the genotypes. Analysis of data sets can be complex. Many software packages are available. There is still a need for a comprehensive and user-friendly software packages that would integrate different data set for analysis and generate reliable and useable information about genetic relationship. Equally important in genetic diversity studies is the need for a genetic resource centre. Studies should incorporate utilization of genetic diversity information in developing genetic resource centre accessible to breeders.

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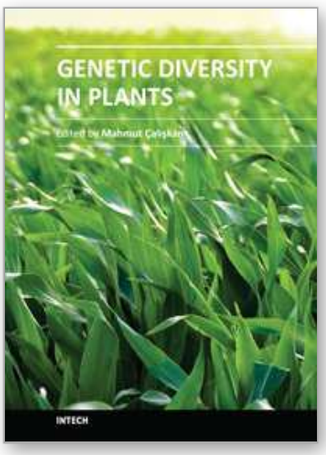
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**Genetic Diversity in Plants**

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Genetic diversity is of fundamental importance in the continuity of a species as it provides the necessary adaptation to the prevailing biotic and abiotic environmental conditions, and enables change in the genetic composition to cope with changes in the environment. Genetic Diversity in Plants presents chapters revealing the magnitude of genetic variation existing in plant populations. The increasing availability of PCR-based molecular markers allows the detailed analyses and evaluation of genetic diversity in plants and also, the detection of genes influencing economically important traits. The purpose of the book is to provide a glimpse into the dynamic process of genetic variation by presenting the thoughts of scientists who are engaged in the generation of new ideas and techniques employed for the assessment of genetic diversity, often from very different perspectives. The book should prove useful to students, researchers, and experts in the area of conservation biology, genetic diversity, and molecular biology.

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