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# Genetic Variability of Agronomic Traits of Low Nitrogen Tolerant Open-Pollinated Maize Accessions as Parents for Top-Cross Hybrids

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**Abstract.** Discrimination among parental accessions maintains the agronomic performance and purity of hybrids. Low nitrogen tolerant open-pollinated maize were screened for variation in agronomic characters, parents' identification and grouping using principal component and single linkage analyses, Pearson's correlation and bi-plot methods. The accessions were genetically distinct and diverse. BR9928-DMRSRLN proved intercrop-able and drought tolerant. LNTP-WC3, Sint-Marzoca-Larga and ART/98/SW5-OB were best pollen materials. Pairs of days to anthesis and silking, total number of leaves and ear height, leaf length or width and number of leaves below uppermost ear, veination index and leaf width, and shell and field weights can be improved simultaneously. First four PCs accounted for 80% variability. PC I had the strongest discriminating ability but insufficiently distinguished the accessions. First three PCs identified anthesis-silking-interval, plant height, number of veins, ear height, total number of leaves, leaf width, veination index, field and shell weights, days to anthesis and silking as discriminators. The accessions clustered into three. LNTP-YC6 and ILE-1OB flowered earlier; BR99TZL-Comp4DMSRSR and Sint-Marzoca-Larga yielded highest.

**Key words**: Diversity, genetic distance, open pollinated, top-cross hybrid, *Zea mays*.

## Introduction

Maize is one of the major cereal crops in tropical Africa. It is cultivated mostly by resource poor farmers in the rainforest and the derived savannah zones of Nigeria (Iken and Amusa, 2004). The crop is consumed in a variety of ways as processed food, feeds and raw materials for industries. Maize yields in farmers' fields in Africa range from 1 to 2 t ha$^{-1}$ in contrast to the higher yields of about 5 to 7 t ha$^{-1}$ reported on breeding stations or about 8.6 t ha$^{-1}$ realizable in farmers' fields in developed countries (FAO, 2001; Fakorede *et al.,* 2003). The poor yield is attributed to poor soil fertility, poor nutrient management and high cost of farm inputs (Azeez and Adetunji, 2007).

Nitrogen (N) is the major limiting essential nutrient to maize production (Badu-Apraku *et al.,* 2010; Ismaila *et al.,* 2010). Its deficiency in the soil is due to the rapidity by which it is taken up or lost from the soil through erosion, volatilization or leaching. A possible approach to reduce N deficiency in soil is to lower crop demand for N through selection for low N tolerance (Smith *et al.,* 1994). This has brought about the development of inbred lines, open pollinated (OP) and hybrid varieties capable of utilizing the available N in the soil. The improved varieties could enhance productivity in N poor soils and reduce reliance on inorganic N fertilizers. Wide range of genetic diversity that exists among cultivated species of maize is usually harnessed to improve the crop. Open pollinated maize varieties are useful in generating low N tolerant top cross hybrids. Resource-poor farmers prefer top crosses to pure inbred line hybrids because the production of top cross hybrids seeds is cheaper than those of other hybrids. Hence, seed price of top-cross hybrids is cheaper than conventional hybrids' seeds (Correjado and Magulama, 2008).

Numerous techniques have been proposed to estimate genetic divergence in crop accessions. Among such techniques are coefficient of racial likeness proposed by Pearson (1926), principal component analysis (PCA) proposed by Gower (1966) and numerical taxonomic (Sneath and Sokal, 1973). PCA is useful for reducing and interpreting large multivariate data sets with underlying linear structures and for discovering previously unsuspected relationships among crop characters. It is used for identifying patterns in data and expressing the data by highlighting their similarities and differences. However, PCA alone would not give an adequate character representation in terms of relative importance when numerous characters are considered simultaneously (Shalini *et al.,* 2003; Oduwaye *et al.,* 2010). There is, therefore, the need to complement PCA for this purpose. Single linkage cluster analysis (SLCA) with principal component analysis has been used to classify the variation among crop genotypes (Oduwaye *et al*., 2010; Nwanguruka *et al.,* 2011; Denton and Nwanguruka, 2012; Rao *et al.,* 2012). SLCA summarizes the position of accessions by sorting them into distinct groups.

Information on the genetic variability among genotypes and its potential use is vital in the crop's genetic improvement. This will enhance the identification of useful genes and their behaviour in breeding programmes. In view of this, data on differences among the OP maize accessions were found useful in top cross varieties development. This will guide plant breeders in defining their breeding goals. Therefore, this study evaluated low N tolerant OP maize accessions to determine the major plant characters responsible for variation, identify potential parental stocks for top-cross hybrids and group the accessions using PCA and SLCA techniques.

## Materials and methods

### Collection and agronomic practices of the open pollinated maize accessions

A total of 10 low nitrogen tolerant OP maize accessions were obtained and evaluated in this study. The collection represented eight accessions obtained from International Institute of Tropical Agriculture and two obtained from Institute of Agricultural Research and Training (IAR&T), both in Ibadan, Nigeria.

The 10 maize accessions were evaluated in Ibadan (3° 56′ E and 7° 33′ N 168 m above sea level) during the early and late seasons of 2011 using agronomic practices recommended by IAR&T (2010). Mean rainfall of the experimental site were 1890 and 970 mm per annum during the early and late planting season respectively. Seeds of each accessions were sowed at 75 cm × 50 cm at two per hill in two rows, (each row was 5 m long) in an experiment laid out in a randomized complete block design with three replicates. A compound fertilizer of 40 kg $ha^{-1}$ 20:10:10 NPK was applied at four weeks after sowing to boost growth of the plants according to Bänziger *et al.* (2000). Ears of all crops were harvested when the bracts were dry.

## Data collection and statistical analysis

Observation was made on days to 50% tasselling (DTA) and silking (DTS), anthesis-silking interval (ASI), plant height (PH), ear height (EH), number of leaves below the uppermost ear (NoL), total number of leaves per plant (TNoL), leaf length (LLT) and width at mid-way along the length(LWD), venation index (VI) estimated by dividing the number of veins mid-way along the ear leaf by the leaf width, field weight per plot (FWT) (kg) and shelled grain weight (SWT) per plot (kg). These parameters were taken according to (IBPGR & CIMMYT, 1991) procedures.

Data collected were pooled across seasons and were analyzed according to Steel and Torrie (1980) method using SAS vesrion 8.0 (SAS 1999). Pearson correlation analysis was performed to determine the associations that existed between all possible pairs of character. The extent of genetic variation and percentage dissimilarity within accessions were determined using PCA and SLCA. Eigen-values and factor loadings were obtained from PCA, and were used to determine the relative discriminative power of the axes and their associated characters. The relationship between the first two PCs and the accessions was described with bi-plot procedure. The position of genotypes was summarized into cluster with a dendogram generated from SCLA.

## Results

### Botanical diversity of low N tolerant OP maize accessions

A wide variability existed in the botanical characteristics of the OP maize accessions (Table 1). All the plants were green with medium to high staying green attributes at maturity. The orientation of the leaves of the plants was erect only in BR9928-DMRSRLN. The BR9928-DMRSRLN, TZL-Com1C6LN and R99TZL-Comp4DMSRSR were densely pubescent whereas the pubescence of other accessions was intermediate. Tassel types in all the accessions were primary-secondary. The LNTP-WC6, Sint-Marzoca-Larga and ART/98/SW5-OB had large tassel size compared to all other accessions with medium tassel size.

### Means and association between pairs of some relevant characters of OP accessions

Means, standard errors, ranges and coefficients of variation of vegetative, flowering and grain yield characters are presented in Table 2. Minimum ASI was -1.0 day. Table 3 shows the association between pairs of some relevant agronomic characters of the accessions. Some pairs of the characters considered were highly significant whereas many were not significant. All the pairs of characters were significantly correlated, with one another positively except ASI and FWT which were negatively correlated (-0.38: $P<0.05$) showing a strong relationships among these pairs of characters. DTA was highly correlated with DTS (0.89; $P<0.01$) and DTS correlated significantly with ASI (0.38; $P<0.05$). FWT correlated with both VI (0.64) and SWT (0.62) both at $P<0.01$.

### Principal component analysis of variance in low N tolerant OP maize accessions

The results of the PCA are shown in Table 4. Only four out of 11 principal component axes had eigen values greater than 1.0. These four PC accounted for over 80 % of the total variation. The first four PCA (I, II, III and IV) recorded 3.26, 3.20, 2.74 and 1.24 respectively, thereby accounted for 25.06 %, 24.64 %, 21.08 % and 9.58 % in that order of occurrence. PCA I was highly correlated with EH (0.82), TNoL (0.87) and NoV (0.69) when the characters loaded the axis. ASI (0.61), PH (0.58), LWD (0.63), VI (0.78), FWT (0.69) and SWT (0.82) loaded PCA II whereas DTA (0.89), DTS (0.85) and NoL (0.57) loaded PCA III. LLT (0.58) loaded PCA IV.

## Classification of the low N tolerant OP maize accessions

The clusters of the accessions generated with the bi-plot on PCA I and PCA II (Figure 1) showed that component I best described LAPOSTA-SEQUIA-C6, ILE-1OB and ART/98/SW5-OB whereas component II best described BR99TZL-Comp4DMSRSR and Sint-Marzoca-Larga. The dendogram (Figure 2) describes the relationships among the 10 low N OP accessions. The accessions differed at 100 % variability, but clustered into three and only two respectively at 50 % and 70 % variability. ART/98/SW5-OB was more distant to BR9928-DMRSRLN. The accessions when ranked according to the vegetative, flowering and grain yield characters show wide variability (Figure 3). Variations were obtained due to various developmental factors making LNTP-YC6, Sint-Marzoca-Larga and ART/98/SW5-OB performed above average in vegetative characters. The LNTP-YC6 and ILE-1OB were superior in earliness in flowering but Sint-Marzoca-Larga and BR99TZL-Comp4DMSRSR were ranked below average in grain weight.

## Discussion

A wide genetic variability in crop species creates options from plant breeders to draw materials for use in breeding programmes. The wide variation in the botanical characteristics of the accessions signifies that the accessions are morphologically highly distinct and diverse. It is possible that the differences in the qualities affect the growth and general performance of the accessions differently. The greenish colour of the plants in all the accessions means that the plants effectively harnessed the available N in the soil thereby nourishing the plants adequately. The BR9928-DMRSRLN that has erect leaf orientation can be a good candidate for intercropping and planting under drought. The surface area of the leaves of the accession exposed to direct solar radiation is reduced; hence solar radiation interception is enhanced while evapo-transpiration through the leaves is reduced. The accession may contain genes useful for developing varieties suitable for intercropping and or drought tolerance. High stay green at maturity quality of all the accessions also indicates that they have high nitrogen use efficiency. This together with the plant greenish colouration proves the accessions to be low N tolerant varieties. This confirms the observation of Bänziger *et al.* (2000) that low N tolerant accessions have high stay green quality and that genes responsible for low N and drought tolerance are linked. The accessions could use the little N available in the soil and can be selected for drought tolerance. However, BR9928-DMRSRLN and ILE-1OB can be selected only where they are relatively abundant or easy to obtain because their stay green quality was medium contrary to the high rating of the other accessions. Though other accessions had medium tassel size, LNTP-WC3, Sint-Marzoca-Larga and ART/98/SW5-OB had large tassel size which may make them produce more pollen. Thus, any of the three accessions are selectable as male parents because of high pollen potential.

The PH, EH, FWT and SWT are effective in creating options for breeders for maize improvements because of their high standard errors and coefficients of variation (CV). Similarly, ASI and LLT respectively had high CV and standard error. High standard error and CV describe wide variations. Thus, any of these traits as good selection index either individually or combined. The correlation coefficients of pairs of most traits of the crop were not significant, denoting weak or non-association among these morphological attributes and their potential usefulness as discriminators in low N tolerant maize genotypes. But the pairs of DTA and DTS, TNoL and EH, LLT and NoL, LWD and NoL, VI and LWD as well as SWT and FWT were highly and positively correlated (r> 60 %). This suggests that either of each pair can be simultaneously selected or improved for the other and that they are potentially vital taxonomic descriptors of the crop. Simultaneous selection saves costs and time in breeding programmes. LWD demonstrates its importance when it positively correlated with NoL, LLT and VI. Based on this relationship LWD is a versatile trait for selection. The r values between grain yield and any other trait were low and not significant except ASI that negatively correlated with FWT. It can then, be concluded that selection based on vegetative traits cannot be reliably inferred for yield. ASI did not affect the crop's yield but may be useful in choosing parents for effective pollination.

The results of the PCA proved the effects of PC on variability among the accessions by showing that different characters contributed to the total variation. Lezzoni and Pritts (1991); Oduwaye *et al.* (2010); Daniel *et al.* (2012) have also reported that genetic variability among genotypes can be estimated from the contributions of characters of accessions to each of the components based on the eigen vectors. The first three PCs had very strong discriminating ability to identify the characters that contributed to the variation in the accessions suggesting their potential usage for accessions discrimination using morphological attributes of the maize. PC I, which accounted for the variations due to EH, TNoL and NoV, had the strongest ability but did not sufficiently distinguish the accessions because PC I accounted for only about 25.1 % of the total variation. The PC II that accounted for about 24.6 % associated with more characters than any of the four axes. It was loaded by ASI, PH, LWD, VI, FWT and SWT. The DTA and DTS correlated with PC III which accounted for 21.1 %. This conforms to the finding of Khavari *et al.* (2011); Rao *et al.* (2012) that total number of leaves and yield associated with PCA I and II respectively. The contributions of these first three PCs were close and collectively accounted for a total of about 70 % variation. This agrees with the observation of Daniel *et al.* (2012) that plant characters associated with these axes can be used to distinguish the accessions. Any of the characters considered except NoL and LLT is capable of revealing the differences in maize accessions. Besides, EH, TNoL, NoV, VI, FWT and SWT contributed large variability (eigen vectors= 0.82, 0.87, 0.69, 0.78, 0.69 and 0.82 respectively) suggesting that their potential usage for effective discrimination among the maize accessions.

The PC I best described LAPOSTA-SEQUIA-C6, ILE-10B and ART/98/SW5-0B whereas PC II best described BR99TZL-Comp4DMSRSR and Sint-Marzoca-Larga. The marked differences in the PC scores of the characters, the eigen values of the first three PCs (3.26, 3.20 and 2.70 respectively) that were close and the wide distance between clusters (0-70 %) of the accessions revealed by the dendogram suggest a great variability among the accessions. The BR9928-DMRSRLN and ART/98/SW5-OB were the most distinct and diverse (up to 70 % of the distance between clusters) makes the accessions very vital sources of materials for improvement of the accessions. However, BR99TZL-Comp4DMSRSR and BR9928-DMRSRLN may contain similar genes and can be used to achieve similar purposes in breeding programmes. The LNTP-YC6, Sint-Marzoca-Larga and ART/98/SW5-0B were above average based on vegetative characters. The LNTP-YC6 and ILE-1OB ranked below average due to earliness in flowering and Sint-Marzoca-Larga and BR99TZL-Comp4DMSRSR below average due to grain yield.

## Conclusions

Information useful for plant breeding programmes were generated from the study as follows:

- All the accessions were both botanically and genetically distinct and diverse. Variation exists in the usefulness of each of the accessions due to their traits in breeding programme, thereby grouping the accessions into three and showing BR9928-DMRSRLN and ART/98/SW5-OB as the best sources of materials for maize improvement for yield and low N tolerance.
- Leaf width is a versatile trait for selection. Pairs of days to 50 % anthesis and 50 % silking, total number of leaves and ear height, leaf length or leaf width and number of leaves below uppermost ear, veination index and leaf width, as well as shell weight and field weights were found to suitable for simultaneous improvement.
- The first three PCs identified the effective discriminators by accounting for variations due to flowering and vegetative traits. Thus, LNTP-YC6, Sint-Marzoca-Larga and ART/98/SW5-OB ranked high for vegetative characters whereas LNTP-YC6 and ILE-1OB recorded earliness in flowering; BR99TZL-Comp4DMSRSR and Sint-Marzoca-Larga were high yielding materials.

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**Table1. Botanical description of the plants of low N tolerant open pollinated maize accessions**

| Accession name | Source | Description |
|---|---|---|
| **LNTP-YC6** | IITA | Green plant, pendant leaves, intermediate sheath pubescence high stay green at maturity; primary-secondary tassel typ and medium tassel size. |
| **LNTP-WC3** | IITA | Green plant, pendant leaves, intermediate sheath pubescence high stay green at maturity; primary-secondary tassel typ and large tassel size. |
| **TZPB Prol C3** | IITA | Green plant, pendant leaves, intermediate sheath pubescence high stay green at maturity; primary-secondary tassel typ and medium tassel size. |
| **BR9928-DMRSRLN** | IITA | Green plant, erect leaves, dense sheath pubescence, mediu stay green at maturity; primary-secondary tassel type an medium tassel size. |
| **TZL Com1C6LN** | IITA | Green plant, pendant leaves, dense sheath pubescence, hig stay green at maturity; primary-secondary tassel type an medium tassel size. |
| **LAPOSTA SEQUIA C6** | IITA | Green plant, pendant leaves, intermediate sheath pubescence high stay green at maturity; primary-secondary tassel typ and medium tassel size. |
| **Sint Marzoca Larga** | IITA | Green plant, pendant leaves, intermediate sheath pubescence high stay green at maturity; primary-secondary tassel typ and large tassel size. |
| **BR99TZLComp4DMSRSR** | IITA | Green plant, pendant leaves, dense sheath pubescence, hig stay green at maturity; primary-secondary tassel type an medium tassel size. |
| **ILE-10B** | IAR&T | Green plant, pendant leaves, intermediate sheath pubescence medium stay green at maturity; primary-secondary tasse type and medium tassel size. |
| **ART/98/SW5-0B** | IAR&T | Green plant, pendant leaves, intermediate sheath pubescence high stay green at maturity; primary-secondary tassel typ and large tassel size. |

**Table 2. Means, standard errors and coefficients of variation of some relevant traits of low N tolerant open pollinated maize accessions**

| Trait | Mean±se | Min. | Max | C.V.(%) |
|---|---|---|---|---|
| Days to tasselling | 54.7±0.29 | 52.0 | 58.5 | 2.89 |
| Days to silking | 56.6±0.31 | 53.0 | 60.0 | 3.12 |
| Anthesis-silking interval | 1.9±0.14 | -1.0 | 3.0 | 39.11 |
| Plant height (cm) | 210.8±3.74 | 158.8 | 264.4 | 9.76 |
| Ear height (cm) | 107.1±2.65 | 73.3 | 139.7 | 13.54 |
| No. of leaves below uppermost ear | 7.1±1.00 | 6.0 | 8.3 | 7.62 |
| Total No. of leaves per plant | 13.1±0.16 | 11.3 | 15.0 | 6.81 |
| Leaf length (cm) | 99.9±1.24 | 84.8 | 110.4 | 6.79 |
| Leaf width (cm) | 10.1±0.14 | 8.2 | 11.8 | 7.47 |
| Number of veins | 25.5±0.30 | 22.8 | 29.5 | 6.44 |
| Venation index | 2.5±0.03 | 2.2 | 3.0 | 7.28 |
| Field weight per plot (kg) | 0.6±2.36 | 0.4 | 0.9 | 20.25 |
| Shell weight per plot (kg) | 0.5±2.82 | 0.2 | 0.8 | 30.60 |

se=standard error, Max.= maximum, Min.= minimum, C.V.=coefficient of variation

**Table 3. Association between pairs of some relevant traits of low N tolerant open pollinated maize accessions**

| | DTA | DTS | ASI | PH | EH | NoL | TNoL | LLT | LWD | VI | FWT |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **DTS** | 0.89** | | | | | | | | | | |
| **ASI** | -0.08 | 0.38* | | | | | | | | | |
| **PH** | -0.01 | 0.19 | 0.44** | | | | | | | | |
| **EH** | 0.07 | 0.23 | 0.37* | 0.58** | | | | | | | |
| **NoL** | 0.32 | 0.26 | -0.10 | 0.35 | 0.29 | | | | | | |
| **TNoL** | -0.02 | 0.10 | 0.27 | 0.50** | 0.72** | 0.51** | | | | | |
| **LLT** | 0.27 | 0.32 | 0.22 | 0.57** | 0.51** | 0.71** | 0.57** | | | | |
| **LWD** | 0.13 | 0.14 | 0.03 | 0.24 | 0.26 | 0.63** | 0.25 | 0.53** | | | |
| **VI** | 0.19 | 0.19 | 0.03 | -0.07 | 0.20 | 0.33 | 0.09 | -0.17 | 0.64** | | |
| **FWT** | 0.20 | 0.01 | -0.38* | -0.01 | -0.09 | -0.05 | 0.02 | -0.17 | -0.13 | 0.36 | |
| **SWT** | -0.01 | -0.04 | -0.07 | -0.10 | 0.11 | 0.02 | 0.23 | -0.05 | 0.06 | 0.32 | 0.62** |

DTA=days to tasselling, DTS=days to silking, ASI=anthesis-silking interval, PH=plant height, EH=ear height, NoL=number of leaves below topmost ear, TNoL=total number of leaves per plant, LLT=leaf length, LWD=leaf width, VI=venation index. FWT=field weight per plot, SWT=shell weight per plot.

Table 4. Principal component analysis of variation among 10 low N tolerant open pollinated maize accessions

| Trait | PC Axis I | PC Axis II | PC Axis III | PC Axis IV |
|---|---|---|---|---|
| Days to tasselling | 0.08 | -0.31 | 0.89 | 0.16 |
| Days to silking | 0.19 | -0.02 | 0.85 | 0.47 |
| Anthesis-silking interval | 0.19 | 0.61 | -0.27 | 0.53 |
| Plant height (cm) | 0.44 | 0.57 | 0.34 | 0.21 |
| Ear height (cm) | 0.82 | -0.01 | -0.32 | 0.10 |
| No of leaves below uppermost ear | 0.53 | 0.00 | 0.57 | -0.05 |
| Total No. of leaves per plant | 0.87 | -0.01 | -0.14 | -0.34 |
| Leaf length (cm) | 0.52 | -0.19 | 0.42 | -0.58 |
| Leaf width (cm) | 0.41 | -0.63 | -0.50 | 0.30 |
| Number of veins | 0.69 | 0.44 | -0.33 | 0.17 |
| Venation index | 0.27 | 0.78 | 0.19 | -0.15 |
| Field weight per plot | -0.54 | 0.69 | 0.12 | -0.12 |
| Shell weight per plot | -0.05 | 0.82 | -0.12 | -0.26 |
| Eigen value | 3.26 | 3.20 | 2.74 | 1.24 |
| % variance | 25.06 | 24.64 | 21.08 | 9.58 |
| Cumulative % variance | 25.06 | 49.70 | 70.78 | 80.36 |

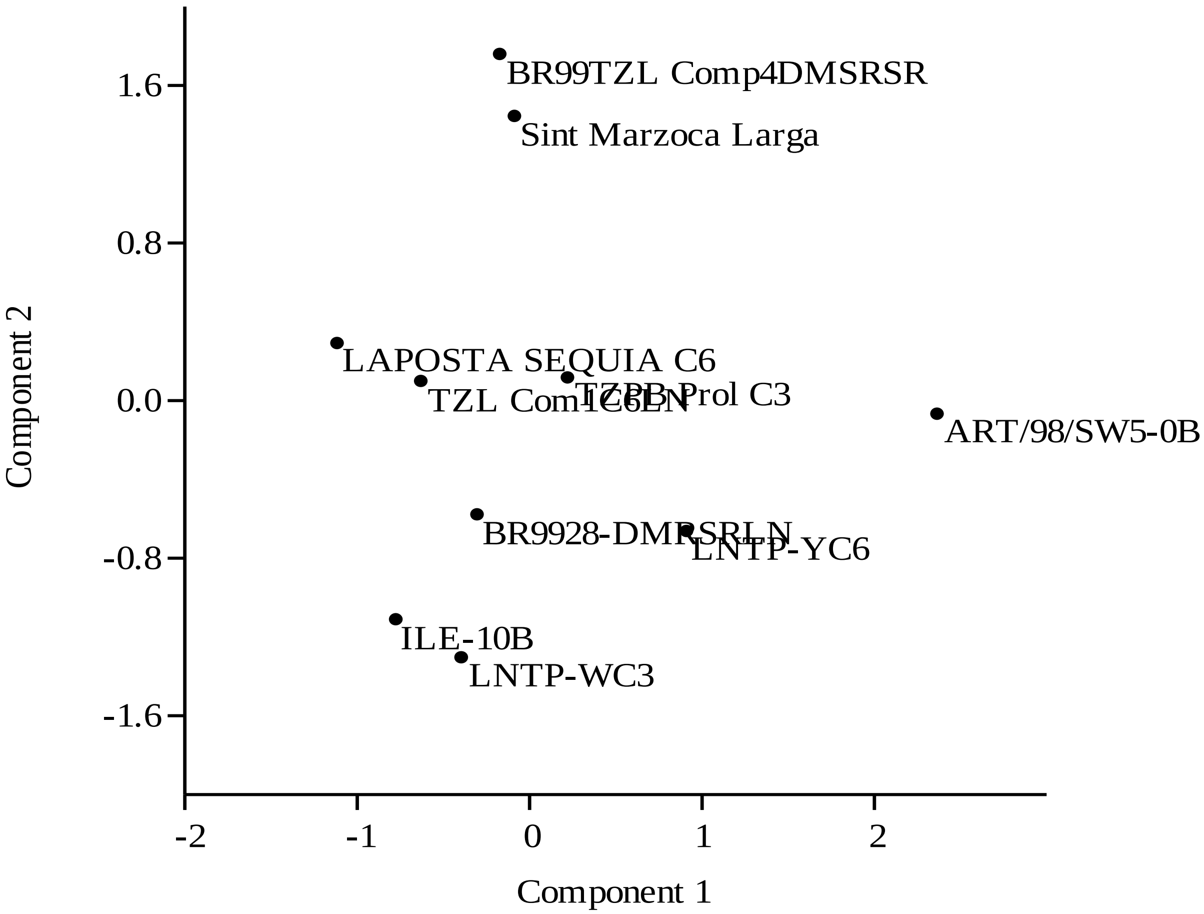

**Figure 1. A bi-plot of principal axes I and II showing the distance among low N tolerant open pollinated maize accessions**

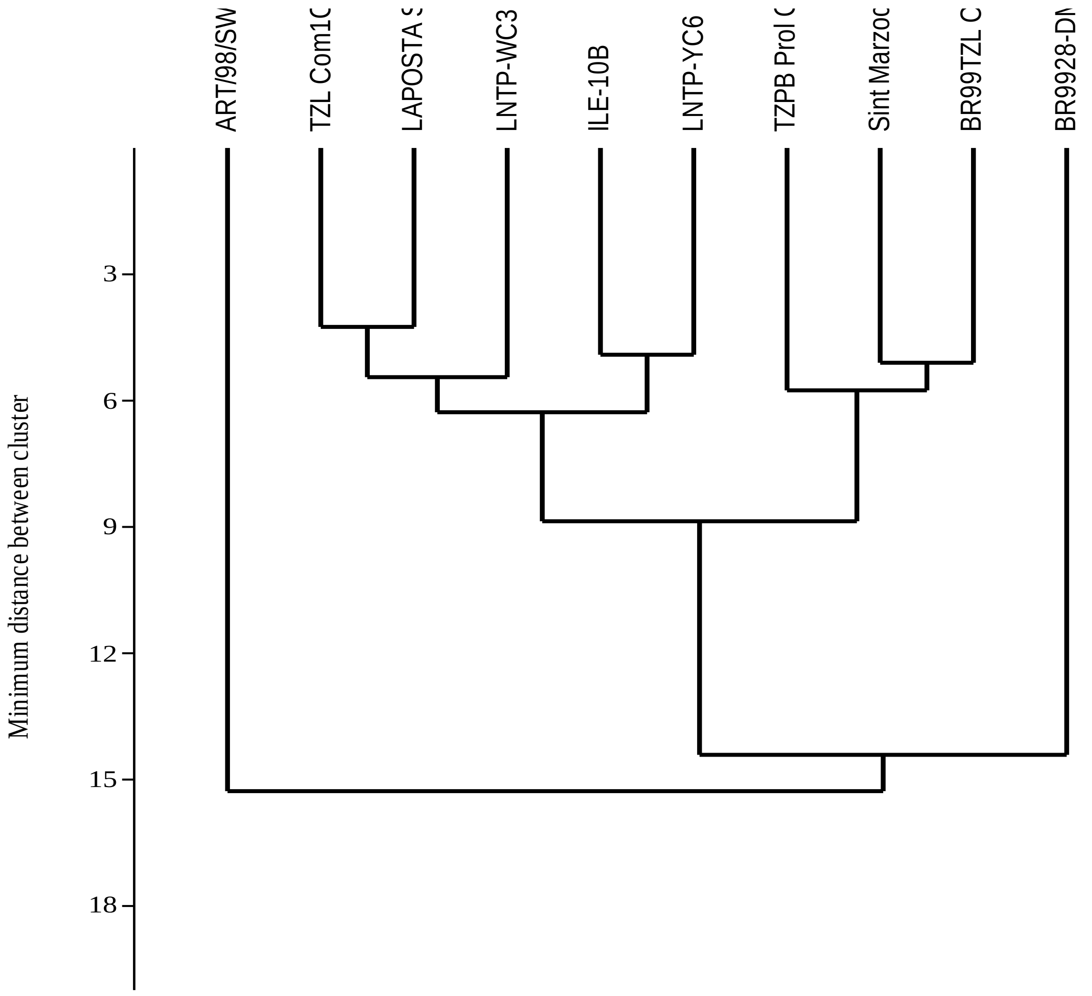

**Figure 2. Dendogram showing the distance among 10 low N tolerant open pollinated maize accessions**

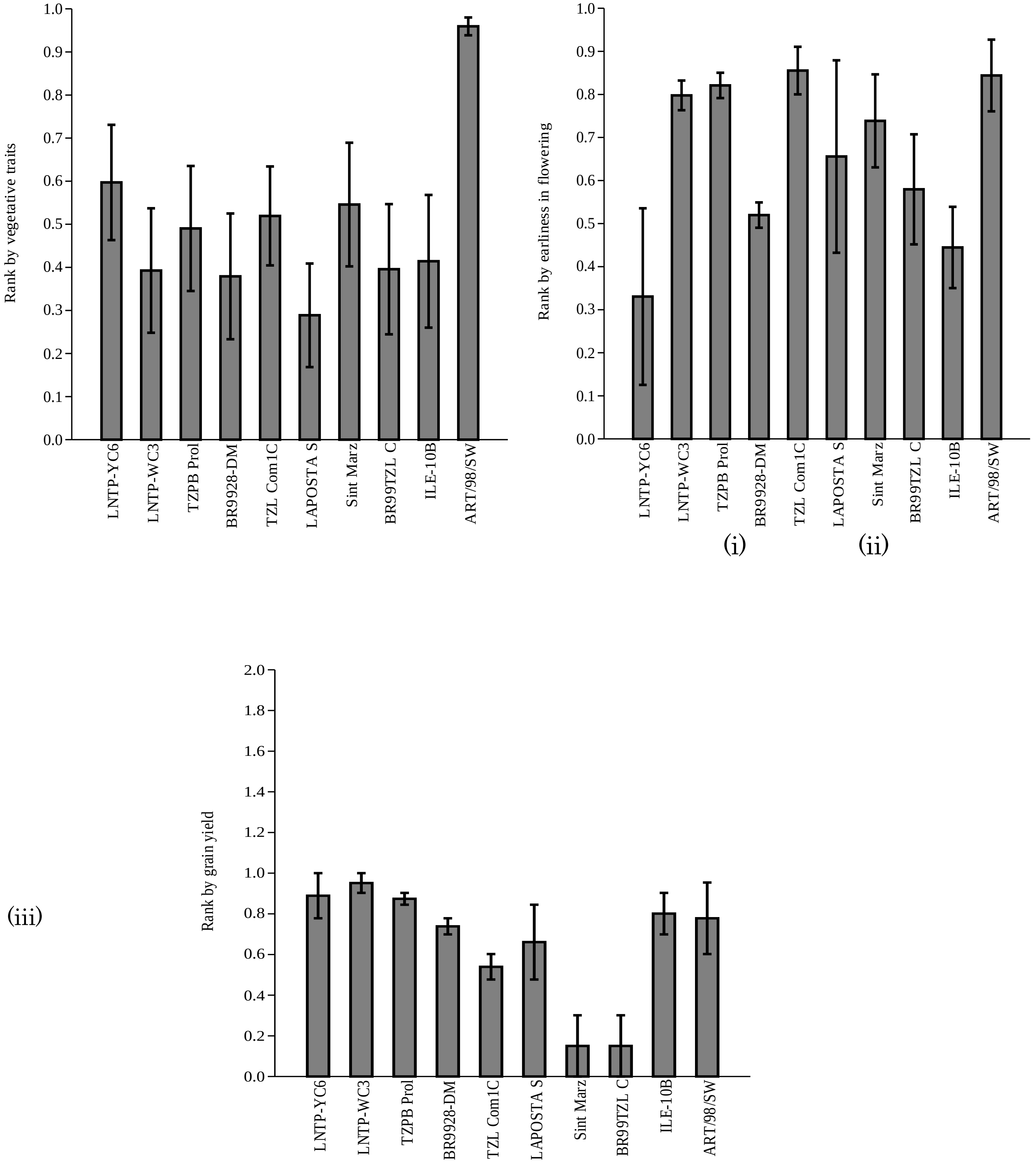

**Figure 3. Ranking the low N tolerant open pollinated maize accessions based on (i) vegetative (ii) flowering and (iii) grain yield potential.**