Genetic Variations in Field Condition Clonally Replicated Sugarcane (*Saccharum officinarum* L.) Cultivars on the Basis of Morphological and Quality Traits

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Abstract This study is an initiative approach to assess the genetic variation on basis of enormous morphological and qualitative attributes for future breeding selection. A set of 16 sugarcane clones were evaluated in the fields of Sugar Crops Research Institute (SCRI), Mardan- Pakistan during the spring cropping season of 2011-12. The clones were analyzed for 40 morphological (qualitative and quantitative) traits in a randomized complete block (RCB), design with four replications. Variations were measured for 16 morphological quantitative traits (1st germination, 2nd germination, 1st tillering, 2nd tillering, 1st plant height, 2nd plant height, leaf length, leaf width, leaf area, number of nodes per cane, internode length, weight of five unstripped canes, weight of five stripped canes, yield, weight of trash and tops and number of millable canes), 20 morphological qualitative traits (cane height, cane color, hardness, thickness, leaf colour, attitude, leaf shape, legule size, dewlap color, pith, bud shape, lodging, streaks, wax, tillering, pubescence, growth, maturity, tops and trash) and four qualitative laboratory traits (corrected brix percentage, purity percentage and recovery percentage). The data were analyzed using descriptive statistics (means, standard deviations, standard errors, variances and ranges) which showed considerable diversity among the studied sugarcane clones. The analysis of variance (ANOVA) exhibited highly significant difference (p<0.01) for 1st and 2nd germination, 1st and 2nd tillering, 1st and 2nd plant height, yield, number of millable canes, number of nodes per plant, internode length, weight of five unstripped canes, weight of five stripped canes. Moderately, significant differences (0.01< p <0.05) were recorded for leaf length, leaf area, as well as sugar purity and recovery. Cluster analyses were performed on the 40 different traits which divided the 16 sugarcane clones in to two main clusters (cluster I and cluster II) and two sub-clusters (IIA and IIB) which might reflect the genetic variations. Principal component analysis, reduced morphological variables to five independent linear combinations. The principal components of variables had Eigen values >1 and accounted for 84.04% of the total variance in the data. Analysis of the principal components inferred that 1st and 2nd tillering, 1st and 2nd plant height, weight of five unstripped canes, leaf area, millable canes, 1st and 2nd germination were the major parameters of variation. High degrees of variations were observed for most of the traits. Our analysis has described that the promising performance for all the traits and spatially for yield and sugar has shown by the genotype MS-99-HO-317 followed by the genotype MS-91-CP-238. It is concluded from this research that these two genotypes will be released for farmers community in the next year after approval from the VAC (Variety Approval Committee). Moreover, the breeders can use these genotypes in their future breeding program to achieve some better performing lines.

Keywords Genetic variations; Morphological; Sugarcane; Quantitative and qualitative traits

Introduction Sugarcane (*Saccharum officinarum* L.) belongs to family Gramineae and is a complex hybrid of five different species of the genus *Saccharum*. There are two main groups within the genus; the thin, hardy types like the north Indian *S. barberi* (2n = 81 to 124) and the Chinese *S. sinense* (2n = 111 to 120) and the thick, juicy noble canes like *S. officinarum* (2n = 80). *S. robustum* (2n = 60 to 80) is the closest wild relative and putative ancestor of *S.*
officinarum. It is believed that the origin of S. officinarum is the Indo-Myanmar -Chinese border. However, New Guinea is the main centre of diversity today. Flowering of S. officinarum is hard to predict and is rare in Pakistan therefore, most sugarcane varieties are maintained as vegetative clones. The maintenance of the purity, homogeneity and identity of clones is usually achieved by morphological marker analyses.

Morphological trait scores have been widely used as genetic markers in breeding and plant germplasm management. Although morphological markers are limited in number, their assays neither require sophisticated equipment nor complicated procedures (Singh and Singh, 1992). Unlike physiological, anatomical and molecular characterization, morphological characterizations are generally simple, rapid and inexpensive to score (Ghafoor, 1999). Until recently scientific classification of plants was based entirely on morphological traits Stuessy, 1999). Morphological characterization of sugarcane cultivars, landraces and wild species is fundamental to breeders, researchers and growers for practical and scientific purposes and for the development of new varieties. Sugarcane characterization is often based on morphological traits and growth characters like growth habit (stool weights), tops weight, trash amount, drought index, leaf blade and sheath shape, auricle, dewlap, ligule, internode, node and bud number. Morphological characterizations are used for the protection of new varieties of sugarcane and the associated intellectual property rights (UPOV, 1998). Growers undertake morphological characterization in order to maintain the purity and uniformity of their cultivated clonal varieties. Morphological characterization can also aid breeders and researchers in many ways including, identifying varieties, cultivars or related species, building phylogenetic relationships among related lines and developing selection criteria as parameters associated with desired traits of economic importance. The main traits desired are higher yields, pest and disease resistances. The identification of phenotypic traits for new genetic variants are especially important (Moore, 1987). Traditional tools for sugarcane breeders to identify different varieties depend on anatomical and these morphological characters (Skinner, 1972). At various international centers, the breeder used morphological descriptors such as stalk wax, leaf sheath wax, leaf sheath margin, leaf sheath hair (pubescence), dewlap appearance, stalk color, auricle size and color, and other distinguishing characteristics in the evaluation and selection of the best clones. However, breeders from other locations or researchers in other disciplines may not be familiar with scoring and evaluating these morphological traits. The problem of reproducibility is especially keen for traits for which differential expression has already been known to be strongly influenced by the environment (Pan, 2010).

Information about genetic variation based on morphological trait is a prerequisite for any breeding program. Shuffling and selecting new genetic diversity enables breeders to develop cultivars which can tolerate changing environments, new diseases, pests and climatic conditions. Studies of genetic divergence using different multivariate techniques such as cluster, canonical and principal component analyses have been used for the last two decades in several crops (Singh, 1981). The technique most often used is cluster analysis with group sampling units like varieties, lines and clones. The aim is, to obtain groups with a high degree of within- group homogeneity that consequently are heterogeneous in relation to one another (Johnson and Wichern, 1982; Viana et al., 1991).

The present study was conducted to evaluate 16 sugarcane genotypes for various morphological parameters in Pakistan using cluster, and principal component analysis.

1 Results and Discussions
Basic statistics for the quantitative traits are presented in Table 2 which showed maximum values of variance for 1st and 2nd germination, 1st and 2nd tillering, 1st and 2nd plant height, leaf length, leaf area and millable cane. Therefore, there is scope for further improvement in these traits. For number of nodes, internode length, leaf width, weight of five unstrapped canes, weight of five strapped canes, weight of trash and tops, yield (tons per ha), C. brix, pol percentage, purity percentage and recovery also showed genetic variations, however, these were statistically non significant (p ≥ 0.05).

1.1 Morphological attributes analysis
1.1.1 Qualitative traits
The distinct groups formed morphological traits are presented in Table 1. Maximum variations were observed in
Table 1 Frequency distribution of qualitative traits of 16 sugarcane clones

| Traits     | Frequency | % Frequency | Traits     | Frequency | % Frequency |
|------------|-----------|-------------|------------|-----------|-------------|
| Cane Height|           |             | Pith       |           |             |
| Tall       | 3         | 18.75       | Absent     | 8         | 50          |
| Medium     | 8         | 50          | Moderate   | 4         | 25          |
| Dwarf      | 5         | 31.25       | Pithy      | 4         | 25          |
| Cane Color |           |             | Bud Shape  |           |             |
| White      | 3         | 18.75       | Rounded    | 3         | 18.75       |
| Yellow     | 3         | 18.75       | Oval       | 4         | 25          |
| Green      | 8         | 50          | Pointed    | 9         | 56.25       |
| Purple     | 2         | 12.5        | Lodging    |           |             |
| Hardness   |           |             | Low        | 7         | 43.75       |
| Soft       | 1         | 6.25        | Medium     | 9         | 56.25       |
| Medium     | 8         | 50          | Streaks    |           |             |
| Hard       | 7         | 43.75       | Nil        | 13        | 81.25       |
| Thickness  |           |             | Few        | 3         | 18.75       |
| Thick      | 3         | 18.75       | Wax        |           |             |
| Medium     | 5         | 31.25       | Medium     | 9         | 56.25       |
| Thin       | 8         | 50          | Strong     | 7         | 43.75       |
| Leaf Color |           |             | Tillering  |           |             |
| Light green| 6         | 37.5        | Poor       | 4         | 25          |
| Green      | 4         | 25          | Moderate   | 8         | 50          |
| Dark green | 6         | 37.5        | Good       | 4         | 25          |
| Attitude   |           |             | Pubescence |           |             |
| Erect      | 3         | 18.75       | Sparse     | 11        | 68.75       |
| Semi-erect | 7         | 43.75       | Medium     | 5         | 31.25       |
| Horizontal | 4         | 25          | Growth     |           |             |
| Droopy     | 2         | 12.5        | Upright    | 4         | 25          |
| Leaf Shape |           |             | Intermediate| 5       | 31.25       |
| Broad      | 3         | 18.75       | Bent       | 7         | 43.75       |
| Medium     | 11        | 68.75       | Maturity   |           |             |
| Narrow     | 2         | 12.5        | Early      | 6         | 37.5        |
| Ligule Size|           |             | Medium     | 5         | 31.25       |
| Small      | 9         | 56.25       | Late       | 5         | 31.25       |
| Medium     | 6         | 37.5        | Tops       |           |             |
| Large      | 1         | 6.25        | Light      | 5         | 31.25       |
| Dewlap Color|          |             | Moderate   | 8         | 50          |
| White      | 4         | 25          | Heavy      | 3         | 18.75       |
| Yellow     | 1         | 6.25        | Trash      |           |             |
| Light Green| 1         | 6.25        | Self       | 8         | 50          |
| Green      | 1         | 6.25        | Moderate   | 8         | 50          |
| Light red  | 9         | 56.25       | Clinging   | 0         | 0           |

plant height, cane color, hardiness, thickness, attitude, legule size, dewlap color, pith, bud shape, tillering, growth, maturity and tops. For these characters, selection for various genetic markers may be utilized by breeders in future breeding programs. However, for the other seven traits where low variability was observed, the clones with rare traits might be acquired by breeding program or to have more samples collected from the centers of genetic diversity.

1.1.2 Quantitative traits
The estimation of range, mean, standard deviation, variance and standard error are shown in Table 2. High degree of variations was observed for most of the traits. A wide range of variations were observed for 1st and 2nd
germination, 1\textsuperscript{st} and 2\textsuperscript{nd} tillering, 1\textsuperscript{st} and 2\textsuperscript{nd} plant height, leaf length and leaf area.

1.2 Analysis of the variance (ANOVA)

The ANOVA results (Table 3 and 4) showed highly significant differences (p<0.01) for 1\textsuperscript{st} germination and 2\textsuperscript{nd} germination among the clones. Maximum buds germination (1\textsuperscript{st} and 2\textsuperscript{nd} germination) observed for genotype Mardan-93 i.e., 89 and 99, respectively where as minimum 1\textsuperscript{st} germination and 2\textsuperscript{nd} germination (41 and 48) was exhibited by genotype S-96-SP-1215. Number of tillers is playing a vital role in increasing the final yield of sugarcane. Highly significant differences (p<0.01) were observed for 1\textsuperscript{st} tillering and 2\textsuperscript{nd} tillering. Maximum number of 1\textsuperscript{st} tillering and 2\textsuperscript{nd} tillering (250.75 and 378) were exhibited by the genotype MS-91-CP-238, whereas minimum numbers of 1\textsuperscript{st} tillering and 2\textsuperscript{nd} tillering (60.5 and 128.25) were recorded for genotypes S-96-SP-1215 and Hoth127, respectively. Differences in the performances of the clones reflect the genetic variations. 1\textsuperscript{st} and 2\textsuperscript{nd} plant height data showed highly significant differences (p<0.01), the highest 1\textsuperscript{st} and 2\textsuperscript{nd} plant height (172.4 cm and 252.5 cm) being exhibited by the genotype MS-99-HO-391 whereas the lowest (102.35 and 171.55) were recorded for the clone Mardan-93. All the genotypes displayed highly significant variations (p<0.01) for cane yield. Cane yield for all genotypes ranged between 62.39 and 121.19 tons per ha. Maximum cane yield (113.38 tons per ha) was recorded for the genotype MS-99-HO-317 whereas minimum yield (67.51 tons per ha) was obtained from the genotype Mardan-93. Highly significant differences (p<0.01) were exhibited by the clones for Millable cane which ranged between 80.5 and 129.25. Highest Millable canes (129.25) were exhibited by the genotype MS-99-HO-391 whereas lowest Millable canes (80.5) were shown by the genotype Mardan-93. Analysis of variance displayed highly significant differences (p<0.01) for number of nodes per plant. Maximum number of nodes per plant (29) were recorded for the genotype Hoth-127 whereas minimum (18) was displayed by the genotype MS-99-HO-675. This may due to diverse nature of clones used in the present studies. Highly significant differences (p<0.01) were observed among the genotypes for internode length. Highest internode length (20.8) was showed by the genotype MS-94-CP-15 whereas lowest internode length (13.6) was exhibited by the genotype Hoth-127. Highly significant differences (p<0.01) were exhibited by all genotypes for weight of five unstrapped

Table 2 Basic Statistics for different quantitative traits in 16 sugarcane clones

| Traits                                | Mean  | Std. Error | Std. Deviation | Variance | Range | Minimum | Maximum |
|---------------------------------------|-------|------------|----------------|----------|-------|---------|---------|
| 1st germination                       | 67.28 | 3.69       | 14.75          | 217.58   | 48.00 | 41.25   | 89.25   |
| 2nd germination                       | 80.73 | 3.79       | 15.17          | 230.02   | 51.00 | 48.25   | 99.25   |
| 1st tillerings                        | 142.08| 11.77      | 47.09          | 2217.61  | 190.25| 60.50   | 250.75  |
| 2nd tillerings                        | 225.14| 14.39      | 57.57          | 3313.74  | 249.75| 128.25  | 378.00  |
| 1st Plant height                      | 128.71| 3.99       | 15.96          | 254.83   | 70.05 | 102.35  | 172.40  |
| 2nd Plant height                      | 204.25| 4.81       | 19.25          | 370.42   | 80.95 | 171.55  | 252.50  |
| Number of nodes per plant             | 21.46 | 0.79       | 3.16           | 9.98     | 11.07 | 17.93   | 29.00   |
| Inter node length                     | 17.15 | 0.57       | 2.28           | 5.19     | 7.20  | 13.57   | 20.77   |
| Leaf length(cm)                       | 155.60| 7.74       | 30.98          | 998.63   | 113.87| 114.53  | 228.40  |
| Leaf width(cm)                        | 4.55  | 0.15       | 0.61           | 0.37     | 2.31  | 3.83    | 6.15    |
| Leaf area(cm\(^2\))                   | 711.21| 47.22      | 188.86         | 35668.92 | 641.96| 492.61  | 1134.57 |
| weight of five unstrapped canes (kg)  | 8.17  | 0.36       | 1.43           | 2.05     | 4.83  | 6.00    | 10.83   |
| weight of five stripped canes (kg)    | 6.43  | 0.32       | 1.26           | 1.60     | 4.17  | 5.00    | 9.17    |
| Weight of trash and tops              | 1.74  | 0.10       | 0.41           | 0.17     | 1.50  | 1.00    | 2.50    |
| Milliable cane                        | 105.11| 3.18       | 12.71          | 161.63   | 48.75 | 80.50   | 129.25  |
| Yield (tons per ha)                   | 84.87 | 1.37       | 10.97          | 120.37   | 58.81 | 62.39   | 121.19  |
| C. Brix                               | 20.51 | 0.08       | 0.33           | 0.11     | 1.17  | 19.74   | 20.90   |
| Pol percentage                        | 16.95 | 0.15       | 0.59           | 0.34     | 1.61  | 16.07   | 17.68   |
| Purity percentage                     | 82.56 | 0.44       | 1.77           | 3.15     | 5.21  | 79.76   | 84.97   |
| Recovery                              | 10.62 | 0.13       | 0.51           | 0.27     | 1.36  | 9.89    | 11.25   |
canes. Highest weight of five unstrapped canes (10.8 kg) was shown by the genotype MS-99-HO-317 whereas lowest (6.0 kg) was displayed by the genotype MS-91-CP-272. Analysis of variance for weight of five strapped canes showed highly significant differences (p<0.01) among all the genotypes. Highest weight of five strapped canes (9.2 kg) was displayed by the genotype MS-99-HO-317 whereas lowest (5.0 kg) was represented by the genotype MS-91-CP-272. Significant differences (p<0.05) were observed among all the genotypes for leaf length. Highest leaf length (228.40 cm) was displayed by the genotype S-97CP-288 whereas lowest (114.53 cm) was shown by the genotype Hoth-127. Significant differences (p<0.05) among all the genotypes were observed for leaf area. The highest (1134.6 cm²) leaf area was displayed by the clone MS-99-HO-93 whereas the lowest (492.6 cm²) was exhibited by the clone MS-99-HO-93. The analysis of variance showed significant differences among all the genotypes for purity percentage. These results are in agreement with the finding of Tai and Miller, (2002). The highest range for this parameter recorded was 85%, exhibited by the clone MS-99-HO-93 whereas the lowest (79.8%) was shown by the clone MS-99-HO-388. Significant differences were recorded among all the clones for recovery as well. The highest recovery (11.3) was exhibited by the clone MS-99-HO-93 whereas the lowest (9.9) was observed for the clone MS-91-CP-238. Non-significant differences were observed among the traits i.e., weight of trash and tops, leaf width, pol percentage and C. Brix percentage.

Table 3 Mean squares for 1<sup>st</sup> germination (F. Ger), 2<sup>nd</sup> germination (S. Ger), 1<sup>st</sup> tillering (F. T), 2<sup>nd</sup> tillering (S. T), 1<sup>st</sup> plant height (F. Ph), 2<sup>nd</sup> plant height (S. Ph), Yield (tons per ha), Millcane (MC), Nodes plant<sup>-1</sup> (NPP) for 16 Sugarcane clones

| SOV | D. F | Ger | F. T | S. T | F. Ph | S. Ph | Yield | MC | NPP |
|-----|------|-----|------|------|------|------|-------|----|-----|
| Reps | 3 | 270.27<sup>**</sup> | 352.80<sup>**</sup> | 380.52<sup>**</sup> | 267.30<sup>**</sup> | 142.44<sup>**</sup> | 182.54<sup>**</sup> | 185.51<sup>**</sup> | 1626.76<sup>**</sup> | 2.54<sup>**</sup> |
| Genotypes | 15 | 870.33<sup>**</sup> | 920.08<sup>**</sup> | 8870.42<sup>**</sup> | 13254.97<sup>**</sup> | 1019.32<sup>**</sup> | 1481.69<sup>**</sup> | 345.13<sup>**</sup> | 646.53<sup>**</sup> | 38.73<sup>**</sup> |
| Error | 45 | 217.49 | 302.81 | 1161.21 | 425.81 | 199.98 | 368.86 | 1843.47 | 151.11 | 1.90 |
| C.V% | 21.92 | 21.55 | 9.17 | 9.17 | 10.99 | 9.40 | 7.55 | 11.70 | 6.41 |

Note: *Significant at 5% level of probability, ** Significant at 1% level of probability; Ns: Non significant, D.F: Degrees of freedom, C.V: Coefficient of variation

Table 4 Mean squares for Intermediate Length (IL), Weight of Five Unstrapped Canes (WFUSC), Weight of Five Strapped canes (WFSC) , Weight of Trash and Tops (WTAT), Leaf Length (LL), Leaf Width ( LW), Leaf Area (LA), Corrected Brix(C. Brix), Pol percentage, Purity percentage (Pur %) and Rec (Recovery) of 16 Sugarcane clones

| SOV | D. F | IL | WUSC | WSFC | WTAT | LL | LW | LA | C. Brix | Pol % | Pur % | Rec |
|-----|------|----|------|------|------|----|----|----|---------|-------|-------|-----|
| Reps | 3 | 2.325<sup>**</sup> | 1.50<sup>**</sup> | 0.90<sup>**</sup> | 2.92<sup>**</sup> | 2078.42<sup>**</sup> | 0.64<sup>**</sup> | 17965.44<sup>**</sup> | 0.969* | 5.152** | 49.13** | 4.156** |
| Genotypes | 15 | 20.75<sup>**</sup> | 8.19<sup>**</sup> | 6.39<sup>**</sup> | 0.68<sup>**</sup> | 3838.50<sup>**</sup> | 1.47<sup>**</sup> | 17965.44<sup>**</sup> | 0.427<sup>**</sup> | 1.381<sup>**</sup> | 12.59* | 1.059* |
| Error | 45 | 1.21 | 0.96 | 0.533 | 0.49 | 1900.19 | 0.85 | 140719.73 | 0.335 | 0.77 | 5.36 | 0.543 |
| C.V% | 6.41 | 11.97 | 11.36 | 40.26 | 28.01 | 20.22 | 35.88 | 2.82 | 5.16 | 2.80 | 6.94 |

Note: *Significant at 5% level of probability, ** Significant at 1% level of probability; Ns: Non significant, D.F: Degrees of freedom, C.V: Coefficient of variation

1.3 Cluster analysis

Cluster I consisted of three genotypes Hoth-127, S-97CP-288 and MS-99-HO-391 (Figure 1). Cluster II comprised 13 genotypes MS-91-CP-272, MS-94- CP-15, MS-91-CP-238, MS-92-CP-979, MS-99-HO-317, RS-97-N-45, MS-99-HO-388, MS-99-HO-675, MS-99-HO-93, S-96-SP-1215, CP-89-831, CP77/400 and Mardan-93. The cluster II is again divided in to two sub-clusters. Sub-cluster IIA comprised 7 clones MS-99-HO-675, RS-97-N-45, MS-99-HO-93, CP-89-831, S-96-SP-1215, CP77/400 and Mardan-93 clones whereas-sub cluster IIB consists of 6 clones MS-91-CP-272, MS-94- CP-15, MS-91-CP-238, MS-92-CP-979, -92-CP-979 and MS-99-HO-317. Mean values along with standard deviation of two clusters are presented in the Table 5. Cluster I consisted of genotypes which were similar in both quantitative and qualitative traits. Cluster II included genotypes showing intra-cluster diversity. Sub-cluster IIA included genotypes of sugarcane which varied from each other consistent with the diverse origin of these sugarcane clones. The genotype with lowest sugar recovery was present in sub-cluster IIB, whereas the highest recovery was found for the genotypes present in sub-cluster IIA. So genotypes in sub-cluster IIA can be used to develop cultivars with more sugar recovery. The highest yield (t ha<sup>-1</sup>) was found in sub-cluster IIB whereas the lowest was found sub-cluster IIA. The clones with higher tillering capability were found in the sub-cluster IIB whereas poor tillering features was observed in cluster IIA. Most genotypes with early maturity
were in sub-cluster IIB, followed by sub-cluster II. Later maturing clones were mostly in cluster IIA. These results indicated the scope for selection from various clusters for the desired traits could be done which may be used for further crop improvement program.

1.4 Principal Component Analysis

Only five of fifteen principal components had Eigen values greater than 1.0 (Table 6 and 7). The primary four principal components had Eigen value of 5.74, 4.38, 2.98 and 2.00, and jointly accounted for 75.51% of the total variation among the genotypes. The first five principal components together explained over 85% of the total variation among the 20 quantitative traits. The first principal component (PC1) accounted for 28.72% of the total variance and had high contributing factor loadings from 1st plant height (0.822), leaf length (0.739), 2nd plant height (0.727), leaf area (0.718), weight of five unstripped canes (0.700), weight of five stripped canes (0.627), milliable cane (0.617), nodes plant\(^{-1}\) (0.515) and weight of trash and trash (0.509). The second principal component (PC2) accounted for 21.92 % variation and indicated the importance of 1st tillering (0.853), 2nd tillering (0.838), 2nd germination (0.609) and 1st germination (0.519). The traits associated with the third principal component (PC3) were nodes plant\(^{-1}\) (0.685), 2nd germination (0.667), 1st germination (0.519), yield (tons ha\(^{-1}\)) (0.491) and milliable cane (0.481). PC 4, these accounted for 9.98 % of the total variance and showed positive loadings for 1st plant height (0.336) and leaf width (0.314). The principal component 5, which accounted for 8.56% of the total variability and described the traits leaf width (with a high loading of 0.706), followed by leaf area (0.574), 1st germination (0.478), internode length (0.411) and leaf length (0.319). (Figure 2-5)
Table 5 Inter cluster variation for different quantitative and qualitative characters in sugarcane

| Traits                        | Cluster I          | Cluster II         |
|-------------------------------|--------------------|--------------------|
| 1st Germination               | 56.83±9.87         | 69.69±3.82         |
| 2nd Germination               | 76.58±12.30        | 81.69±3.99         |
| 1st Tillering                 | 126.83±22.89       | 145.59±13.69       |
| 2nd Tillering                 | 155.75±13.83       | 241.15±14.04       |
| 1st Plant height              | 146.82±12.90       | 124.52±3.21        |
| 2nd Plant height              | 227.88±13.11       | 198.79±3.97        |
| Yield                         | 8.40±0.22          | 8.50±0.28          |
| Millable Cane                 | 118.17±5.55        | 102.09±3.21        |
| Number of node plant          | 25.71±2.15         | 20.47±0.59         |
| Internode length              | 15.28±1.13         | 17.57±0.60         |
| Weight of five stripped canes | 10.00±0.25         | 7.74±0.33          |
| Weight of five unstripped canes| 7.89±0.24         | 6.08±0.31          |
| Weight of trash and tops      | 2.11±0.20          | 1.65±0.10          |
| Leaf length                   | 187.89±36.74       | 148.14±3.95        |
| Leaf width                    | 4.76±0.24          | 4.49±0.17          |
| Leaf area                     | 909.32±207.16      | 665.49±29.16       |
| C. Brix percentage            | 20.46±207.16       | 20.51±0.16         |
| Pol percentage                | 16.82±0.32         | 16.97±0.16         |
| Purity percentage             | 82.20±0.78         | 82.64±0.52         |
| Recovery                      | 10.51±0.27         | 10.64±0.14         |
| Cane height                   | 1.33±0.33          | 2.30±0.17          |
| Cane color                    | 2.33±0.88          | 3.69±0.52          |
| Hardness                      | 3.00±0.00          | 2.23±0.16          |
| Thickness                     | 1.33±0.33          | 2.53±0.18          |
| Leaf color                    | 1.67±0.67          | 2.07±0.23          |
| Attitude                      | 3.67±0.33          | 2.1±0.19           |
| Leaf shape                    | 1.67±0.33          | 2.1±0.16           |
| Legule size                   | 1.33±0.33          | 1.53±0.18          |
| Dewlap color                  | 3.67±1.33          | 3.61±0.48          |
| Pith                          | 2.33±0.67          | 1.61±0.21          |
| Bud shape                     | 1.67±0.67          | 2.53±0.18          |
| Lodging                       | 2.00±0.00          | 1.46±0.14          |
| Streaks                       | 1.33±0.33          | 1.15±0.10          |
| Wax                           | 2.67±0.33          | 2.38±0.14          |
| Tillering                     | 1.33±0.33          | 2.15±0.19          |
| Pubescence                    | 1.67±0.33          | 1.23±0.12          |
| Growth                        | 3.00±0.00          | 2.1±0.22           |
| Maturity                      | 2.33±0.67          | 1.84±0.22          |
| Tops                          | 1.33±0.33          | 2.1±0.19           |
| Trash                         | 1.67±0.33          | 1.46±0.14          |

Table 6 Eigen values, % total and cumulative variance of the five most important characters from factor analysis

| Principle components | Eigen value | % Total variance | Cumulative |
|----------------------|-------------|------------------|------------|
| 1                    | 5.74        | 28.72            | 28.72      |
| 2                    | 4.38        | 21.92            | 50.64      |
| 3                    | 2.98        | 14.89            | 65.53      |
| 4                    | 2.00        | 9.98             | 75.51      |
| 5                    | 1.71        | 8.56             | 84.07      |
### Table 7: Eigen vectors (loadings) of the first five principal components

| Traits                  | PC1  | PC2  | PC3  | PC4  | PC5  |
|-------------------------|------|------|------|------|------|
| 1st Germination         | -0.132 | 0.600 | 0.519 | 0.225 | 0.478 |
| 2nd Germination         | 0.013 | 0.609 | 0.667 | 0.283 | 0.214 |
| 1st Tillering           | 0.132 | 0.853 | 0.356 | -0.097 | 0.149 |
| 2nd Tillering           | -0.264 | 0.838 | 0.023 | -0.333 | 0.226 |
| 1st Plant height        | 0.822 | -0.104 | 0.179 | 0.336 | 0.003 |
| 2nd Plant height        | 0.727 | -0.296 | 0.181 | 0.282 | -0.036 |
| Yield                   | 0.181 | 0.115 | 0.491 | -0.717 | 0.016 |
| Milliable Cane          | 0.617 | 0.205 | 0.481 | -0.412 | -0.057 |
| Nodes plant             | 0.515 | -0.193 | 0.685 | 0.081 | -0.346 |
| Internode length        | -0.148 | -0.097 | -0.524 | -0.462 | 0.411 |
| Weight of unstrapped 5 canes | 0.700 | -0.478 | 0.151 | -0.318 | 0.001 |
| Weight of stripped 5 canes | 0.627 | -0.437 | 0.117 | -0.486 | -0.015 |
| Weight of Trash and Tops | 0.509 | -0.322 | 0.164 | 0.384 | 0.050 |
| Leaf length             | 0.739 | -0.037 | -0.229 | -0.212 | 0.319 |
| Leaf width              | 0.253 | -0.284 | -0.119 | 0.314 | 0.706 |
| Leaf area               | 0.718 | -0.180 | -0.214 | 0.003 | 0.574 |
| C. Brix percentage      | -0.554 | -0.480 | 0.479 | 0.011 | 0.171 |
| Pol percentage          | -0.629 | -0.604 | 0.423 | -0.068 | 0.193 |
| Purity percentage       | -0.603 | -0.639 | 0.334 | -0.100 | 0.184 |
| Recovery                | -0.630 | -0.617 | 0.400 | -0.084 | 0.193 |

**Figure 2** Scattered diagram for 16 genotypes of sugarcane by quantitative traits

**Figure 3** Scattered diagram for 16 genotypes of sugarcane by quantitative traits
In our results we used different types of statistical tests. Each approach resulted in appreciable magnitude of genetic variations such as descriptive statistics for the quantitative traits showed maximum values of variations for 1st and 2nd germination, 1st and 2nd tillering, 1st and 2nd plant height, leaf length, leaf area and millable cane. Therefore, the scope for further improvement in these traits can never be ignored. For number of nodes per cane, internode length, leaf width, weight of five unstrapped canes, weight of five strapped canes, weight of trash and tops, yield (tons per ha), C. brix, pol percentage, purity percentage and recovery also showed appreciable magnitudes of genetic variations.

Maximum variations were observed in plant height, cane color, hardiness, thickness, attitude, legule size, dewlap
 Highly significant variations were reported among the genotypes for germination, tillering, plant height, cane yield, millable canes, number of nodes per cane, internode length, weight of five unstrapped canes, leaf length, leaf area, weight of five strapped canes, weight of trash and tops while significant difference were recorded for parameter e.g., leaf length, leaf area, purity percentage and recovery. Previous research also revealed similar pattern of results.

In the present finding for more precise results germination, tillering and plant height data were recorded twice. Tahir et al., 2014 also conducted such type of research. He conducted research on 25 sugarcane clones and observed considerable magnitude of genetic variations. Similarly Alam et al., (2011), recorded results in support of our research for germination, they reported significant differences among 10 crosses of sugarcane genotypes and observed variations in results for germination. The previous research work of Khan et al., (2007) revealed differences in tillering, plant height and leaf length, and leaf area which showed harmony to our results. Differences in cane yield among sugarcane clones was reported by Bahadar et al., (2002) which supported our findings. The highest millable canes were recorded for genotype MS-99-HO-391 whereas lowest Millable canes were shown by the genotype Mardan-93. Difference in millable canes were also detected by Anbanandan and Saravanan (2010). Similarities in performance in number of nodes per cane was displayed by the previous research work of Arain et al., (2011). This contradiction may be due to the high adoptability of the clones in the Thatta area which is considered more suitable for sugarcane cultivation and breeding. Kashif and Khan (2007) reported the results which are in agreement with our findings. In our experiment highest internode length (20.8) was shown by the genotype MS-94-CP-15 whereas lowest internode length (13.6) was exhibited by the genotype Hoth-127. Differences in performances of clones for weight of unstrapped five cane and weight of stripped five canes were recorded by the was observed by Suggo et al., (2010), who are in agreement to our findings for the same parameters. Tai and Miller (2002) and Bahadur et al., (2002) reported results which exhibited differences in performances of the clones for purity percentage and sugar recovery and supported our findings. Non-significant differences were observed among the traits i.e., weight of trash and tops, leaf width, pol percentage and C. Brix percentage. These results are in contrary with the findings of Guerra et al., (2009). These differences in results may be due to the multi-environmental trials which were conducted by the reported researchers. The cluster analysis classified the 16 sugarcane clones on the basis of 40 traits in to two main clusters (cluster I and cluster II) and two sub-clusters (IIA and IIB) which might reflect the genetic variations. Principal component analysis, reduced morphological variables to five independent linear combinations. The principal components of variables had Eigen values >1 and accounted for 84.04% of the total variance in the data. Analysis of the principal components inferred that 1st and 2nd tillering, 1st and 2nd plant height, weight of five unstrapped canes, leaf area, millable canes, 1st and 2nd germination were the major parameters of variation. High degrees of variations were observed for most of the traits. Tahir et al., (2013) performed cluster analysis and PCA in 25 clone and observed 3 main cluster. Furthermore, they reported two main principle components i.e., vigor and quality which explained 93.34% and 7.36% of variations, respectively.

2 Conclusion
It is concluded that high degree of variations were observed for most of the traits in the present studies. Our analysis has described that the promising performance for all the traits and spatially for yield and sugar yield has shown by the genotype MS-99-HO-317 followed by the genotype MS-91-CP-238. It is suggested that these two
genotypes will be released for farmers community in the next year after approval from the VAC (Variety Approval Committee). Moreover, the breeders can use these genotypes in their future breeding program to achieve some better performing lines. The rest of clones should be utilized in future breeding programs to draw some conclusive results.

3 Materials and Methods

3.1 Plant material and statistical analysis

To assess the genetic variations among sugarcane clones, a set of 16 genotypes were sown at the fields of Sugar Crops Research Institute (SCRI), Mardan- Pakistan during the spring cropping season of 2011-12. The experiment was laid out in a randomized complete block (RCB), design with four replications. The plot size was 67 m² with 10 m row length and 6.7 m row width and plant to plant distance was kept 90 cm. Three budded double sets were used as sowing materials. The list of sugarcane clones and their origin is given in Table 8. The Recommended dose of fertilizer was used (N, P, and K at 150, 100, and -100 Kg per ha from Urea, DAP and SOP). It was applied as 4.5 bags (50 kg) DAP per ha at planting time and 5 bags (50 kg) SOP plus 2.5 bags (50 kg) urea per ha in March-April. In addition urea was applied as 2.5 bags urea/ha at earthing up. Pesticides and other inputs were applied at specific times necessary to raise a good crop that season. Forty traits were brought under the current studies in which 20 were morphological qualitative, four were quantitative measures of cane juice quality and 16 were morphological quantitative traits. Data were recorded on five plants randomly selected within each plot. All the morphological qualitative data were recorded at maturity. The cane juice quality parameters were evaluated at Sugar Crops Analytical Laboratory (Mardan, Pakistan) from the fresh cane samples after harvesting the crop. The list of qualitative attributes and further types of each trait are given in Table 9. The quantitative attributes in plants were recorded as the mean of five plants and the data were taken from the central row only. The list of quantitative attributes is given in Table 10.

Table 8 List of 16 sugarcane genotypes and their source used for morphological characterization at SCRI, Mardan, during 2011-12

| S. No | Genotypes      | Source                                                                 |
|-------|----------------|------------------------------------------------------------------------|
| 1     | MS91CP272      | USDA-ARS stations at Canal Point, USA                                   |
| 2     | MS94CP15       | USDA-ARS stations at Canal Point, USA                                   |
| 3     | MS91CP238      | USDA-ARS stations at Canal Point, USA                                   |
| 4     | MS92CP979      | USDA-ARS stations at Canal Point, USA                                   |
| 5     | MS99HO391      | USDA-ARS stations, Houma, Louisiana, USA                               |
| 6     | S97CP288       | USDA-ARS stations at Canal Point, USA                                   |
| 7     | MS99HO317      | USDA-ARS stations, Houma, Louisiana, USA                               |
| 8     | RS97N45        | South African Research Institute, Natal (South Africa)                 |
| 9     | MS99HO388      | USDA-ARS stations, Houma, Louisiana, USA                               |
| 10    | MS99HO675      | USDA-ARS stations, Houma, Louisiana, USA                               |
| 11    | MS99HO93       | USDA-ARS stations, Houma, Louisiana, USA                               |
| 12    | S96SP1215      | São Paulo (Brazil)                                                     |
| 13    | Hoth127        | USDA-ARS stations, Houma, Louisiana, USA USA and Sugarcane Research Institute, Thatta |
| 14    | CP89831        | USDA-ARS stations at Canal Point, USA                                   |
| 15    | CP77400 (Check-I) | USDA-ARS stations at Canal Point, USA                                   |
| 16    | Mardan93 (Check-II) | USDA-ARS stations at Canal Point, USA                                  |

Note: MS: Mardan Selection, Hoth: Houma-Thatta, SP: São Paulo, HO: Houma, N: Natal; USDA-ARS: United States Department of Agriculture-Agriculture Research Service

3.2 Statistical analysis

The averaged data were analyzed for simple statistics including mean, standard deviation, variance, range, standard error and frequency distribution, using computer software MS EXCEL, Windows 7. ANOVA was performed by using computer software MSTATC package version 1.2 (Freed, 1990). Morphological, qualitative and quantitative data were subjected to dendrogram analysis to determine the genetic divergence among the genotypes by complete linkage cluster analysis using the SAHN (Sequential, Agglomerative, Hierarchical, And Nested) option of NTSYS-pc 2.2 version (Rohlf, 2005). Principal Component Analysis was carried out with the
Table 9 List of morphological qualitative traits of 16 sugarcane clones

| Morphological qualitative traits of 16 sugarcane clones |
|---------------------------------------------------------|
| **Cane Height**                                         |
| i) Tall; ii) Medium; iii) Dwarf                         |
| **Cane Color**                                          |
| i) White; ii) Yellow; iii) Light green; iv) Green; v) Light red; vi) Red; vii) Purple |
| **Hardness**                                            |
| i) Soft; ii) Medium; iii) Hard                          |
| **Thickness**                                           |
| i) Thick; ii) Medium; iii) Thin                         |
| **Leaf Color**                                          |
| i) Light green; ii) Green; iii) Dark green              |
| **Attitude**                                            |
| i) Erect; ii) Semi-erect; iii) Horizontal; iv) Drooping |
| **Leaf Shape**                                          |
| i) Broad; ii) Medium; iii) Narrow                      |
| **Ligule Size**                                         |
| i) Small; ii) Medium; iii) Large                       |
| **Dewlap Color**                                        |
| i) White; ii) Yellow; iii) Light Green; iv) Green; v) Light Red; vi) Red; vii) Purple |
| **Pith**                                                |
| i) Absent; ii) Moderate; iii) Pithy                    |
| **Bud Shape**                                           |
| i) Rounded; ii) Ovate; iii) Pointed                    |
| **Lodging**                                             |
| i) Low (Tolerant); ii) Medium; iii) High               |
| **Streaks**                                             |
| i) Nil; ii) Few; iii) Moderate; iv) Many               |
| **Wax**                                                 |
| i) Weak; ii) Medium; iii) Strong                       |
| **Tillering**                                           |
| i) Poor; ii) Moderate; iii) Good                       |
| **Pubescence**                                          |
| i) Sparse; ii) Medium; iii) Profuse                    |
| **Growth**                                              |
| i) Upright; ii) Inter med; iii) Bent                   |
| **Maturity**                                            |
| i) Early; ii) Medium; iii) Late                        |
| **Tops**                                                |
| i) Light; ii) Moderate; iii) Heavy                     |
| **Trash**                                               |
| i) Self; ii) Moderate; iii) Clinging                   |

Table 10 List of morphological qualitative and cane juice quality traits

| Morphological qualitative parameters                  |
|-------------------------------------------------------|
| **1st germination**                                  |
| Number of buds germinated per 150 buds of the central row was recorded after 30 days of plantation. |
| **2nd germination**                                  |
| This attribute was recorded as number of buds germinated per 150 buds of the central row after 30 days of the 1st germination. |
| **1st tillering**                                    |
| Count of the number of tillers in the central row in the 1st week of April. |
| **2nd tillering**                                    |
| Count of the number of tillers in the central row one month after the 1st tillering. |
| **1st plant height (cm)**                            |
| Recorded July by with the help of a meter rod measurement from soil to top. |
| **2nd plant height (cm)**                            |
| Recorded exactly 30 days after 1st plant height in the same manners. |
| **Leaf length(cm)**                                  |
| Measured from the leaf axil of the base leaf to the terminal point. |
| **Leaf width(cm)**                                   |
| Measured at the widest point of the leaf.            |
| **Leaf area(cm²)**                                   |
| Calculated through the following formula             |
| Leaf area = Leaf length × leaf width × K             |
| Where K (factor) = Actual leaf area/L×W               |
| **Number of nodes cane⁻¹**                           |
| At cane maturity the count of the number of buds cane⁻¹. |
| **Inter node length (cm)**                           |
| The distance between two nodes.                      |
| **Weight of five unstrapped canes (kg)**             |
| Weight of five canes was determined with the help of a scale. |
| **Weight of five strapped canes (kg)**               |
| Weight of five stripped canes was determined with the help of a scale after removing the tops and the trash from the canes. |
| **Yield (tons ha⁻¹)**                                 |
| X ×10,000/Plot size x 1000 Where “X” is sugarcane yield |
| **Weight of trash and tops**                          |
| Subtracting the weight of stripped cane from weight of canes with trash and tops. |
| **Number of millable cane**                          |
| This parameter was recorded by actually counting the number of millable canes (i.e. excluding the tillers which have not developed in to mature canes). |

| Cane juice quality parameters                         |
|-------------------------------------------------------|
| **Brix**                                              |
| It is the total soluble solids in cane juice, expressed in percentage. Brix contains sugars as well as non-sugars substances. Brix was measured either in the field in standing cane crop using a hand refractometer or in the cane laboratory with the help of a hydrometer. |
| **Pol percentage**                                    |
| The juice sucrose percent is the actual cane sugar present in the juice. It was measured by using polarimeter. Sucrose content is also referred to as pol percent. |
help of computer software “Statistica version-7”. Different attributes were measured by different units, so the average values of the parameter were standardized before the PC analysis to eliminate differences in values.

Authors’ contributions
Muhammad Khalid designed this research idea, carried out the analysis of data using different computer softwares and drafted this scientific article. All authors Hidayt ur Rahman, Farhatullah, Amanullah participated in the reviewing of this article. Ashiq Rabbani has done a tremendous job in the analysis of data and the last but not the least Dr. David A. Lightfoot has polished scientific article. All authors Hidayt ur Rahman, Farhatullah, Amanullah participated in the reviewing of this article. Ashiq Rabbani has done a tremendous job in the analysis of data and the last but not the least Dr. David A. Lightfoot has polished scientific article.

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Continuing table 10

| Cane juice quality parameters | Purity percentage was determined with the help of the following formula | Sugar Recovery was calculated with the help of the following formula: |
|-------------------------------|-----------------------------------------------------------------------------|---------------------------------------------------------------------|
| Purity percentage             | Purity % = (Pol% / Corrected brix) x 100                                     | Sugar Recovery (%) = [Pol % – 0.5(brix – Pol %)] x 0.70              |
| Sugar Recovery percentage     |                                                                              |                                                                     |