Phenotypic Characterization on Selected Kenyan and Tanzanian Rice (Oryza sativa L) Populations Based on Grain Morphological Traits

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Abstract

Phenotypic characterization of rice varieties is a good approach for assessing genetic and phenotypic variability among varieties and is key in grading of rice varieties. The objective of this study was to determine the major determinants of phenotypic diversity and the strength of segregation among aromatic and non-aromatic rice (Oryza sativa L) populations collected from Kenya and Tanzania. Multivariate analyses including principal component analysis (PCA) and cluster analysis were carried out assess the overall patterns of morphological variation. Using Principal component analysis, it was found that grain length, kernel length, grain weight and kernel length/breadth ratio are major drivers of the huge phenotypic diversity observed. Cluster analysis was found to effectively distinguish the majority of aromatic from non-aromatic varieties based on the grain quality traits evaluated where two distinct clusters were formed. The results obtained from this study demonstrated that phenotypic trait measurement can be relied upon in diversity studies among diverse and closely related genotypes. We conclude that this research which forms first part of rice grading gives an insight in to the general patterns of phenotypic diversity and finds out the most important distinguishing characters. This will be validated with subsequent molecular analysis.

Keywords: Rice; Phenotypic diversity; Cluster analysis

Abbreviation: PCA: Principal Component Analysis

Introduction

Rice (Oryza sativa L) is a member of the grass family (Gramineae) belonging to the genus Oryza. The genus Oryza includes 23 wild species and 2 cultivated species. of the two cultivated species, African rice (Oryza glaberrima) is highly grown in West Africa whereas the Asian rice (Oryza sativa L) has spread over time and is grown in all continents in the world. Being able to grow in a wide spectrum of climates and conditions, rice is a staple food for one third of the world’s population [1].

Rice (Oryza sativa L) is regarded as one of the major cereal crops with high agronomic and nutritional importance. The current global production of rice is about 738.1 million metric tones per year. This constitutes more than a quarter of all cereal grains. Of these, Asia accounts for the largest production totaling to about 584 million tones, whereas Africa produces approximately 21.9 million tones. In Kenya, rice is the third most important staple food after maize and wheat. The local production is estimated at between 45,000 to 80,000 tones whereas its consumption is about 300,000 tones. This huge production - consumption gap is met through imports. About 80% of the rice grown in Kenya is from irrigation schemes in Mwea, Ahero, Bunyala, West Kano and Yala swamp. The remaining 20% is produced under rain fed conditions [2].

Landraces are the local or traditional varieties of a domesticated plant species which have developed over time through adaptation to their natural environment [3]. The demand for productive and homogeneous crops has led to development of a small number of standard, high yielding varieties. This has consequently resulted to tremendous loss of heterogeneous traditional cultivars through genetic erosion. Landraces preserve much of this lost diversity and are known to harbor great genetic potential for breeding new crop varieties that can cope with environmental and demographic changes. There are more than 400,000 rice varieties worldwide but the major categories include; indica, japonica, basmati and glutinous. These varieties differ in their grain qualities which include: milling quality, grain shape, cooking quality, nutritional quality and aroma. These traits are crucial determinants of cooked rice grain quality [4].

Kenya is home to many varieties of rice varieties and land races. These varieties were developed through selection based on agronomic traits. This resulted in a wide spectrum of varieties that are highly valued both in domestic and foreign markets. In Kenya, rice consumers prefer the aromatic rice, which is high in quality, and hence price. Unscrupulous traders often blend this fragrant rice which has good cooking quality traits with low quality non-fragrant rice to make more profit from their trade. Various methods routinely used to evaluate and grade rice varieties are inconsistent and have failed to address these concerns due to low sensitivity, time consumption and large sample volume requirement. Therefore, phenotypic characterization based on grain morphological traits that describe the uniqueness of a variety is imperative in morphological distinction between aromatic and non-aromatic rice varieties [5].

Materials and Methods

Plant material

A total of 500 g of thirteen different rice varieties were collected from

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Received October 06, 2015; Accepted November 17, 2015; Published November 20, 2015

Citation: Kioko WF, Mawia MA, Piero NM, Muriira KG, Wavinya ND, et al. (2015) Phenotypic Characterization on Selected Kenyan and Tanzanian Rice (Oryza sativa L) Populations Based on Grain Morphological Traits. J Rice Res 3: 155. doi:10.4172/2375-4338.1000155

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Mwea Irrigation Agricultural Development (MIAD) and Kilimanjaro Agricultural Training Center (KATC). The names and attributes of the rice varieties and the names of the corresponding sources are detailed in Table 1. The rice seeds were stored in Molecular Biology laboratory at Kenya Bureau of Standards, Nairobi, Kenya (Table 1).

Determination of phenotypic diversity

A total of seven traits were measured in this study. They included; grain length (GL), grain breadth (GB), grain length/breadth (G-L/B), grain weight (GW), kernel length (KL), kernel breadth (KB), kernel length/breadth (K-L/B). 100 randomly selected raw rice grains and kernels from each rice variety were measured for their length and breadth traits using a digital vernier caliper. The measurements were repeated 10 times in each and thus an average of 10 replicates was recorded. The grain weight of 100 randomly counted rice kernels from each variety was determined using a weighing balance (METTLER TOLEDO) and an average recorded. The grain and kernel length/breadth ratio (measure of slenderness) for each variety was obtained by dividing length/breadth.

Data Analysis

The phenotypic data was analysed using Analysis of Variance (ANOVA) followed by Tukey’s post hoc statistical tools as implemented in Minitab 15 software package (State College, Pennsylvania). A dendrogram was obtained from the mean values of the seven traits across all the test varieties with the help of Minitab 15 software package. Principal Component Analysis (PCA) was carried out to investigate the overall pattern of phenotypic diversity and the individual trait contributions to observed phenotypic diversity [6].

Results

Seven grain and kernel trait measurements were found to vary across the 13 studied rice varieties as shown in Table 2 of all the traits, the highest variation was observed in grain weight where most of the rice varieties significantly differed (P<0.05). Supa rice variety showed the highest grain weight followed by IR 2793 and IR 54 whereas BS 370, ITA 310 and BS 217 showed the lowest grain weight mean values. It was observed that short and bold grains were heavier compared to long and slender grains. High grain length coupled with grain breadth was associated with high weight values for Supa and most of improved rice varieties. However, Kahogo, Soro 5, Kilombero and Red Afaa had no significant variation in grain weight (P>0.05; Table 2). Moderate variation was observed in kernel length where dimensions ranged from 6.052 mm to 7.586 mm. Based on this trait, Supa and Wahiwahi which showed the highest kernel length mean values were significantly different from the rest of the test varieties (P<0.05; Table 2). The lowest kernel length mean values were identified in ITA 310 and Red Afaa and the two varieties significantly differed from other varieties (P<0.05; Table 2). All the varieties that had high and low values for grain length also showed high and low values for kernel length (Table 2).

Low variation was observed in grain and kernel breadth traits where grain breadth dimensions across the rice varieties ranged from 1.846 to 2.055 mm. The highest grain breadth mean values were observed in Supa, followed closely by Red Afaa and IR 54 and they significantly differed from the rest of the varieties (P<0.05); Table 2). The lowest grain breadth mean values were observed in BS 370, BS 217 and ITA 310 respectively and based on this trait, they were significantly different from other test varieties (P<0.05; Table 2).

On the other hand, kernel breadth dimensions ranged from 1.64 mm to 1.87 mm where the highest mean values were observed in Red Afaa, Supa and Kilombero. The three rice varieties had almost similar kernel breadth dimensions but differed significantly when compared to the rest of the varieties in this study (P<0.05; Table 2). The lowest kernel breadth mean values for were identified in BS 217, BS 370 and ITA 310. These results indicated that there was an association between grain and kernel breadth traits since similar varieties showed consistency in high and low kernel breadth values.

Grain length measurements ranged from 8.999 mm to 10.666 mm. Wahiwahi had the longest grain size followed by Supa and Kilombero. Unlike other traits, the three rice varieties that had the longest grain sizes were significantly different from each other (P<0.05; Table 2). On the other hand, ITA 310 and IR 64 had the shortest grain sizes and were significantly different from other rice varieties. It was observed that aromatic landraces had the longest grains among the test varieties and shared a common source, Tanzania. On the other hand, non-aromatic improved varieties were found to have the shortest grains and shared a common origin, as shown by the IR codes which indicates are improved varieties from Philippines.

Grain length/breadth ratio was calculated and the highest mean values were observed in Wahiwahi, BS 217 and Soro 5 varieties. The lowest values were observed in Red Afaa, IR 54 and IR 2793. Combination of the two traits depicted IR 54 and IR 2793 as short and bold grains. Kernel length/breadth ratio which is the measure of slenderness mean values ranged from 3.45 mm to 4.34 mm. The highest mean values for this trait were observed in BS 217, BS 370 and Wahiwahi where BS 217, an improved aromatic variety from Kenya, was the most slender kernel and significantly differed from the rest of the rice varieties (P<0.05; Table 2). On the other hand, Red Afaa, IR 64 and IR 2793 had the lowest mean values for kernel length/breadth ratio.

Red Afaa, IR 64 and IR 2793 with KL/B ratio of less than 3.80 was categorized as short grain varieties whereas Kahogo, IR 54, ITA 310, and BW 196 rice varieties having KL/B ratio of less than 4.0 were considered as medium grain varieties. BS 217, BS 370, Kilombero, Soro 5, Wahiwahi and Supa showed a KL/B ratio greater than 4.0 and were categorized as long grain varieties. From these results, it was inferred that basmati varieties had long and slender rice kernels followed by Supa, Wahiwahi, Kilombero and Soro 5 which had medium grain sizes. Red Afaa, IR 64 and IR 2793 varieties had short and bold kernels.

High variability was revealed by analysis of variance of the seven traits across all the varieties are shown in Table 2. The ANOVA table

| Sr.no | Genotype | Source        | Attribute       |
|-------|----------|---------------|-----------------|
| 1     | IR 2793  | Kenya         | Improved variety|
| 2     | BS 217   | Kenya         | Improved variety|
| 3     | BS 370   | Kenya         | Improved variety|
| 4     | BW 196   | Kenya         | Improved variety|
| 5     | ITA 310  | Kenya         | Improved variety|
| 6     | Red Afaa | Tanzania      | Landrace        |
| 7     | IR 54    | Tanzania      | Improved variety|
| 8     | Kilombero| Tanzania      | Landrace        |
| 9     | IR 64    | Tanzania      | Improved variety|
| 10    | Kahogo   | Tanzania      | Landrace        |
| 11    | Soro 5   | Tanzania      | Improved variety|
| 12    | Wahiwahi | Tanzania      | Landrace        |
| 13    | Supa     | Tanzania      | Landrace        |

Table 1: Profiles of rice varieties used in the study.
 Principal component analysis

The principal component analysis (PCA) was carried out to investigate the morphological traits that played a key role in phenotypic diversity among the rice varieties. It provided the Eigen values and percent of variation for 7 principal component axes in 13 rice varieties. Table 3. It was found that the first three principal components jointly accounted for 99.5% of the total variation among all the studied varieties. Combination of the first and the second principal components accounted for 95.2% of the total variation among the rice varieties.

Principal component 1 (PC1) had 84.6% of the total variation where all the traits; grain length, grain breadth, grain length/breadth ratio, kernel length, kernel length/breadth ratio and grain weight contributed positively. Of all, three traits; grain length, kernel length and grain width had a notably major contribution to PC1. In the case of Principal Component 2 (PC2), three traits; grain length/breadth ratio, kernel length/breadth ratio and kernel length contributed positively and accounted for 10.6% of the total morphological variability.

On the other hand, grain length, grain breadth, kernel breadth and grain weight traits were negatively associated with PC2. Figure 1 shows a plot for the first two vectors of PCA. A total of 4.30% variation was could be explained by the third principle component and was greatly negatively characterized by grain breadth, kernel length, kernel breadth, kernel length/breadth ratio and grain weight. However, it was found that two traits; grain length and grain length/breadth ratio contributed positively to Principal Component 3 (PC3).

The first two principal components efficiently separated most of the improved varieties from landraces with varieties possessing long grains clustering close together as shown in the scatter plot, Figure 1. Basmati improved varieties from landraces with varieties possessing long grains clustering close together as shown in the scatter plot, Figure 1. Basmati ± 0.06 2.05 ± 0.11 4.989 ± 0.29 7.586 ± 0.54 1.849 ± 0.11 4.108 ± 0.25 32.7 ± 0.03 0.73 -0.456 0.408 -0.235 0.093 0.153 0.029 -0.281 -0.113 -0.247 0.048 -0.517 -0.76 0.294 0.099 0.489 0.568 -0.143 -0.177 -0.3 0.562 0.455 0.742 0.271 0.029 0.188 -0.12 0.027 -0.261 -0.15 0.356 -0.03 -0.729 0.499 0.25 0.645 -0.064 -0.591 -0.016 -0.352 0.211 0.323 -0.112 -0.064 -0.105 -0.985 0.04 -0

Table 3: Eigen values and percent of variation for 7 principal component axes in 13 rice varieties.

## Table 2: Analysis Of Variance (ANOVA) of seven grain and kernel traits of the 13 studied rice genotypes.

| TRAITS | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 | PC7 |
|--------|-----|-----|-----|-----|-----|-----|-----|
| [TRAITS] | [84.6] | [95.2] | [99.9] | [100] | [100] | [100] |
| [Eigen values] | [0.519] | [0.0647] | [0.0265] | [0.0026] | [0.00025] | [2E-05] | [0] |
| [Proportion of variance] | [84.6] | [10.6] | [4.3] | [0.4] | [0] | [0] | [0] |
| [Cumulative % variance] | [84.6] | [95.2] | [99.5] | [99.9] | [100] | [100] | [100] |

| TRAITS | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 | PC7 |
|--------|-----|-----|-----|-----|-----|-----|-----|
| [Eigen values] | [0.73] | [-0.456] | [0.408] | [-0.235] | [0.093] | [0.153] | [-0.76] |
| [GL] | [0.029] | [-0.281] | [-0.113] | [-0.247] | [0.048] | [-0.517] | [-0.76] |
| [GB] | [0.294] | [0.099] | [0.489] | [0.568] | [-0.143] | [-0.177] | [-0.3] |
| [GL/B] | [0.562] | [0.455] | [-0.742] | [0.271] | [0.029] | [0.188] | [-0.12] |
| [KL] | [0.027] | [-0.261] | [-0.15] | [0.356] | [-0.03] | [-0.729] | [0.499] |
| [KB] | [0.25] | [0.645] | [-0.064] | [-0.591] | [-0.016] | [-0.352] | [0.211] |
| [KLB] | [0.323] | [-0.112] | [-0.064] | [-0.105] | [-0.985] | [0.04] | [-0] |
| [GW] | [0.73] | [-0.456] | [0.408] | [-0.235] | [0.093] | [0.153] | [-0.76] |
| [GL] | [0.029] | [-0.281] | [-0.113] | [-0.247] | [0.048] | [-0.517] | [-0.76] |
| [GB] | [0.294] | [0.099] | [0.489] | [0.568] | [-0.143] | [-0.177] | [-0.3] |
| [GL/B] | [0.562] | [0.455] | [-0.742] | [0.271] | [0.029] | [0.188] | [-0.12] |
| [KL] | [0.027] | [-0.261] | [-0.15] | [0.356] | [-0.03] | [-0.729] | [0.499] |
| [KB] | [0.25] | [0.645] | [-0.064] | [-0.591] | [-0.016] | [-0.352] | [0.211] |
| [KLB] | [0.323] | [-0.112] | [-0.064] | [-0.105] | [-0.985] | [0.04] | [-0] |

Table 3: Eigen values and percent of variation for 7 principal component axes in 13 rice varieties.
Among these two groups, *Supa*, an improved aromatic variety, clustered close together with *Kilombero*, a semi aromatic variety in group i. In group ii, Saro 5, an improved aromatic variety clustered together with two other varieties from the same origin. In sub cluster CIC, improved aromatic Basmati genotypes clustered together in with a similarity coefficient of 0.43.

Cluster II contained four improved rice varieties and only one landrace from both source countries where IR 310 showed parentage to the rest of the varieties on the pedigree. Two improved varieties from Kenya in this cluster, IR2793 and BW 196 were the most similar with a similarity coefficient of 0.21. The relationship among the 13 rice varieties was revealed by the dendrogram as shown in (Figure 2).

**Discussion**

Analysis of variance of the grain and kernel traits measurements indicated a wide spectrum of variation among all the rice varieties for all the characters. The means of the characters measured varied significantly across all the varieties and this demonstrated that based on these traits, the varieties were distinct from each other. The highest variability was observed in grain weight ranging from 18.2 g to 32.7 g. The maximum grain weight was recorded in *Supa* with 32.7 g followed by IR 2793 with 28.9 g and then IR 54 with 28.5 g. The lowest grain weight was observed among basmati varieties, BS 370 and BS 217 with 18.2 g and 23.5 g, respectively. These results concur with the report of who reported immense variation in grain weight among Bangladesh rice varieties. Low grain weight among BS varieties can be explained by the fact that these varieties possess longer and slender grains. This study observation relates to the report of [6] who found that most non aromatic landrace varieties had large and heavier grains. On the other hand, majority of aromatic varieties had small and lighter grains using a set of Indian aromatic and non-aromatic rice varieties.

On the other hand, varieties showed moderate variation in kernel length ranging from 6.052 mm for *ITA 310* to 7.586 mm for *Supa* with landrace varieties having the highest grain and kernel length. The same varieties also showed very high values for grain and kernel breadth ranging from 1.804 mm for *Kilombero* to 1.849 mm for *Supa*. Maximum grain length coupled with grain breadth gave these rice varieties highest grain weight above the other varieties in this study. These results agree with the findings of [7] who reported moderate variability for kernel length among Indian rice varieties. However, these results contrast with the report of who did not find any association between grain/kernel length and grain/kernel breadth.

Low variation was observed in grain and kernel breadth. The results clearly indicated that BS 217 and BS 370, both of which are aromatic varieties had a small grain breadth that contributes to [8] slender appearance. *Red Afia*, IR 54 and IR 2793 had the lowest values of grain length/breadth ratio. Combination of these two traits depicted IR 54 and IR 2793 as short and bold grains. *Supa*, an improved aromatic variety and *Kilombero*, an aromatic landrace showed the highest grain length and breadth mean values and this corresponded well with their long and bold rice grains. High grain lengths coupled with high grain breadths contributed round appearance of these varieties. Generally, most non-aromatic varieties had low variability in GB and KB traits hence appeared as short and bold. These results agree with earlier findings of who reported the existence of long bold aromatic varieties [6].

In the case of grain and kernel length/breadth ratio (a measure of slenderness) the highest mean values were observed in BS 217 with 4.336 followed by BS 370 with 4.177 and *Kilombero* with 4.169. The rice varieties with a KL/B ratio of >4.0 were categorized as slender and long grains [9]. Most slender rice varieties are desirable and preferred by consumers [10]. It was observed that most of aromatic landraces and improved varieties belonged to this category with KL/B ratios ranging from 4.075 for *Saro 5* to 4.336 BS 217. Generally, Basmati varieties showed the highest kernel L/B values. Similar observations were made by who reported Basmati varieties as being slender and light using Indian rice varieties. This type of rice is long and slender in shape and possess most desirable grain and cooking quality traits. *Wahiwahi* and *Kilombero* were the most slender landrace varieties with a KL/B ratio of 4.180 and 4.139 respectively. On the other hand, non-aromatic varieties had the lowest KB/L values of <4.0-hence had bold appearance.

The varieties with high grain and kernel length/breadth ratios may be utilized as sources of these traits in breeding for long grain varieties. Immense variation in grain and kernel L/B ratios has also been reported by [11]. Grain shape and size are important traits that determine the market value of rice. These traits are highly considered by breeders in developing new varieties for commercial release [12,13].

Principal Component Analysis (PCA) provided an insight of the contribution of each of the trait towards divergence among the
Characteristics of the rice varieties. Principal component analysis grouped the rice varieties into three clusters indicating the presence of considerable phenotypic diversity among the varieties. The first two principal components were utilized for principal component analysis because they expressed better total variability (95.2%) of the plant material. The most predominant traits that contributed to the observed phenotypic diversity were: grain length, kernel length, grain weight, and kernel length/breadth ratio. This analysis validates their use as the main discriminating traits among the test varieties. The other traits were found to have a minimal contribution to variability. These results are consistent with the report of [10] who found major contributions of grain length, kernel length, grain weight and grain length/breadth ratio to phenotypic diversity in a set of Indian rice. In contrast [7] reported that grain breadth, grain length and grain weight were the major contributors of phenotypic diversity. This discrepancy could be due to use of different set of rice varieties. The major contribution of grain length, kernel length, grain weight, and kernel length/breadth ratio could be perhaps due to the fact that they are the most important agronomic traits selected to variation by farmers and breeders over time [13,15].

Cluster analysis provided a good opportunity to identify and group the rice varieties into distinct categories with respect to similarity levels based on the phenotypic traits. The dendrogram showed two major clusters, I and II. Cluster I contained eight rice varieties from both source countries. All the aromatic rice varieties, both improved and landraces were grouped in cluster I. This indicated the extent in which most aromatic varieties share phenotypic grain quality traits. On the other hand, cluster II contained five rice varieties from both source countries where all were non aromatic varieties, representing both improved and landrace varieties. Cluster analysis indicated that there was no association between the observed pattern of variation and the geographical origin of the rice varieties. Similar observations were made by [6] where cluster analysis effectively distinguished the majority of aromatic from non-aromatic landraces. However, these results disagree with the findings of who [11] reported an association between the pattern of phenotypic variation with geographical origin of the varieties.

Cluster analysis grouped the basmati varieties, BS 217 and BS 370 together and close to other aromatic varieties from different sources and this presented aroma as a grain quality trait that distinguished rice varieties into different categories. This also suggests a possibility that the widely preferred basmati type of rice may have evolved through natural mutation from non-basmati genotypes. These results are consistent with the findings of who observed clustering together of the basmati group based on morphological traits using aromatic and non-aromatic rice varieties from India [6,14,15].

Only one improved aromatic variety, Saro 5, clustered with an improved non-aromatic variety IR 54, and Kahogo, a landrace semi aromatic variety, which is rational since they are collections from Kilimanjaro Agricultural Research Training College and possibly have common ancestors. These varieties had similar phenotypic traits such as grain length, kernel breadth and grain weight but differed in grain breadth, grain length/breadth ratio and kernel length/breadth ratio. These differences contribute to the slender nature of Saro 5 and distinguish it from other varieties with almost similar size. Other long grain non-aromatic varieties fell in one sub-cluster corresponding well with their grain characters. These varieties, although have varying cooking and eating qualities, lack the desirable basmati traits. Generally, cluster analysis gave an insight into the diversity of the rice varieties as shown in the dendrogram in Figure 2. Variety groups were primarily associated with phenotypic differences among them and with variety type. These results agree with earlier report of [16], who also found that in cluster analysis, varieties grouped together with greater phenotypic similarity but the groups did not essentially include varieties from the same origin [17,18].

Conclusion

From this study, it can be concluded that phenotypic analysis of the 13 studied rice varieties revealed an enormous diversity across all the varieties for all the traits evaluated. The most distinct phenotypic traits among the studied Kenyan and Tanzanian rice varieties were GI, KL, GW and KL/B ratio. This analysis recommends their use as the main discriminating traits among the test varieties and this can be validated by follow up molecular analysis. Based on these traits, at least three landrace rice varieties; Kilombero, Wahiwahi and Kahogo were found to possess good grain quality traits. These promising landraces should be conserved as reservoir of beneficial gene pool for improvement of grain quality traits in rice varieties. From this study it is recommended that agro- morphological traits can be employed as a common approach for assessing genetic and phenotypic variability among varieties. Hybridization of different distant related rice varieties from different clusters should be carried out to obtain segregants with high degree of hybrid vigor for the traits studied.

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