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Journal of Plant Breeding and Crop Science

January-March 2020
ISSN 2006-9758
DOI: 10.5897/JPBCS
www.academicjournals.org

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Vol. 12(1), pp. 1-7, January-March 2020
DOI: 10.5897/JPBCS2019.0842
Article Number: EB069B462808
ISSN 2006-9758

http://www.academicjournals.org/JPBCS

Journal of Plant Breeding and Crop Science

Full Length Research Paper

Identification of single nucleotide polymorphism (SNP) markers closely linked with powdery mildew resistance gene *Pm5e* in wheat

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Received 14 September, 2019; Accepted 23 December, 2019

Wheat (*Triticum aestivum* L.) is one of the most important food crops worldwide. Powdery mildew (Pm), caused by *Blumeria graminis* f. sp. *tritici* is a severe disease in wheat production. Gene *Pm5e*, from a Chinese wheat cultivar Fuzhuang 30 has proven to be a valuable resistance source for Pm in breeding. To further map this gene and develop Kompetitive allele-specific PCR (KASP) assays for marker-assisted selection (MAS), a F_2 population containing 395 individuals was first phenotyped for Pm resistance, a bulked segregant analysis (BSA) was used to identify polymorphic SNPs using the 35K wheat SNPs chip. 27 polymorphic SNPs between bulks in the *Pm5e* region were identified and were converted into KASP assays to map *Pm5e*. A genetic linkage map of *Pm5e* was constructed with 2 SNP and 2 SSR molecular markers. *Pm5e* was mapped to a 9.5 cM interval and the two SNP markers AX-95000860 and AX-94638908 were the two closet flanking markers, which delimited *Pm5e* into a 14 Mb region. Identification of the molecular markers and development of the two KASP assays laid a solid base for MAS of gene *Pm5e* in breeding.

Key words: Linkage map, marker assisted selection, SNP marker, wheat

INTRODUCTION

Wheat is an adaptable and widely distributed world food crop, which provide about 21% of food calories and 20% of protein for the human (He et al., 2018). Powdery mildew (Pm) is a disease caused by the biotrophic fungus *Blumeria graminis* f. sp. *tritici* (*Bgt*), which often occurs in wheat production areas with cool and humid climates (Cowger et al., 2012). In China, this foliar disease is endangering most regions of winter wheat and spring wheat productions (Liu et al., 2016).

Use of Pm resistance genes to develop resistant cultivars is the most effective way to control the epidemics of Pm and reduce the economic losses (Hulbert et al., 2001) however, the development of Pm-resistant wheat cultivars requires resistance genes. To date, seventy-eight designated and many other temporarily designated Pm resistance genes or alleles have been identified in wheat. Some of these genes have single alleles, while some of them have multiple alleles (e.g., *Pm1*, *Pm2*, *Pm3*, *Pm4*, *Pm5*, and *Pm54* loci) (Wu et al., 2018; Zhang et al., 2016).

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Pm5 was a recessive Pm resistance gene located on the long arm of 7B (Lebsock and Briggle, 1974). It is widely contributed in the cultivars and landraces of China and Europe (Huang et al., 1997; Zeller et al., 1998). Five alleles at the *Pm5* locus have been reported (Huang et al., 2000; Hsam et al., 2001). *Pm5e*, from Fuzhuang 30, a cultivar developed from the cross of two Chinese landraces, has proven to be a valuable Pm resistance source for breeding (Huang et al., 1997, 2003). The resistance gene in Fuzhuang 30 was first mapped to 7B (Huang et al., 2000), and Wang et al. (2000) designated the gene as *Pm5e*. Huang et al. (2003) mapped this gene to the distal end of 7BL by SSR markers.

Bulked segregant analysis (BSA) has been widely used to identify polymorphic molecular markers in genetic mapping by traditional molecular markers, such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP) (Tsilo et al., 2009; Xu et al., 2018). However, these markers cannot meet the demand for fine mapping of a gene as well as MAS due to inadequate density. The crop genomics landscape has been revolutionized due to the next generation sequencing (NGS) technologies, which provides a large amount of sequencing information with great improvements in coverage, time, and costs (Bevan and Uauy, 2013; Rasheed et al., 2017). These technologies facilitate the development of chip-based marker platforms for genotyping in an ultra-high-throughput fashion. In wheat, the 9K, 90K, 660K, 820K, and 35K wheat genotyping chip have been developed and widely used in genetic study (Manish et al., 2017; Rasheed et al., 2017; Windju et al., 2016; Xu et al., 2018).

Kompetitive allele-specific PCR (KASP) is a proprietary technology that can distinguish alleles at variant loci. KASP is a cost-effective single-step genotyping technology, cheaper than SSRs and more flexible than genotyping-by-sequencing (GBS) or chip-based genotyping, and thus has been widely used in linkage mapping, QTL mapping, and MAS (Liu et al., 2014; Patil et al., 2017; Semagn et al., 2014; Steele et al., 2018).

In a former study, using a F_2 population derived by crossing Fuzhuang 30 with Chancellor, we mapped *Pm5e* to 7BL, and identified two flanking SSR markers, *Xwmc364* and *Xbarc065* (Zhu et al. 2008). The objective of this study is to: (1) identify SNP markers closely linked with *Pm5e*, and (2) develop KASP assays that can be widely used in MAS of *Pm5e* to improve Pm resistance in wheat.

MATERIALS AND METHODS

Plant materials

A population of 395 F_2 was derived from a cross between the Pm-resistant parent Fuzhuang 30 and a Pm-susceptible parent Chancellor. Seeds from 212 randomly selected F_2 individuals were harvested to produce 212 F_3 families. Two susceptible cultivars Huixianhong and Mingxian 169 were used as the susceptible controls.

Pm inoculation and resistance identification

E09, a dominant local isolate of *Blumeria graminis* f. sp. *tritici* in China was used to identify the resistance at the seedling stage under artificial climate chamber conditions at 22°C day/18°C night with 60% relative humidity and a 12-h light/12-h dark photoperiod. Fuzhuang 30, Chancellor, Huixianhong, Mingxian 169, 395 F2 plants, and at least 15 plants from each F_3 family were tested for Pm resistance. Inoculation and resistance identification followed the methods described by Liu et al. (1999). Seedlings at one leaf stage were inoculated with E09 by dusting conidiospores that were multiplied on the susceptible plants of Huixianhong. Infection types (ITs) of all plants were scored on a 0–4 scale 15 days after inoculation. The inoculated plants with ITs 0-2 were divided into a resistant group with those of 0-4 to a susceptible group (Liu et al., 1999). The genotype of each F_2 individual for *Pm5e* was determined by the phenotype of the corresponding F_3 family.

DNA extraction and BSA analysis

Leaf tissue was harvested at the three-leaf stage of each F_2, dried in a SCIENTZ-18 freezer dryer (Ningbo Scientz, China) for 3 days, and ground to powder in a G200 mixer mill (Coyote Bio, China) for 3 min with the aid of a metal bead in each tube. DNA was isolated using a modified CTAB method (Liu et al., 2014; Saghai-Maroof et al., 1984). Bulked segregant analysis (BSA) was used to screen potential polymorphic single nucleotide polymorphism (SNP) markers associated with Pm resistance. Each of the two bulks consisted of 25 highly Pm-resistant and 25 highly Pm-sensitive F_2 individuals, respectively, from the F_2 population of Fuzhuang 30/Chancellor and screened by the 35K Axiom® Wheat Breeder Genotyping Array (Allen et al., 2017).

SSR, KASP analysis and map construction

Two SSR markers *Xwmc364* and *Xbarc065*, which have been identified to be linked with *Pm5e* were also screened in this population following the method described by Zhu et al. (2008). Polymorphic SNPs identified by 35K Axiom® Wheat Breeder Genotyping Array between the two bulks were converted into KASP assays and run across the F_2 population for linkage mapping.

KASP assay followed the method described by Liu et al. (2014). In brief, a 6 µL reaction was used for KASP assay, which includes 3 µL of 2× reaction mix, 0.106 µL of assay mix (LGC Genomics, Beverly, MA) and 3 µL of genomic DNA at 15 ng/µL. PCR and fluorescent endpoint readings were carried out using an ABI Quant StudioTM 12K Flex Real-Time PCR System (Life Technology, Grand Island, NY). PCR thermal cycling profile followed the manufacturer's manual (http://www.kbioscience.co.uk/ reagents/ KASP_manual.pdf).

Linkage mapping was performed using JoinMap 3.0 software (Van Ooijen and Voorrips 2001). Recombination fractions were converted into centiMorgans (cM) using the Kosambi function (Kosambi, 1944). Map construction followed the methods described by Liu et al. (2014).

BLAST analysis

Sequences containing SNPs linked with *Pm5e* were used as

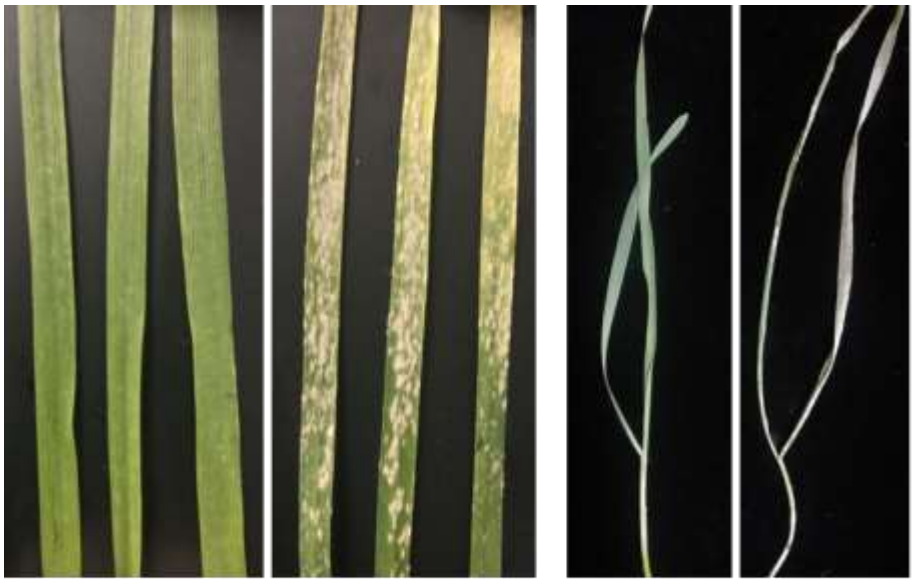

Figure 1. The powdery mildew infection phenotype of Fuzhuang30 (RP), Chancellor (SP) and F_2 resistant (R) and (S) susceptible individuals.

queries to search in the Chinese Spring genome sequence database released by the International Wheat Genome Sequencing Consortium (IWGSC) RefSeq1.0 (Rudi et al., 2018, https://urgi.versailles.inra.fr/) using BLAST to identify the physical positions of the SNPs on 7B. A significant match was declared when at least 98% nucleotide identity was identified with an e-value lower than e^{-20} (Liu et al., 2014).

RESULTS

Pm resistance evaluation

Fuzhuang 30 was immune to Pm, while Chancellor was highly susceptible (Figure 1). Among the 395 F_2 individuals, 108 were resistant and the remaining 287 were susceptible. The chi-square test showed that the segregation of resistant and susceptible F_2 plants fit to a 1:3 ratio (χ^2=2.256 <$\chi_{0.05}{}^2$=3.841). Among the F_3 families, 47 were resistant, 125 were segregating, 50 were susceptible and fit to a 1:2:1 ratio (χ^2=3.466<$\chi_{0.05(2)}{}^2$=5.991), indicating that Fuzhuang 30 carries a single recessive gene for Pm resistance to *Bgt* isolate E09.

Polymorphic SNP detected by 35K chip

After screening of the two bulks using the 35K Axiom® Wheat Breeder Genotyping Array, 1548 probes were identified as polymorphic between the two bulks. Blasting of these probes in the IWGSC wheat genome sequence database RefVer1.0 showed that these polymorphic probes were located on all wheat chromosomes (74 in average); however, the probes located on 7B (191) was significantly more than other chromosomes (Figure 2), indicating *Pm5e* was probably located on 7B.

Among the 191 probes on 7B, 98 were on 7BL, and only 27 were located between two wheat ESTs CJ729392 and CJ584170 (*Pm5e* region) (Table 1). According to the IWGSC wheat genome sequence database RefVer1.0, this region was a 41 Mb interval from 687 Mb to 728 Mb on 7BL.

KASP and SSR marker analysis

The 27 polymorphic SNPs were converted into KASP assays and run in two parents and randomly selected 40 F_2 individuals, only 2 assays AX-95000860 and AX-94638908 detected polymorphism between the parents and can separate the F_2 plants clearly. The primers for AX-95000860 KASP assay includes CAGGATTGGACTCGGCTGGAAAC as the AX-95000860-FAM forward primer, CAGGATTGGACTCGGCTGGAAAT as the AX-95000860-HEX forward primer, and ATGTCAGGTCACCACGATGC as the common reverse primer. The primers for AX-94638908 KASP assay include ATGATAACATGCTGCGCATGAC as the AX-94638908-FAM forward primer, ATGATAACATGCTGCGCATGAT as the AX-94638908-HEX forward primer, and TACACAAACTAGGTGGAGGTACAAC as the common reverse primer. The two assays were further run across

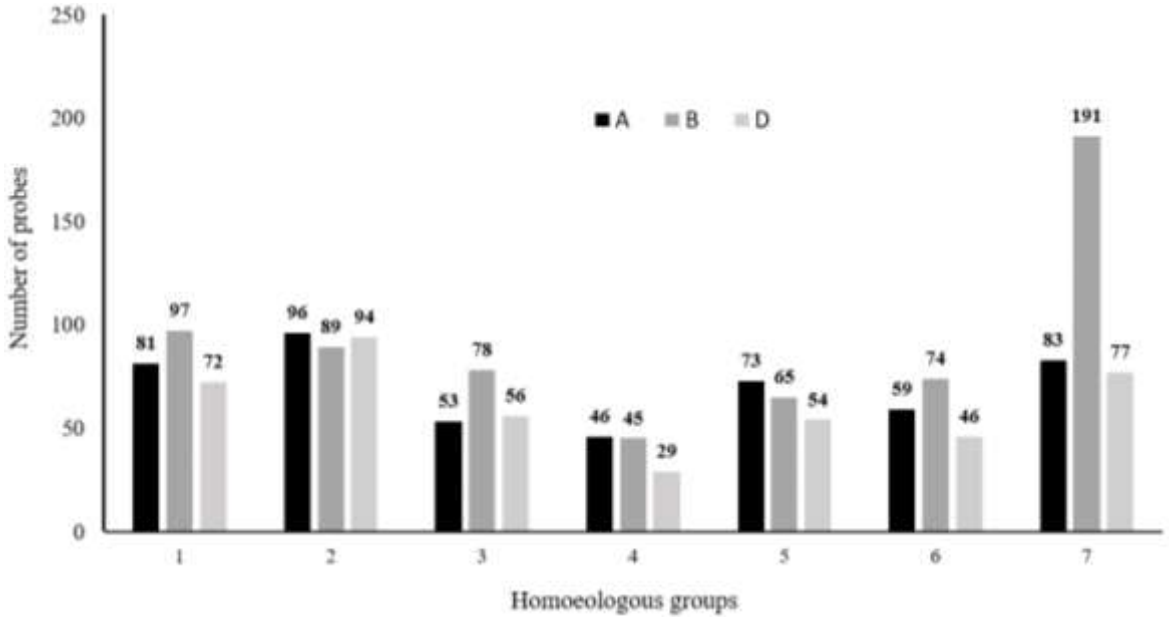

Figure 2. Distribution of polymorphic probes on wheat chromosomes.

Table 1. Polymorphic probes between the resistant and susceptible bulks on 7BL in the interval 687-728 Mb on 7BL.

Probe name	Resistant bulk	Susceptible bulk	Polymorphic SNP
AX-94418014	C/C	C/G	C/G
AX-94459856	C/G	C/C	G/C
AX-94463979	C/C	T/C	C/T
AX-94472687	C/C	C/G	C/G
AX-94535041	T/T	T/C	T/C
AX-94584717	T/T	T/C	T/C
AX-94596410	T/T	T/C	T/C
AX-94614297	C/C	A/C	C/A
AX-94628121	G/G	A/G	G/A
AX-94638908	C/C	T/C	C/T
AX-94667120	C/C	T/C	C/T
AX-94677860	G/G	C/G	G/C
AX-94677963	A/A	A/C	A/C
AX-94750259	C/G	C/C	G/C
AX-94826552	A/A	A/G	A/G
AX-94831799	T/T	T/C	T/C
AX-94878591	G/G	A/G	G/A
AX-94931476	T/C	T/T	C/T
AX-94960851	G/G	A/G	G/A
AX-94977792	T/T	T/C	T/C
AX-94999423	A/G	A/A	G/A
AX-95000860	C/C	T/C	C/T
AX-95140096	C/C	T/C	C/T
AX-95163625	C/C	T/C	C/T
AX-95186295	T/C	T/T	C/T
AX-95188770	T/G	T/T	G/T
AX-95652788	A/G	A/A	G/A

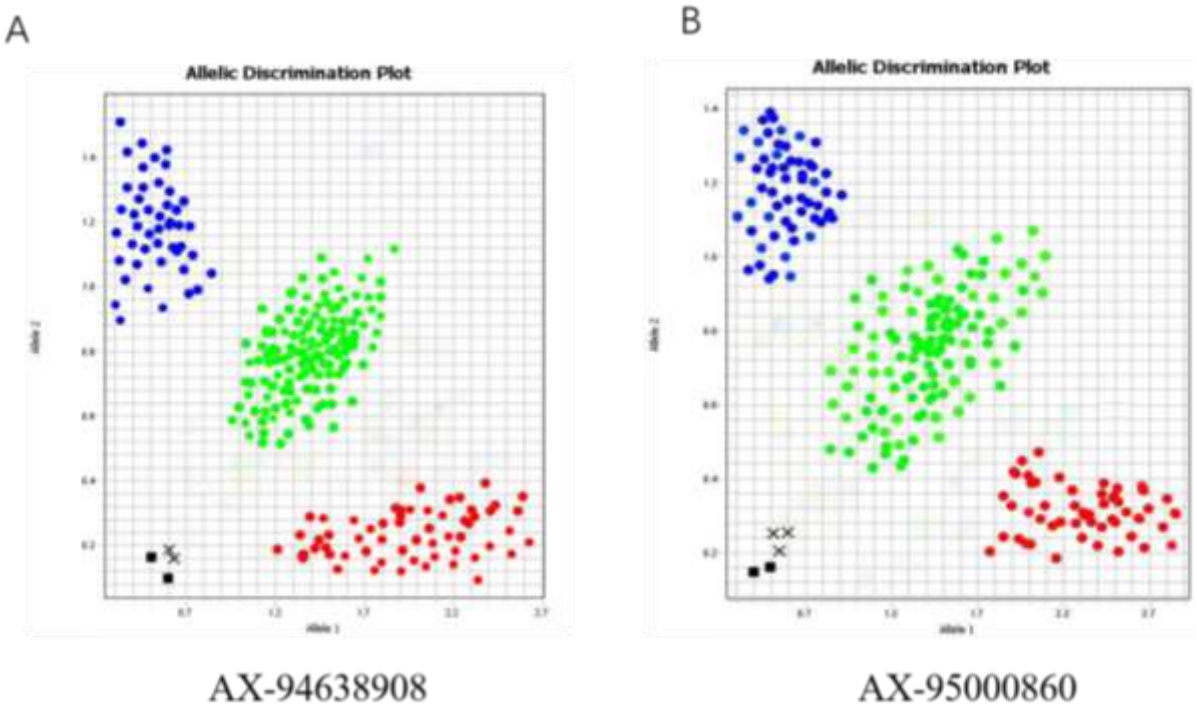

Figure 3. KASP assay of single nucleotide polymorphism (SNP) AX-94638908 (A) and AX-95000860 (B) in F_2 population. Allele X (KASPHEX, red) shows the T (A) and T (B) nucleotide, and allele Y (KASPFAM, blue) shows the C (A) and C (B) nucleotide. The green color dots indicate heterozygotes. The black squares and X in the left bottom are water and blank controls, respectively.

the F_2 population and can clearly separate the population into three groups with homozygous resistant, heterozygous and homozygous susceptible (Figure 3). The two SSR markers, *Xwmc364* and *Xbarc065*, which have been identified to be linked with *Pm5e* were also run across the F_2 population and can separate the genotypes of the F_2 individuals clearly (Figure 4).

Map construction

A linkage map including *Pm5e*, two SNP, and two SSR markers was obtained with a total genetic distance of 20.2 cM (Figure 5). Among them, the SNP markers were the two closet flanking markers of *Pm5e* with genetic distances of 4.2 cM and 5.3 cM apart from *Pm5e*, respectively. *Xwmc364* and *Xbarc065* were farther apart with *Pm5e*. Based on the IWGSC RefVer1.0 sequence, the two flanking SNP markers AX-95000860 and AX-94638908 delimited *Pm5e* to a 14 Mb interval from 707 Mb to 721 Mb on 7BL.

DISCUSSION

Since gene *Pm5e* gene was successfully excavated, the predecessors have successfully developed some molecular markers linked to it, including SSR markers and EST markers (Huang et al., 2003; Zhu et al., 2008; Xie, 2016). However, the mapped markers are far apart from *Pm5e*. Closely linked flanking markers are urgently needed for effective marker-assisted transfer of *Pm5e* to new wheat cultivars by MAS.

SNPs are the most abundant DNA sequence polymorphisms in a genome. In the last decade, next-generation sequencing (NGS) technologies have advanced rapidly and have become the cheapest and fastest technology for identification of genome-wide SNPs (Manish et al., 2017). SNP arrays have been developed and used for a variety of genetic and breeding applications including genome-wide association analysis and genomic selection in many crops (Manish et al., 2017; Allen et al., 2017).

In this study, BSA and wheat SNP chip was used to identify SNP markers closely linked with *Pm5e*, segregation of Pm resistance of resistant and susceptible individuals in the F_2 population showed a 1:3 ratio, indicating that Fuzhuang 30 carries a single recessive gene for Pm resistance to *Bgt* isolate E09, which is consistent with the former study (Huang et al., 2003). Based on the Pm resistance identification, a resistant and susceptible bulk was made and a 35K wheat SNP chip was used to screen the resistant and susceptible bulks to identify polymorphic SNPs between the two bulks, and

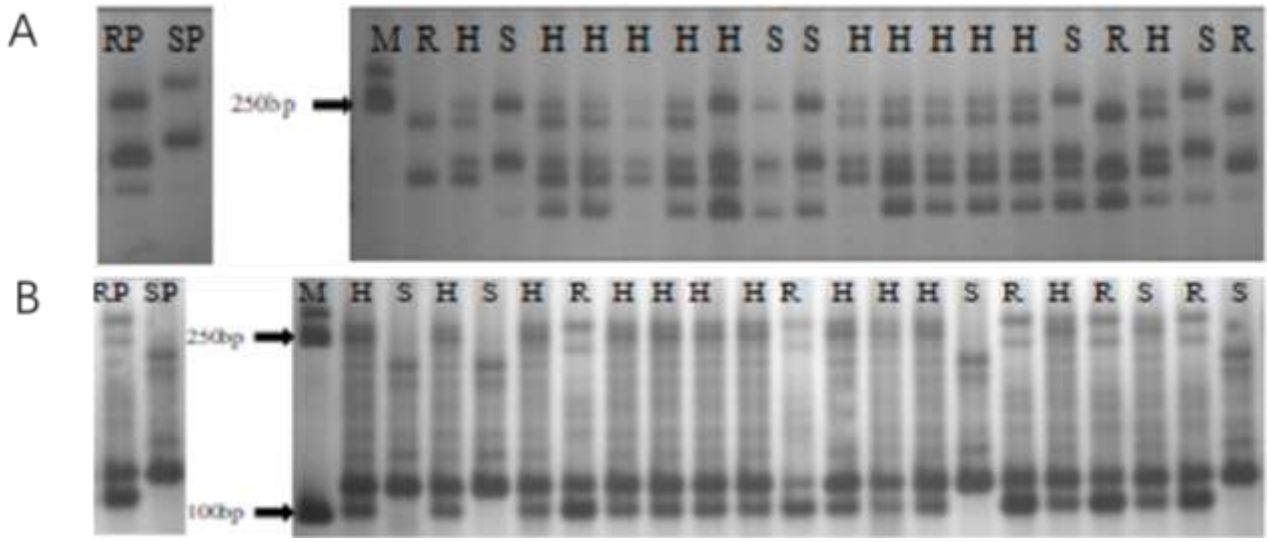

Figure 4. Segregation of SSR marker *Xwmc364* (A) and *Barc065* (B) in the Fuzhuang 30/Chancellor F_2 population. R, S and H indicate resistant, susceptible and heterozygous genotype and the marker locus. RP and SP indicate resistant and susceptible parent. M, marker ladder.

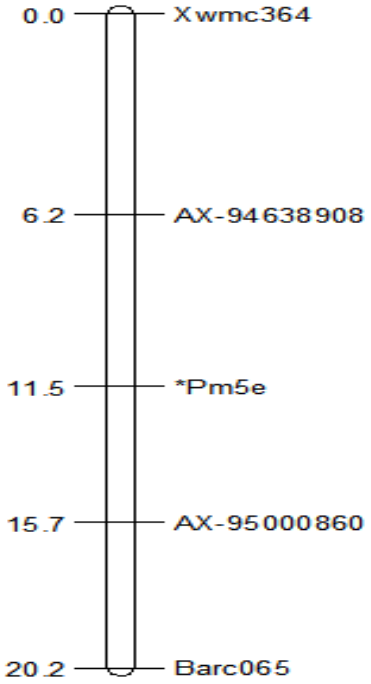

Figure 5. Linkage map of gene *Pm5e*.

1548 polymorphic SNPs were identified. Because *Pm5e* has been mapped on 7BL and two flanking wheat ESTs, CJ729392 and CJ584170, have been identified, we can easily delimit the physical interval of *Pm5e* according to the wheat reference genome sequence. Only 27 polymorphic SNP between the two flanking ESTs were used to develop KASP assays to fine map *Pm5e.* Two KASP markers, AX-95000860 and AX-94638908, were identified closely linked to *Pm5e* with genetic distance of 4.2 cM and 5.3 cM apart from *Pm5e*, which were much closer than the formally mapped markers, and the interval of the *Pm5e* region was decreased from 41 Mb to 14 Mb (Zhu et al., 2008; Xie, 2016), which indicated that using SNP chip to identify SNP markers for a specific gene was very effective.

Wheat SNP chip may not be cost effective for breeding selection due to high cost per sample if only a few SNPs are interested. KASP assay, however, is a time saving and cost-effective genotyping method for single SNP screening and has been successfully used in wheat genetic and breeding studies (Semagn et al., 2014; Rasheed et al., 2017). In this study, the identified polymorphic SNPs between the two bulks were converted into KASP assays to map *Pm5e* and two KASP assays closely linked with *Pm5e* were mapped, which can be used in MAS of *Pm5e*.

Conclusions

i) Twenty-seven polymorphic SNPs between the resistant and susceptible bulks in *Pm5e* genomic region were identified using the 35K wheat SNPs chips.
ii) KASP assays of the polymorphic SNPs were developed and a genetic linkage map of *Pm5e* was constructed together with 2 SNP and 2 SSR markers.
iii) *Pm5e* was mapped to a 9.5 cM interval and the two KASP markers AX-95000860 and AX-94638908 were the two closet flanking markers, which delimited *Pm5e* into a 14 Mb region and laid a solid base for MAS of *Pm5e* in breeding.

ACKNOWLEDGMENTS

This project is funded by the National Key Research and Development Programs of China (2016YFD0101802), and the Shandong Province Agricultural Fine Seeds Project (2016LZGC023).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Vol. 12(1), pp. 8-15, January-March 2020
DOI: 10.5897/JPBCS2019.0839
Article Number: 3F3CAE062810
ISSN 2006-9758

http://www.academicjournals.org/JPBCS

ACADEMIC JOURNALS

Journal of Plant Breeding and Crop Science

Full Length Research Paper

Genotype by environment interaction and yield stability analysis of open pollinated maize varieties using AMMI model in Afar Regional State, Ethiopia

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Received 31 August, 2019; Accepted 7 January, 2020

The present study attempts to analyze the magnitude of GxE interaction and evaluates the adaptability and stability of open pollinated maize genotypes for grain yield, using AMMI (Additive Main Effects and Multiplicative Interaction) model. The field experiment was conducted for two consecutive years during the off seasons of 2016/17 and 2017/18 at three locations namely, Awra, Dalifage and Dubti. The experiment in each location was laid out using RCBD with three replications. The pooled analysis of variance over environments for AMMI model was highly significant (P<0.01). The results revealed the existence of considerable variation among the genotypes and the environments for grain yield, indicating the differential performance of genotypes across the environments. Based on the AMMI model genotypes Melkassa-2 and Melkassa-7 were the most stable varieties with lower Interaction (IPCA) score and lowest ASV rank. Genotypes Melkassa-3 and Melkassa-4 had shown specific adaptation to environment Awra and Dalifage, respectively; indicating that these genotypes were more sensitive to environmental changes and have better adaptation for specific locations. The results of AMMI biplots were also in agreement with the results of ASV. Thus, the whole analysis generally suggested that maize grain yield was highly influenced by environments and G x E interaction. Thus, testing OPV maize varieties in more seasons and locations could enhance breeding efficiency with respect to genotypic stability and adaptation across environments.

Key words: AMMI, ASV, G x E interaction, IPCA, open-pollinated maize.

INTRODUCTION

Maize (*Zea mays* L.) (2n=20), which is also known as corn, belongs to the family *Poceaceae*. Maize is the most important crop worldwide and basic trade product recurring ingredient for millions of people in Sub-Saharan Africa (Nzuve et al., 2013). It is the third most significant cereal crop in the world, after wheat and rice, in terms of cultivated area, production and grain yield. Maize is a multipurpose crop that acclimates effortlessly to a wide variety of production set of conditions (FAO, 2015). Thus, maize is one of the most important cereals in Sub-Saharan Africa (SSA) and a staple food for an estimated 50% of the population. It is an important source of carbohydrate, protein, iron, vitamin B, and minerals (Apraku and Akinwale, 2011).

The genetic diversity of maize, as cross pollinated crop, is very wide for management in its genetic improvement,

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because landraces reveal important phonological and morphological distinction and allelic polymorphism (Anley et al., 2013).

One of the most exigent issues in plant breeding progress is to perfectly dissect genotype x environment (G x E) interaction, because it is based on figures from multi-environment experiments. In most trails, the G x E interaction is witnessed and then modeled statistically and elucidated. Genotype x environment interaction adjusts the reasonable grain yield of genotypes in diverse environments and makes it hard to select the better ones (Miah and Uddin, 2016).

Clarification of genotype x environment (G x E) interaction can be more supported by statistical modeling. Models can be linear formulations such as joint-regression, multivariate clustering techniques, multiplicative formulations such as additive main effects and multiplicative interaction (AMMI) or nonparametric methods (Albert, 2004). Modeling G x E interaction in Meta environments assists to clarify consistency of breeding materials, however, this thought has been well predetermined in various ways, and a number of stability parameters have been developed. Selection processes in plant breeding depends critically on the quality of phenotype predictions (Malosetti et al., 2013). The phenotype is classically predicted as a function of genotypic and environmental information. Models for phenotype prediction contain a mixture of statistical, genetic and physiological elements (Yong-Jian et al., 2010; Bustos-korts et al., 2016).

Although a number of methods are employed for genotype by environment interaction (GEI) and phenotypic stability analysis, Additive Main Effect and Multiplicative Interaction (AMMI) model is more suitable and simplify instantaneous choice of genotypes for stability. The model helps in establishing the relationship of genotypes, environment and their interaction (Giridhar et al*.,* 2016). The AMMI model has been intensively used recently, since it incorporates both the classical additive main effects for GEI and the multiplicative components into an integrated least square analysis and thus become more effective in selection of stable genotypes (Dewi et al., 2014; Frutos et al., 2015).

However, the AMMI model alone does not provide measure for a quantitative stability. For this reason, AMMI Stability Value (ASV) was proposed by Purchase (1997). The lower the ASV value, the lower the genotype's interaction to the environment and consequently the variety is said to be more stable. The most stable and adapted genotypes can be identified using ASV as that of Lin and Binns (1986) method.

Maize is one of the most important crops both in terms of production area and productivity and the basic staple food for Afar pastoral community of Ethiopia. Maize is produced mostly by small holder resource poor farmers under irrigation. In spite of this, the production of maize in farmer's fields in the region is low. The average grain yields of maize are around 18.9 tons ha^{-1} (Solomon et al., 2008). A number of maize varieties were developed and released to the rift valley areas by different research centers, but most of them failed to adapt due to the dynamics of the growing environment and climate change effects in the area. In spite of this; adaptation of released varieties has to be conducted in multi environment before they are distributed to the farmers. However, limited efforts have been made to the adaptation of released varieties of maize in the low-land agro-ecologies where it is widely produced and utilized by the community. Hence, G x E interaction analysis or testing genotypes for wide and specific adaptation to a micro environment is a paramount for yield stability of maize varieties. Therefore, the present study was undertaken to analyze the magnitude of GEI and evaluate the adaptability and stability of open pollinated maize genotypes for grain yield, using Additive Main Effects and Multiplicative Interaction (AMMI) model.

MATERIALS AND METHODS

Description of the study area

The study was conducted in three locations, namely, Awra, Dalifage and Dubti of the Afar Regional State. Afar region is situated in the great rift-valley, the topography of the region is predominantly arid and semi-arid flat-land characterized by lowland climate. Pastoralism is the predominant economic and social mainstay of the population of Afar with around 88% of the total population livelihood depending on rearing, and moving with livestock herds. Agro-Pastoralism (estimated at 12%) involves production of crops, including maize, sorghum, vegetables and fruits to a lesser extent, using some permanent and temporary rivers in the region. The geographic descriptions of the study area are summarized follows: Awra is located at coordinate of 11°36'N and 39°59'E, located 208 km away from Samara with an altitude of 939 masl. The mean Max and Min temperature is 33.3 and 21°C having hot and dry weather with annual rain fall of 410 mm and a predominant soil type of silty-clay (WARC and APARI, 2007).

Dalifage is located in 11° 03'N and 40°13'E, in (Zone-5) of Afar Regional State and is found 235 km west of Samara. The elevation of the area is 695 masl, with low and erratic rainfall. The weather is hot and dry with mean Max and Min temperature ranging between 37 and 23°C (WARC and APARI, 2007).

Dubti is one of the districts in Zone-1 of Afar Regional State, located in 11° 33' N and 40° 44' E. The Max-Min temperature during the main rainy season is 42 to 31°C with annual rain fall of 100-200 mm and the predominant soil type is fluvisol (WARC and APARI, 2007).

Experimental materials and design

Six open pollinated maize genotypes namely Melkassa-1, Melkassa-2, Melkassa-3, Melkassa-4, Melkassa-7 and Melkassa-6Q were collected from Melkassa National Maize Research Coordinating Center and planted at three locations: Awra, Dalifage and Dubti Pastoral and A/pastoral Research Centers in 2016/2017 and 2017/2018 off seasons. In each location, the experiment was laid-out in randomized complete block design (RCBD) with three replications. Each plot was 11.25 m^2 size having 5 rows of 3 m long

with row spacing of 0.75 m. The harvested plot size was 6.75 m^2 (3-rows from the center of each plot). Agronomic and cultural practices, like fertilizer, weeding and irrigation were applied as required based on recommendations.

Data collection

Ten competitive plants were randomly selected from the middle rows of each plot and the following morphological data were recorded on plant basis: days of silking (DSL), days of maturity (DM), ear per plant (EPL), ear length (EL), leaf per plant (LPL), plant height (PLH), cob weight (CW), row kernel number (RKN), number of kernel per row (NKPR), hundred kernel weight (HKW) and grain yield per hectare (GYPH). Mean grain yield was estimated for each genotype at each location and season.

Statistical methods and data analysis

The data on grain yield and yield related traits in six environments were subjected to pooled analysis of variance using Crop Stat 7.2 (IRRI, 2009). The AMMI model is a hybrid model incorporating both ANOVA (for additive component) and PCA (for multiplicative component) for analysing two way (G x E) data structures. To show a clear insight into specific GEI combination and the general pattern of adaptation, a biplot of varieties and environments was done. The AMMI biplot is developed by placing both genotype and environment means on the abscissa (X- axis) and the respective PCA axis, eigen vector on the Y- axis. In the AMMI model, the contribution of each genotype and each environment to the G x E interaction is valued by using the Biplot graphic representation as suggested by Zobel et al. (1988). The equation for AMMI model is represented as:

$$Y_{ij}=\mu + g_i + e_j + \Sigma\lambda k + \alpha ikyjk + Rij$$

Where, Y_{ij} is the yield of i^{th}-genotypes in j^{th}-environment; μ is the overall mean; g_i is the effect of the i^{th} genotype; e_j is the effect of the j^{th} environment; λk is the eigen value of the PCA for axis k. Then α_{ik} and y_{jk} are the genotype and environment principal component scores for axis k, respectively, and R_{ij} is the residual term. Environment and genotype PCA scores are expressed as unit vector times the square root of λk.

In order to rank the genotypes in terms of stability, AMMI stability value (ASV) was employed for each genotype following the procedure proposed by Purchase (1997) as follows:

$$ASV = \sqrt{\left[\frac{IPCA1SS}{IPCA2SS} \times IPCA1score\right]^2 + [IPCA2score]^2}$$

Where, ASV AMMI Stability Value; IPCA1and IPCA2 are Interaction Principal Component Axis one and Axis two; SS = sum of squares.

The ASV is the distance from zero in a two dimensional scatter-gram of IPCA1 scores against IPCA2 scores. Since the IPCA1 score contributes more to G x E sum of squares, it has to be weighted by the proportional difference between IPCA1 and IPCA2 scores to compensate for the relative contribution of IPCA1 and IPCA2 to total G x E sum of squares (SS).

RESULTS AND DISCUSSION

AMMI analysis

Additive Main Effect and Multiplicative Interaction (AMMI) analysis of variance for the data on yield (t/ha) showed that all the three variance components genotype (G), environment (E) and GEI were highly significant at P< 0.01; indicating the existence of considerable variability among the tested varieties (Table 1). Similar results were reported by Solomon et al. (2008) and Anley et al. (2013). However, the variance due to environments accounts for 55% of the total variation and was about 4 times greater than that of the variance due to genotypes. The higher proportion of environmental variance may be due to the high variation in soil types and weather conditions among the environments. This suggested that the yield potential of OPV maize is greatly influenced by environmental factors.

Genotype x environment interaction (GEI) was further partitioned into two Interaction Principal Component Axes. The AMMI result also showed that the first and second Interaction Principal Component Axis (IPCA1 and IPCA2) explained about 86.2% of the interaction sum squares, indicating that the first two IPCA are sufficient to explain GEI in grain yield of maize genotypes. This result is in harmony with some previous findings (Nzuve et al., 2013; Kumar and Singh, 2015; Kumar et al., 2014; Miah and Uddin, 2016); they indicated that AMMI with only two interaction principal component axes was the best predictive model. IPCA1 captures about 91.0% of the interaction sum of squares and the rest 9% were captured by IPCA2. IPCA scores of genotypes and environments were both positive and negative, thus, representing the principal source of variation for any crossover interaction.

Mean performance of open pollinated maize genotypes

Average environment grain yield varied between 4.46 t/ha for Dubti-1 and 6.18 t/ha for Dalifage-1 (Table 2). Melkasa-4 was the highest yielding genotype with average grain yield of 5.85 t/ha, followed by Melkasa-7 with average grain yield of 5.62 t/ha. The lowest yielding genotype was Melkasa-1 with average grain yield of 4.91 t/ha (Table 3). The genotypes showed varied performance in response to the test environments, thus contributed to greater variation in GEI; similar results were reported by Giridhar et al. (2016). GEI diminishes the efficacy of genotypes by confounding their yield potential, which indicates the relevance of evaluating the adaptability and stability of genotypes across multi environments.

ASV analysis

Table 3 also presents the AMMI stability value (ASV) and ranking with IPCA1 and 2 scores for each maize variety. In ASV method, a variety with high mean yield and least ASV score is the most stable (Purchase et al., 2000).

Table 1. The combined analysis of variance for AMMI model.

Source of variation	Degree of freedom	Sum of squares	Mean squares	Sum squares explained	
				% total	% G x E
Reps within E	12	3.50	0.292*		
Genotypes	5	12.46	2.493**	13.44	
Environments	5	50.59	10.118**	54.59	
G x E	25	18.24	0.73**	19.69	
IPCA 1	9	10.95	1.217**	11.81	60.03
IPCA 2	7	4.74	0.677**	5.12	25.99
Residual	60	7.88	0.131		
Total	107	92.68			
Grand mean =	5.33	CV (%) = 6.79			

**, * indicate highly significant and significant at 1 and 5% probability level, respectively.

Table 2. IPCA 1, IPCA 2 scores and environment means of grain yield over 3 locations and 2 seasons.

Environment	Mean (t/ha)	Graph ID	Rank	IPCA1	IPCA2
Awra1	6.111	E1	2	-0.96611	0.58784
Awra2	5.039	E2	4	-0.46100	-0.92503
Dalifage1	6.148	E3	1	-0.01283	0.00842
Dalifage2	5.642	E4	3	0.54950	0.19317
Dubti1	4.456	E5	6	0.26314	0.13627
Dubti2	4.568	E6	5	0.62729	-0.00067
Grand mean	**5.33**				

IPCA= Interaction Principal Component Axis, E_{1-6} = Environment 1-6.

Table 3. IPCA 1, IPCA 2 scores and genotype means of six OPV maizes tested at 3 locations and 2 seasons.

Genotype	Mean (t/ha)	GraphID	Rank	IPCA1	IPCA2	ASV	Rank
Melkasa1	4.905	1	6	0.57705	-0.06313	0.58	4
Melkasa2	5.335	2	4	0.51133	0.04777	0.51	3
Melkasa3	5.420	3	3	-0.46125	0.94381	1.05	5
Melkasa4	5.851	4	1	-1.01292	-0.52266	1.14	6
Melkasa6Q	4.921	5	5	0.15338	-0.15481	0.22	1
Melkasa7	5.605	6	2	0.23241	-0.25099	0.34	2
Grand mean	**5.33**						

IPCA=Interaction Principal Component Axis, ASV=AMMI Stability Value.

Accordingly, the variety Melkasa-7 had higher mean yield (above the grand mean) with lower ASV value and was considered as the most stable across all environments, followed by Melkasa-2. Whereas, Melkasa-3 and Melkasa-4 were the most unstable varieties, as they exhibited largest ASV ranks. Though these genotypes, having higher mean yield over the grand mean, are suited to specific environments, this result is incongruence with the result of AMMI biplot. However, the remaining varieties, whatever ASV rank they have, since they had under average yield performance, were considered as unsuitable to any environment.

Biplot analysis

The results of AMMI analysis further enlightened the relative contribution of the first two IPCA axes to the

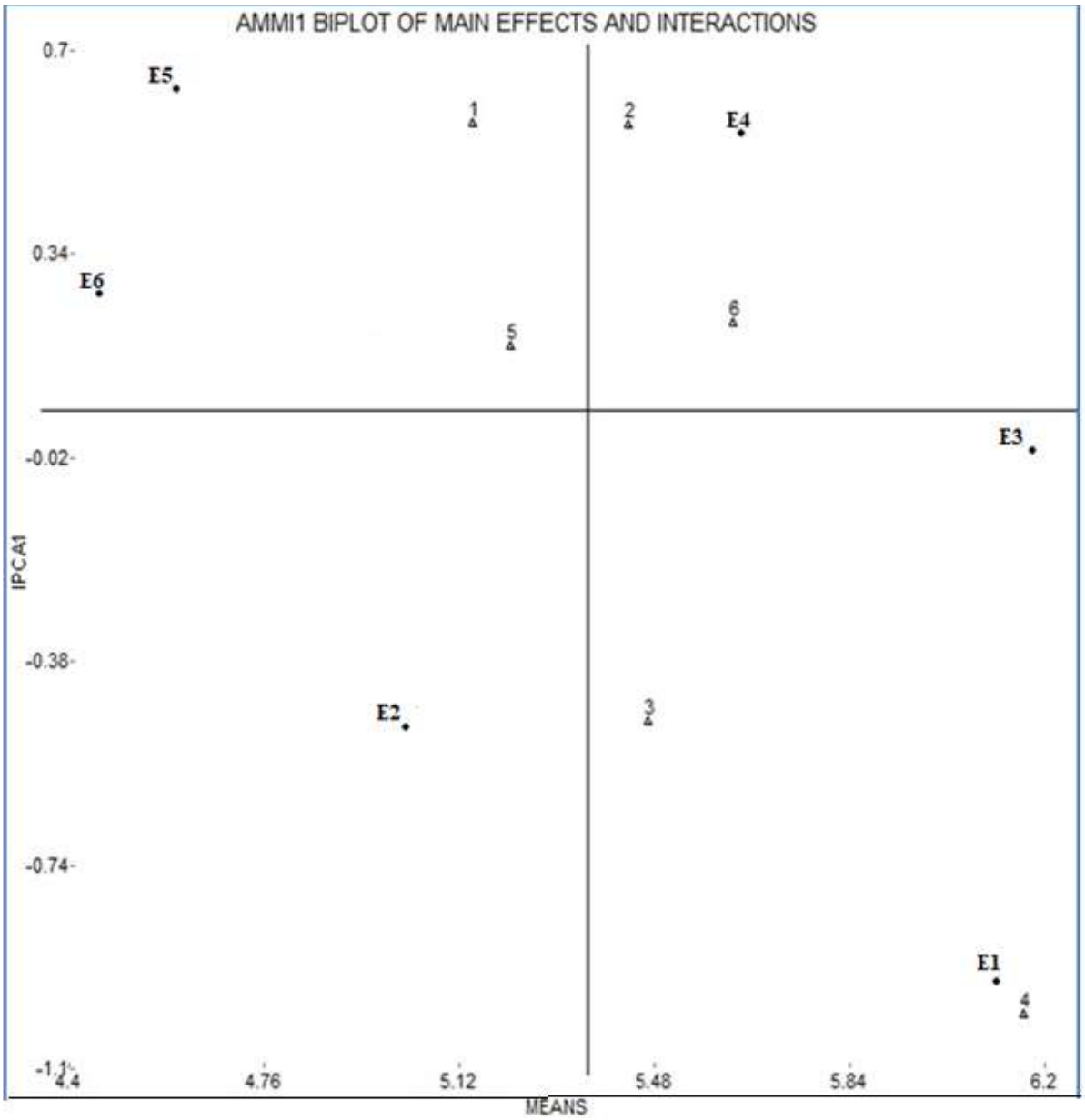

Figure 1. AMMI-1 biplot for grain yield (t/ha) showing the means of genotypes and environments (X-axis) and IPCA1 scores on (Y-axis).

interaction effects by plotting with genotype and environment means as presented in Figures 1 and 2.The mean performance and PCA1 scores for both the varieties and environments used to construct the biplots are presented in Tables 2 and 3. In the biplot, environments are designated by the letter 'E' followed by numbers 1 to 6 as suffix (Table 2 and Figure 1), while genotypes represented by numbers from 1 to 6 (Table 3, Figure 1). The quadrants in the graph represented (QI and QII) higher mean, (QIII and QIV) lower mean, (QI and QIV) +ve IPCA1 and (QII and QIII) –ve IPCA1 scores (Figure 1). When a variety and environment have the same sign on PCA1 axis, their interaction is positive and if opposite, their interaction is negative. Thus, if a variety has a PCA1 score near to zero, it has small interaction effect and was considered as stable over wide environments. Conversely, varieties with high mean yield and large PCA scores were considered as explicitly adapted to specific environments (Abdi and Williams, 2010; Askari et al., 2017; Mustapha and Bakari, 2014).

Accordingly, Dalifage-1 (E3) was the most stable environment having highest mean and lowest PCA score. Dalifage-2 (E4) was the next stable environment with higher mean yield and moderate interaction effects.

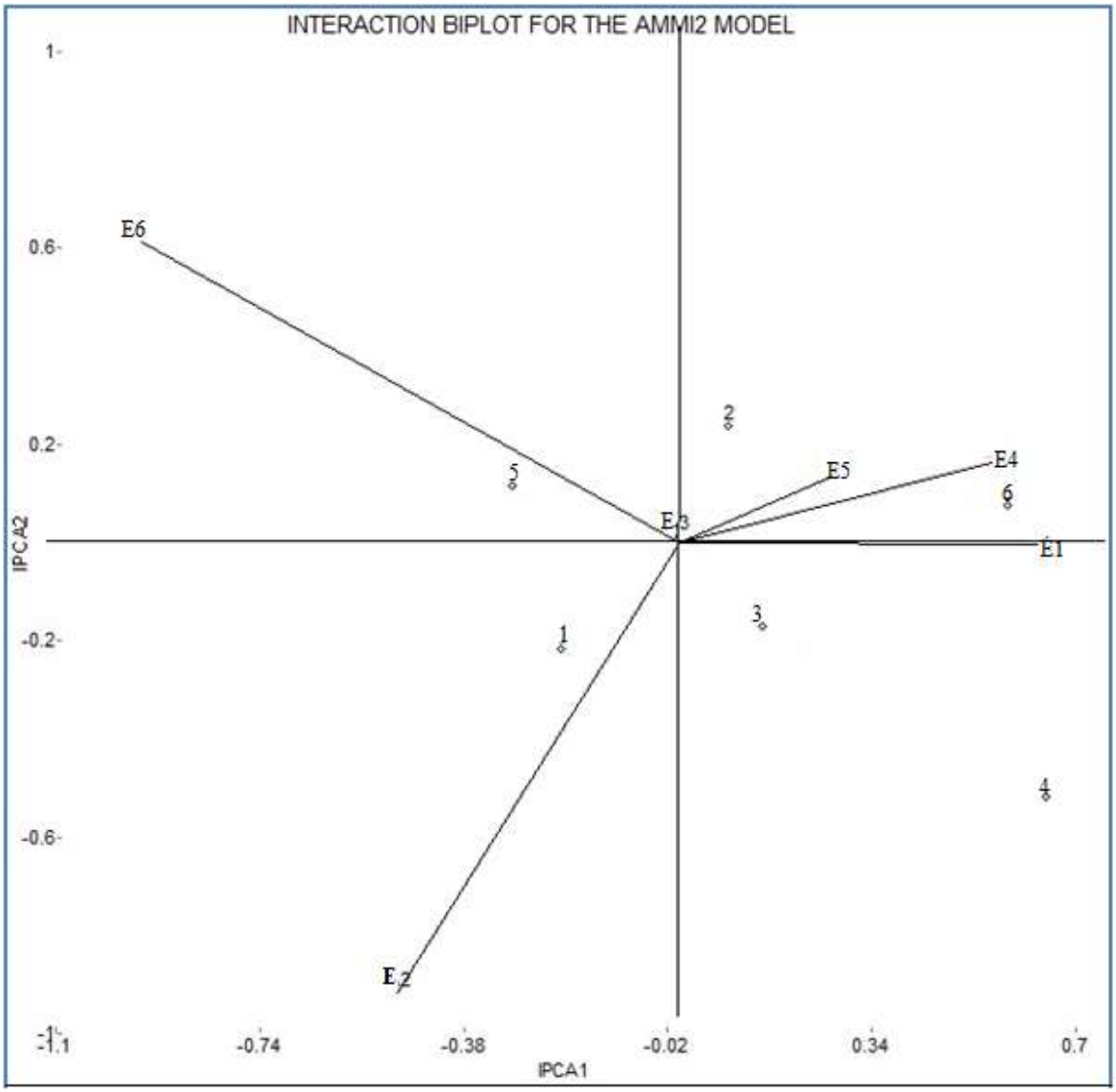

Figure 2. AMMI-2 biplot for grain yield (t/ha) showing the IPCA1 *vs* IPCA2 for genotypes and environments.

These environments are most suitable for synthesizing hybrids due to low interaction effects. However, environments Dubti-1 (E5) and Dubti-2 (E6) showed lower mean yield and high interaction effects, hence they were considered as unfavorable for the present set of genotypes. Similarly, Awra-2 (E2) had negative interaction effects with most of the genotypes with mean yield below the grand mean and was considered as unstable environment (Figure 1). Although Awra-1 (E1) had above average grain yield, since it interacted negatively with most of the genotypes, it is suitable for specific adaptation with high mean yield. Hence, it is more favorable for Melkasa-4. Similar results were reported by Nzuve et al. (2013) and Ndhlela et al. (2014).

Regarding the scattered plot of genotypes, Melkassa-7 (genotype no.6) had higher mean yield with very low interaction effects and it can be regarded as the most stable for seed yield across environments, which is consistent with the ASV result. The two high yielder varieties: Melkasa-3 and Melkasa-4 (no. 3 and 4) had higher mean yield above the grand mean, but since they exhibited high interaction effects, they are desirable for specific adaptation in favorable environments with high mean yield; whereas, Melkasa-2 (no.2) was most favored in Awra1 (E1). As it interacted negatively with most of the environments, it is best suited for unfavorable environments with high yield. However, the rest genotypes, since they had below average mean yield, were not selected to any environment for grain yield (Figure 1). Different authors (Haruna et al., 2017; Kumar and Singh, 2015) have also used AMMI biplot to discriminate among OPV maize varieties.

AMMI-2 relationships among genotypes and environments

In AMMI2 biplot, the IPCA1 and IPCA2 scores of genotypes and environments were plotted against each other, depicted easy visualization of differences in interaction effects (Figure 2). The AMMI2 biplot graph showed that Dalifage1 (E_3) was the most favorable and ideal environment for the low-land OPV maize varieties; whereas, Dalifage-2 (E_4) and Awra-1 (E_1) were the average environments for OPV maize varieties. However, Awra-2 (E_2) and Dubti-2 (E_6) were found to be unfavorable environments for the present set of genotypes. The AMMI2 biplot graph also showed that varieties Melkasa-7 and Melkasa-2 were the most stable genotypes across location, which supports the results of AMMI1 biplot and ASV analysis. Whereas, Melkasa-3 and 4 were highly interactive and unstable genotypes which are then suited for high yielding favorable environments (Figure 2). However, genotype1 and 5 (Melkasa-1 and Melkasa-6Q) were not suitable to any of the environment. Similar results were reported by Sumathi et al. (2017) and Bose et al. (2014).

Genotypes located near the origin had lower interaction effects than the genotypes farther from the center of the vector. Moreover, genotypes that are closer to each other tend to manifest similar adaptability pattern and *vice versa*. Further information about the discriminating power of environments, together with a representation of their mutual relationships, can be obtained by the environment-vector of the AMMI2 biplot. In this case, a long environmental vector reflects a high capacity to discriminate the genotypes (Askari et al., 2017). Accordingly, Awra-2 and Dubti-2 had the longest vector and genotypes Melkasa-3 and Melkasa-4 are still came out the best performing genotypes in Dalifage-1 and Awra-1, respectively. These genotypes showed the highest ASV and identified as the most unstable but high yielding genotypes. The closer the genotypes to the center in AMMI2 biplot are assumed to be more stable than the genotypes far away from the center. AMMI model does not provide a quantitative stability measure and is indispensable to quantify and rank genotypes in terms of yield and stability; however, ASV quantifies and ranks genotypes (Kumar and Singh, 2015; Yong-Jian et al., 2010; Shiri, 2013; Sumathi and Govintharaj, 2017; Mortazavian et al., 2014; Miah and Uddin, 2016).

Conclusion

The present study revealed that the varieties Melkasa-7 and Melkasa-2 were identified to be the most stable open pollinated maize genotype across all location having greater yield above the grand mean, and are recommended for wider adaptation across diverse agro-ecologies of the Afar Regional State. Whereas, Melkasa-3 and Melkasa-4 were the most unstable across the test environments with outstanding grain yield and recommended for specific adaptation. The AMMI analysis also revealed that environment-3 (Dalifage-1) was the most favorable and ideal environment for best yield performance of OPV maize varieties, while Dalifage-2 and Awra-1 were average environments for better grain yield of OPV maizes. Moreover, the results of the different AMMI components were consistent in identifying the stable genotypes for specific and wide adaptation. However, yield performance in maize was greatly influenced by environment and GEI, which contributed more to the phenotypic variation. Generally, the first two IPCAs of the AMMI model were more efficient to discriminate GEI in grain yield of maize genotypes. Further testing of these OPV maize genotypes in different environments could enhance breeding efficiency with respect to genotypes' stability and adaptation across environments.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The author is grateful to the Afar Pastoral and Agro-pastoral Research Institute (APARI) for funding this research work.

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Vol. 12(1), pp. 16-24, January-March 2020
DOI: 10.5897/JPBCS2019.0815
Article Number: D036F7462847
ISSN 2006-9758

http://www.academicjournals.org/JPBCS

ACADEMIC JOURNALS

Journal of Plant Breeding and Crop Science

Full Length Research Paper

Fusarium oxysporum Race 1 resistance and quality traits variations in apple banana germplasm

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Received 28 April, 2019; Accepted 23 December, 2019

***Musa* species, AAB genome group, commonly known as Sukali Ndizi (SND) in Uganda, has attained a substantial commercial value in the recent past owing to its superior fruit quality attributes and better prices. However, its sustainable production and productivity are highly threatened by *Fusarium* wilt. To facilitate large scale area expansion of this important dessert banana, the present study was carried out to identify the near-ideotypic lines of best quality fruit traits that are also resistant to *Fusarium wilt*. Nineteen SND ecotypes were subsequently collected from nine key SND growing districts of Uganda and evaluated in the field and laboratory for different fruit quality attributes and response to *Fusarium* wilt. Results showed a wide diversity among SND ecotypes for fruit-quality traits (fruit pulp texture, flavor and taste). The ecotypes were, however, not significantly different ($p > 0.05$) for susceptibility to FOC race 1. Cluster analysis based on organoleptic and physio-chemical properties grouped the 19 ecotypes into two major-clusters, each of which was also split into two sub-clusters. Individual sub-clusters summarize levels of similarity amongst the different ecotypes. The study confirmed the presence of diversity in SND germplasm that could be exploited for SND genetic improvement of the crop through hybridization and selection.**

Key words: Sukali Ndizi, fruit-quality traits, *Fusarium* wilt, ecotypes, desert banana, diversity.

INTRODUCTION

Apple banana is the most widely distributed dessert banana cultivar in Uganda (Gold et al., 2002). It is locally known as Sukali Ndizi (SND) in central Uganda, Kabaragara in the Western region of Uganda and Kamaramasenge in Rwanda (Nsabimana and van Staden, 2006).

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The banana is most popular due to its sensorial and nutritional characteristics. It has a small fruit with a thin peel and a slightly acidic apple like taste of the pulp, which is its unique character (Van Asten et al., 2010). It is commonly sold and consumed fresh, but can also be processed for shelf-life improvement and value addition (Van Asten et al., 2010). This makes it fit well in the Uganda government policy of value addition of agricultural products (MAAIF and MFPED, 2000). Owing to its superior characters, SND has big potential regional and export markets (Akankwasa, 2007).

Although SND is important to the farmers in the East African region, it is susceptible to most banana pests and diseases, especially weevils, nematodes, black Sigatoka, yellow Sigatoka, banana bacterial wilt and *Fusarium* wilt. *Fusarium* wilt, also known as Panama disease, is the most important lethal disease of dissert bananas (Butler, 2013; Dale et al., 2017). It is a fungal disease caused by *Fusarium oxysporum f*. sp. *cubense* (FOC) (Ploetz, 2015). Foc race 1 is the primary cause of Fusarium wilt disease of SND in Uganda (Karangwa et al., 2016). It causes an estimated yield loss of >60% to this type of bananas (Tushemereirwe et al., 2000). In addition, the low yield of about 7.9 kg/bunch weight (Onyango, 2007) and non-uniformity in physio-chemical attributes among the cultivars make it difficult for the variety to sustainably penetrate the export market.

A sustainable and user-friendly strategy to control pests and diseases is by use of resistant cultivars, either by selection within existing germplasm or by introgressing resistance into the population ecotypes (Amorim et al., 2011). Diversity in any germplasm could arise as a result of occurrence of genetic variations or be induced artificially under *in vitro* conditions through use of mutagens (Bhagwat and Duncan, 1998). In bananas, production of somaclonal variants is common naturally due to meiotic instability (Withers, 1992). Whereas rates are very low, discovery of a single variant can be of great interest.

Surveys of traditional plant growing areas have been of great importance in the discovery of desirable variants which otherwise would have gone unnoticed in nature. For example, variants have been discovered in banana that exhibit desirable characters such as dwarfness (Tang and Hwang, 1998), resistance to *Fusarium* wilt disease (Ploetz, 1994) and banana cultivars of good fruit size and shape suitable for export in Brazil (Ferreira and Silver, 2002). A good understanding of genetic diversity within SND germplasm would be a useful tool in the genetic improvement of SND as it would boost the discovery and use of genes of commercial value in its improvement program.

Banana breeders have improved yield, host plant resistance to diseases and other agronomic aspects but have not targeted improvement of sensory fruit quality. To respond to consumers' needs, crop breeders must know the potential genetic variability and influence of environment on the quality traits. In this context, emphasis has shifted towards choice of parents with high performance from diverse groups. Selecting parents based on performance and genetic diversity to obtain better recombinants can provide a great opportunity to the breeder. Therefore, the present study was aimed at assessing the diversity within the SND germplasm for organoleptic, physio-chemical and Fusarium wilt race 1 resistance traits. The information generated will guide breeders to develop desirable and market preferred SND cultivars.

MATERIALS AND METHODS

Plant germplasm collection

Indigenous germplasm of SND ecotypes were collected from the major SND growing areas, representing diverse germplasm from the different eco-geographic parts in Uganda based on promising performances with regard to consumer preferences for the fruit as well as yield performance and evaluated for the traits.

Mature green SND fruits and banana plant suckers were collected from Mbarara, Masaka, Lira, Hoima, Singo, Dokolo and Mbale representing the major SND growing areas of Uganda.

Field screening of Sukali Ndizi ecotypes for resistance to *Fusarium oxysporum* f. sp. *Cubense (FOC)*

Experimental fields were established in a randomized complete-block design with four replicates at National Agricultural Research Laboratories (NARL), Kawanda between the periods of 10/09/2016 to 8/08/2017. Kawanda is located in Central Uganda at 32°36'E and 0°25'N, 1210 m above sea level (Tumuhimbise et al., 2016). During the Foc screening period, the mean annual rainfall was 1322.7 mm and temperatures ranged from 17.8 to 29°C. Kawanda is a hotspot for many pathogens and pests, including FOC race 1, weevils and nematodes. To produce enough planting materials for the experiment, the various banana suckers collected from different sources were multiplied *in vitro* (Faturoti et al., 2002). Tissue culture-derived plantlets of each ecotype, Yangambi (Km5), 1026 hybrid and Pisanglilan from International Musa Germplasm Transit Centre (ITC) (resistance to FOC) and "Kawanda-local " (susceptible to FOC) were planted in lines of seven plants per ecotype. Manuring, spacing and inoculation was done as described in Buregyeya et al. (2018). The data collected from the trials included pseudostem splitting on a scale of 1-3 and corm discoloration on a scale of 0-6 as described by Smith et al. (2008), but with some modifications, where 0= no discoloration of tissue of stellar region of corm or surrounding tissue, 1 = no discoloration of stellar region of corm; discoloration at junction of root and corm, 2 = trace to 5% of stellar region discolored, 3 = 6-20% of stellar region discolored, 4 = 21-50 of stellar region discolored, 5 = more than 50% of stellar region discolored and 6 = discoloration of the entire corm stele. Disease severity assessment based on pseudostem splitting was done using a scale of 1-3, where 1 = no cracking of the pseudostem, 2 =slight cracking of the pseudostem and 3 = advanced cracking of the pseudostem.

Physio-chemical assays on extracted juice

Juice samples from the ripe banana fruits were extracted using a commercial fruit blender (8011E Model 38BL41 Made USA). Fifty

Table 1. Severity scores for pseudostem splitting and corm discoloration due to Foc race 1 in the Uganda Sukali Ndizi ecotypes.

Zone	District	Code	Site	Pseudostem splitting scale 1-3	Corm discoloration index scale 0-6
Central	Lwengo	A	Kyazanga-Luyembe	2.19±0.8[a]	4.89± 0.33[a]
Central	Lwengo	KYA-I	Kyazanga–Kyakanyenya	2.07 ± 0.20[a]	4.57 ± 0.32[a]
Central	Lwengo	B	Kyazanga- Rwebigali	1.83 ± 0.19[a]	4.60 ± 0.32[a]
Central	Lwengo	KYA-II	Kyazanga–Mukapochi	1.69 ± 0.18[a]	4.72 ± 0.31[a]
Central	Masaka	K	Kinoni	1.93 ± 0.19[a]	4.69 ± 0.32[a]
Central	Masaka	M	Kyasonko	1.89 ± 0.19[a]	4.52 ± 0.35[a]
Central	Masaka	MSK-TN	Nzizi	2.35 ± 0.15[a]	5.11 ± 0.25[a]
South-western	Mbarara	C	Nyakayojo	2.07 ± 0.19[a]	4.76 ± 0.31[a]
South-western	Mbarara	O	Kashaka	2.04 ± 0.18[a]	4.71 ± 0.37[a]
South-western	Mbarara	F	Nyaihanga	2.00 ± 0.19[a]	5.00 ± 0.27[a]
South-western	Mbarara	Mmb-Bw	Biharwe	1.93 ± 0.21[a]	4.28 ± 0.35[a]
South-western	Mbarara	RT-mb	Mwizi	2.21 ± 0.18[a]	4.77 ± 0.32[a]
South-western	Mbarara	D	Rubaya	2.07 ± 0.19[a]	4.69 ± 0.31[a]
Western	Kiboga	N	Lwamatta	1.93± 0.18[a]	4.69 ± 0.42[a]
Western	Hoima	L	Bukwili	2.12 ± 0.18[a]	5.04 ± 0.22[a]
Eastern	Mbale	G	Bufumbo	1.96 ± 0.18[a]	4.63 ± 0.34[a]
Northern	Lira	H	Akokoro	2.10 ± 0.17[a]	4.52 ± 0.37[a]
Northern	Dokolo	J	Lwala	2.07 ± 0.18[a]	4.53± 0.31[a]
Northern	Lira	I	Agwata	2.07 ± 0.17[a]	4.90 ± 0.30[a]
Northern	Lira	Lira'J'	Boroboro	1.86 ± 0.19[a]	4.86 ± 0.27[a]
ITC	Wakiso	Km5	NBRP-kawanda	1.00 ± 0.00[b]	0.00 ± 0.00[b]
ITC	Wakiso	Psanglilin	NBRP-kawanda	1.00± 0.19[b]	0.00 ± 0.00[b]
Central	Wakiso	E (1026-hybrid)	NBRP-kawanda	1.00 ± 0.20[b]	0.00 ± 0.29[b]
Central	Wakiso	L12-1	NBRP-kawanda	2.10 ± 0.20[a]	4.79 ± 0.28[a]

Means with different letters in the same column are significantly different at α = 5%.

(50) grams of fresh banana sample were diluted in 50 mL of distilled water and blended for 1 min until homogenized and turned juicy. The mixture was centrifuged (6000 rpm, 6 min) using the Hitachi centrifuge (Hitachi Germany).

The physio-chemical assays involved the determination of the titratable acidity (% malic acid) using 0.1 M NaOH (AOAC, 2016), total soluble solutes (%Brix) using a refractometer (WZS 50 brix meter YANHE Shangai Chain) and pH using handheld pH meter (Model TDS Made China), fruit texture (pulp firmness) in kgf (Soltani et al., 2010), and the sugar/acid ratio, which was calculated using Equations 1 and 2, and 0.0067, a factor for malic acid multiplied since malic acid is the dominant acid in dessert bananas (AOAC, 2016).

$$Percentage\ Acid = \frac{\text{Titre X Acid Factor X 100}}{\text{10 (ml Juice)}} \quad (1)$$

$$Sugar\ acid\ ratio = \frac{\text{°Brix Value}}{\text{Percentage Acid}} \quad (2)$$

Organoleptic assay

Sensorial acceptance test for fruits was conducted with a panel of untrained assessors selected from staff of the National Agricultural Research Laboratories, Food Bioscience Research Department, staff from companies involved in the SND export, and SND consumers in urban markets of Kampala. The test involved individual assessment in isolated testing conditions and panelists were not permitted to discuss outcomes. The panelists were asked to assess pulp flavor, sweetness, pulp texture, pulp color and overall acceptance on a six-point scale following the method described in Micham et al. (2003).

Statistical analysis

One-way analysis of variance (ANOVA) was applied with XLSTAT 2018 to determine whether there were any statistical significant differences between the studied quality attributes and host plant resistance to FOC. Complete linkage cluster and heat –map analyses were performed in R-version 3.3.1(2016) to assess similarities in the different SND ecotypes based on their taste and physio-chemistry characteristics. Multivariate analysis of variance (MANOVA) was also performed in (R-version 3.3.1(2016) to measure the strength of the relationships among the quality determining traits.

RESULTS AND DISCUSSION

Response of ecotypes to Fusarium oxysporum *f. sp.* cubense race 1

There were no significant differences (P > 0.05) in mean scores of the all ecotypes for corm discoloration and pseudostem splitting as measures of Foc race 1 severity (Table 1). Yangambi-Km5, the highly resistant check,

showed the lowest mean scores for both pseudostem splitting and corm discoloration.

There were no significant variations among the ecotypes in their reaction to Fusarium wilt (Table 1). The only observed variation in Table 1 was as a result of genotypes Km5, Pisang lilin and E which are the known Fusarium wilt race 1 resistant genotypes. The results suggest that all ecotypes used in the study lack host plant resistance to FOC Race 1, and such resistance would have to be introgressed in from a resistant parent.

Variability of Sukali Ndizi ecotypes for chemical attributes and pulp texture

Differences between ecotypes were significant (p < 0.0001) for five physio-chemical quality traits that were studied (Figure 1A-E). The traits were total soluble sugar (TSS), texture, sugar/acid ratio, titratable acidity (TA) and pH. Highly significant differences (F_r=2020.055, p<0.0001) existed for TSS levels which ranged from 14% Brix in RT-MB ecotype to 29.1% Brix in F ecotype (Figure 1A). The results from this study indicated significant variability that could be used in a breeding program to develop improved SND varieties with varying sugar levels to target different consumer groups. For example, ecotypes with high TSS content are desirable for fruit processing.

The fruit pulp texture is an important quality trait, as the markets prefer SND fruits within a certain range (0.6 to 1.5 kgf Figure 1B) of pulp texture that is not very soft and neither very hard texture. The trait showed significant differences (F_r=22.576, p<0.0001) for which pulp texture was low (0.610 kgf) in ecotype A and high (2.089 kgf) in Km5 (Figure 1B). In Figure 1C, significant difference (F_r=14,236, p<0.0001) was observed in sugar/acid ratios from low 69.847 in C ecotype to high 160.158 in N ecotype. Since the flavor of any fruit is also contributed by the sugar/acid ratio, then the variability in this may cause the variations flavor which causes the variability in the general acceptability of the fruit.

In Figure 1D, significant variation (F_r =11.830, p<0.0001) was observed in titratable acid levels which ranged from 1.49611 g/l in the RT-MB ecotype to a maximum of 3.216 g/l in the C ecotype (Figure 1D). SND has a slightly acidic apple like taste of the pulp which is its unique characteristic, and titratable acidity may contribute towards this trait. This is mainly observed in ecotypes with high levels 3.216 g/l highly acceptable than ecotype with low levels 1.49611 g/l and this variation can be exploited by selecting and promoting the highly preferred SND ecotypes from the present germplasm.

In Figure 1E, pH lowest value 3.1 was recorded for KYA 1 ecotype while the highest was 5.3 in Km5 genotype which portrayed statistically significant difference (F_r.=5172.157, p<0.0001). The pH of the pulp may be contributing towards the overall acceptability as it is observed that Km5 genotype with very high pH is not liked by the market. These significant differences among ecotypes for the quality attributes indicated that existence of variability to have an effective selection, thus producing market friendly ecotypes.

Organoleptic analysis of the ecotypes

In Figure 1F, a significant difference (F_r=5.835, p<0.0001) in opinion regarding mouth feel was recorded. On a scale of 1-6, the panelists rated ecotype RT-MB highest at 5.421 and ecotype D lowest at 3,000. Regarding mouth feel, > 75% of the ecotypes had averages above 4, and less than 25% had less than 3.9, which meant that the ecotypes were very good apart from the 4 (D, J, E and H) as far as mouth feel was concerned. These wide phenotypic variations in mouth feel (pulp texture) is of great importance to the breeder, for example, breeding with the best preferred pulp texture varieties would be preferable to choosing RT-MB and A as putative parents.

Results for taste showed significant variation (F_r=6.327, p<0.0001) in opinion regarding the different ecotypes. The highest score on a scale of 1 – 6 was 5.625 for ecotype A and lowest (2.875) for ecotype D (Figure 1G). According to the results, all the ecotypes tasted very well as they had averages above 4 apart from the 3 ecotypes, which had averages less than 3.9. The present results confirm the long time claim by traders and consumers of the presence of different SND clones that differ in terms of fruit quality.

Aroma (odor) is one of the unique traits responsible for the popularity of SND dissert bananas. There was a statistically significant difference (F_r=2.981, p<2.981) in opinion regarding aroma Figure 1H. On a scale of 1-6, the panelists rated ecotype K highest at 5.385 and ecotype J the lowest at 3.615. Up to 16 ecotypes were above 4 implying that most of the ecotypes had the unique flavor characteristic of SND. On the contrary, the 3 ecotypes were below average 4 thus not liked by the panelists.

Of the group of panelists, Figure 1I recorded a highly significant differences (F_r=4.413, p<0.0001) views in the likeability of the color of the fruit pulp for the different ecotypes. On a scale of 1 – 6, they rated ecotype K highly at 5.385, while ecotype D was rated lowest at 3.675. The results show that 75% of the ecotypes' color was liked by the panelists and 25% of the ecotypes less appealing to the panelists.

Figure 1J overall organoleptic analysis revealed a wide diversity among SND ecotypes with most ecotypes having average general acceptability above 4, that is, 15 ecotypes and 4 ecotypes had less than 4 meaning that the largest percentage of ecotypes were more acceptable to the panelists. The results agrees with those reported by Reis et al. (2016) who reported a linear correlation between the sensorial attributes and the overall

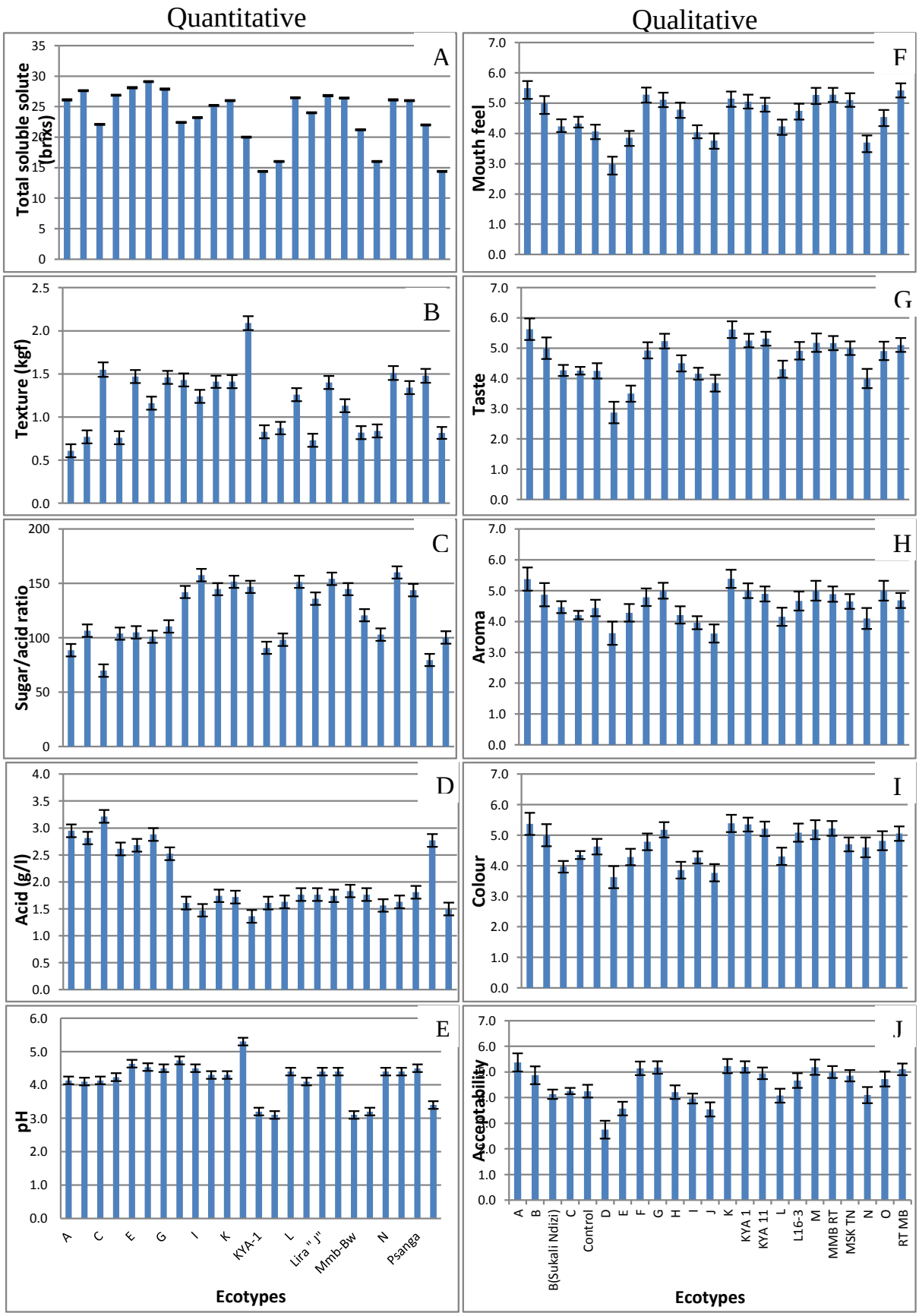

Figure 1. Variability in organoleptic and physio-chemical attributes of the ecotypes at 5% significance level.

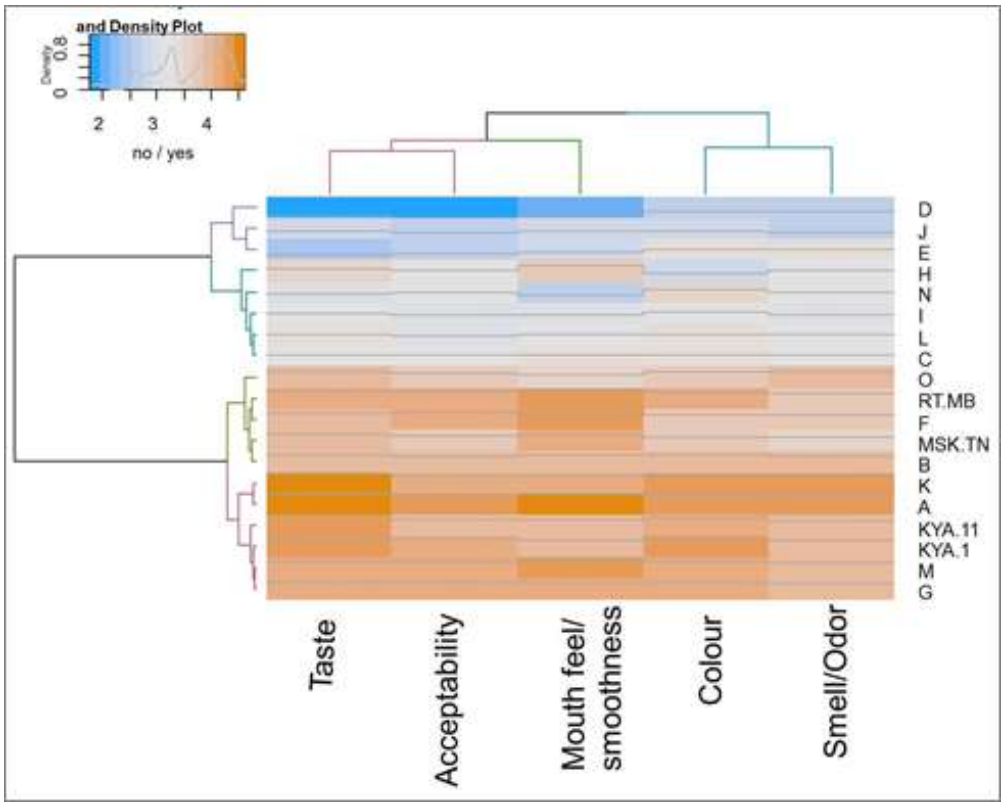

Figure 2. Comparative clustering with heat-map of Sukali Ndizi ecotypes for organoleptic fruit quality attributes.

acceptance of the bananas. Basing on this, it was observed that ecotypes K and A rated highest on almost all the attributes consistently and ecotype D scored the least on almost all the attributes and was the least liked. The wide range observed in all the characters of interest is a clear sign of variability amongst the ecotypes irrespective of origin. Donors for different SND quality characters can be selected from this germplasm but it would require an understanding of the amount of environmental variance.

Considering the regions, most of the ecotypes in Central region (Greater Masaka) were consistently rated best on all the organoleptic attributes. This could be due to selection pressure whereby farmers have resorted into planting location specific ecotype in central region. Ecotypes that were least rated on organoleptic attributes were from Northern and South-Western. Notably, no single region contributed 100% of ecotypes in the same class of rating, thus presence of variability within each region. Multivariate analysis of variance on characters of various ecotypes (both organoleptic and physio-chemical) showed very significant difference (F_r=1168.5, p<2.2 e-16) confirming the presence of wide variations in the studied traits of the ecotypes. This trait diversity evident among the Uganda SND germplasm suggests presence of opportunities for genetic improvement through selection directly from the germplasm and or selection of diverse parents for hybridization programs.

Clustering of ecotypes and diversity

Considering cluster classification for SND organoleptic quality attributes, grouped 19 SND ecotypes into four distinct clusters (Figure 2), whereby the whole group was first divided into two sub-groups as indicated by the brown and bluish colors which were finally divided into four clusters. That is brown color is divided into two smaller sub-clusters, the lower brown with 6 ecotypes and the upper one light-brown with 5 ecotypes. The lower brown contains ecotypes with average rating of all organoleptic attributes of 5 and above while the upper light-brown has most of ecotypes with average rating of all organoleptic attributes of ≤5. The bluish sub-cluster is also further divided into two groups: the very light bluish which is lower one with 5 ecotypes and the upper one bluish which has 3 ecotypes. The lower very light-bluish has most of ecotypes with average rating of all organoleptic attributes >4 and bluish upper group with ecotypes of average rating of all organoleptic attributes of <4. The brown sub-cluster is made up of the highly preferred ecotypes and the preference diminishes

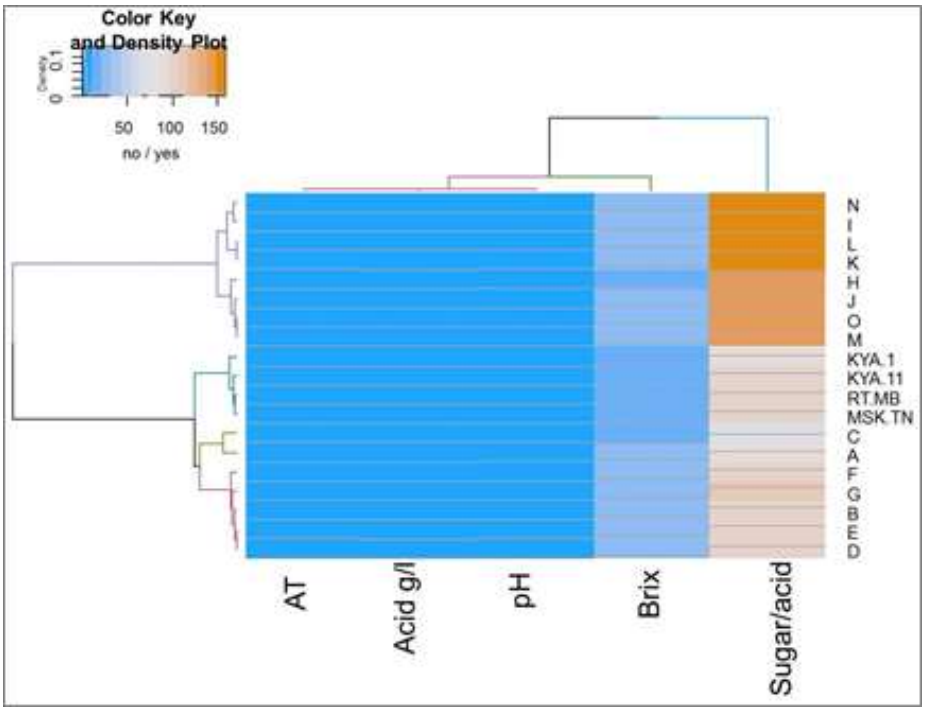

Figure 3. Comparative clustering with heat-map of Sukali Ndizi ecotypes for physio–chemical fruit quality attributes.

upwards until the bluish cluster of 3 which were not preferred by the taste panelists. With regard to the proportionate contribution of geographical origins, our clusters for organoleptic quality attributes, and the 19 ecotypes from the 5 regions were grouped into four clusters (Figure 2).

The contribution per cluster varied from 83.3 to16.7%. In this regard, large (83.3%) amounts of ecotypes were contributed to cluster I by central region, cluster II (60%) by South-western region, cluster III by (40%) South-western and cluster IV (33.3%) by Northern region. In most cases, it was difficult to see the ecotypes that were collected from one geographical origin in the same cluster, implying that they were clustered in mixture of geographical origin, which could be attributed to the free movement of planting materials among geographical origins. This might be explained as gene flow in SND attributed to human interference (since most bananas are sterile) but could only be confirmed based on molecular markers analysis clustering.

Cluster analysis based on fruit chemical quality traits grouped 19 SND ecotypes into four distinct clusters (Figure 3), whereby the whole group was first divided into two sub-groups that were finally divided into four clusters. The respective first, second, third and fourth clusters consisted of 7 ecotypes (37%), 4 ecotypes (21%), 4 ecotypes (21%) and 4 ecotypes (21%) of total ecotypes. This indicates that SND ecotypes of the same cluster group were at least with similar quality chemical attributes. The ecotypes' distribution pattern in four clusters confirmed the existence of diversity among the SND germplasm. Looking at cluster classification for SND physio-chemical quality attributes, the ecotypes were clustered into 4 groups (Figure 3). Clusters I, II, III and IV (Figure 3) were characterized by relatively high mean values of average titratable acidity and total soluble solutes, low average titratable acidity, pH and total soluble solutes, very high pH and high sugar/acid ratio respectively. For contribution of geographical origins over clusters for physio-chemical quality attributes, the results of the 19 ecotypes showed the presence of variation within the same location of collection (Figure 3). Accordingly, the ecotypes from central region were distributed into 4 clusters (I, II III and IV). It can be understood that these ecotypes are quite different for physio-chemical quality attributes though they were from the same geographic origins, suggesting a high diversity within each geographical location. Therefore, there is no need to go for geographic origins to collect genetically diverse plants in breeding for such quality traits. The possible explanation for this could be the wide divergence in the features created within each geographic origin through selection. Conversely, Western region contributed 50% of all ecotypes into cluster IV for physio-chemical quality attributes. Based on these results, Central region has wider genetic variability as compared to Western region. By the fact that Western region's ecotypes fall in the same cluster IV, implying they were similar for physio-

Table 2. Cluster means for chemical attributes.

Trait	Cluster				SD
	I	II	III	IV	
Titratable acidity	4.23	2.35	2.6	2.45	0.73
Alkalinity / acidity of pulp	4.33	3.22	4.55	4.4	1.34
Total soluble solute	26.81	15.1	25	25.44	4.84
Sugar/acid ration	97.94	98.09	143.9	155.2	32.32

chemical quality attributes, ecotypes within the cluster are similar. There are no ecotypes from the same region that occupied up to 100% of the cluster without shearing with other regions. These findings indicate that the SND germplasm from same region were diverse in physio-chemical quality attributes. Our results showed that several ecotypes were clustered together despite being collected from different geographical location, as shown in the clusters I, II, III and IV. For example, ecotypes collected from different places such as South-western, Central and Eastern regions were grouped in cluster I. Likewise, ecotypes collected from Northern, South-Western and Central regions were also clustered together in cluster III. Cluster II included ecotypes from Central and Western regions. Finally, ecotypes collected from Western, Northern and Central regions were grouped in cluster IV. The observed mix-up could be explained by the unrestricted movement of the SND planting materials from one region to another by farmers.

There were SND ecotypes with different physio-chemical quality attributes spread over the clusters, and crossing of clusters would give positive response to quality improvement. This is great opportunity in selection and breeding program to improve location-specific varieties and promote production of known SND sugar/acid ration quality profile, plus selection of appropriate parents for future breeding programs. These phenotypic analysis data are of great importance for SND breeders as they could assist in selection of appropriate ecotypes as putative parents in future breeding programs. For example, for breeding Foc race 1 resistant with consumer preferred attributes Ndizi hybrid, it would be preferable to choose female parents in cluster II (Figure 3) and cross them with diploid Foc race 1 resistant male parent.

The diverse mean values of different characters (in respect to quality chemical attributes) for different clusters (Table 2) shows that there exists a substantial level of divergence among ecotypes investigated. In fact, as regards most of the evaluated characters, the diversity amongst all the clusters is big because the mean values do differ very much. The exhibiting of difference in cluster means for various characters indicates that there is option available for identification of donors for different traits to be proposed for inclusion in hybridization program.

Conclusion

The findings of the study demonstrate the existence of diversity within SND germplasm in Uganda for quality attributes of physio-chemical and organoleptic traits. No variability was observed within SND germplasm for resistance to FOC race 1. Characterization of germplasm ecotypes based on quality traits using the hierarchical cluster analysis resulted in grouping of the germplasm ecotypes into four clusters. Most of the cluster means were significantly different, indicating the presence of variability which can be exploited through selection and hybridization. Ecotypes belonging to cluster I bear desired values for various quality traits. These ecotypes could be promoted for export.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Vol. 12(1), pp. 25-33, January-March 2020
DOI: 10.5897/JPBCS2019.0844
Article Number: 91062BC62915
ISSN 2006-9758

http://www.academicjournals.org/JPBCS

Journal of Plant Breeding and Crop Science

Full Length Research Paper

Effect of low temperature stress on field performance of highland sorghum (*Sorghum bicolor* (L.) Moench) at flowering stages

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Received 17 September, 2019; Accepted 7 January, 2020

Sorghum is a C4 grass native in the semi-arid environments of the African sub-Saharan and consequently chilling stress can affect the performance of the crop, especially at the reproductive stages. Moreover, a significant delay of flowering and maturity was observed when sorghum grows under low temperatures regions, and consequently farmers in highland areas of Uganda face yield penalties. Forty genotypes were evaluated in 2017B and 2018A seasons under non-stress (Kabanyolo) and cold stress (Kachwekano and Zombo) field conditions. Data were recorded on: Days to 50% flowering, days to physiological maturity, culm height, panicle length, panicle weight, kernel weight per panicle, and thousand grain weight. Mean comparison of most agronomic traits recorded indicated high significant differences for season-by-genotype, location-by-genotypes, and the three-way interaction (GxLxS). This indicates that cold stress significantly affects yield components. Significant positive correlation was obtained between days to 50% flowering, days to maturity, and culm height, which suggested that simultaneous improvement of these traits is possible. Some genotypes (IESV 91003LT, IESV 91105LT and IS 29376) were best ranked in normal environment but poorly performed in cold environments, which indicates lack of adaptation in highland. BM6, Cytanobe, IESV 91018, IESV 91609, IS 25563 showed generally good performance and stability in all locations. Therefore, these genotypes can be used as parental lines for further breeding process.

Key words: Sorghum, cold stress, flowering, maturity, yield component.

INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) is among the most important food and animal feed grain crop wordwide and can be considered as the best bioenergy source in this era of global climate change (Reddy et al*.,* 2008), owing to various merits in terms of tolerance to abiotic stresses (Tari et al*.,* 2013). As a C4 grass native in the As a C4 grass native in the semi-arid environments of the African Sub-Saharan regions, the crop is well adapted to hot and dry conditions. However, its gradual introduction into regions characterized by low temperatures has led to

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the evolution of adapted cold tolerant sorghum (Maulana and Tesso, 2013). Although some progress has been made, numerous abiotic stresses, including cold stress, continue to present challenges in most sorghum producing areas.

Cold stress is a major determinant in the rate of plant growth and development, as well as distribution of plant genotypes in various regions of the planet (Sharma and Solanke and Sharma, 2008; Ramankutty et al., 2008; Yadav, 2009). Sorghum genotypes differ in their growth and development at the threshold temperature of around 15°C (Singh, 1985; Maiti, 1996). Therefore, a genotype can mature faster in non-stress environment, while in cold environment maturity is delayed. This is because gene expression patterns responsible for growth and development are altered under cold environment, and therefore protein stability is compromised which impairs stem and leaf growth (Rymen et al., 2007; Janmohhamadi et al., 2015). Chilling stress was found to cause a significant decline in key cellular functions and photosynthetic activity (Allen and Ort, 2001; Rapacz et al., 2008).

The effect of cold stress on plant growth rate and days to flowering varies among sorghum genotypes (Maulana and Tesso, 2013). However, earliness was found to be affected by both genetic background, environmental conditions, or the interaction of both. Recent studies reported six maturity genes and 40 QTL with small additive effects on flowering time (Rooney and Aydin, 1999; Mace et al., 2013). Hence, development of early maturing sorghum genotypes is a paramount goal for numerous breeding programs due to the fact that harvest can be done before the new season of cold and rainy weather resumes, thereby allowing famers to increase productivity and as well reduce yield penalties.

In Uganda, sorghum is grown in almost all agricultural regions, including the higher altitude regions that cover 25% of the arable land with high population density compared to the national density (Kasozi et al., 2005). To avoid the effects of seasonal cold temperatures, farmers in highland regions of Uganda plant sorghum 4 to 6 weeks before the beginning of the cold period that usually start from February to July. However, farmers are still using unimproved varieties with a longer maturity period of about eight months. Therefore, farmers would benefit from having genotypes with reduced maturity period for production twice per annum in order to alleviate issues of malnutrition and food insecurity. The objective of this study was to evaluate the effect of cold temperatures on plant development, flowering, maturity and yield components of sorghum genotypes in order to identify genetic sources of early maturity under cold stress.

MATERIALS AND METHODS

Genetic plant materials

The forty highland sorghum genotypes used in this research study were acquired from International Crops Research Institute for Semi-Arid Tropics (ICRISAT, Nairobi – Kenya), including various breeding lines, released varieties and local landraces. The names, origins and characteristics of sorghum genotype used in the present study are given in Table 1.

Experimental design

Sorghum genotypes were evaluated during two consecutive seasons (2017B and 2018A) at three locations: Kachwekano field farm (1° 15'S, 29° 57'E, 2,200 m.a.s.l.), located in the highland of South Western region of Uganda, is characterized by a bi–modal rainy season with an annual average rainfall of 1,300 mm, and has a sandy clay loam soil; Zombo (2° 40'S, 30° 54'E; 1,705 m.a.s.l.) situated in the northern region of the country, has heavy clay loam soil with an annual temperature and cooler temperature amplitude; while Kabanyolo (0° 32'N, 32°37'E, 1,240 m.a.s.l) is mid-altitude region characterized by relative optimum temperature ranges (19 - 28°C) for sorghum growth (Table 2).

A 4 × 10 alpha lattice design was used for this experiment with three replications. Plots of 3 m by 2.25 m were laid with spacing of 30 cm within row, and 75 cm between rows. Seeds were planted at 2 cm depth and agronomic practices were applied when necessary. Insecticides (Cypermethrin) were also applied regularly in order to control stem borer and shoot flies.

Data collection and statistical analysis

Data collection included: days to 50% flowering, days to physiological maturity, culm height, panicle length, number of leaves, panicle weight, kernel weight per panicle and thousand kernel weight. Days to 50% flowering were determined as the mean number of days from planting to half-bloom stage. Days to maturity was measured as the average number of days from planting to when the grains on the lower one-third section of the panicles have reached physiological maturity (formed black layer called aleuron). Culm height was recorded as the length of the plant from the ground to the beginning of the panicle, while the panicle length was measured as the length from the beginning to the tip of the panicle. After completion of physiological maturity, panicles were detached, dried and kernel threshed to measuring yield components. Panicle weight was measured as the weight of panicles from individual plants. Kernel weight per panicle was determined as the mean weight of kernels threshed from the individual panicle. A thousand kernel weight was measured and determined from each panicle.

A Restricted Maximum Likelihood (ReML) analysis was used to generate analysis of variance (ANOVA) for single site analysis, using Genstat 18th edition (VSN International, England). For multiple interactions (Genotype x Location x Season), data were analyzed as Randomized Complete Block Design (RCBD) in which replications, locations and seasons were considered as random and genotypes as fixed effects. Means were separated by Fisher's protected least significance difference at 5% probability level. Pearson correlation was used to determine relationship among traits recorded in this experiment.

RESULTS

Growth and phenological parameters

The pooled analysis of variance including genotype, locations and seasons and their interactions, is presented in Table 3a and b. Genotypes, locations and seasons

Table 1. List of sorghum accessions, origins and characteristics used the study.

Accession name	Origin	Status	Subspecies	Seed color
ABALESHYA	Rwanda	Fixed line	Caudatum	Red
AMASUGI	Rwanda	Fixed line	Durra	Red
BM 16	Uganda	Fixed line	Caudatum	Red
BM 21	Uganda	Fixed line	Bicolor-caudatum	Red
BM 27	Kenya	Fixed line	Caudatum	Red
BM 29	Kenya	Fixed line	Bicolor-caudatum	Red
BM 6	Kenya	Fixed line	Caudatum	Red
CYTANOBE	Uganda	Fixed line	Bicolor	Red
NYUNDO	Rwanda	Fixed line	Caudatum	Red
E 1291	Kenya	Fixed line	Bicolor-caudatum	Red
IESV 90015 LT	Kenya	Breeding line	Bicolor	Red
IESV 90042 LT	Kenya	Breeding line	Caudatum	Red
IESV 91003 LT	Kenya	Breeding line	Caudatum	White
IESV 91018 LT	Kenya	Breeding line	Kafir	Red
IESV 91054 LT	Kenya	Breeding line	Kafir	Red
IESV 91069 LT	Kenya	Breeding line	Caudatum	Red
IESV 91071 LT	Kenya	Breeding line	Bicolor	Red
IESV 91073 LT	Kenya	Breeding line	Bicolor-caudatum	Red
IESV 91075 LT	Kenya	Breeding line	Caudatum	Red
IESV 91105 LT	Kenya	Breeding line	Caudatum	White
IKINYARUKA	Rwanda	Fixed line	Caudatum	Red
IS 11141	Kenya	Breeding line	Bicolor	Red
IS 11612	Kenya	Breeding line	Bicolor	Red
IS 11721	Kenya	Breeding line	Caudatum	Red
IS 11838	Kenya	Breeding line	Bicolor-caudatum	Red
IS 25546	Kenya	Breeding line	Caudatum	Red
IS 25547	Kenya	Breeding line	Caudatum	Red
IS 25557	Kenya	Breeding line	Bicolor-caudatum	Red
IS 25558	Kenya	Breeding line	Bicolor	Red
IS 25561	Kenya	Breeding line	Caudatum	Red
IS 25562	Kenya	Breeding line	Durra	Red
IS 25563	Kenya	Breeding line	Kafir	Red
IS 29415	Kenya	Breeding line	Kafir	Red
IS 25545	Kenya	Breeding line	Bicolor	Red
MB 30	Kenya	Fixed line	Caudatum	Bright orange
N 12	Uganda	Fixed line	Bicolor-caudatum	Red
N 2	Uganda	Fixed line	Bicolor	Red
NDAMOGA	Uganda	Fixed line	Caudatum	Red
S 87	Kenya	Breeding line	Caudatum	Red
IS 11442	Kenya	Breeding line	Bicolor	Red

significantly affected culm height and panicle length. Although, the average reduction of culm height was about 12 cm and 17 cm at Zombo and Kachwekano compared to the optimal growth condition of Kabanyolo, respectively, there was marked variation among sorghum lines. Results showed that late maturing genotypes such as IS 11442, IS 25545, IS 11612 and IS 11721, recorded the tallest culm height (> 250 cm) across environments (Appendix 2). Generally, culm height was greatly affected by cold temperatures, since the non-stress environment of Kabanyolo (Mean: 180.9 cm; range: 100.8 to 323 cm) recorded the higher average compared to Zombo (Mean: 168.5 cm; range: 89.6 to 295.7 cm) and Kachwekano (Mean: 163.3 cm; range: 85.3 to 281.3 cm) (Table 4).

Days to 50% flowering and maturity period were both affected by cold stress, as indicated by the significant interaction of genotype x location x season (Table 3a). As expected, Kachwekano had the longest days to 50%

Table 2. Data on climatic conditions of the field experiments at three locations.

Location	Season	Trial date (sowing-harvest)	Mean temp. (°C)	Mean max temp. (°C)	Mean min. temp. (°C)	Precipitations (mm)
Kachwekano	2017B	August-April	16.4	23.1	11.6	543
	2018A	February-October	15.3	21.9	10.8	466
Zombo	2017B	August-February	18.4	25.7	14.4	572
	2018A	February-September	19.1	25.5	13.8	508
Kabanyolo	2017B	August-January	22.4	29.2	16.6	612
	2018A	February-July	21.7	28.7	15.3	487

flowering (Mean: 138.5 days; range: 117.8 to 167.3 days) followed by Zombo (Mean: 113.2 days; range: 88.6 to 153.5 days) while the non-stress environment of Kabanyolo recorded the shortest days to flowering (Mean: 75.8 days; range: 58.2 to 108.3 days). A significant genotype x season interaction indicated that flowering took slightly longer in the second season as compared to the first season. A similar trend was observed in days to physiological maturity, since additional 56 days at Zombo, and 73 days at Kachwekano were required to complete this stage, as compared to the non-cold stress environment of Kabanyolo. Generally, genotypes IESV 90015 LT, IESV 90042 LT and IESV 91003 LT flowered earlier than others across environments, while AMASUGI, IS 255545 and IS 11442 matured later (Appendix 2). Moreover, sorghum lines such as IESV 91054 LT, and IS 29415 failed to reach their reproductive stages due to their cold susceptibility under Kachwekano and Zombo environment.

Yield components

As expected, highly significant differences on all yield components evaluated in this study were observed and the genotypes and locations contributed significantly as sources of variation (Table 3a and b). Except at Kabanyolo, the season 2018A recorded relatively inferior yield components values because of extended periods of lower temperatures that occurred from March to August 2018 at Kachwekano and Zombo (Table 4). Overall, the cold weather of Kachwekano reduced 3 to 31.6% across sorghum genotypes, as compared to Kabanyolo. Except IESV 91105 LT that ranked first in the non-cold stress environment (Mean panicle: 144.8 g) and failed to reach maturity in the cold environments of Kachwekano and Zombo, results showed that AMASUGI, BM 6, CYTANOBE and IESV 91018 LT expressed higher panicle weight across environments, however, variation among other sorghum lines were marked.

Thousand kernel weight (TKW) and kernel weight per panicle averaged 25.2 and 73.2 g, respectively, at Kabanyolo, while it decreased at Zombo (TKW: 23.3 g; Kernel weight: 63.2 g) and Kachwekano (TKW: 22.6 g; Kernel weight: 60.8 g). As expected, highest kernel weight per panicle was recorded at MUARIK (IESV 91105 LT: 121.5 g), while the maximum at Zombo and KAZARDI was 102.3 g for IESV 91105 LT and 82.6 g for BM 6, respectively. Although there was marked variation in sorghum lines across locations and seasons (significant genotype x location x season), IESV 91105 LT recorded the highest kernel weight per panicle (Mean: 121.5 g), while the maximum at Zombo and Kachwekano was 102.3 g for IESV 91105 LT and 82.6 g for BM 6, respectively. Although there was marked variation in sorghum lines across locations and seasons (significant genotype x location x season), IESV 91003 LT and IESV 91105 LT expressed a higher TKW but were partially tolerant to cold, since they were unable to survive the weather conditions of Kachwekano in the season B. However, three sorghum genotypes recorded the lowest TKW, less than 17 g, at Kachwekano (BM21, IESV 91071 LT, IS 25561), Zombo were (BM16, IS 11721, IS 29376), while AMASUGI, IS 11612, and IS 11721 were ranked as the last at Kabanyolo.

Relationship among observed traits in the field trials

At Kachwekano, days to flowering was positive and highly significantly correlated to days to maturity (r = 0.95), culm height (r = 0.63), but negatively significant correlated to thousand kernel weight (r = -0.57) and slightly correlated to panicle weight (r = -0.29) (Table 5). Days to maturity was also highly significant correlated with culm height (r = 0.65) and thousand kernel weight (r = -0.54), and slightly correlated with panicle weight (r = -0.29) but non-significant with panicle length (r = 0.12) and kernel weight (r = -0.29). Moreover, panicle weight was also highly correlated to kernel weight (r = 0.96).

A similar trend was observed at both Kabanyolo and

Table 3. (a) Mean squares of recorded traits and their interactions across all locations and seasons; (b) Mean squares of the evaluated sorghum traits and their interactions partitioned into 2017B and 2018A seasons.

Source of variation	d.f.	Days to 50% Flowering	Days to Maturity	Culm Height (cm)	Panicle Length (cm)	Panicle Weight (g)	Kernel Weight (g)	T KW (g)
Location (L)	2	239,321.2***	332,489.2***	23,827***	698.2***	14,481.9***	12,140***	354.8***
Season (S)	1	6,076.9***	10,351.5***	14.3^{ns}	27.7*	9.3^{ns}	190.8^{ns}	203.8***
L x S	2	765.8***	525.6***	511.5^{ns}	2.3^{ns}	624.4**	424.4*	81.2***
L x S/Rep	12	21.41	19.42	138.14	4.4	76.64	66.06	3.43
Genotype (G)	39	1,758.96***	1,779.43***	55,845.01***	343.23***	3,305.36***	2,240.98***	267.51***
G x L	76	140.19***	225.41***	463.90*	14.10^{ns}	143.27*	132.16*	13.89*
G x S	39	29.02^{ns}	51.71^{ns}	488.84*	10.32^{ns}	96.51^{ns}	102.00^{ns}	24.94***
G x L x S	72	49.62***	51.30***	285.45***	12.21***	90.82***	87.07***	9.42***
Pooled error	452	12.48	7.54	77.57	3.98	24.66	17.55	2.53

Source	d.f.	Days to 50% Flowering	Days to Maturity	Culm Height (cm)	Panicle Length (cm)	Panicle Weight (g)	Kernel weight (g)	TKW (g)
				2017 (Season B)				
Location (L)	2	120,707.82***	175,535.33***	9,071.24***	293.07***	5,925.23***	6,295.18***	62.08*
Rep/L	6	11.25	13.7	239.88	5.6	82.07	54.57	6.88
Genotype (G)	39	1,166.68***	997.11***	27,198.13***	161.52***	2,000.21***	1,383.22***	249.16***
G x L	73	122.46***	147.25***	308.35***	14.69***	144.59***	120.97***	9.70***
Error	224	11.92	5.76	59.74	3.23	26.28	18.07	2.63
s.e.d.		2.82	1.96	6.31	1.47	4.18	3.47	1.32
CV (%)		3.34	1.65	4.52	6.05	6.29	6.52	7.09
				2018 (Season A)				
Location (L)	2	154,288.36***	210,705.73***	1,6140.15***	377.703***	11,287.35***	8,392.96***	652.59***
Rep/L	6	31.75	19.22	101.9	3.7	142.17	117.24	1.968
Genotype (G)	49	1,170.87***	1,052.39***	29,552.40***	161.97***	2,498.08***	1,762.67***	343.93***
G x L	63	134.52***	186.87***	393.36***	10.47***	85.79***	75.75***	17.63***
Error	224	11.39	8.76	77.39	4.593	25.91	19.66	2.71
s.e.d.		2.75	2.41	7.18	1.75	4.15	3.62	1.34
CV (%)		3.1	1.94	5.14	7.27	6.18	6.64	6.73

*, **, *** Significant at P ≤ 0.05, P ≤ 0.01, and P ≤ 0.001, respectively; TKW= Thousand Kernel weight.

Zombo, where days to flowering was significantly correlated to days to maturity (r = 0.90 and r = 0.93, respectively), culm height (Kabanyolo: r = 0.53; Zombo: r = 0.56) and thousand kernel weight (Kabanyolo: r = -0.46, Zombo: r = -0.37). Days to maturity was significantly correlated to culm height (Kabanyolo: r = 0.53, Zombo: r = 0.56). Panicle length was only significant correlated to culm height (Zombo: r = 0.28; Kabanyolo: r= 0.36) and thousand kernel weight (Kabanyolo: r = -0.35; Zombo: r = -0.31).

Panicle weight was highly significant correlated

Table 4. Descriptive statistics of phenological parameters and yield related traits at three locations.

Phenological parameter	Statistics	Kabanyolo		Zombo		Kachwekano	
		2017B	2018A	2017B	2018A	2017B	2018A
Days to 50% flowering	Min	55.33	61.00	86.33	91.00	112.67	122.95
	Max	112.00	104.67	145.33	161.67	162.33	172.33
	Mean	74.85	76.78	109.94	116.49	134.11	143.06
	SD	10.93	8.51	11.56	13.51	10.55	9.91
Days to maturity	Min	93.67	101.00	129.67	135.33	158.67	166.83
	Max	151.00	141.67	190.67	218.00	208.33	219.33
	Mean	109.29	113.46	154.06	163.28	180.13	189.49
	SD	10.78	8.20	12.82	15.47	10.12	9.94
Culm height (cm)	Min	105.67	101.00	86.00	93.33	84.67	84.32
	Max	317.00	329.00	297.00	294.33	278.00	284.67
	Mean	192.28	195.67	180.23	180.07	175.83	173.44
	SD	56.29	61.32	55.26	56.55	53.89	56.31
Panicle length (cm)	Min	22.57	21.00	20.17	17.63	19.07	16.67
	Max	45.93	46.33	42.80	38.60	43.83	41.17
	Mean	31.95	31.78	29.38	28.85	28.98	28.50
	SD	4.80	5.34	4.90	4.51	4.56	4.23
Panicle weight (g)	Min	54.40	58.23	47.37	48.70	50.10	44.53
	Max	137.80	151.93	123.33	107.00	101.57	96.64
	Mean	84.87	88.29	76.72	75.31	72.78	70.08
	SD	16.58	17.23	15.08	13.13	12.74	12.21
Kernel weight (g)	Min	46.07	48.90	33.77	39.83	40.10	34.07
	Max	113.70	129.40	102.83	90.20	83.20	80.02
	Mean	69.43	71.99	58.24	60.81	58.52	56.48
	SD	13.43	14.74	13.28	11.29	10.19	10.42
Thousand kernel weight (g)	Min	15.59	16.38	14.53	16.34	16.11	16.13
	Max	38.87	40.77	37.29	38.72	35.64	34.57
	Mean	21.81	24.09	20.88	21.83	20.59	20.55
	SD	4.26	4.58	4.35	4.70	4.01	4.00

to kernel weight (Zombo: r = 0.96; Kabanyolo: r= 0.97), and thousand kernel weight (Zombo: r = 0.53; Kabanyolo: r = 0.54), but non-significant to panicle length (Table 5). Moreover, kernel weight per panicle was significantly correlated to thousand kernel weight at Kabanyolo (r = 0.54) and Zombo (r = 0.55).

DISCUSSION

Effect of cold stress to flowering time

Being a C4 plant native in the tropical regions, sorghum is sensitive to temperature below 15°C at all growth and developmental stages (Solanke and Sharma, 2008). Delays in both flowering time and days to physiological maturity are the most frequent phenomena found in cool weather environments (Kapanigowda et al*.,* 2013), especially in the African highland regions. In the present study, we noted that sorghum grown under cool weather (Kachwekano and Zombo) delayed significantly to reach days to 50% flowering and physiological maturity, even for the cold tolerant lines. This is because cold stress acts on key cellular functions, metabolism and photosynthetic activity (Rymen et al., 2007; Zhu et al*.,* 2007; Liu et al., 2019). Therefore, plants responded by slowing the growth rate during the vegetative stage, except for susceptible sorghum lines that died at early developmental stages.

Flowering time and physiological maturity are

Table 5. Phenotypic correlation among observed traits for 40 sorghum lines across locations.

Trait	Location	Days to flowering	Days to maturity	Culm height	Panicle length	Panicle weight	Kernel weight
Days to maturity	Kachwekano	0.95***					
	Zombo	0.93***					
	Kabanyolo	0.90***					
Culm height	Kachwekano	0.63***	0.65***				
	Zombo	0.64***	0.55***				
	Kabanyolo	0.70***	0.53***				
Panicle length	Kachwekano	0.19^{ns}	0.12^{ns}	0.24^{ns}			
	Zombo	0.06^{ns}	0.05^{ns}	0.28*			
	Kabanyolo	0.27^{ns}	0.28	0.36*			
Panicle weight	Kachwekano	-0.29*	-0.29*	-0.20^{ns}	-0.14^{ns}		
	Zombo	0.10^{ns}	0.11^{ns}	-0.17^{ns}	-0.21^{ns}		
	Kabanyolo	0.09^{ns}	0.12^{ns}	-0.15^{ns}	-0.19^{ns}		
Kernel weight	Kachwekano	-0.25^{ns}	-0.26^{ns}	-0.17^{ns}	-0.15^{ns}	0.96***	
	Zombo	0.04^{ns}	0.05^{ns}	-0.19^{ns}	-0.21^{ns}	0.96***	
	Kabanyolo	0.05^{ns}	0.09^{ns}	-0.13^{ns}	-0.21^{ns}	0.97***	
Thousand kernel weight	Kachwekano	-0.57***	-0.54***	-0.52***	-0.14^{ns}	0.30*	0.28*
	Zombo	-0.37**	-0.28*	-0.54***	-0.31*	0.53***	0.55***
	Kabanyolo	-0.46***	-0.32*	-0.61***	-0.35*	0.54***	0.54***

ns=non-significant, *, **, *** Significant at 0.05, 0.01 and 0.001 levels, respectively.

characteristics controlled by the genetic make-up of the plant and other environmental factors, especially temperatures (Andres and Coupland, 2012). Therefore, different sorghum genotypes can show variable responses under different temperature regime. Since the beginning of 20th century, maturity has been an important trait and one of the main focus for sorghum breeding programs (Quinby et al., 1974). This focus is because knowledge about genetic mechanism that regulate flowering and environmental factors that affect this trait (Murphy et al*.,* 2011), especially temperatures that are responsible for the plasticity in different environments (Marais et al*.,* 2013), could play an important role in the optimization of sorghum production in the highland regions.

Kabanyolo (non-stress environment) was the best environment to identify early maturing sorghum lines, since both cold tolerant and susceptible genotypes were able to reach the final maturity stages. IESV 91003 LT, IESV 91054 LT, and IESV 91105 LT showed early maturity attributes, but showed partial tolerance to cold stress at Kachwekano. This indicates that those sorghum lines could possess recessive alleles for genes responsible for maturity, since they reduce days to flowering (Wang et al*.,* 2015). However, this hypothesis needs to be tested through molecular and genetic analyses.

Generally, sorghum lines delayed by 37 and 63 days, at Zombo and Kachwekano, respectively, compared to non-stress weather conditions of Kabanyolo. Towards the end of the raining period, temperatures rose and cold stress was relieved, thus plants were able to reach their final plant height and complete maturity stage. Although all growth and phenological parameters decreased in all sorghum lines, cold-sensitive sorghum lines were seriously affected compared to tolerant variants. Moreover, physiological maturity was also affected since the grain filling period was longer in both cold environments, whereby the delay caused by this abiotic stress averaged 47 days at Zombo, and 73 days at Kachwekano.

Effects of cold stress on yield components

In tropical native plants like sorghum and maize, low temperature stress cause significant reduction in photosynthetic activity and biomass accumulation, which are the main sources of grain yield (Tari et al., 2013; Fiedler et al., 2014; Ortiz et al., 2017). In fact, cold stress negatively affects chlorophyll function, and consequently photosynthetic activities are significantly decreased (Allen

and Ort, 2001; Tari et al., 2013). Furthermore, reproductive organs of plants grown cool environments can be seriously damaged and consequently cause reduction in the yield and yield components, when cold stress coincides with the grain filling period (Pereira da Cruz et al., 2006; Maulana and Tesso, 2013). In case where cold temperatures coincide with male and female organs formation, it may cause irreversible damages such as reduction anthesis rate, failed fertilization, reduced grain filling, which lead to the insufficient grain number per panicle and consequently low grain yield (Clarke and Siddique, 2004; Thakur et al*.,* 2010; Maulana and Tesso, 2013).

The comparison between the non-stress (Kabanyolo) and cold stressed environments (Kachwekano and Zombo) indicated that yield related components were reduced for all evaluated genotypes, although some marked differences were identified whereby some genotypes could only yield a half of their actual performance as compared to Kabanyolo. However, cold tolerance is mainly determined by the levels of expression of cold tolerant responsive genes in the line *per se* (Janmohammadi et al*.,* 2015). Cold tolerant genotypes have developed adaptation strategies to withstand cold stress through cold acclimation, whereby plants adjust to cold tolerance by exposing them to low but non-freezing temperatures (Thomashow, 1999, Chinnusamy et al*.,* 2007). Genetic variability exists in sorghum adapted to high altitude areas of Africa, including the Eastern-African highland regions, which are considered as an important source of cold tolerant sorghum gene pool (Balota et al*.,* 2010).

Conclusion

Low temperatures that coincide with vegetative period can affect various metabolic pathways, slowing growth rate and reduce photosynthetic activities. Consequently, susceptible plants would not survive, while tolerant genotypes with taller plant height can reach flowering and physiological maturity later. In the present study, sorghum genotypes with shorter stature coupled with tolerance to coldness were best ranked as early maturing in the cold environments of the highlands regions of Uganda, and thus can be used as parental lines for future breeding research based on line *per se* performance. Therefore, sorghum breeders need to constantly improve the genetic materials as far as flowering and grain filling period are concerned, as well as other agronomic traits based on farmer's preferences, since this strategy would result in reducing yield penalty and contribute to enhancement of food security.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors are grateful to Makerere University for providing education and technical expertise, and ICRISAT (Kenya) for providing sorghum lines used in this research study. This research was funded by the Alliance for Green Revolution in Africa (AGRA).

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Vol. 12(1), pp. 34-49, January-March 2020
DOI: 10.5897/JPBCS2019.0836
Article Number: C3727B962917
ISSN 2006-9758

http://www.academicjournals.org/JPBCS

Journal of Plant Breeding and Crop Science

Full Length Research Paper

Agro-morphological characterization of pigeonpea (*Cajanus cajan* L. Millspaugh) landraces grown in Benin: Implications for breeding and conservation

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Received 19 August, 2019; Accepted 7 January, 2020

Pigeonpea (*Cajanus cajan* L. Millspaugh) is a neglected and under-utilized crop consumed in several regions of word. In order to assess performance of pigeonpea landraces grown in Benin for useful breeding programs, 50 accessions were collected from 39 villages. These accessions were characterized by using 12 qualitative and 11 quantitative traits. Based on the seeds morphological characteristics, the 50 accessions were grouped in 12 morphotypes. However, 8 morphological classes were obtained with cluster analysis based on the unweighted pair group method with arithmetic average method using qualitative traits, whereas in principal component analysis only 5 clusters have been obtained using quantitative traits. The association/correlation among quantitative characters showed that grain yield was negatively correlated with pod width, days to 50% flowering and physiological maturity while it was positively correlated with pod length, pods per plant, branches per plant and number of seeds per pod. Based on four quantitative traits (number of pods per plant, number of seeds per pod, 100 seed weight, and early maturity), the 23 accessions from cluster 3 of whom kk5 (*Ekloui*), kk8 (*Nontchiovi kloui*), kk15 (*Otili founfoun*), kk18 (*Klouékoun wéwé*), kk22 (*Otili*), kk23 (*CA monlikoun*) and kk28 (*Hounkoun wéwé*) have been recommended as good sources of germplasm for improving the pigeonpea productivity. Further characterization using molecular techniques as well as conservation attention should be conducted to confirm the present result and maintain the germplasm for future breeding programs.

Keywords: Benin, Cluster analysis, morphological diversity, pigeonpea, quantitative characters, selection.

INTRODUCTION

Pigeonpea (*Cajanus cajan* L. Millspaugh) is a shrub, which plays an important role in food security, nutritional

balance and poverty alleviation in sub-Saharan Africa (Rao et al., 2002). It is predominantly cultivated in the developing countries of tropical and subtropical environments (Suman et al., 2017). Africa, with 19.03% of the world's total production represents the second producer followed by Americas (3.15%) and behind Asia (77.82%) (Anon, 2017a). In Benin, though this legume is not considered by farmers as a priority crop, pigeonpea is the sixth-largest legume crop with a cultivated area of 3027 ha with an average yield of 1843 tons, behind groundnut, cowpea, soybeans, bambara groundnut and Kersting's groundnut (Anon, 2017b).

Various parts of pigeonpea plant are used for food consumption, as medicine for cure diseases. Leaves are used in traditional medicine to cure diseases such as malaria and fever, in Benin (Dansi et al., 2012; Ayenan et al., 2017; Zavinon et al., 2018), in Nigeria (Aiyéloja and Bello, 2006; Oladunmoye et al., 2011) and in South Africa (Mander et al., 1996). In most African countries, seeds are used in human nutrition as food in combination with cereals and in commercialization (Odeny, 2007; Dansi et al., 2012; Ayenan et al., 2017). In Benin, seeds are highly consumed in the Adja cultural area in the South-East (Dansi et al., 2012). Pigeonpea also has a strong potential to contribute to food security through market possibilities and by using it to make up for the shortage of cowpea, maize and other staple foods during lean season (Ayenan et al., 2017). The plant is also useful in soil conservation and weed management (Versteeg and Koudokpon, 1993; Aihou, 2003; Dansi et al., 2012).

The potential yield of pigeonpea is estimated at 2500 kg/ha, while the yields obtained on farmer's fields is estimated at 736.2 kg/ha in Africa and 620 kg/ha in Benin (Dutta et al., 2011; Anon, 2017b). The relatively lower yield obtained is due to biotic and abiotic constraints and as well lack of quality seed (Ayenan et al., 2017). Moreover, these constraints can cause yield penalty of pigeonpea and could be involved in the long term process disappearance of some landraces. In fact, the evaluation of genetic diversity is essential for efficient use and conservation of pigeonpea genetic resources (Shende and Raut, 2013). It is therefore important to know genetic variability among pigeonpea landrace in Benin for future breeding research and conservative management.

In Benin, various landraces of pigeonpea are grown across different ecological zones and their vernacular names were given by farmers to distinguish them. However, pigeonpea's vernacular names usually vary from one ethnic group to another, from one village to another within the same ethnic area and sometimes from one household to another within the same village (Ayenan et al., 2017). In this context a cultivar across villages may be designated by different names while different cultivars can sometimes be designated by the same name (Otoo et al., 2009; Agre et al., 2015). For instance, in the Guinean and Sudano-Guinean zones of Benin, pigeonpea is called Hounkoun, Kloué or Klouékoun by farmers belonging to Fon and Mahi sociolinguistic groups while in the Guinean and Sudanian zones, pigeonpea is called Otili by farmers belonging to Nago and Dendi sociolinguistic groups (Kinhoégbè et al., 2019). This constitutes a bias in the estimation of pigeonpea diversity. Characterization of existing landraces germplasm is a prerequisite step for identifying potential germplasm to be used in breeding program and also avoid duplication in the germplasm collection.

Different methods can be used to access genetic variability in plant species, such as pedigree data, morphological and molecular markers. The use of agro-morphological traits is the most common approach utilized to estimate relationships between genotypes and provide information for plant breeding programs (Bajracharya et al., 2006; De, 2019). Data obtained by landrace description are futher statistically processed. Multivariate analysis such as cluster analysis, Principal Component Analysis (PCA) and discriminate analysis is the most commonly used approach for genetic variability estimation to illuminate the patterns of variation in germplasm collections. Among multivariate techniques, PCA and cluster analysis are preferred tools for morphological characterization of genotypes and their grouping on similarity basis (Mohammadi and Prasanna, 2003). Cluster analysis is used to reveal the association between landraces while relationships between traits are statistically analyzed using PCA. Landraces can be grouped together based on informative data and be used directly in a breeding program. In Africa, many studies have been conducted to examine patterns of genetic diversity among pigeonpea accessions using both qualitative and quantitative agro-morphological descriptors (Silim et al., 2005; Manyasa et al., 2008; 2009; Gwata and Slim, 2009; Vange and Egbe, 2009; Kundy et al., 2015). Unfortunately, in Benin, very scarce study has been done to characterize pigeonpea landraces (Quenum et al., 2016). This study however based on the evaluation of the pigeonpea seeds quality, allowed a partial characterization of the plants of the different morphotypes consequently, different landraces agronomic performances were not evaluated and conservation strategy of this genetic resource has not been developed in Benin. The objectives of this study were to classify the different pigeonpea landraces under cultivation in Southern and Central region of Benin and evaluate the agronomic performance of these accessions.

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MATERIALS AND METHODS

Description of experimental site

The present study was carried out in the experimental site of the Laboratory BIORAVE (Center for Research, Training, Incubation, Technological Innovation and Seed Production for Agricultural Development) at Massi (9°55'0" N and 1°28'0" E) in the municipality of Zogbodomè (Benin Republic) during the cropping season of 2017 to 2018 (April 2017 to January 2018). The site benefits a sub-equatorial climate with two dry seasons and two rainy seasons. The long rainy season extends from March to July and the short one from September to November. As for the dry seasons, they cover the period from December to March, and from July to September (Adam and Boko, 1993). The average annual temperature varies between 26 and 28°C (Yabi and Afouda, 2012) and the annual rainfall varies between 800 and 1,200 mm (Adam and Boko, 1993). The soil is ferruginous type dominated by sandy-clay sediments.

Plant material

The study was carried out on 50 accessions of pigeonpea, collected from 39 villages belonging to 7 different ethnic groups located in the departments of Southern and Central part of Benin (Kinhoégbè et al., 2019). In fact, 54 accessions were collected during an ethnobotanical survey and according to famers seem to have different agronomical performances. From these 54 accessions, four did not germinate and data were collected on 50 accessions that germinated during the experiment. Among these accessions, 29 were collected from Central region and 21 from South (Table 1).

Field layout

The experimental design used was randomized complete block (RCBD) with three repetitions. We used tree blocks of 50 plots corresponding to the 50 pigeonpea accessions. Plots were 11 m length with 1.5 m and 1 m row spacing. At the time of sowing, three seeds were put in a pouch. The depth of sowing was 3cm. After 30 days, extra plants were removed and the most healthier and vigorous plants were left for phenotyping. The experiment was carried out without application of fertilizer since the soil is naturally fertile enough to support the crop.

Morphological traits/characters studied

Firstly, seed classification was made based on seed's morphological description characteristics (seed colour pattern, seed colour, seed eye colour, seed shape and seed size as described in Loko et al. (2018). Secondly, a total of 23 characters including 12 qualitative (Table 2), 11 quantitative (Table 3) were recorded according to the descriptors of *C. cajan* recommended by IBPGR and ICRISAT (1993). The different traits: plant height (PlHe), stem thickness (StT), branches per plant (BrP), pod length (PL), pod width (PWi), number of pods per plant (PPl), number of seeds per pod (SP), grain yield (GY), 100-seed weight (100SW), days to 50% flowering (D50F), physiological maturity (PhM), growth habit (GH), leaflet shape (LSh), base flower colour (BFCo), pod colour (PCo), pod colour pattern PCoPa), pod shape (PSh), pod form (PFo), seed shape (SSh), seed colour pattern (SCoPa), seed colour (SCo), seed eye colour (SECo), and seed size (SSi); were measured from vegetative stage until harvest according to the nature of each trait. For instance, growth habit and leaflet shape were recorded at preflowering while the base flower colour was recorded at flowering. Seed colour pattern, seed size and seed colour were recorded at the harvest of dried seeds, plant height, stem thickness and branches per plant at the end of flowering, number of pods per plant and number of seeds per pod at the first and second harvest of dried seeds (Tables 2 and 3). Data were recorded on five plants randomly selected from the eight planted in each row except the bordering plants in each row.

Data analysis

To group accessions with homogeneous morphological class, the genetic distance between accessions was calculated according to Nei (1972). The distance matrix obtained served for the construction of a dendrogram by the UPGMA (Unweighted Pair Group Method with Arithmetic average) method using SAHN (Sequential Agglomerative Hierarchical Nested) clustering of the NTSYS-pc software (Rohlf, 2000). Subsequently, using Minitab 16 software, the quantitative characters were initially subjected to a descriptive statistic and secondly to see relation between pairs of quantitative characters, Pearson correlation coefficient was performed. To examine the contribution of each quantitative character to total genetic variation, Principal Component Analysis (PCA) was performed. Then, on the basis of the Principal Component Analysis (PCA), accessions were projected on the first two PCs, in order to group different accession into clusters. In order to determine the differences in performance of the landraces for each agronomic trait, analysis of variance (ANOVA) was performed by using Minitab 16 software. Significant differences between means were observed using Turkey test ($p < 0.05$) (Sangseok and Dong, 2018).

RESULTS

Distribution of phenotypic characters

The 50 accessions were classified in twelve (12) morphotypes according to the seed morphological description characteristics (Figure 1). The number of accession for each group, the accessions and their characteristics are presented in Table 4. Based on this classification, the majority of pigeonpea cultivar grown were of cream seed colour. The analysis of the variability of qualitative characters showed that all the evaluated characters were polymorphic (Table 5). From the results, 34 accessions showed semi-spreading growth habit and 48 lanceolated leaflet shape. Thirty-six landraces showed light yellow colour for base flower and 34 had green pod colour. Sixteen landrace showed right pod shape, 10 cylindrical pod form and 43 oval seed shape. Forty-two showed plain seed colour pattern and 42 accessions showed cream seed colour. Thirty and thirty-nine accessions showed red eye colour and intermediate size, respectively.

The characterization based on the 12 qualitative characters grouped the 50 accessions in 11 morphological type assembled in eight morphological classes named C1 to C8 (Figure 2).

- C1 (4 accessions) is characterized by erect growth habit, lanceolated leaflet, curved and flatted pod totally coloured in green containing oval and cream seeds.
- C2 (2 accessions) is characterized by erect growth

Table 1. List of the 50 studied pigeonpea accessions, their code, corresponding prospected village, districts, locality and sociolinguistic group where accession was collected.

N°	Local name	Codes	Villages	Districts	Localities	Sociolinguistic group
1	Adja Kloui	kk34	Fangnonhoué	Lalo	Southern	Adja
2	CA Monlikoun	kk23	N'gbèhouédo	Ouèssè	Central	Mahi
3	Carder Ekloui	kk33	Toimey	Klouékanmè	Southern	Adja
4	Carder Ekloui	kk35	Toimey	Klouékanmè	Southern	Adja
5	Ekloui	kk2	Dékpo	Aplahoué	Southern	Adja
6	Ekloui	kk5	Hélétoumey	Aplahoué	Southern	Adja
7	Ekloui	kk6	Hélétoumey	Aplahoué	Southern	Adja
8	Ekloui	kk38	Djowé	Aplahoué	Southern	Adja
9	Ekloui Ri	kk12	Golouhoué	Klouékanmè	Southern	Adja
10	Ekloui Ri	kk39	Golouhoué	Klouékanmè	Southern	Adja
11	Hounkoun Wéwé	kk28	Adaklamè-Dénou	Kétou	Southern	Mahi
12	Kloué	kk16	N'gbèhouédo	Ouèssè	Central	Mahi
13	Kloué	kk29	Atomey-Kpodji	Aplahoué	Southern	Adja
14	Kloué	kk36	Towé	Pobè	Southern	Yorouba
15	Klouékoun Vôvô	kk47	Hèlontèdji	Zangnannado	Central	Fon
16	Klouékoun Wéwé	kk4	Kpakpassa	Savalou	Central	Mahi
17	Klouékoun Wéwé	kk14	Soclogbo	Dassa-Zoumè	Central	Mahi
18	Klouékoun Wéwé	kk18	Kpakpassa	Savalou	Central	Mahi
19	Klouékoun Wéwé	kk20	Katakou	Savè	Central	Fon
20	Klouékoun Wéwé	kk37	Kèmondji	Zakpota	Central	Fon
21	Klouékoun Wéwé	kk40	Gossoé	Zangnannado	Central	Fon
22	Klouékoun Wéwé	kk41	Kèmondji	Zakpota	Central	Fon
23	Klouékoun Wéwé	kk43	Sohoungo	Zakpota	Central	Fon
24	Klouékoun Wéwé	kk44	Hounsso	Covè	Central	Fon
25	Klouékoun Wéwé	kk45	Hèlontèdji	Zangnannado	Central	Fon
26	Klouékoun Wéwé	kk46	Abahogo	Zangnannado	Central	Fon
27	Klouékoun Wéwé	kk48	Gossoé	Zangnannado	Central	Fon
28	Klouékoun Wéwé	kk49	Gossoé	Zangnannado	Central	Fon
29	Klouékoun Wéwé	kk50	Gbihoungon	Djidja	Central	Fon
30	Klouékoun wlanwlan	kk17	Monsourou	Djidja	Central	Fon
31	Klouékoun wlanwlan	kk27	Gbihoungon	Djidja	Central	Fon
32	Klouékoun wlanwlan	kk42	Gossoé	Zangnannado	Central	Fon
33	Nontchiovi Kloui	kk8	Hélétoumey	Aplahoué	Southern	Adja
34	Otili	kk22	Olata	Ouèssè	Central	Nago
35	Otili Founfoun	kk15	Towé	Pobè	Southern	Yorouba
36	Otili Founfoun	kk19	Oké-Ola	Kétou	Southern	Holly
37	Otili Founfoun	kk21	Chaffou	Pobè	Southern	Yorouba
38	Otili Founfoun	kk25	Ayétédjou	Dassa-Zoumè	Central	Holly
39	Otili Founfoun	kk32	Kèmon	Ouèssè	Central	Nago
40	Otili Founfoun Kékélé	kk7	Ferme Gbagba	Savè	Central	Biali
41	Otili Founfoun Lakoun	kk11	Monsourou	Djidja	Central	Fon
42	Otili Kpoukpa	kk13	Oké-Odja	Pobè	Southern	Yorouba
43	Otili Kpoukpa	kk30	Towé	Pobè	Southern	Yorouba
44	Otili Kpoukpa	kk31	Ayétédjou	Dassa-Zoumè	Central	Holly
45	Otini Founfoun	kk26	Ayétédjou	Dassa-Zoumè	Central	Holly
46	Otini Kpoukpa	kk1	Ayétédjou	Dassa-Zoumè	Central	Holly
47	Otini Kpoukpa	kk3	Oké-Ola	Kétou	Southern	Holly
48	Otini Kpoukpa	kk24	Oké-Ola	Kétou	Southern	Holly
49	Otini Tchofiti	kk9	Ayétédjou	Dassa-Zoumè	Central	Holly
50	Wlétchivé Kloui	kk10	Djowé	Aplahoué	Southern	Adja

Table 2. Qualitative morphological characters evaluated.

Character	Codes	Period of observation	Variables and score
Growth habit	GH	Preflowering	Erect (1) Semi-spreading (2) Spreading (3)
Leaflet shape	LSh	Preflowering	Oblong-lanceolate (1) Lanceolate (2)
Base flower colour	BFCo	Flowering	Light yellow (1) Yellow (2) Orange-yellow (3)
Pod colour	PCo	Harvest of green seeds	Green (1) Purple (2) Mixed (3)
Pod colour pattern	PCoPa	Harvest of green seeds	Total (1) Spots or bands dark rose (2) Pigmentation on the surface or in the cavities of the pod (3)
Pod shape	PSh	Harvest of green seeds	Right (1) Curve (2)
Pod form	PFo	Harvest of dried seeds	Flat (1) Cylindrical (2)
Seed shape	SSh	Harvest of dried seeds	Oval (1) Globular (2) Square (3)
Seed colour pattern	SCoPa	Harvest of dried seeds	Plain (1) Mottled (2)
Seed colour	SCo	Harvest of dried seeds	Cream (1) Blackish (2) Red (3) Brown (4) Light-red (5)
Seed eye colour	SECo	Harvest of dried seeds	Red (1) Black (2) No one (3)
Seed size	SSi	Harvest of dried seeds	Small (1) Intermediate (2) High (3)

habit, lanceolated leaflet, curved and flatted pod totally coloured in green containing globular and high cream seeds having red eyes.

- C3 (23 accessions) is characterized by erect growth habit, lanceolated leaflet, curved and flatted pod totally coloured in green containing globular and cream seeds having red eyes and intermediate size.
- C4 (5 accessions) is similar to the previous (C3) with the only difference by grouping seeds with small size.
- C5 (6 accession) is characterized by semi-spreading growth habit, lanceolated leaflet, light yellow base flower, right and flat pods having mixed colour with pigmentation on the surface or in their cavities, containing oval and mottled seeds having intermediate size.
- C6 (2 accessions) is characterized by spreading growth habit, oblong lanceolated leaflet, light yellow base flower, cylindrical and right pod having mixed colour with pigmentation on the surface or in theirs cavities, containing globular and mottled seeds having intermediate size
- C7 (3 accession) is characterized by semi-spreading growth habit, lanceolated leaflet, light yellow base flower, right and cylindrical pod shapes having purple colour with spots or bands dark rose, containing squared seeds entirely coloured in light-red having intermediate size.
- C8 (5 accessions) is characterized by semi-spreading growth habit, lanceolated leaflet, light yellow base flower, right and cylindrical pod having purple colour with spots

Table 3. Quantitative morphological characters evaluated.

Character	Code	Period of observation	Unit
Plant height	PlHe	End of flowering	m
Stem thickness	StT	End of flowering	mm
Branches per plant	BrPl	End of flowering	unity
Pod length	PL	Harvest of dried seeds	mm
Pod width	PWi	Harvest of dried seeds	mm
Days of 50% flowering	D50F	Flowering	Days
Physiological maturity	PhM	Physiological maturity	Days
Number of pods per plant	PPl	1st and 2nd harvest of dried seeds	unity
Number of seeds per pod	SP	1st and 2nd harvest of dried seeds	unity
Grain yield	GY	Harvest of dried seeds	tonnes/ha
100-seed weight	100SW	1st and 2nd harvest of dried seeds	g

Figure 1. Pictures of different groups obtained from seeds classification.

Table 4. Accessions corresponding of each group obtained based on morphological characteristics.

Group	Number of cultivars	Cultivar	Seed colour pattern	Seed colour	Seed eye colour	Seed shape	Size
G1	2	kk44; kk48	Plain	Cream	Black	Oval	Intermediate
G2	23	kk5; kk8; kk12; kk15; kk18; kk20; kk21; kk22; kk23; kk25; kk28; kk29; kk33; kk34; kk38; kk39; kk40; kk41; kk43; kk45; kk46; kk49; kk50	Plain	Cream	Red	Oval	Intermediate
G3	2	kk35; kk36	Plain	Cream	No one	Oval	High
G4	5	kk9; kk10; kk17; kk27; kk42	Highly mottled	Cream	No one	Oval	Intermediate
G5	1	kk11	Mottled	Cream	No one	Oval	Intermediate
G6	1	kk31	Plain	Brown	No one	Oval	Intermediate
G7	3	kk1; kk24; kk30	Plain	Red	No one	Oval	Intermediate
G8	3	kk3; kk13; kk47	Plain	Light red	No one	Square	Intermediate
G9	1	kk32	Plain	Blackish	No one	Oval	Intermediate
G10	5	kk4; kk7; kk14; kk16; kk37	Plain	Cream	Red	Oval	Small
G11	2	kk19; kk26	Plain	Cream	Red	Globular	High
G12	2	kk2; kk6	Mottled	Cream	No one	Globular	High

Table 5. Frequency of appearance of qualitative variables in set of collection

Character	Variables and score	Number of accession
Growth habit	Erect	14
	Semi-spreading	34
	Spreading	2
Leaflet shape	Oblong-lanceolate	2
	Lanceolate	48
Base flower colour	Light yellow	36
	Yellow	8
	Orange-yellow	6
Pod colour	Green	34
	Purple	8
	Mixed	8
Pod colour pattern	Total	34
	Spots or bands dark rose	8
	Pigmentation on the surface or in the cavities of the pod	8
Pod shape	Right	16
	Curve	34
Pod form	Flat	40
	Cylindrical	10
Seed shape	Oval	43
	Globular	4
	Square	3
Seed colour pattern	Plain	42
	Mottled	8
Seed colour	Cream	42
	Blackish	1
	Red	3
	Brown	1

Table 5. Contd.

	Light-red	**3**
Seed eye colour	Red	30
	Black	2
	No one	18
Seed size	Small	5
	Intermediate	39
	High	6

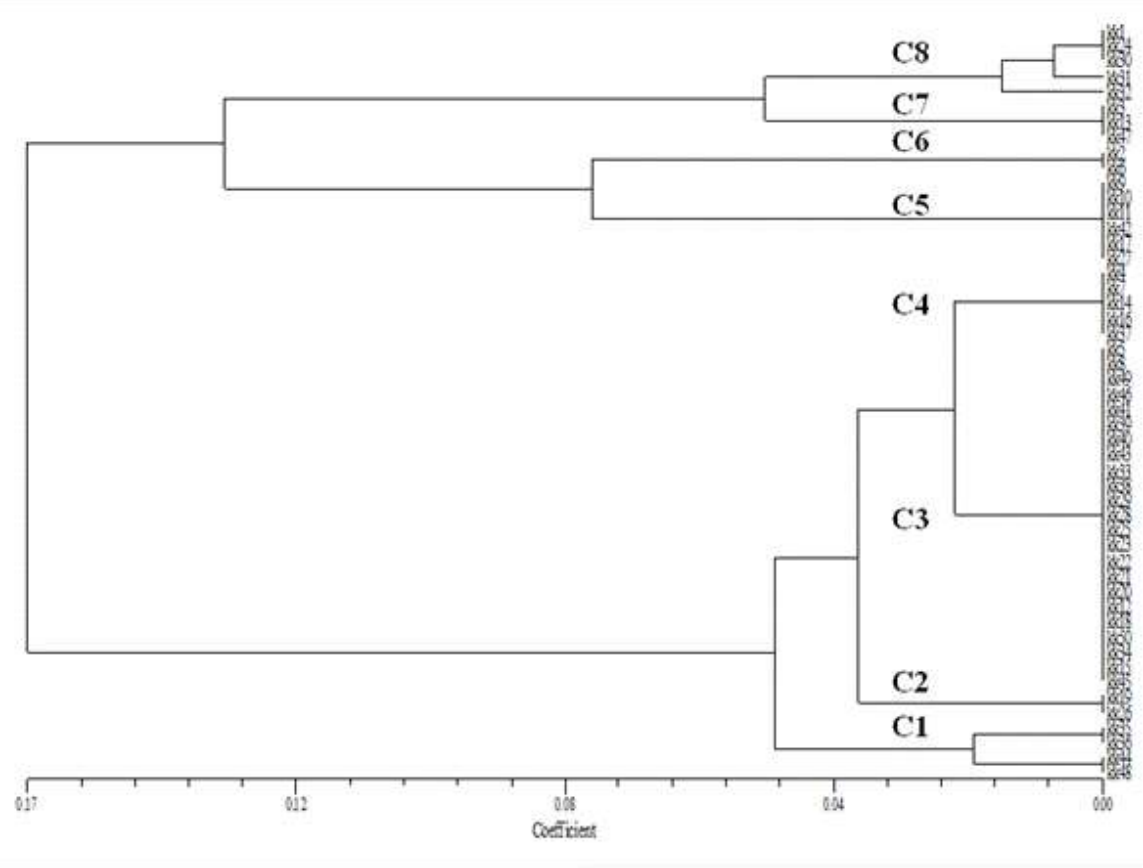

Figure 2. Dendrogram showing different morphological types assembly in morphological classes of pigeonpea in Benin using UPGMA method.

or bands dark rose, containing oval seeds entirely coloured, having intermediate size and without pigmentation and seeds eyes.

Agro-morphological evaluation based on quantitative traits

The results (Table 6) showed that branches per plant, number of pods per plant, pod width and grain yield were the most variable when referring to their coefficient of variation. The plant height ranged from 1.86 m (kk31) to 3.35 m (kk15) with an average of 2.93 m. The stem thickness ranged from 26.20 mm (kk31) to 66.20 mm (kk21) with an average of 51.93 mm. Mean number of branches per plant was 33.79 unities. The length of the pods ranged from 41.80 mm (kk17) to 71.33 mm (kk15), with an average of 61.74 mm and coefficient of variation of 16.06% while the width of the pods ranged from 3.48 mm (kk15) to 8.14 mm (kk19; kk26), with an average of 5.70 mm and a coefficient of variation of 37%. The number of pods per plant ranged from 134.60 unities

Table 6. Descriptive statistics of the quantitative characters evaluated.

Character	Mean	Min	Max	CoeffVar	StDev
PlHe	2.93±0.08	1.86	3.35	18.64	0.55
StT	51.93±1.93	26.20	66.20	26.32	13.67
BrPl	33.79±1.80	5.00	45.83	37.68	12.73
PL	61.74±1.40	41.80	71.33	16.06	9.92
PWi	5.70±0.30	3.48	8.14	37.00	2.11
PPl	1340.30±88.40	134.60	1956.30	46.62	624.80
SP	5.14±0.12	3.40	5.83	15.87	0.82
GY	3.73±0.17	0.55	4.74	32.55	1.21
100SW	10.84±0.21	7.54	12.50	13.84	1.50
D50F	135.21±3.95	109.00	185.00	20.67	27.94
PhM	174.77±2.78	156.00	228.00	11.24	19.64

Min: Minimal; Max: Maximal; CoeffVar: Coefficient of Variation; StDev: standard deviation; PlHe: Plant height; StT: Stem thickness; BrPl: Branches per plant; PL: Pod length; PWi: Pod width; PPl: Number of pods per plant; SP: Number of seeds per pod; GY: Grain yield; 100SW: 100-seed weight; D50F: Days of 50% flowering; PhM: Physiological maturity

Table 7. Correlation matrix among quantitative characters.

Character	PlHe	StT	BrP	PL	PWi	PPl	SP	GY	100SW	D50F	PhM
PlHe	1										
StT	0.96***	1									
BrP	0.37*	0.22ns	1								
PL	0.92***	0.82***	0.58***	1							
PWi	-0.54***	-0.39*	-0.89***	-0.79***	1						
PPl	0.91***	0.94***	0.42**	0.82***	-0.56***	1					
SP	0.89***	0.77***	0.56***	0.99***	-0.79***	0.76***	1				
GY	0.98***	0.95***	0.47**	0.89***	-0.56***	0.93***	0.85***				
100SW	0.14ns	0.36*	-0.43**	-0.18ns	0.48***	0.34*	-0.26ns	0.19ns	1		
D50F	-0.27ns	-0.27ns	-0.75***	-0.34*	0.67***	-0.58***	-0.28ns	-0.39*	-0.10ns	1	
PhM	-0.46***	-0.45**	-0.77***	-0.47***	0.66***	-0.70***	-0.40**	-0.58***	-0.16ns	0.95***	1

PlHe: Plant height; StT: Stem thickness; BrP: Branches per plant; PL: Pod length; PWi: Pod width; PPl: Number of pods per plant; SP: Number of seeds per pod; GY: Grain yield; 100SW: 100-seed weight; D50F: Days to 50% flowering ; PhM: Physiological maturity; Significant correlations at *p < 0.05, **p < 0.01, ***p < 0.001; ns: not significant.

(kk32) to 1956.25 unities (kk15) with an average of 1340 unities. The mean of number of seed per pod was 5.14 unities. Grain yield ranged from 0.55 tons/ha (kk32) to 4.74 tons/ha (kk15; kk22 and kk25) with an average of 3.73 tons/ha. The 100-seed weight ranged from 7.54 g (kk4) to 12.5 g (kk19; kk21 and kk24) with an average of 10.84 g. The days to 50% flowering ranged from 109 days (kk15 and kk22) to 185 days (kk32) with an average of 135.21 days. Physiological maturity ranged from 156 days (kk20 and kk25) to 228 days (kk32) with an average of 174.77 days.

Correlation between/among quantitative characters

The coefficient of correlation between quantitative characters is presented in Table 7. The results showed that number of branches per plant (BrP) was positively correlated with pod length (PL) ($r = 0.58^{***}$), number of pods per plant (PPL) ($r = 0.42^{**}$), number of seeds per pod (SP) ($r = 0.56^{***}$) and grain yield (GY) ($r = 0.47^{**}$) while negatively correlated with pod width (PWi) ($r = -0.89^{***}$), 100-seed weight (100SW) ($r = -0.43^{**}$), days to 50% flowering ($r = -0.75^{***}$) and physiological maturity (PhM) ($r = -0.77^{***}$). Pod length (PL) was positively correlated with number of seeds per pod (SP) ($r = 0.99^{***}$), number of pods per plant (PPL) ($r = 0.82^{***}$) and grain yield (GY) ($r = 0.89^{***}$) while it was negatively correlated with pod width (PWi) ($r = -0.79^{***}$), days to 50% flowering ($r = -0.34^{*}$) and physiological maturity (PhM) ($r = -0.47^{***}$). Pod width (PWi) was negatively correlated with the number of pods per plant (PPL) ($r = -0.56^{***}$), the number of seeds per pod (SP) ($r = -0.79^{***}$) and grain yield (GY) ($r = -0.56^{***}$) while it is positively correlated with 100-seed weight (100SW) ($r = 0.48^{***}$), days to 50% flowering ($r = 0.67^{***}$) and physiological

Table 8. Correlations between characters and the first three factorial axes

Character	PC1	PC2	PC3
PlHe	0.34***	0.25**	0.14
StT	0.32***	0.36***	0.01
BrPl	0.26**	-0.46***	-0.05
PL	0.35***	0.04	0.27**
PWi	-0.31***	0.37***	-0.12
PPl	0.35***	0.20**	-0.15
SP	0.34***	0.02	0.34***
GY	0.35***	0.21***	0.03
100SW	0.00	0.48***	-0.54***
D50F	-0.23**	0.31***	0.50***
PhM	-0.28**	0.21**	0.46***
Eigen value	6.98	2.18	1.59
Proportion (%)	0.64	0.20	0.15
Cumulative proportion (%)	0.64	0.83	0.98

PlHe: Plant height; StT: Stem thickness; BrPl: Branches per plant; PL: Pod length; PWi: Pod width; PPl: Number of pods per plant; SP: Number of seeds per pod; GY: Grain yield; 100SW: 100-seed weight; D50F: Days of 50% flowering; PhM: Physiological maturity; * degree of correlative value with the axe.

maturity (PhM) ($r = 0.66^{***}$). The number of pods per plant (PPL) was positively correlated with grain yield (GY) ($r = 0.93^{***}$) while it was negatively correlated with days to 50% flowering ($r = -0.58^{***}$) and physiological maturity (PhM) ($r = -0.70^{***}$) while the number of seeds per pod (SP) was positively correlated with grain yield (GY) ($r = 0.85^{***}$) and negatively with physiological maturity (PhM) ($r = -0.40^{*}$). Grain yield (GY) was negatively correlated with physiological maturity (PhM) ($r = -0.58^{***}$). Days to 50% flowering (D50F) was positively correlated with physiological maturity (PhM) ($r = 0.95^{***}$).

Principal component analysis

The Principal Component Analysis performed using the 11 quantitative characters showed that the first two PC had an Eigen value higher than 1 and accounted for 83% of the total variability (Table 8). Plant height (PlHe), stem thickness (StT), branches per plant (BrPl), pod length (PL), number of pods per plant (PPl), number of seeds per pod (SP) and grain yield (GY) were positively correlated with PC1. The 100-seed weight (100SW) was negatively correlated with the 3rd PC and positively correlated with the 2nd axis. The correlation of the characters with the first two PCs is represented in Figure 3. The fifty accessions have been grouped in 5 clusters (Figure 4).

The landrace accessions of the cluster I (12 accessions; 3 from Central and 9 from Southern) are characterized by the high 100-seed weight (100SW) and pod width (PWi). The cluster III (23 accessions; 12 from Central and 11 from Southern) seems to group accessions with high good parameters of yield: pods per plant (PPl), number of seeds per pod (SP) and grain yield (GY). The cluster IV (5 accessions; all from Central) seems to group accessions witch maturing late. The cluster V (2 accessions, all from central) grouped accessions that have opposite performances to accessions of the cluster III. The cluster II (8 accessions; 7 from Central and 1 from Southern) group accessions with performance values close to the mean of those of fourth and cluster V.

The comparison of the means of the different groups for each character revealed significant differences ($p < 0.001$) between the 5 clusters for all the 11 considered characters. The characteristics of each cluster are presented in Table 9. Indeed, the cluster I had high pod width (PWi), stem thickness (StT) and 100-seed weight (100SW) accessions and in addition number of seeds per pod (SP) beyond the mean. The cluster II had accessions of 100-seed weight (100SW) similar to the ones of the cluster I while the plant height (PlHe), stem thickness (StT), pod length (PL) and the number of seeds per pod (SP) are very low. The cluster III grouped accessions with maximum number of pods per plant (PPl), number of seeds per pod (SP) and in addition to high yielding and rapid maturing but the plants have the weakness of being tall. The cluster IV and the cluster V grouped the accessions which were late maturing.

Distances between clusters

Inter clusters Euclidian distances varied from 60.48 to 519.79. The highest inter cluster distance (60.48) was

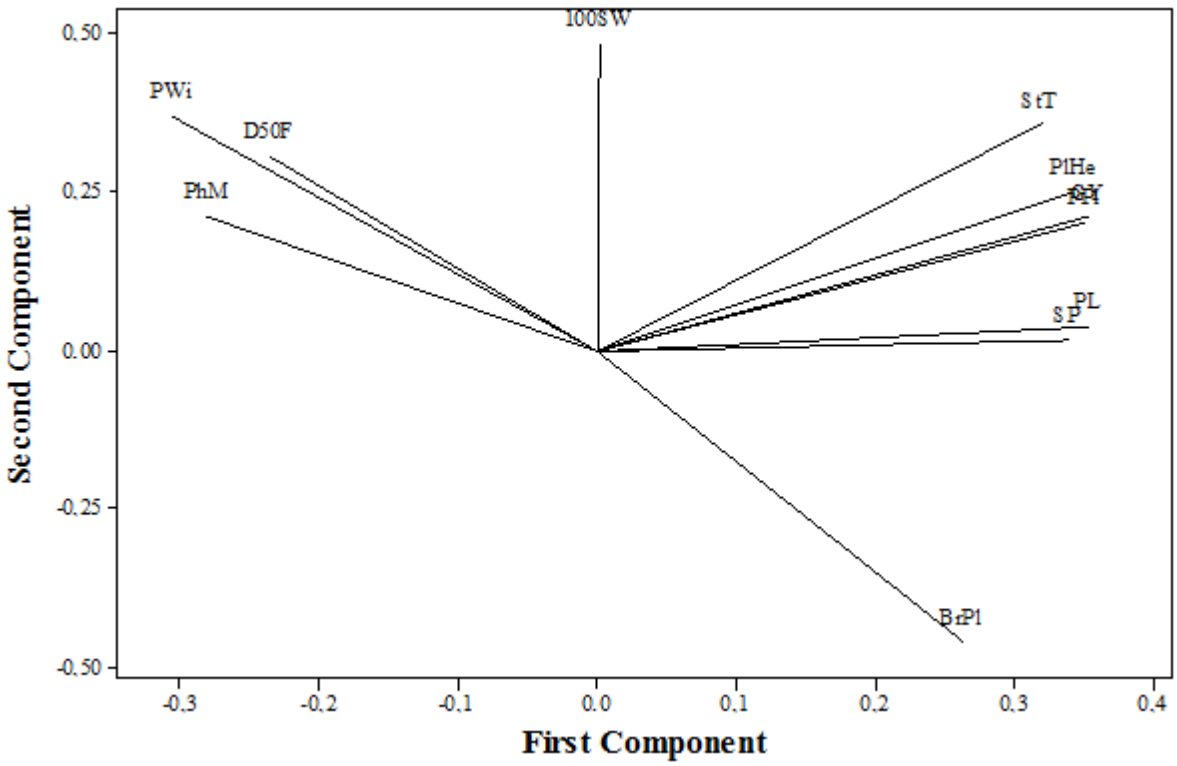

Figure 3. Projection of 11 quantitative characters on the first two components (axis 1 and axis 2) of the PCA.

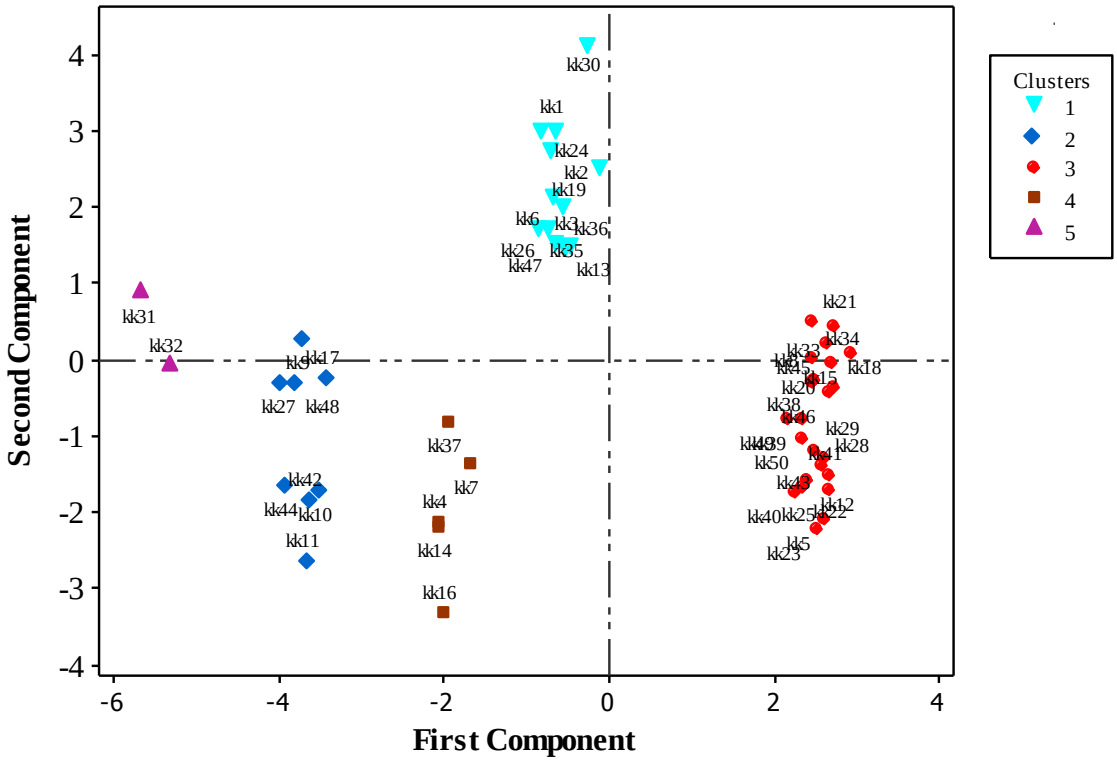

Figure 4. Projection of 50 pigeonpea accessions on the 2 first axes of PCA based on 11 quantitative variables.

Table 9. Comparison of the means of each variable between the five clusters using ANOVA one way and Turkey test.

Variable	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
PlHe	3.20±0.08^{b}	1.95±0.03^{d}	3.30±0.03^{a}	2.60±0.10^{c}	1.87±0.01^{d}
StT	61.70±0.96^{a}	30.50±0.06^{c}	60.10±2.28^{a}	35.42±0.03^{b}	26.30±0.14^{b}
BrP	17.75±0.92^{d}	29.05±0.72^{c}	44.80±0.77^{a}	40.64±0.12^{b}	5.25±0.35^{e}
PL	61.00±0.94^{b}	42.50±0.39^{d}	70.00±0.68^{a}	60.76±0.20^{b}	50.50±0.07^{c}
PWi	8.10±0.03^{a}	8.00±0.05^{b}	3.60±0.06^{e}	5.32±0.05^{d}	7.12±0.03^{c}
PPl	1488.90±3.36^{b}	570.15±0.10^{c}	1855.20±22.76^{a}	329.86±0.66^{d}	135.20±0.85^{e}
SP	5.05±0.11^{c}	3.50±0.06^{e}	5.80±0.03^{a}	5.21±0.02^{b}	4.50±0.00^{d}
GY	4.20±0.15^{b}	1.86±0.02^{d}	4.60±0.08^{a}	2.86±0.03^{c}	0.57±0.03^{e}
100SW	12.40±0.06^{a}	11.70±0.04^{a}	10.60±0.78^{b}	7.57±0.04^{d}	8.97±0.01^{c}
D50F	165.00±1.01^{c}	124.58±0.88^{d}	110.50±0.57^{e}	174.60±0.21^{b}	184.63±0.53^{a}
PhM	190.00±4.77^{c}	173.00±4.45^{d}	157.40±0.61^{e}	199.75±0.10^{b}	227.88±0.18^{a}

PlHe: Plant height; StT: Stem thickness; PrBr: Number of primary branches; SeBr: Number of secondary branches; PL: Pod length; PWi: Pod width; PPl: Number of pods per plant; SP: Number of seeds per pod; GY: Grain yield; 100SW: 100-seed weight; D50F: Days of 50% flowering; PhM: Physiological maturity; Averages that have no common letters are statistically different ($p < 0.05$).

Table 10. Inter clusters Euclidian distances.

Cluster	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
Cluster 1	0				
Cluster 2	277.57	0			
Cluster 3	112.43	387.73	0		
Cluster 4	349.65	74.76	460.56	0	
Cluster 5	408.53	133.64	519.79	60.48	0

observed between the cluster III and cluster V, followed by cluster III and the cluster IV (460.56), cluster I and cluster V (408.53), cluster II and cluster III (387.73), cluster I and cluster V (349.65). The lowest inter cluster distance was between cluster IV and cluster V (60.48) (Table 10).

DISCUSSION

Classification of seeds based on their morphological characteristics is the main criteria in folk taxonomy (Akohoue et al., 2018). In the present study of pigeonpea landraces grown in Benin, a real link has been observed between seed classification based on its morphological characteristics and those using morphological qualitative characteristics by grouping accessions in a similar way. This suggests that the morphological characteristics of seeds are important in the evaluation of pigeonpea diversity (Muniswamy et al., 2014). Similar observations have been made on characterization of other legumes such as common bean (Loko et al., 2018), cowpea (Gbaguidi et al., 2013), and Kersting groundnut (Assogba et al., 2015; Akohoue et al., 2018). This confirm that folk taxonomy is not obsolete and can remain for a long time an important preliminary step in the characterization of cultivated genetic resources for further researches.

Our study revealed that seed colour was the highest polymorphic trait. Similar result was found on pigeonpea characterization by Upadhyaya et al. (2007) in Kenya but contrary to those of Manyasa et al. (2008) in Tanzania. This difference can be explained by the fact that the accessions are of different origin. Cream colour and oval-shaped seeds were found to be dominant among pigeonpea landrace grown in Benin. This suggests that landraces with the mentioned traits have been selected by farmers for a long period of time, because of their acceptability by consumers who constitute a key link in the value chain of cultivated genetic resources. Similar observation on seed colour was made on pigeonpea grown in Tanzania (Manyasa et al., 2008; Rao et al., 2012; Kimaro et al., 2017) and Malawi (Rao et al., 2012). This preference for cream seed colour was also observed on other legumes such as Kersting groundnut (Assogba et al., 2015). These characteristics can therefore be considered as varietal preference criteria and should be taken into account by any breeding program of pigeonpea genetic resources in Benin. Majority of pigeonpea landraces showed a strong tendency to semi-spreading growth habit, lanceolate leaflet shape, light yellow base flower colour, and plain seed colour pattern. Similar results have already been reported in the morphological

variability of Tanzanian pigeonpea germplasm (Manyasa et al., 2008) and world-wide collection (Rupika and Bapu, 2014). Thus, in spite of the influence of environmental factors, qualitative variables can be used to characterize pigeonpea genetic resources.

Analysis of the genetic characterization of pigeonpea collection based on qualitative characteristics revealed that according to their local names, accessions named differently were grouped into the same morphological class. For instance, landraces kk9 called *Otini tchofiti* (Holly sociolinguistic group), kk10 called *Wlétchivé kloui* (Adja sociolinguistic group), kk11 called *Otili founfoun lakoun* (Fon sociolinguistic group) and, kk17 called *Klouékoun wlanwlan* (Adja sociolinguistic group) grouped in the morphological class C5 on the one hand, and kk35 called *Carder ekloui* (Adja sociolinguistic group), kk36 called *Kloué* (Adja sociolinguistic group) and kk48 called *Klouékoun wéwé* (Fon sociolinguistic group) grouped in the morphological class C1 on the other hand suggests the existence of duplicates in the collection. This fact is not surprising since in the folk nomenclature, the same cultivar through the villages can be designated by different names, which constitute a bias to the estimation of diversity (Agre et al., 2015; Loko et al., 2018). As the identification of duplicates is becoming a priority for genebank managers, molecular genetic characterization would be an efficient approach to discriminate among collection of pigeonpea germplasm (Le clerc et al., 2005; Rana et al., 2015) in order to establish equivalences of names between cultivars (Gbaguidi et al., 2013), but also to reduce the cost of conservation (Horna et al., 2010).

Analysis of the quantitative data showed high level of variation among the 50 accessions with regards to branches per plant, number of pods per plant, pod width and grain yield. This finding suggest the existence of genetic diversity in the pigeonpea landraces grown in Southern and Central parts, which can offer opportunities for genetic improvement in component traits through selection (Pal et al., 2018).

The average grain yield, in our collection (3.73 tonnes/ha) was higher than those obtained in similar studies on pigeonpea (Mergeai et al., 2001; Atta et al., 2008). However, our finding is similar to those observed by Ojwang et al. (2016) and confirm the fact that pigeonpea grain yield can reach up to 5 tons/ha under optimum environmental conditions (Van Der Maesen, 2006) and considering the influence of the environment on certain yields components (Chalak et al., 2018). The average number of seeds per pod estimated at 4.52 was lower than those observed by Kundy et al. (2015). However this number is higher than those observed by Muniswamy et al. (2014) on pigeonpea in India. According to Choudary et al. (2011), the physiological maturity of the cultivars observed in the present study reveal the existence of cultivars with medium and late physiological maturity day.

The correlation analysis of quantitative data revealed strong positive correlation between days to 50% flowering and physiological maturity. Similar results were also reported by Singh et al. (2016); Meena et al. (2017) and Pal et al. (2018) for physiological maturity, on pigeonpea.

These results suggested possibility of indirect selection in correlated traits (Silva et al., 2016) *viz.,* days to 50% flowering cannot be prioritized in selection without effects on physiological maturity. Moreover, the positive significant association between grain yield and plant height, number of branches per plant, pod length, number of pods per plant and number of seeds per pod indicates that these traits are important yield contributing traits in pigeonpea. Thus, should be put into consideration when selecting for yield potential (Ojwang et al., 2016). However, strong negative correlation was observed between physiological maturity and grain yield. Similar finding was observed on pigeonpea in Kenya by Ojwang et al. (2016). This negative correlation between grain yield and physiological maturity should be explained by the lack of enough time by plants to accumulate biomass (Vange and Egbe, 2009; Cheboi et al., 2016) which suggests the presence in our pigeonpea collection of some accessions with short grain filling period. So direct selection for long grain filling periods may increase yield for pigeonpea in Benin. Also high temperatures, low rainfall and high pest infestations constituted such as many factors which involve flower abortion involving low number of pods per plant and 100-seed weight thus lowering the grain yield. Moreover, grain yield is a complex character which is highly influenced by the environment and is the result of interrelationships of its various yield components (Grafius, 1960). Thereby, the negative significant correlation exhibited between plant height, number of branches per plant and number of pods per plants with physiological maturity, implies that plants in our pigeonpea collection mature early and justify the fact that the lack of enough time by plants to accumulate biomass could have been a result of negative correlation observed between physiological maturity and grain yield rather than abiotic (high temperatures and low rainfall) and biotic stress (pest infestations).

This study allowed grouping the 50 accessions into 12 morphotype according to the seed characteristics while the qualitative variables grouped them in 11 morphological types and the Principal Component Analysis grouped them into five clusters. These findings suggested that both qualitative variables and quantitative variables data can reveal diversity providing different but complementary information.

Our results revealed that clustering pattern of the pigeonpea accessions from different origin were frequently present in same cluster. Thus, there was no clear relationship between accessions and geographical diversity. This could be attributed to free exchange of materials that may have overlapped in the previous diversity distribution pattern of the domesticated species (Jaradat and Shahid, 2006; Aghaee et al., 2010). These

findings suggest that geographical isolation may not be the only factor causing genetic diversity (Rekha et al., 2011). Therefore, for any hybridization programs in Benin, the choice of suitable diverse parents based on genetic divergence analysis would be more fruitful than the choice based on the geographical distances.

Considering the mean performance for different earliness and yielding traits, the promising genotypes that can be used as parents in hybridization program are those of cluster 3. The high variation of inter clusters Euclidian distances observed in the present study indicated enormous diversity among the genotypes. The highest inter cluster distance was observed between the cluster III and the cluster V suggesting that accessions from these clusters were too much genetically different. However the lowest inter cluster distance between the cluster IV and the cluster V indicated the closer relationship among the genotypes between these clusters. Selection of genotypes from these clusters may not be desirable to get higher yield benefits (Muniswamy et al., 2014; Rupika and Bapu, 2014).

Conclusion

Despite the high diversity in terms of qualitative and quantitative traits, from 23 accessions, kk5 (*Ekloui*), kk8 (*Nontchiovi kloui*), kk15 (*Otili founfoun*), kk18 (*Klouékoun wéwé*), kk22 (*Otili*), kk23 (*CA monlikoun*) and kk28 (*Hounkoun wéwé*) were identified in this study. Our results indicated that the higher level of genetic diversity observed within collected accessions will enable efficient utilization and pigeonpea improvement in breeding programs. Further characterization using molecular techniques as well as conservation attention for these local germplasms should be conducted.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

This study was carried out by the Laboratory of Biotechnology, Genetic Resources and Plant and Animal Breeding. The authors thank the following individuals: Marcelin AGOSSOU and Thomas TONON for establishing and maintaining field trials; Paulin SEDA, MSc researchers at the Laboratory of Molecular genetic and genomes analysis (LGMAG) of the Faculty of Sciences and Technology of the University of Abomey-Calavi for technical assistance during accession characterization.

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Vol. 12(1), pp. 50-57, January-March 2020
DOI: 10.5897/JPBCS2019.0831
Article Number: F31C83863241
ISSN 2006-9758

http://www.academicjournals.org/JPBCS

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Journal of Plant Breeding and Crop Science

Full Length Research Paper

Genetic variability, heritability and correlation of quantitative traits for Arabusta coffee (*C. arabica* L. X Tetraploid *C. canephora* Pierre)

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Received 12 July, 2019; Accepted 18 October, 2019

The biennial bearing and the long productive nature of coffee makes it difficult to release coffee variety within a short time span. This study aimed at evaluating the yield performance of the Arabusta hybrids and its backcrosses developed by Coffee Research Institute of the Kenya Agricultural and Livestock Research Organization (KALRO-CRI) using the morphological traits. Nineteen coffee genotypes were evaluated at Siaya ATC and KALRO-Alupe using randomized complete block design with three replications and the morphological data for growth and yield was recorded during the year 2018. The results indicated that there was significant difference in yield among the coffee genotypes and between the sites. Yield had positive significant associations with parentage berries per node (r= 0.61), berries on the longest primary (r= 0.58) and berries per node on the longest primary(r=0.60). The genotypic coefficient of variation (GCV) values for the morphological traits varied from 6.50 to 31.01%. Broad sense heritability ranged from 0.15 to 0.61 with bean yield recording heritability of 0.31. The number of berries on the longest primary had high broad sense heritability and high genetic advance indicating the presence of additive genes that can be used in coffee improvement through selection.

Key words: Environment, Genetic advance, Robusta, Response, Selection, Variation

INTRODUCTION

The world coffee production increased in the year 2018/2019 to 168.77 million bags which is 1.6% higher higher than the year 2017/2018. From the coffee produced, 109.41 million bags were exported and these exports also were higher by 10.2% when compared to the year 2017/2018. Both Robusta and Arabica coffee exports increased during the year 2018/2019 and from the total exports, 64% was Arabica coffee while 36% was

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Robusta coffee (ICO, 2019b). Coffee in Kenya is the fourth most important source of foreign earnings of US$230 million, after horticulture, tourism and tea supporting livelihoods of about 800,000 farmers. An estimate of 80% of Kenya's workforce are being engaged in agriculture either directly or indirectly with about 30% employed in the coffee industry (ICO, 2019a). Over 90% of the total Kenyan coffee acreage is under Arabica coffee (*Coffea arabica* L.), while the rest is occupied by Robusta coffee (*Coffea canephora* Pierri) (Omondi et al., 2001). However, the performance of coffee in Kenya has been declining since the 1980's where the total production was about 1.7 million bags to the current annual production of 900,000 bags (Karanja and Nyoro, 2002, ICO, 2019a) This has been due to increased cost of production, pests and diseases as well as increased population within urban centers which has paved way of agricultural land under coffee for housing.

Kenya has developed interspecific hybrids between tetraploid Robusta and Arabica coffee termed as Arabusta hybrids. The expectation is to generate a high yielding coffee variety that is disease resistant and with good cup quality coffee that outperforms Robusta coffee and also adapted to low altitude zones which include areas around the Lake Victoria region and lower coastal regions. The backcrosses were carried out for introgression of diseases resistant genes to Arabica coffee which is susceptible to coffee berry disease. Coffee is a biennial crop and because of its productive nature, one generational cycle takes 8 years. This makes it difficult to breed for a variety within a short time span since it may take up to 30 years for release. It is therefore important to identify genotypes with good growth characters that relate positively to increased yield during the early years of production in order to reduce the time span during selection and minimize resources. Growth and yield characters have been shown to have an influence on yield stability in coffee as it has been in a number of other crops (Gichimu and Omondi, 2010).

Assessing the variation of quantitative traits during selection is important to ensure a successful breeding program since it is key to determining the response to selection due to genetic diversity. The genotypic and phenotypic coefficients of variation have been used in breeding in identifying variation found within genotypes (Solomon et al., 2009). Heritability indicates the effectiveness in selection based on phenotypic performances of genotypes. The usefulness of heritability therefore increases when it is combined together with high genetic advance which indicates the degree of gain of a trait during selection (Dyulgerova and Valcheva, 2014). This will provide an indication on the genetic improvement required in maximizing the potential of a specific genotype (Weldemichael et al., 2017). Measuring heritability guides in predicting the breeding value of a phenotype (Tazeen et al., 2009).

Various studies on Ethiopian coffee by Yigzaw (2005) and Atinafu et al. (2017) have shown high heritability on morphological traits including hundred bean weight, number of secondary branches, plant height, internode length and number of primary branches. The genetic correlation, which is the proportion of variance that two traits share due to genetic causes, is useful in studying the genetic relationships among traits under selection (Anim-Kwapong and Adomako, 2010). The study is aimed at identifying growth and yield traits that correlate highly with yield having genetic variation in terms of high heritability and genetic advance for selection of best performing genotypes.

MATERIALS AND METHODS

Experimental materials

Nineteen materials including seven Arabusta hybrids, and six different backcross derivatives of Arabica to Arabusta hybrids were evaluated together with the three Arabusta varieties, Robusta, *C. arabica* (Batian) and *C. arabica* (Ruiru 11) as shown in Table 1.

Description of the experimental site and design

The trials were laid down in Siaya ATC (Siaya County) and Alupe (Busia County) in the year 2015 (Table 2). The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications in KALRO-Alupe and Siaya ATC. Five coffee trees were planted per genotype with a spacing of 3 m × 3 m per plot measuring 855 m^2 and all recommended agricultural practices were applied. Data on growth and yield parameters were collected and recorded during the third year after establishment.

Growth parameter measurements

The growth and yield parameters were recorded as described by Walyaro (1983). They include:

(i) Percentage of berries per node. The bearing nodes with berries, flowers or flower buds were counted and expressed as percentage of the total number of nodes on the same tree and this was collected from five trees per plot.
(ii) Total number of berries on the three longest primaries and the mean was derived.
iii) Number of bearing primaries, recorded as the total number of primaries carrying berries, flowers or flower buds from five trees per plot.
(iv) Number of berries per node, was obtained as the mean number of berries per node on the selected four primaries from five trees per plot.
(v) Tree height was recorded as length from base to the tip of the tree (cm) from five trees per plot and mean calculated.
(vi) Total number of laterals (number of secondary branches). This was derived by counting all lateral per tree from the five trees per plot and the mean calculated.
(vii) Length of the longest primary was measured from the five trees per plot and mean calculated.
(viii) Number of berries on the highest bearing node from the longest primaries derived from the five trees per plot.
(ix) Number of bearing nodes on the longest primaries from five trees per plot.
x) Mean of number of primaries from five trees per plot.

Table 1. Description of backcross progenies and varieties for yield and morphological evaluation at Alupe and Siaya.

Code	Pedigree information	Genotype description
ARH1	B11 2415 = CATURRA X B6. 1834 = (SL 28 X UT 6)	Arabusta Hybrid
ARH2	B11 2554 = CATURRA X B6. 1834 = (SL 28 X UT 6))	Arabusta Hybrid
ARH3	B11 2406 = CATURRA X B6. 1834 = (SL 28 X UT 6)	Arabusta Hybrid
ARH4	B11 2407 = CATURRA X B6. 1757 = (SL 34 X UT 6)	Arabusta Hybrid
ARH5	B11 2556 =CATURRA X B6. 1757 = (SL 34 X UT 6))	Arabusta Hybrid
ARH6	B13 2271 = SL 28 X B6. 1835 = (SL 34 X UT 6)	Arabusta Hybrid
ARH7	B14 1140 = SL 28 (SL 34 X UT 8)	Arabusta Hybrid
BC01	B13 2400 = SL 34 X B6. 1764 = (SL 34 X UT 6)	Backcross
BC02	B13 2567 = SL 28 X B6. 1778 = (SL 28 X UT 6)	Backcross
BC03	B13 2286 = SL 28 X B6. 1836 = (SL 28 X UT 6)	Backcross
BC04	B13 2617 = SL 34 X B6. 1616 = (SL 34 X UT 6)	Backcross
BC05	B13 2806 = SL 34 X B6. 1756 = (SL 34 X UT 6)	Backcross
BC06	B14 1108 = SL 28 (SL 28 X UT 8)	Backcross
ARV1	PL 4 CONGUSTA 161 CRAMER	Cultivar
ARV2	PL 4 CONGENSIS 263 CRAMER	Cultivar
ARV3	PL 4 169, 177, 178 ARABUSTA	Cultivar
Robusta	Pure line	Robusta
Ruiru 11	Hybrid	Arabica
Batian	Pure line	Arabica

Table 2. Description of the experimental sites.

Parameter	Siaya ATC	Alupe
Longitude and latitude	0º 30 N' and 0º 45' E	0º 30 N' and 34º 30' SE
Altitude (asl) (m)	1,135 to 1,500	1241 to 1343
Mean rainfall (mm)	1,500	1400
Annual mean temperature range (ºC)	20.9 and 22.7	26 and 29
Soils	Chromic/Orthic acrisols and ferrasols	Dolerites and Andesites

The soils are as described by Jaetzold et al. (2009) and Rachilo and Michiela (1991).

(xi) Mean of 100 berry weight (g) from five trees per plot.
(xii) The red ripe cherry was harvested during peak harvesting period of May to July and from September to November in 2017 and 2018. The cherry from five trees of each genotype per replication bulked weighed and yield data, which is the weight of the cherry in grams, was recorded and expressed in grams per tree.

Statistical analysis

The yield and growth characters' data was subjected to Analysis of Variance (ANOVA) using GENSTAT statistical software and effects declared at 5% significant level General Linear Model (GLM) was used (Jansen, 1993). Least significance difference (LSD) was used to separate the means (Martin et al., 1978). Separate as well as combined analysis of variance was performed on data from the two sites. The correlation was calculated to show the relationship between growth and yield characters using the Pearson's Correlation Coefficient. Genotypic and phenotypic variances were calculated using the formula by Baye (2002) as follows

(i) Genotypic variance, GV = (MSg – MSe) / r, where MSg = mean square of genotypes, MSe = mean square of error, and r = number of replications.
(ii) Phenotypic variance, PV = GV + MSe, where GV = genotypic variance and MSe = mean square of error.

Phenotypic and genotypic coefficient of variation as suggested by Singh and Chaudhary (1985) can be calculated as

(i) Phenotypic coefficient of variation, PCV = (PV/X) × 100, where PV = phenotypic variance and X = mean of the character.
(ii) Genotypic coefficient of variation, GCV = (GV/X) × 100, where GV = genotypic variance and X = mean of the character.

Heritability (broad sense heritability) was calculated as suggested by Falconer (1989) using H = GV/PV, where GV = genotypic variance and PV = phenotypic variance; also, Genetic advance (GA) expected and GA as percent of the mean assuming selection of the superior 5% of the genotypes was estimated as per Assefa et al. (1999)

$GA = K \times (PV/X) \times H$

GA (as % of the mean) = (GA/X) × 100, where K is a constant (which varies depending upon the selection intensity and, if the

latter is 20%, stands at 1.40), PV/X is phenotypic standard deviation, H is heritability and X refers to mean of the character being evaluated. Expected response to selection (Re) was estimated as (2) $Re = i\sqrt{Vph2}$, where i = 1.40 at 20% selection intensity, V_p = phenotypic variance for a trait, and h^2 = broad-sense heritability for a specific trait (Singh and Chaudhary, 1985).

RESULTS

Growth and yield traits

There was variation amongst the coffee genotypes with regard to the growth and yield traits recorded at Busia and Siaya over the two-year period. The berries on the longest primary were significantly (P≤0.05) different amongst the genotypes at Siaya where genotype BC05 recorded 22 berries while genotype ARV1 recorded 12 berries (Table 3). There was significant (P≤0.05) difference on berries per node on the longest primary where in Siaya ARV3 recorded the highest number (22) while genotype ARH3 recorded the least (9). In Siaya, berries per node was significantly (P≤0.05) different amongst the genotypes varying from one to six berries where genotype AVR1 recorded largest number and genotype ARH3 recorded the least. The height ranged from 135 to 217.7 cm in both sites where the genotype Ruiru 11 recorded the shortest plants at both sites while BC05 recorded high values at Busia compared to other genotypes (Table 3). The yield varied in the two sites ranging from 728 to 4580 g/tree in Busia, while in Siaya it ranged from 2005 to 8227 g/tree.

Correlation

The correlation coefficients amongst the twelve different traits were measured for both sites from combined mean analysis. The percentage berries per node had significant positive correlations with berries on the longest primary=0.69), berries per node on the longest primary (r=0.90), berries per node (r=0.65), nodes with highest number of berries (r=0.64) and yield (r=0.61) (Table 4). Berries on the longest primary had positive significant associations with berries per node (r=0.46), nodes with highest number of berries (r=0.48) and yield (r=0.48) (Table 4). All traits except 100 berry weight, berries per node, longest primary, and total number of primaries showed significant positive correlations with yield. Longest primaries showed positive associations to nodes with highest number of berries, total number of primaries and yield although they were not significant.

Yield performance

The genotypes performed significantly (P≤0.05) different from each other. ARH1 was the best performing genotype in Busia followed closely by ARH4 and ARH5. In Siaya, the best performing genotypes were genotype ARH4, followed closely by genotypes BC06, ARV2 and BC04. Production in Siaya was high when compared to Busia. (Figure 1) and genotypes ARH2 and ARH3 performed poorly in Busia and Siaya respectively.

Genotypic and phenotypic parameters

Estimation of the genotypic and phenotypic variances was calculated and this showed that the coffee genotypes evaluated expressed different level of variations in the morphological traits measured. Genotypic coefficient of variation (GCV) varied between the morphological traits the values scored varying from 6.50 to 31.01%. The trait with high GCV value was berries on the longest primary with 31.01%, followed closely by total number of laterals with 30.58% and berries per node scoring 29.79%. The values scored for the phenotypic coefficient of variation (PCV) ranged between 11.03 to 70.51% with yield scoring the highest value (Table 5)

On genetic advance (GA), yield scored a higher value of 699.3 and berries per node on the longest primary recorded the least with 0.42. The percentage mean of GA varied from 0.8 to 21.42%, yield (g/tree) scoring the highest percentage while the percentage berries per node scored the least. The broad sense heritability (H) was calculated for the morphological traits measured in the experiment. The values for the broad sense heritability ranged from 0.15 to 0.61 within the traits. The morphological traits that showed a higher broad sense heritability (>0.50) were berries on the longest primary, length of longest primary and height which scored 0.61, 0.59 and 0.59 respectively (Table 5). The percentage berries per node scored a low broad sense heritability of 0.008, yield had the highest value on response to selection of 1328.14 while berries per node was the least with 1.196.

DISCUSSION

ANOVA showed that there was significant (p<0.05) differences amongst the growth and yield traits for the 19 coffee genotypes assessed except for percentage berries per node, number of bearing primaries and total number of primaries. The variability within the genotypes in terms of the growth traits is important for an efficient selection of coffee genotypes thus the possibility of improvement through selection and crossing. The selection efficiency for yield can be enhanced by considering various growth parameters and components of yield, such as, percentage of bearing nodes, number of berries per node and percentage of bearing primaries as reported by Van der Vossen (1985). This can be confirmed by the results of this study whereby the site where higher number of bearing primaries, berries on the longest primaries,

Table 3. Growth and yield traits taken from coffee genotypes taken at KALRO-Alupe (Busia) and Siaya ATC.

Genotype	%BN		BELP		BNLPR		BPR		B/N		H		LAT		LPR		NHB		PR		Yield	
	Busia	Siaya	Busia	Siaya	Busia	Siaya	Busia	Siaya	Busia	Siaya	Busia	Siaya	Busia	Siaya	Busia	Siaya	Busia	Siaya	Busia	Siaya	Busia	Siaya
ARH1	57.1	53.3	120.1	88.3	17.1	14.3	51.7	54.6	4	3.3	205	199	8.6	12.7	108	100	12.4	14.5	71.3	67.4	4580.8	2329.2
ARH2	53.8	63.9	79.6	115.3	13.6	16.7	47.3	55.3	3.2	4.4	165	177	7.9	13.1	89.8	95.2	11.5	15.7	72.8	70	1240	2004.9
ARH3	61	36.9	78.7	32.2	17.3	8.8	58.3	50	2.7	1.4	179	161	10.7	12.8	90.1	85.3	9.9	7.7	77.3	62.1	1245.1	2668.2
ARH4	68.3	68.1	127	177.8	19.8	18.6	56.6	53.6	4.4	6.6	155	160	4.4	9.3	87.9	92.6	14.7	22.5	69.2	69.9	4550	8227.8
ARH5	66.3	61.6	140.1	134	20.5	17.2	50.3	54.7	4.6	4.8	152	170	4.9	8.8	96.1	97	15.9	18.9	65.3	68.3	3930.1	3677.6
ARH6	58.2	65.2	87.9	102.3	16.9	17.8	46.9	54.7	3	4	190	161	6.8	9.8	107	104	10.6	14.1	64.6	65.7	1422.9	3044.7
ARH7	33.2	54.5	60.2	50.1	11.5	14.7	50.3	49.6	1.7	1.9	174	170	7.3	10.4	104	106	8.3	10.4	63.3	62.9	1616.7	1977.1
BC01	56.5	62.6	122.1	125.4	16.4	17.2	51.8	58.6	4.3	4.6	182	198	8.3	11.1	110	108	18.8	18.3	66.3	71.9	2778.2	3724.2
BC02	46.8	52	79.8	98.4	12.7	13.9	47.4	59.1	3	3.7	194	192	11.4	10.9	106	108	8.4	11.2	71.6	73.8	2830.8	3167.8
BC03	49.4	56.3	103.5	79.4	13.9	14.6	51	55.4	3.6	3.1	200	199	8.3	13.4	100	101	10.6	15.8	71.2	67.7	2708.3	2860.8
BC04	51.4	70.7	135.6	139.3	14.9	19.3	48.6	54.4	4.6	4.9	196	196	5.3	8	118	117	12.3	16.1	65.1	63.4	1459.7	7235
BC05	70.8	46.9	166.8	48.2	21.8	13.3	55.2	49.3	5.3	1.7	218	185	4.7	17.8	109	102	16.2	9.2	75.8	64.9	2029.2	2115
BC06	58.5	66.8	82.9	95.9	19.3	21.1	49.4	57.1	2.5	3.1	153	145	9.7	14.6	102	108	8.7	17.5	64.4	66.8	969.5	6613.5
ARV1	44.7	61.8	119.3	153.4	12.1	16.4	47.9	58.8	4.4	5.9	176	166	5.5	5.2	95.5	96.9	16.7	22.2	70.3	71.9	2056.7	2932.5
ARV2	62.4	63.6	94	125.6	16.6	14.7	50.8	47	3.5	5.5	189	180	3.1	8.3	95.3	92.4	14	20.6	70.1	61.7	2597.9	7666.9
ARV3	60.6	75.1	176.6	167.6	19.7	21.9	52.1	56.7	5.5	5.8	195	196	6.8	9.6	123	128	16	20	70.9	66.6	2861.7	4261.5
Robusta	55.6	53.1	117	116.3	13.9	15.6	37.6	61	4.6	4	178	198	5.2	6.6	95	132	12.7	14.9	68.9	74.3	727.8	6940.3
Ruiru	63.8	72.7	113.3	97.2	19.1	19.8	41.4	48.8	3.8	3.6	138	135	6.2	10.4	89.2	83.3	14.3	14.6	59.1	62.1	2550	4747.8
Batian	50.2	58.6	95.9	63.6	14.9	17.3	44.7	54.8	3.2	2.2	163	184	6.1	16.2	84.4	99.8	9.2	10.4	62.1	62.2	1848.8	3883.3
LSD	24.8	19.7	88.2	65.4	7.5	5.8	15.1	12.9	3	2.3	28.7	36.8	4.1	4.1	18.4	18.4	7.6	7.7	2.6	12.6	1968.9	3577
%CV	14.2	9.9	27.2	12.4	15.6	9.8	13	0.6	25.7	11.2	0.1	1.7	11.8	5.7	1.6	2.1	16.9	8	4.8	2.1	17.2	30.4
Ftest	NS	NS	NS	S	NS	S	NS	NS	NS	S	S	S	S	S	S	S	NS	NS	S	S	S	S

% BN=percentage bearing nodes BELP=Number of berries on the longest primaries, BPR= Number of bearing primaries bearing, B/N=Number of berries per node, H (cm) = Height. LAT=Number of laterals, LPR (cm) =Length of longest primaries, NHB= Number of nodes with the highest number of berries, BNLPR=Number of bearing nodes on the longest primary, PR= Number of primaries, Yield (g/tree).

berries per node on the longest primary, berries per node and laterals lead to increased yield. Arabusta hybrids ARH4 were the best performing genotype across the two locations. The result of the study is in agreement with those of Gichimu and Omondi (2010), who reported significant phenotypic variations with the use of the different quantitative characters in coffee and Olika et al. (2011) who also observed variations amongst the longest primaries, bearing nodes, height, number of laterals, yield (g/tree), bearing nodes, berries per node among other traits.

There was a significant positive correlation between yield and percentage berries per node, berries on the longest primary, berries per node on the longest primary, berries per node and nodes with highest number of berries. The traits that associated positively and significantly with that associated positively and significantly with yield can be used in indirect selection for yield, thus allowing efficiency in selection. The selection of potentially superior genotypes can be done by disregarding the undesirable genotypes early during evaluation reducing the time and resources in breeding. The traits that showed negative associations can impede the indirect selection gains for yield. The results agree with those of Gichimu and Omondi (2010) who reported a highly significant correlation between the number of berries and bearing primaries, nodes on bearing

Table 4. Pearson's correlation analysis for growth and yield traits for coffee genotypes Siaya ATC and KALRO- Alupe.

Correlation	%BN	BELP	BNLPR	%BPR	B/N	BW	H	LAT	LPR	NHB	PR	Yield (g/tree)
%BN	-	0.6984***	0.9011***	-0.0709	0.6513**	0.3099	-0.3181	-0.3284	0.0138	0.6452**	-0.235	0.6124**
BELP		-	0.6071**	0.2745	0.9687***	0.2199	0.1197	-0.6052**	0.3716	0.8607***	0.2026	0.5852**
BNLPR			-	0.0219	0.4651*	0.0862	-0.3411	-0.1205	0.1767	0.4859*	-0.331	0.4844*
%BPR				-	0.2329	-0.4099	0.3373	0.246	0.2664	0.2387	0.6206**	0.0373
B_N					-	0.2678	0.1276	-0.6906**	0.2661	0.898***	0.2798	0.6009**
BW						-	0.1935	-0.4703*	0.0623	0.1355	-0.2925	0.3829
H							-	0.0931	0.6122**	-0.0568	0.4662*	-0.1499
LAT								-	-0.0862	-0.6477**	0.0738	-0.49968*
LPR									-	0.0944	0.1705	0.002
NHB										-	0.1724	0.5538*
PR											-	-0.1049
Yield (g/tree)												-

*** indicates significance at p≤ 0.001; ** indicates significance at p≤ 0.01 and * indicates significance at p≤ 0.05. % BN= percentage bearing nodes BELP=berries on the longest primary, BNLPR= bearing nodes on longest primary, BPR= percentage bearing primaries, B/N= berries per node, BW= 100 berry weight (g),H= height (cm), LAT=laterals, LPR=longest primary (cm), NHB= node with highest berries, and PR=Number of primaries and yield.

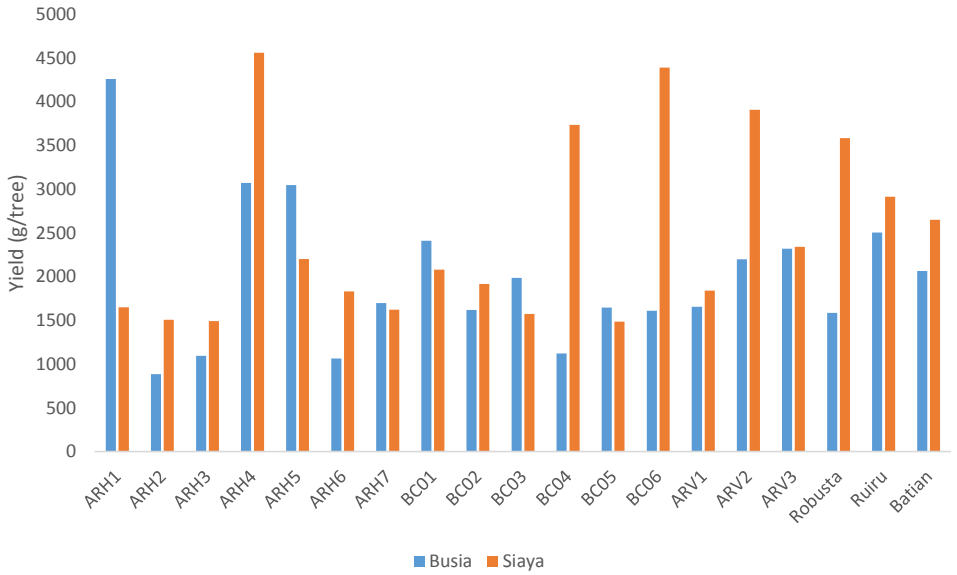

Figure 1. Performance of the coffee genotypes across the two locations (Busia and Siaya) over the two-year period.

Table 5. Estimate of genotypic and phenotypic parameters estimated from combined Analysis of Variance of twelve growth and yield traits.

Morphological trait	GCV (%)	PCV (%)	H	GA	GA (% of mean)	Re
%BN	7.61	26.48	0.08	0.471478	0.810099	4.604276
BELP	31.01	55.89	0.61	14.565	13.46119	34.89299
BNLPR	13.58	29.99	0.21	0.425273	2.583678	1.197311
BPR	11.50	15.68	0.22	0.53748	1.035207	3.970031
B/N	29.79	54.30	0.30	0.479482	12.42181	1.195774
H	13.28	17.23	0.59	4.399551	2.468884	24.61089
LAT	30.58	43.65	0.49	1.171933	13.09422	2.846638
LPR	12.99	16.93	0.59	2.404388	2.361874	13.75135
NHB	28.07	44.32	0.40	1.555225	11.02996	4.116001
PR	4.29	11.03	0.15	0.828803	1.223867	3.020778
Yield (g/tree)	39.11	70.51	0.31	699.3102	21.41838	1328.146

primaries and bearing nodes. The significant and negative correlation observed in the study between yield and laterals was also reported by Olika et al. (2011), however Dessalegn (2005) reported that except for the number of primary branches almost all the characters measured showed positive phenotypic correlations.

The traits with a higher GCV and PCV value (>20%) were, berries on the longest primary, berries per node, total number of laterals, nodes with high number of berries, and yield (g/tree). These values indicate that there exists a wide genetic variation within the genotypes that affects their phenotypic performance. The traits with medium GCV and PCV value (10-20%) were, number of berries on the longest primary, height and the length of the longest primary. There were high GCV and PCV values for yield indicating that there was a high environmental variation. The low GCV values for most traits could have resulted from the varying environmental conditions. Berries on the longest primary, total number of lateral, nodes with high number of berries and yield had a higher mean percentage of GA, the same observation as reported by Olika et al. (2011) and Bayetta (2007). Malau and Pandiagan (2018) also reported low to moderate GA for most of the plant vigor and yield traits

The quantitative traits with high heritability (>50%) were height, berries on the longest primary and number of longest primary while the rest had heritability values less than 50%. High heritability and low genetic advance observed implies that apart from the environmental effects, the additive and non-additive genes also contributed to trait expression (Abate et al., 2015). There was narrow gap between the GCV and PCV values for traits with high heritability, implying that the influence by the environment was minimal thus the high heritability expressed (Getachew et al, 2017). Traits with lower heritability are controlled by more genes, which in turn complicate the selection process by slowing it down (Sousa et al., 2019). The results indicate that the berries on the longest primary and the total number of longest primaries which also correlated highly with yield can be used in selection for yield. Similar findings on heritability were reported by Bayetta (2001) and Dessalegn (2005) who found the high heritability on height (0.59), however, Beksisa and Ayono (2016) reported low heritability on plant height. Kebede and Bellachew (2005) reported high broad sense heritability and Getachew et al. (2013) reported moderate heritability for all the traits respectively.

Conclusion

For an effective selection, the use of heritability and genetic advance is key to determining the degree of genetic gain from selection of a trait. The selection efficiency for yield can be obtained by identifying traits that exhibit high GA and heritability and also show positive correlations with yield. The variation within the traits means that there is possibility of maximizing on gains during crop improvement. Total number of berries on the longest primary, number of bearing primaries, berries per node and laterals can be utilized well during early selection for yield.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors appreciate the director general- KALRO for sponsoring the researcher's studies at the University of Nairobi as well as staff at the Coffee Breeding Unit for support during establishment of trials and data collection.

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Vol. 12(1), pp. 58-69, January-March 2020
DOI: 10.5897/JPBCS2019.0840
Article Number: 2C6A83763304
ISSN 2006-9758

http://www.academicjournals.org/JPBCS

ACADEMIC JOURNALS

Journal of Plant Breeding and Crop Science

Full Length Research Paper

Variability, Association and Path Coefficient Analysis of Green Pod Yield and Yield Components of Hot pepper (*Capsicum annuum L.)* Landraces at Mereb Lehke, Northern Ethiopia

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Received 4 September, 2019; Accepted 8 November, 2019

Understanding the genetic variability and diversity of crops is the basis for breeding and improving of crops. Sixty four Ethiopian hot pepper genotypes were evaluated in 8×8 simple lattice design for genetic variability in green fruit yield and yield related traits at Axum Agricultural Research Center during 2018/2019 under irrigation. Data were collected on green pod yield and yield related characters. The analysis of variance showed significant amount of variations among genotypes in their mean performances of studied traits. High heritability and genetic advance were observed for average fruit weight (145.03, 97.11), fruit length (97.43,98.62), number of fruits per plant (78.54,95.78), number of branches per plant (77.65, 98.64), green pod yield per plant (74.26,99.80) and fruit pericarp thickness (63.61,97.76), respectively. This indicates that these traits are predominantly governed by additive gene action. From correlation study fruit yield per plant exhibited highly significant positive association with average fruit weight (0.72, 0.71), fruit length (0.69, 0.68) and fruit diameter (0.61, 0.60)) at both genotypic and phenotypic levels, respectively. Fruit length had the highest direct effect (0.46) on fruit yield per plant, followed by average fruit weight (0.36). In general, result of this study indicated that average fruit weight, fruit length, fruit diameter and fruit pericarp thickness showed high heritability, genetic advance, positive correlation and high positive direct effects. Hence, these traits can be used as indirect selection criteria for hot pepper yield improvement program.

Key words: GCV, genetic advance, heritability, PCV, pod yield, variability.

INTRODUCTION

The genus Capsicum belongs to the family Solanaceace and it includes 30 species, including five domesticated and commercially cultivated species (*Capsicum annuum* L., *Capsicum baccatum* L., *Capsicum chinensis* Jacq., *Capsicum frutescence* L. and *Capsicum pubescence*) (Dagnoko et al., 2013). Among them, *C. annuum* L. is the most widely cultivated species worldwide (Pickersgill, 1997). It is the world's most important vegetable after tomato and used as fresh, dried or processed products, as vegetables and spices or condiments (Berhanu et al.,

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2011a). Nutritionally, hot pepper like any other *Capsicum* species is rich in vitamin A and C, calcium, phosphorus and potassium. It has been reported that peppers are highly appreciated for their spicy flavor and nutritional value (Amare, 2013). Currently, it is produced in many parts of the country, because food is tasteless without hot pepper for most Ethiopians. In addition, Capsicum species have been used as medicines and lachrymatory agents (Shimeles, 2018). In Ethiopia, it is a high value crop due to its high pungency which serves as food consumption and source of cash earning for smallholder farmers in both green and dry form (Amare, 2013).

According to CSA (2017), the national average yields of hot pepper are 6.3 t ha^{-1} for green pod and 1.8 t ha^{-1} for the dry pod, which is far below the dry pod yield (2.5-3.7 t ha^{-1}) of improved varieties harvested at research fields of Ethiopia (MoANR, 2016) and world average yield of 3 - 4 t ha^{-1} (FAO, 2015). At farmers level the green pod yield is less than 5-6 t ha^{-1}. The gap between research plot yield and farmer's field yield could be associated with many biotic and abiotic factors such as lack of high yielding varieties, non-availability of quality seeds, imbalanced fertilizer use, lack of irrigation facilities, lack of proper disease and insect pest management and other agronomic practices, low storability, and lack of proper marketing facilities (Shimeles, 2018).This calls for urgent breeding work in order to develop varieties with better yield potential. For efficient and effective breeding work investigation and better understanding of the variability of existing genotypes is essential.

The first step in the development of varieties is assessing the genetic variability of available genotypes for the characters of interest (Rosmaina et al., 2016).Naturally, the genetic variation or diversity for most of the yield attributes is considerably high in pepper. There is a need for improvement in complex quantitative trait such as yield. The wide range of distribution of peppers has created an opportunity for local germplasm leading to varieties and landraces to exist. Landraces are important genetic resources because they have unique gene pools and serve as important reservoirs of genetic diversity for breeding and conserving biodiversity (Shimeles, 2018). The use of morphological characterization for studying genetic diversity of local pepper germplasm, including landraces, accessions and cultivated varieties, has long been used for identifying the potential for breeding to meet desirable traits. High genetic advancement coupled with high heritability estimates offers the most suitable condition for selection (Johnson et al., 1955).The presence of variability, heritability and genetic advance in different yield related characters of hot pepper has been reported by Berhanu et al. (2011a), Birhanu (2017) and Shimeles (2018). However, no variability studies have been conducted on hot pepper in the study area.

Fruit yield is a complex trait and highly influenced by many genetic factors and environmental fluctuations whereas yield component traits are less complex in inheritance and influenced by the environment to a lesser extent. In plant breeding programme, direct selection for fruit yield as such could be misleading (Abrham et al., 2017). A successful selection depends upon the information on the genetic variability and association of morpho-agronomic traits with fruit yield. Correlation studies along with path coefficient analysis can provide a better understanding of the association of different traits with fruit yield. Path coefficient analysis separates the direct effects from the indirect effects through other related traits by partitioning the correlation coefficient (Berhanu et al., 2011b). Hence, the present study was undertaken with the objectives to estimate phenotypic and genotypic variations, heritability and expected genetic advance of agronomically important traits in the hot pepper genotypes and to assess the extent of associations among yield and yield related traits and to identify traits for indirect selection criteria for hot pepper breeding program in the study area.

MATERIALS AND METHODS

Experimental site

The field experiment was conducted at Rama in Mereb Leke District of Central Administrative Zone of Tigray region, northern Ethiopia, during the 2018/2019 cropping season under irrigation. Rama is located at 14° 22'25" N latitude and 038°47'32" E longitude at an elevation of 1390 meters above sea level. It lies in the dry agro-ecological zone and its soil is sandy clay loam. The mean annual rainfall in the area ranges from 400 to 600 mm and the rainfall distribution is mono-modal with an erratic distribution beginning late in June and ending in the last week of August. The mean maximum and minimum temperatures of Rama during the 2018/2019 growing season were 33.9 and 18.7°C, respectively.

Experimental materials and design

Sixty-three local hot pepper Ethiopian landraces along with one released variety Mareko fana as a check were used in this study. The landraces were collected from farmer's fields in major hot pepper growing regional states of Ethiopia, namely Amhara, B/Gumuz, Oromiya, SNNPRS and Tigray varying in altitude, rainfall, temperature, and soil form and from Shire-Maitsebri Agricultural Research Center and Ethiopian Biodiversity Institute (EBI). The accession numbers and sources of the genotypes are shown in Tables 1 and 2.

The experiment was laid out in 8×8 simple lattice design with two replications. The seeds of 64 genotypes were sown in plastic plug trays containing mixture of soil, filter cake, compost and sand in the ratio of 2:2:1:1by volume, respectively inside the naturally ventilated polyhouse. The seedlings were transplanted into the main field 38 days after sowing when the seedlings attained 15 cm height. The plot size of each genotype was 8.4 m^2 (3 m x 2.8 m) planted with inter and intra-row spacing of 0.7 m and 0.3 m. Fertilizer, Di-ammonium phosphate (DAP) as a source of Phosphorus was applied at the rate of 200 kg ha^{-1} during planting and nitrogen fertilizer was applied in the form of Urea at the rate of 150 kg ha^{-1} in split half during transplanting and the rest as side dressing at 45 days after transplanting. Furrow irrigation method scheduled at 7 days interval (AxARC, 2016) was used. Weeding, hoeing and other

Table 1. List of qualitative characters considered with their codes and descriptions as per IPGRI (1995) *Capsicum annum* descriptor.

S/N	Character	Description and code
1	Plant growth habit	Prostrate (3), Compact (5) and Erect (7)
2	Leaf color	Yellow (1), Light green (2), Green (3), Dark green (4), light purple (5), Purple (6), Variegated(7)
3	Branching habit	Sparse (3), compact (5), Dense (7)
4	Tillering	Sparse (3), Intermediate (5), Dense (7)
5	Leaf density	Sparse (3), Intermediate (5), Dense (7)
6	Fruit set	Low(3) Intermediate(5) High(7)
7	Fruit colour at mature stage	Orange (6), Light red (7), Red (8), Dark red (9), Purple (10), Brown(11), Black (12)
8	Fruit Shape	Elongate (1), Almost round (2), Triangular (3),Campanulate (4), Blocky (5)
9	Fruit shape at pedicel attachment	Acute (1), Obtuse (3), Truncate (5), Cordate (7), Lobate (9)
10	Fruit shape at blossom end	Pointed(1) Blunt(2) Sunken(3) Sunken and pointed(4)

field management and crop protection activities were done as required.

Data recording

Data were collected on days to germination, flowering and fruiting and total fruit yield t ha^{-1} on plot basis. Five randomly selected plants from the central rows of each plot were used for data collection on plant height, canopy width, stem diameter(mm), number of flowers, leaves, branches and pods per plant, pod weight(g) and green pod yield(g) per plant. The pod length (cm) and width (cm) and pericarp thickness (mm) were measured from 10 pods harvested from each plot following the method adapted from IPGRI (1995).

Data analysis

Data for quantitative characters were subjected to analysis of variances (ANOVA) for simple lattice design using proc lattice procedure of SAS version 9.2(SAS Institute Inc., 2010) to test the presence of significant differences among genotypes; mean separations were estimated using Tukey Test at 5% probability level.

Genotypic and phenotypic variance and coefficient of variation

The variability present in the population was estimated by simple measure, namely range, mean, phenotypic and genotypic variance and coefficient of variation. The phenotypic and genotypic variance and coefficient of variation was estimated according to the method suggested by Burton and DeVane (1953) as follows: Genotypic Variance (σ^2g) = $\frac{MSg-MSe}{r}$,Phenotypic variance (σ^2p) = [σ^2g + (σ^2e/r)], Phenotypic coefficient of variation (PCV) = $\frac{\sqrt{\sigma 2p}}{\bar{x}} * 100$, Genotypic coefficient of variation (GCV) = $\frac{\sqrt{\sigma 2g}}{\bar{x}} * 100$, where, r = number of replication; MSg = mean square of genotypes and Mse = mean square of error, σ^2p = phenotypic variance, σ^2g = genotypic variance and $\bar{x}$ = grand mean of the character under consideration. Both phenotypic and genotypic coefficients of variations were categorized depending up on cut points suggested by Deshmukh et al. (1986) as low (<10%), moderate (10-20%) and high (>20%).

Estimate of broad sense heritability

(H^2) of all traits were calculated according to the formula as described by Allard (1960) as follows: h^2_{bs}= [(σ^2G) / (σ^2P)] × 100. According to Singh (2001) that heritability values ≥80% were very high, values from 60-79% were moderately high, values from 40-59% were medium and values less than 40% were low.

Genetic Advance (GA) for selection intensity (K) at 5% was computed according to Allard (1960) as given:

$$GA = K*\sigma p*H^2$$

Where, K = the standardized selection differential at 5% selection intensity (K = 2.063), σp = is phenotypic standard deviation on mean basis and H^2 = heritability in the broad sense.

The genetic advance as percentage of population means (GAM) was also estimated with the methods described by Johnson et al. (1955). Genetic advance as % of mean (GAM) was computed as:GAM = $\frac{GA}{\bar{x}} * 100$

Where, $\bar{x}$ = mean of the population. According to Johson et al. (1955) genetic advance as percent of mean was classified as low (<10%), moderate (10-20%) and high (>20%).

Character association

Character associations at genotypic and phenotypic levels were calculated from the genotypic, phenotypic and environmental covariance according to Singh and Chaundhary (1985). In Path analysis, yield per plant was taken as dependent variable while the rest of the characters was considered as independent variables. The direct and indirect effects of the independent characters on fruit yield per plant were estimated by the simultaneous solution of the formula suggested by Dewey and Lu (1959).

Frequency distribution and Shannon-Weaver Diversity Index (H')

Frequency distribution is a systematic way of ordering a set of data

Table 2. Pepper accessions used in the study.

S/N	Accession Name	Origin	Region	Taxonomy	No.	Accession name	Origin	Region	Taxonomy
1	Acc-1	Tselemti	Tigray	*Capsicum annuum L.*	33	Acc-33	Semien Gonder	Amhara	*Capsicum annuum L.*
2	Acc-2	Tanqua Abergelle	Tigray	*Capsicum annuum L.*	34	Acc-34	Ahferom	Tigray	*Capsicum annuum L.*
3	Acc-3	Welkait(Mygiba)	Tigray	*Capsicum annuum L.*	35	Acc-35	Bale	Oromiya	*Capsicum annuum L.*
4	Acc-4	Mekelle	Tigray	*Capsicum annuum L.*	36	Acc-36	Mirab Shewa	Amhara	*Capsicum annuum L.*
5	Acc-5	Ofla(Zata)	Tigray	*Capsicum annuum L.*	37	Acc-37	Semien Gonder	Amhara	*Capsicum annuum L.*
6	Acc-6	Ahferom	Tigray	*Capsicum annuum L.*	38	Acc-38	Mirab Gojam	Amhara	*Capsicum annuum L.*
7	Acc-7	Welkait	Tigray	*Capsicum annuum L.*	39	Acc-39	Mirab Gojam	Amhara	*Capsicum annuum L.*
8	Acc-8	Kilte Awulalo	Tigray	*Capsicum annuum L.*	40	Acc-40	Mirab Gojam	Amhara	*Capsicum annuum L.*
9	Acc-9	Kola Temben	Tigray	*Capsicum annuum L.*	41	Acc-41	Semien Shewa	Amhara	*Capsicum annuum L.*
10	Acc-10	Abergelle	Tigray	*Capsicum annuum L.*	42	Acc-42	Bale	Amhara	*Capsicum annuum L.*
11	Acc-11	Alamata	Tigray	*Capsicum annuum L.*	43	Acc-43	Metekel	B/Gumz	*Capsicum annuum L.*
12	Acc-12	Wojirat	Tigray	*Capsicum annuum L.*	44	Acc-44	Mirab Gojam	Amhara	*Capsicum annuum L.*
13	Acc-13	Welkait	Tigray	*Capsicum annuum L.*	45	Acc-45	Mirab Gojam	Amhara	*Capsicum annuum L.*
14	Acc-14	Embalaje	Tigray	*Capsicum annuum L.*	46	Acc-46	Misrak Gojam	Amhara	*Capsicum annuum L.*
15	Acc-15	Welkait Tsegede	Tigray	*Capsicum annuum L.*	47	Acc-47	Mirab Gojam	Oromiya	*Capsicum annuum L.*
16	Acc-16	Mereb Lehke	Tigray	*Capsicum annuum L.*	48	Acc-48	Guragae	SNNPRS	*Capsicum annuum L.*
17	Acc-17	Illubabor	Oromiya	*Capsicum annuum L.*	49	Acc-49	Guragae	SNNPRS	*Capsicum annuum L.*
18	Acc-18	Misrak Harerge	Oromiya	*Capsicum annuum L.*	50	Acc-50	Mirab Gojam	Amhara	*Capsicum annuum L.*
19	Acc-19	Illubabor	Oromiya	*Capsicum annuum L.*	51	Acc-51	Guragie	SNNPRS	*Capsicum annuum L.*
20	Acc-20	Semien Gonder	Amhara	*Capsicum annuum L.*	52	Acc-52	Mirab Gojam	Amhara	*Capsicum annuum L.*
21	Acc-21	Kembata Alaba	SNNPRS	*Capsicum annuum L.*	53	Acc-53	Mirab Gojam	Amhara	*Capsicum annuum L.*
22	Acc-22	Semien Gonder	Amhara	*Capsicum annuum L.*	54	Acc-54	Mirab Gojam	Amhara	*Capsicum annuum L.*
23	Acc-23	Semien Gonder	Amhara	*Capsicum annuum L.*	55	Acc-55	Mirab Gojam	Amhara	*Capsicum annuum L.*
24	Acc-24	Illubabor	Oromiya	*Capsicum annuum L.*	56	Acc-56	Mirab Gojam	Amhara	*Capsicum annuum L.*
25	Acc-25	Semen Omo	SNNPRS	*Capsicum annuum L.*	57	Acc-57	Butajira	SNNPRS	*Capsicum annuum L.*
26	Acc-26	Misrak Gojam	Amhara	*Capsicum annuum L.*	58	Acc-58	Mereb Lehke	Tigray	*Capsicum annuum L.*
27	Acc-27	Mirab Gojam	Amhara	*Capsicum annuum L.*	59	Acc-59	Abi Adi	Tigray	*Capsicum annuum L.*
28	Acc-28	Semien Gonder	Oromiya	*Capsicum annuum L.*	60	Acc-60	Mirab Gojam	Amhara	*Capsicum annuum L.*
29	Acc-29	Mirab Shewa	Oromiya	*Capsicum annuum L.*	61	Acc-61	Mereb Lehke	Tigray	*Capsicum annuum L.*
30	Acc-30	Illubabor	Oromiya	*Capsicum annuum L.*	62	Acc-62	Mereb Lehke	Tigray	*Capsicum annuum L.*
31	Acc-31	Mirab Shewa	Oromiya	*Capsicum annuum L.*	63	Acc-63	Melkassa	Oromiya	*Capsicum annuum L.*
32	Acc-32	Semien Gonder	Oromiya	*Capsicum annuum L.*	64	Acc-64	Melkassa	Oromiya	*Capsicum annuum L.*

Acc = accession; B/Gumz = Benishangul-Gumz Regional State; SNNPRS = Southern Nation; Nationalities and People's Regional State ; Acc-64; obtained from Melkassa Agricultural Research Center; Acc-64 is a standard check

Table 3. Analysis of Variance (ANOVA) for morphological and green pod yield and pod characters of 64 hot pepper genotypes.

Source	DF	DG	DIF	DFL	DFR	NFLPP	NLPP	NBPP	PHT	CW
Replication	1	0.07	84.5	40.50	2.53	17.04	0.10	1.58	551.12	19.92
Block(Rpn)	14	0.06	15.83	5.48	9.88	45.58	0.83	0.14	16.81	0.56
Genotypes(adji)	63	6.87**	50.46**	34.47**	48.39**	525.88**	2671.2**	16.17**	64.09**	52.10**
Intra block error	49	0.47	11.61	4.95	5.31	70.60	0.72	0.22	21.01	0.79
Source	**DF**	**SD**	**FL**	**FD**	**FPT**	**NFRPP**	**FW**	**GPYPP**	**TY**	
Replication	1	0.74	0.00	0.25	0.04	52.28	5.79	139.03	18699.00	
Block(Rpn)	14	1.39	0.47	0.99	0.02	11.99	1.33	22.55	341.35	
Genotypes(adji)	63	4.15**	25.47**	52.43**	0.46**	611.63**	77.97**	12541.8**	1252.7**	
Intra block error	49	1.04	0.35	1.34	0.01	25.80	2.25	25.30	285.04	

*and** = significant at 5% and 1% probability level, respectively. DF=degree of freedom, DG=days to germination, DIF= days first flowering, DFL= days to flowering, DFR= days to fruiting, NFLPP= number of flowers per plant, NLPP= number of leaves per plant, NBPP=number of branches per plant, PHT=plant height, CW=canopy width, SD=stem diameter, FL=fruit length, FD= fruit diameter, FPT= fruit pericarp thickness, NFRPP=number of fruit per plant, FW= fruit weight, GPYPP=green pod yield per plant, TY= total green pod yield per hectare.

from the lowest to the highest value showing the number of occurrences (frequency) at each value or range of values. The frequency distributions were used to calculate the Shannon-Weaver diversity index (H') for each character (Hennink and Zewan, 1991). The index is defined as:

$$H' = -\sum_{i=1}^{s}(pilnPi)$$

Where H'= diversity index
S= Total number of descriptors in the i^{-th} descriptor, Pi=fraction of individuals belonging to the i^{-th} descriptor state (number of observations/descriptor state in i^{-th} descriptor divided by the total number of characterized plants)

The Shannon weaver index values (H') can range from 0 to ~ 4.6. A value near 0 indicated that every species in the sample is the same and a value near 4.6 indicated the numbers of individuals are evenly distributed between the hot pepper species A low H' indicates unbalance frequency class and lack of diversity for the traits. A higher H' value indicates presence of variability or diversity of genotypes for the trait (Hennink and Zewan, 1991).

RESULTS AND DISCUSSION

Analysis of variance

The analysis of variance for all morphological, yield and fruit characters indicated significant ($P < 0.01$) differences among the genotypes (Table 3). This indicates the existence of substantial amount of variability among the genotypes tested which confirms the possibility to select best genotypes and exploit them for variety development. The significant differences observed for measured quantitative traits in this study were in agreement with the findings of earlier authors (Berhanu et al., 2011b; Birhanu, 2017; Shimeles, 2018) who reported considerable genetic variability within the hot pepper population for yield, fruit and growth characters.

Range and mean performance of accessions

The studied landraces exhibited a wide range of mean values for all traits, particularly for the economically most important traits, that is fruit yield per hectare which ranged from 3.8 to 14.3 tha^{-1}, whereas the mean was 9.3 tha^{-1} (Table 4).The mean, ranges in original units and as percent of the mean for the 17 quantitative traits of the 64 accessions are presented in Table 4. Since the various traits considered here were measured in different units, only variability in percent of the mean was used. The highest range of 296% was observed in fruit weight. Very high ranges were also observed for number of fruits per plant (194.55%), fruit length (172.98%), green pod yield per plant (171.53%), number of branches per plant (159.63%), fruit diameter (152.65%), fruit pericarp thickness (136.81%),number of flowers per plant (119.25%) and total green pod yield per hectare (114.66%). Ranges between 50 and 90% were observed for number of leaves per plant (79%), stem diameter (71%), days to germination (60%), canopy width (58%) and days to first flowering (57%). The remaining traits had low ranges which were between 45.68% for days to fruiting, 45.75% for days to flowering and 48.39% for plant height. This high range and mean value for each trait of interest suggests that great opportunity to improve the various desirable traits through selection as short term strategy and through hybridization as long term strategy. Hence, there is an opportunity to find genotypes having disease resistance and high yielding potential among the tested entries that perform better than the existing varieties to utilize for the future pepper improvement breeding.

Variance components

Estimates of phenotypic (σ^2p), genotypic (σ^2g) and environmental (σ^2e) variances and phenotypic coefficients of variation (PCV) and genotypic coefficients of variation (GCV) along with the mean and the range of various characters investigated in the present study are depicted

Table 4. Mean, range and range in % mean of 17 quantitative traits.

Trait	Mean	Min	Max	Range	Range in % mean
DG(Days)	13.30	10.5	18.5	8	60.13
DIF(Days)	57.61	47	80	33	57.28
DFL(Days)	62.30	54.5	83	28.5	45.75
DFR(Days)	76.44	67.18	102.1	34.92	45.68
NFLPP	76.35	40.95	132	91.05	119.25
NLPP	243.69	160.4	353	192.6	79.03
NBPP	7.45	2.2	14.1	11.9	159.63
PHT(cm)	63.44	47.1	77.8	30.7	48.39
CW(cm)	34.12	25.4	45.1	19.7	57.74
SD(mm)	12.75	9.8	18.86	9.06	71.03
FL(cm)	7.45	1.81	14.7	12.89	172.98
FD(mm)	16.76	7.11	32.7	25.59	152.65
FPT(mm)	1.53	0.64	2.73	2.09	136.81
NFRPP	44.00	16.3	101.9	85.6	194.55
FW(g)	8.62	0.94	26.5	25.56	296.35
GPYPP(g)	219.56	87.4	464	376.6	171.53
TY(tha^{-1})	9.3	3.8	14.3	10.6	11.5

DG=days to germination; DIF= days to first flowering; DFL= days to flowering; DFR= days to fruiting; NFLPP= number of flowers per plant; NLPP= number of leaves per plant; NBPP=number of branches per plant; PHT=plant height; CW=canopy width; SD=stem diameter; FL=fruit length; FD= fruit diameter; FPT= fruit pericarp thickness; NFRPP=number of fruit per plant; FW= fruit weight; GPYPP=green pod yield per plant; TY= total green pod yield per hectare.

in Table 5. For all studied characters, the magnitude of environmental variance was lower than the corresponding genotypic variance. This indicates that the genotypic component of variation was the major contributor to the total variation in the studied characters. According to the categories of Johnson et al. (1955), both GCV and PCV were high for fruit weight (71.34, 72.39), fruit length (47.55, 47.89), number of fruits per plant (38.90, 39.75), number of branches per plant (37.88, 38.14), green pod yield plant (36.03, 36.07), fruit pericarp thickness (31.19, 31.54), fruit diameter (30.15, 30.54) and total green fruit yield per hectare (30.51, 32.04), respectively. The high values of PCV and GCV indicated the existence of substantial variability, ensuring better scope for their improvement through selection of these traits (Rosmaina et al., 2016). The moderate values of GCV and PCV were recorded for number of flowers per plant (19.76,21.24), number of leaves per plant (14.99,15), canopy width (14.85,14.96) and days to germination (13.44, 13.93); while low for stem diameter, plant height, days to first flowering, days to flowering, days to fruiting, respectively. This indicates low sensitivity of most of the traits to the effects of environmental factors, and expressions of these traits are dependent more on genetic factors rather than on environmental conditions. Higher magnitude of phenotypic coefficients of variation (PCV) than genetic coefficient of variation (GCV) indicated the role of environment for expression of the traits. Similar finding was reported by Berhanu et al. (2011a) indicating that days to flowering and days to maturity had low GCV and PCV values, while fruit weight, number fruits per plant, number of primary branches per plant had high GCV and PCV. Razzaq et al. (2016) reported high values of GCV and PCV for weight of red fruit (110.02% and 112.02%) and number of fruits per plant (85.02% and 86.05%). Shimeles et al. (2016) also reported high estimates of GCV and PCV for fruit weight, number of branches per plant and number of fruits per plant. In addition, similar findings were reported by Sharma et al. (2010) and Rosmaina et al. (2016).

Heritability and genetic advance

The effectiveness of selection for any trait depends not only on the extent of genetic variability but also on the extent of transferring genes from one generation to the other (Rosmaina et al., 2016). According to Singh (2001) heritability values greater than 80% are considered as very high, 60-79% as moderately high, from 40-59% as medium and values less than 40% as low. Accordingly, the estimates of heritability of all traits in the current study were moderate to very high. In this study heritability (H^2) varied from 99.97 to 62.21% and the highest estimate of heritability was observed for number of leaves per plant (99.97%) followed by green fruit yield per plant (99.80%), fruit length (98.62%), fruit pericarp thickness (97.76%) and fruit diameter (97.44%) (Table 5). Whereas the estimates heritability was moderately high for green fruit yield per hectare (77.25%), days to first flowering

Table 5. Estimates of Range, Mean, Genotypic, Environmental and Phenotypic variances and Coefficient of variations, Heritability in broad sense, Genetic advance and Genetic advance as percentage of mean for 17 characters of 64 hot Pepper genotypes.

Characters	Ranges	Mean ± SEM	σ^2g	σ^2e	σ^2p	GCV (%)	PCV (%)	H^2 (%)	GA	GAM (%)
DG	10-18.5	13.3±0.49	3.20	0.24	3.43	13.44	13.93	93.15	3.56	26.77
DIF	47-80	57.61±2.41	19.43	5.80	25.23	7.65	8.72	76.99	7.98	13.85
DFL	54.5-83	62.30±1.57	14.76	2.48	17.23	6.17	6.66	85.63	7.33	11.77
DFR	67.18-102.1	76.44±1.63	21.54	2.65	24.20	6.07	6.44	89.03	9.03	11.82
NFLPP	40.95-132	76.35±5.94	227.64	35.30	262.94	19.76	21.24	86.58	28.96	37.93
NLPP	160.4-353	243.69±0.60	1335.26	0.36	1335.62	14.99	15.00	99.97	75.37	30.93
NBPP	2.2-14.1	7.45±0.33	7.97	0.11	8.08	37.88	38.14	98.64	5.79	77.61
PHT	47.1-77.8	63.44±3.24	21.54	10.51	32.05	7.32	8.92	67.21	7.85	12.37
CW	25.4-45.1	34.12±0.63	25.66	0.39	26.05	14.85	14.96	98.49	10.37	30.40
SD	9.8-18.86	12.75±0.72	1.55	0.52	2.07	9.78	11.29	74.99	2.23	17.46
FL	1.81-14.7	7.45±0.42	12.56	0.18	12.73	47.55	47.89	98.62	7.26	97.43
FD	7.11-32.7	16.76±0.82	25.54	0.67	26.21	30.15	30.54	97.44	10.29	61.40
FPT	0.64-2.73	1.53±0.07	0.23	0.01	0.23	31.19	31.54	97.76	0.97	63.61
NFRPP	16.3-101.9	44±3.59	292.91	12.90	305.81	38.90	39.75	95.78	34.55	78.54
FW	0.94-26.5	8.62±1.06	37.86	1.13	38.98	71.34	72.39	97.11	12.51	145.03
GPYPP	87.4-464	219.56±3.56	6258.23	12.65	6270.88	36.03	36.07	99.80	163.04	74.26
TY	37.5-143.3	92.26±11.94	483.83	142.52	626.35	23.84	27.13	77.25	39.88	43.23

DG=days to germination; DIF= days to first flowering; DFL= days to flowering; DFR= days to fruiting; NFLPP= number of flowers per plant; NLPP= number of leaves per plant; NBPP=number of branches per plant; PHT=plant height; CW=canopy width; SD=stem diameter; FL=fruit length; FD= fruit diameter; FPT= fruit pericarp thickness; NFRPP=number of fruit per plant; FW= fruit weight; GPYPP=green pod yield per plant; TY= total green pod yield per hectare; SEM = standard error of the mean; σ^2g = genotypic variance; σ^2e = error variance; σ^2p = phenotypic variance; PCV = phenotypic coefficient of variance; GCV = genotypic coefficient of variance; H^2 = broad sense heritability; GA = genetic advance; GAM = genetic advance as percent of mean.

(76.99%), stem diameter (74.99)and plant height (67.21%). The characters having very high heritability indicated relatively small contribution of the environmental factors to the phenotype and selection for such characters could be fairly easy due to high additive effect.

Heritability alone provides no indication of the amount of genetic improvement that would result from selection of individual genotypes. Hence knowledge about genetic advance coupled with heritability is very useful. A trait exhibiting high heritability may not necessarily give high genetic advance. According to Jonhson et al. (1955) high heritability accompanied by high genetic advance could help to arrive at more reliable conclusion. In the present investigation high to moderate heritability coupled with high to moderate genetic advance as percent of the mean were observed for all the traits. Similar findings were reported by earlier workers for some characters with moderate to high GCV, PCV, heritability and GAM estimates, for fruit yield per plant, fruit diameter, fruit length, average fruit weight and number of fruits per plant (Sharma et al., 2010; Sahu et al., 2016; Razzaq et al., 2016; Pujar et al., 2017).

Character association

Association of fruit yield with yield components was detected (Table 6). Genotypic correlation coefficients were slightly higher than the corresponding phenotypic correlation coefficients. This indicated that there were strong inherent relations among the traits studied. Pod yield per plant had significant and positive genetic and phenotypic correlations with average fruit weight (0.72, 0.71), fruit length (0.69, 0.68), fruit diameter (0.61, 0.60) and fruit pericarp thickness (0.56, 0.55), respectively. However, non- significant positive correlation in case of stem diameter, and plant height at both genotypic and phenotypic levels were observed. Average green fruit weight had also significant positive correlation with fruit diameter (0.89, 0.87), fruit length (0.87, 0.86), and fruit pericarp thickness (0.77, 0.75) at both genetic and phenotypic levels, respectively. This suggested that, selection and improvement of genotypes based on those characters would result in a substantial increment on fruit yield of hot pepper. Similarly, Abrham et al. (2017) and Shimeles (2018) reported higher genotypic correlation coefficients than the phenotypic ones, implying the inherent associations between various characters in Ethiopian Capsicums.

The result further illustrated that plant height was non-significantly correlated with most of the traits at phenotypic level except stem diameter (0.59) and canopy width (0.29); however, at both genotypic and phenotypic levels it was positively and significantly correlated with

Table 6. Estimation of genotypic (rg) (above diagonal) and phenotypic (rp) (below diagonal) correlation coefficients for 14 traits in 64 hot pepper genotypes.

Traits	DFL	DFR	NFLPP	NLPP	NBPP	PHT	CW	SD	FL	FD	FPT	NFRPP	FW	GPYPP
DFL		0.79**	-0.19ns	-0.01ns	0.29*	0.61**	0.30*	0.71**	-0.27*	-0.28*	-0.21ns	0.33**	-0.25*	-0.14ns
DFR	0.75**		-0.02ns	0.02ns	0.46**	0.53**	0.36**	0.67**	-0.46**	-0.46**	-0.31*	0.42**	-0.48**	-0.37**
NFLPP	-0.14ns	-0.04ns		0.56**	0.41**	-0.03ns	0.23*	-0.06ns	-0.33**	-0.30*	-0.13ns	0.25*	-0.36**	-0.12ns
NLPP	0.00ns	0.02ns	0.53**		0.31*	0.02ns	0.33**	0.12ns	-0.19ns	-0.09ns	0.01ns	0.23ns	-0.12ns	0.11ns
NBPP	0.28**	0.43**	0.39**	0.31**		0.17ns	0.56**	0.41**	-0.67**	-0.63**	-0.51**	0.67**	-0.69**	-0.31*
PHT	0.47**	0.39**	-0.01ns	0.01ns	0.16ns		0.32*	0.74**	-0.11ns	-0.11ns	-0.04ns	0.17ns	-0.10ns	0.05ns
CW	0.28**	0.33**	0.22*	0.33**	0.56**	0.29**		0.49**	-0.44**	-0.54**	-0.42**	0.56**	-0.50**	-0.16ns
SD	0.60**	0.57**	-0.06ns	0.10ns	0.36**	0.59**	0.44**		-0.28*	-0.28*	-0.21ns	0.47**	-0.25*	0.01ns
FL	-0.26**	-0.44**	-0.31**	-0.19*	-0.66**	-0.09ns	-0.43**	-0.25**		0.68**	0.63**	-0.61**	0.87**	0.69**
FD	-0.27**	-0.43**	-0.28**	-0.09ns	-0.62**	-0.09ns	-0.53**	-0.25**	0.67**		0.82**	-0.71**	0.89**	0.61**
FPT	-0.19*	-0.28**	-0.13ns	0.01ns	-0.50**	-0.06ns	-0.41**	-0.20*	0.61**	0.80**		-0.58**	0.77**	0.56**
NFRPP	0.30**	0.39**	0.24**	0.23**	0.66**	0.16ns	0.55**	0.42**	-0.60**	-0.69**	-0.57**		-0.70**	-0.40**
FW	-0.25**	-0.46**	-0.34**	-0.12ns	-0.68**	-0.08ns	-0.49**	-0.22*	0.86**	0.87**	0.75**	-0.68**		0.72**
GPYPP	-0.13ns	-0.35**	-0.11ns	0.11ns	-0.31**	0.04ns	-0.16ns	0.01ns	0.68**	0.60**	0.55**	-0.39**	0.71**	

ns= non Significance *and **=significant at 5% and 1% probability levels; respectively. DFL= days to flowering; DFR= days to fruiting; NFLPP= number of flowers per plant; NLPP= number of leaves per plant; NBPP=number of branches per plant; PHT=plant height; CW=canopy width; SD=stem diameter; FL=fruit length; FD= fruit diameter; FPT= fruit pericarp thickness; NFRPP=number of fruit per plant; FW= fruit weight; GPYPP=green pod yield per plant.

days to flowering and days to fruiting.

The study confirmed significant association between branch number and canopy width was significant at both genotypic and phenotypic (0.56, 0.56) levels. Furthermore, branch number had positively significant association with number of leaves per plant, number of flowers per plant, days to fruiting and flowering at both genotypic and phenotypic, levels respectively. Fruit length depicted positive significant correlation at both genotypic and phenotypic levels with fruit width, fruit pericarp thickness and fruit weight. These results are in agreement with those reported by Sharma et al. (2010) and Abrham et al. (2017) who advocated that importance should be given to number of fruits per plant, fruit weight, number of primary branches, fruit length, fruit diameter and plant height during selection process because these characters contribute directly towards the yield.

The study revealed that days to 50% flowering had positive and highly significant association with days to 50% fruiting, Plant height, stem diameter, canopy diameter, number of branches per plant and number of fruits per plant both at genotypic and phenotypic levels (Table 6). The positive correlations between different traits show the possibility of improving hot pepper based on these multiple traits.

Days to fruiting had significant and positive correlation at both genotypic and phenotypic level with number of primary branches per plant, number of fruits per plant, canopy width, stem diameter and plant height. In contrast, days to fruiting exhibited significant negative correlation both at genotypic and phenotypic level with fruit length, fruit diameter, fruit pericarp thickness, average fruit weight and fruit yield per plant. This reveals that early flowered genotypes produced long and large pods with thick pericarp and high fruit yield per plant. Similarly, Sharma et al. (2010) reported a high positive significant correlation of days to 50% flowering and days to fruiting suggesting that early flowering traits would be an appropriate selection criterion to get early fruit yield.

The current result exhibited that green pod yield had significant positive genotypic and phenotypic correlations with fruit length, fruit diameter, fruit pericarp thickness and fruit weight. Hence, these traits were found to be yield contributing characters towards increased fruit yield and weight. This also might indicate complementary gene actions for the traits which could be selected simultaneously.

Table 7. Estimates of direct (bold and diagonal) and indirect effect (off diagonal) of different characters on green pod yield per plant at genotypic level in 64 hot Pepper genotypes.

Traits	DFL	DFR	NFLPP	NLPP	NBPP	PHT	CW	SD	FL	FD	FPT	NFRPP	FW	rg
DFL(Days)	**0.08**	-0.24	-0.01	0.00	0.08	-0.02	0.02	0.23	-0.13	-0.03	-0.01	-0.03	-0.09	-0.14ns
DFR(days)	0.07	**-0.30**	0.00	0.00	0.12	-0.02	0.03	0.21	-0.22	-0.04	-0.01	-0.03	-0.17	-0.37**
NFLPP	-0.02	0.01	**0.07**	0.05	0.11	0.00	0.02	-0.02	-0.15	-0.03	0.00	-0.02	-0.13	-0.12ns
NLPP	0.00	-0.01	0.04	**0.09**	0.08	0.00	0.02	0.04	-0.09	-0.01	0.00	-0.02	-0.04	0.11ns
NBPP	0.02	-0.14	0.03	0.03	**0.27**	-0.01	0.04	0.13	-0.31	-0.06	-0.02	-0.05	-0.25	-0.31*
PHT(cm)	0.05	-0.16	0.00	0.00	0.05	**-0.04**	0.02	0.23	-0.05	-0.01	0.00	-0.01	-0.03	0.05ns
CW(cm)	0.02	-0.11	0.02	0.03	0.15	-0.01	**0.07**	0.16	-0.20	-0.05	-0.02	-0.04	-0.18	-0.16ns
SD(mm)	0.06	-0.20	0.00	0.01	0.11	-0.03	0.03	**0.32**	-0.13	-0.03	-0.01	-0.04	-0.09	0.01ns
FL(cm)	-0.02	0.14	-0.02	-0.02	-0.18	0.00	-0.03	-0.09	**0.46**	0.07	0.02	0.05	0.31	0.69**
FD(cm)	-0.02	0.14	-0.02	-0.01	-0.17	0.00	-0.04	-0.09	0.32	**0.10**	0.03	0.05	0.32	0.61**
FPT(mm)	-0.02	0.09	-0.01	0.00	-0.14	0.00	-0.03	-0.07	0.29	0.08	**0.04**	0.04	0.27	0.56**
NFRPP	0.03	-0.13	0.02	0.02	0.18	-0.01	0.04	0.15	-0.28	-0.07	-0.02	**-0.08**	-0.25	-0.40**
FW(g)	-0.02	0.14	-0.03	-0.01	-0.19	0.00	-0.04	-0.08	0.40	0.09	0.03	0.05	**0.36**	0.72**

*and ** = significant at 5% and 1% probability levels; respectively. DFL= days to flowering; DFR= days to fruiting; NFLPP= number of flowers per plant; NLPP= number of leaves per plant; NBPP=number of branches per plant; PHT=plant height; CW=canopy width; SD=stem diameter; FL=fruit length; FD= fruit diameter; FPT= fruit pericarp thickness; NFRPP=number of fruit per plant; FW= fruit weight; GPYPP=green pod yield per plant; rg = genotypic coefficient of correlation.

Therefore, fruit length, fruit diameter andfruit weight were the most important traits for improving the genotypes for higher fruit yield and may be applied for selection in hot pepper improvement. The results agreed well with Shimeles (2018) who found high positive genotypic correlation of fruit yield with the pericarp thickness. In addition, Razzaq et al. (2016) reported a significant positive correlation between fruit width and fruit length with fruit yield per plant and plant height with fruit length which was in agreement with the current finding. They further suggested that, the presence of such effects of genes lead to the improvement of yield as the improvement made in these characters. Lavinia et al. (2013) confirmed the existence of strong correlation between fruit weight to fruit length and diameter and also weight of fruits per plant. They further concluded that selection made towards increasing the length and diameter of pods can be used as indirect selection criteria to develop varieties with highest fruit weight.

Path coefficient analysis

Significant genetic correlation coefficient between two traits does not always indicate the presence of linkage between them (Sigh, 2001). Path analysis is the partitioning of the correlations into direct and indirect effects. Fruit yield being the complex outcome of various traits was considered to be the resultant variable and the rest of the variables viz; days to flowering, days to fruiting, number of flowers per plant, number of leaves per plant, number of branches per plant, plant height, canopy width, stem diameter, fruit length, fruit diameter, fruit pericarp thickness, number of fruits per plant, fruit weight, green pod yield per plant, were the causal variables. It was observed that each of these traits did influence fruit yield directly or indirectly. The path analysis was done at genetic level and the results are given in Table 7. Fruit length exhibited the highest positive direct effect (0.46) on fruit yield per plant; and had also indirect positive effects on average fruit weight, fruit diameter, and fruit pericarp thickness. The second maximum positive direct effect was exerted by Average fruit weight (0.36) and had positive and significant correlation with fruit yield per plant. This suggests that the correlation has revealed the true relation and direct selection through this trait could be effective. Stem diameter, number of branches per plant, fruit diameter, number of leaves per plant ,number of flowers per plant, canopy width and fruit pericarp thickness had also positive direct effect on green fruit yield per plant. Similar result was reported by Abrham et al. (2017) who found fruit length and diameter could be the most important yield component characters which might be used as selection criteria for yield improvement.

Thus, on the basis of current result, green fruit length, fruit pericarp thickness, average fruit weight, and number of primary branches per plant could be the most important yield components which might be considered as selection criteria for yield improvement. Similar results had been reported by Kumari (2017). Similarly, Shimeles (2018) reported that direct influence of pericarp thickness on fruit yield was very high and positive and its indirect influence through fruit diameter was also positive. However, pericarp thickness showed high negative indirect effect on number of fruits per plant.

Table 8. Frequency distribution, proportion and Shannon-waver diversity index (H') of qualitative traits of 64 hot pepper Landraces.

Characters	Description and codes	Frequency distribution		(H')
		No. of accessions	Percent (%)	
Plant growth habit	Prostrate(3)	13	20.31	0.95
	Intermidate(5)	20	31.25	
	Erect(7)	31	48.44	
Leaf color	Yellow (1)	5	7.8	0.92
	Light green(2)	20	31.25	
	Green (3)	15	23.44	
	Dark green(4)	24	37.51	
Branching habit	compact(3)	6	9.34	0.85
	sparse(5)	28	43.75	
	Dense(7)	30	46.88	
Tillering	Sparse(3)	28	43.75	**0.98**
	Intermediate(5)	17	26.56	
	Dense(7)	19	29.69	
Leaf density	Sparse(3)	2	3.12	0.67
	Intermediate (5)	19	29.69	
	Dense(7)	43	67.19	
Fruit set	Low(3)	13	20.31	0.92
	Intermediate(5)	17	26.56	
	High(7)	34	53.13	
Fruit colour at mature stage	Light red(7)	18	28.13	0.83
	Red(8)	19	29.69	
	Dark Red(9)	26	40.62	
	brown(11)	1	1.56	
Fruit shape	Elongate(1)	50	78.13	**0.45**
	Almost round(2)	1	1.56	
	Triangular(3)	10	15.62	
	Campanulate(4)	1	1.56	
	Blocky(5)	2	3.12	
Fruit shape at pedicel attachment	Acute(1)	9	14.06	0.89
	Truncate(3)	18	28.12	
	Cordate(4)	7	10.93	
	Lobate(5)	30	46.88	
Fruit shape at blossom end	Pointed(1)	37	57.81	0.73
	Blunt(2)	19	29.69	
	Sunken(3)	6	9.38	
	Sunken and pointed(4)	2	3.12	
Overall mean of H'				**0.82**

Days to fruiting had a high direct negative effect on fruit yield per plant (-0.3), but indirect positive effect on average fruit weight, fruit length, fruit diameter, fruit pericarp thickness and number of flowers per plant. This suggested that early fruiting traits would be an appropriate selection criterion to get early fruit yield. Number of fruits per plant had direct negative effect on fruit yield per plant (-0.08), but it showed indirect high positive effect on average fruit weight, fruit pericarp thickness, fruit diameter and fruit length. Similar finding was reported by

Berhanu et al. (2011b) who observed direct positive effects of fruit weight, canopy width, fruit pericarp thickness and number of branches per plant on fruit yield per plant. The set of characters identified as selection indices for fruit yield per plant based on the genetic variability parameters for the characters, their correlations and path coefficient analysis are: fruit length, average fruit weight, stem diameter, number of branches per plant fruit diameter, number of leaves per plant, number of flowers per plant and canopy width.

Frequency distribution and Shannon-Weaver diversity Index (H') analysis of qualitative characters

Frequency distribution patterns, percent of proportion and Shannon-Weaver diversity index were estimated for 64 hot pepper genotypes from 10 qualitative characters and results are presented in (Table 8). Generally, 48.44% of them showed an erect growth habit, 31.25 and 20.31% showed intermediate and prostrate growth habits, respectively. The proportions of genotypes for dense, sparse and compact branching habits were 46.88, 43.75 and 9.34%, respectively. Based on their fruit colour, the genotypes were categorized into dark red (40.62%), Red (29.69%), light red (28%) and brown (1.5%). The predominant leaf colour was dark green (37.51%), light green (31.25%), green (23.44%) and yellow (7.8%). For the fruit shape, 78.13% of the genotypes were elongated, 15.62% triangular and 3.12% were blocky types. In addition, 57.81% of the genotypes have fruits with pointed blossom-end and those showed Blunted and sunken fruit types were 29.69 and 9.38%%, respectively.

The value of Shannon-Weaver diversity index for all characters varied from 0.45 for fruit shape to 0.98 for tillering with an overall mean of 0.82 and also for all of the traits assessed such as plant growth habit (0.95), leaf color (0.92), fruit set (0.92), fruit shape at pedicel attachment (0.89), branching habit (0.85), fruit color at mature stage (0.83) and fruit shape at blossom end (0.73). The overall mean of H' value of 0.82 confirmed the existence of diversity among the accessions.

Furthermore, the diversity indices of all of the quality traits suggested the presence of adequate variability for these traits among genotypes. High Shanon-Waver diversity index with an overall mean of 82% was obtained. The predominant traits that showed wider variations among the genotypes were sparse tillering (98%), followed by erect growth habit (95%) and dark green leaf color (92%). The lowest diversity value of less than the overall mean was recorded for fruit shape (45%) indicating that most of the genotypes used for this study had elongated fruit length. Nsabiyera et al. (2013) reported that the frequency distribution and Shanon weaver-diversity index and observed highly divergent qualitative traits of hot pepper collections. Similar agreement with Shimeles (2018) who found greater level of diversity which ranged from 0.65 to 0.98 among hot pepper quality traits from Bale, Halaba, Assossa, Abshge and Marko parts of the country.

Conclusion

The study showed significant amount of variation among genotypes in most of the studied traits of hot pepper. High heritability and genetic advance were observed in fruit yield per plant, fruit diameter, fruit length, average fruit weight and number of fruits per plant. Yield had significant positive associations with fruit weight, fruit length, fruit diameter and fruit pericarp thickness. From path analysis, fruit length, average fruit weight, stem diameter, number of branches per plant, fruit diameter and fruit pericarp thickness exhibited the highest direct positive effects on fruit yield Overall, the results of this study indicated fruit length and diameter, average fruit weight, number of branches per plant and fruit pericarp thickness can be used as indirect selection criteria in improving hot pepper for green pod production.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGMENT

The authors gratefully acknowledge the Axum Agricultural Research Center for funding the project. It is also time to thank the staff of Horticulture case team in core process of Horticulture and plant sciences for the execution of the experiment.

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Vol. 12(1), pp. 70-90, January-March 2020
DOI: 10.5897/JPBCS2019.0866
Article Number: 385BEEF63332
ISSN 2006-9758

http://www.academicjournals.org/JPBCS

Journal of Plant Breeding and Crop Science

Full Length Research Paper

Evaluating natural infection of fungal, bacterial and viral pathogens to dry bean genotypes under field conditions

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Received 11 December, 2019; Accepted 3 February, 2020

Fungal, bacterial and viral diseases are economic foliar diseases that cause yield losses, between 40 and 100%, in commonly grown dry bean cultivars in the world. Development of disease resistance genotypes is a complex interaction between genetic and environmental factors. This study focused on determining the natural infection of disease-causing pathogens of angular leaf spot, powdery mildew, bacterial blight and bean common mosaic virus in different agro-ecologies in relation to grain yield. Diversity of 211 bean genotypes were tested at two different disease hot spots areas under incomplete block design, with two replications for two cropping seasons in Tanzania. Diseases severity was significantly different ($p<0.001$) for genotypes and their interactions with the environment and season. Higher disease severity was observed at Lyamungo site than Selian site. Effects of genotypes by environment were observed with maximum yield of 2170 kg/ha to low yield of 398 kg/ha with the grand mean of 1151.54 kg/ha. High annual rainfall and relative humidity contributed to disease development among the tested environment. Five genotypes (FEB 189, A774, NUA 16, KG 71-4 and DOR 766) expressed trait of resistance to above diseases and are advised to be incorporated in breeding programs for enhancing dry bean productivity.

Key words: Diseases, losses, productivity, G*E interaction.

INTRODUCTION

Dry bean (*Phaseolus vulgaris* L.) is the most widely grown and significantly consumed grain legume in the world (Broughton et al., 2003; FAOSTAT, 2018). There are two origin centers, Mesoamerican origin in southern Mexico and Guatemala, as well as Andean origin in Peru and Columbia (Landon, 2008; Kwak et al., 2009) where this crop originated from; before spreading across the world (Kwak and Gepts, 2009). Dry bean is the primary food crop with the highest level of variation in adaptation, maturity, growth habit (habitat) and seed characteristics (size, shape and color) (Peters, 1993). Additionally, some geographic regions are favored with producing large size seeds, medium (25 to 40 g per 100 seeds) or large (>40 g per 100 seeds).

Global production is hindered by biotic and abiotic factors resulting in commercial varieties yielding lower

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than their potentiality (De Leque and Creamer, 2014). Based on the economic importance, fungal diseases cause higher losses followed by bacterial and viral diseases (Mahuku and Riascos, 2004). For instance, the percentage of damage caused by fungal diseases is 80% by Angular Leaf Spot (ALS), *Phaeoisariopsis griseola* (Sacc.); 100% by Anthracnose, *Colletotrichum lindemuthianum;* and more than 50% by Powdery mildew (PM), *Erysipelas polygoni*. Bean common blight (*Xanthomonas axonopodis* pv. *phaseoli),* which is a bacterial disease, causes 45% losses (Nkalubo et al., 2007). Viral diseases, such as *Bean common mosaic virus* (BCMV) and *Bean common mosaic necrosis virus* (BCMNV), can cause 100% yield losses (Hillocks et al., 2006; Buruchara et al., 2010; Singh and Schwartz, 2010; Mwaipopo et al., 2016) with their major transmission and survival structures which includes seeds, wind and plant debris.

These diseases (ALS, CBB, PM and BCMV) severely affect farmers' field in Tanzania, which ranks number one in Africa and 6th in the world (FAOSTAT, 2018). The development of foliar disease resistance bean genotypes through understanding of the environmental factors and gene alleles interactions may help gain insight into disease etiology and sub-classification; also, management options would offer better strategies for bean breeding program (Wang et al., 2005). The marked recent improvement in bean breeding program is in the initial stage for biotechnology approaches (Harwell et al., 2011). It has been shown that field crop phenotyping under natural infection assists desirable trait assessments for genetic variability aimed at selecting genotypes with better traits for enhanced improvement (Sankaran et al., 2015). The objectives of this research study are to (a) evaluate the response of dry bean genotypes to ALS, CBB, PM and BCMV diseases at different environments and seasons; (b) screen best genotypes with high yield and resistance traits for breeding purposes to all diseases and their specificity; (c) compare disease occurrence in relation to cropping seasons for actual rainfall and temperature.

MATERIALS AND METHODS

Plant materials

A total of 211 genotypes were collected from different sources: Ethiopia (12), International Center for Tropical Agriculture (CIAT)-Kawanda (184), Kenya (10), Tanzania (3) and Rwanda (2) (Supplement 1) with various market classes evaluated under natural field conditions for 2016/17 and 2017/18 cropping season. From these materials, resistant checks of MEX 54 and G5686 (Mahuku et al., 2009) was used for ALS, Vax 1(Singh et al., 2001) was used for CBB, MAZ 47 was used for BCMV; unfortunately, checks for PM disease was not included.

Experimental area

The experiment was conducted at low to high altitudes, 1407 m above sea level of S03°21.690' and E36°37.879' at Selian Agricultural Research Institute (SARI) and Tanzania Coffee Research Institute commonly called Lyamungo, with 992 m.a.s.l of 03°19.905' and E037°14.067', respectively. The characteristics of this soil area are: Eutrophic Brown Soils on volcanic and Alluvial sediments - Medium texture (loamy soils) (Brady and Weil, 2002; Landon, 1991). The soil contains organic carbon (0.53%), organic matter (0.92%), total nitrogen (0.079%), exchangeable potassium (0.17 cmol (+)/kg) and medium available phosphorous (8.0 mg/kg); this means that the soil fertility status is medium fertility which is moderately suitable for bean cultivation (Kiriba et al., 2020). These selected sites are close to the weather station, from whence respective weather data were collected.

Disease evaluation and grain yield determination

Each disease (ALS, PM, CBB and BCMV) was rated using 1 to 9 scale as described by CIAT (1987) and CIAT-Kawanda (2013); where 1 to 3 refer to resistant, 4 to 6 intermediate, and 7 to 9 susceptible. Grain yield was measured from each plot using digital scale with 11lb (model No. SKS - 006, China). Final grain yield was extrapolated into kg/ha, using the following formula:
Grain yield (kg/ha) =$(dy * 10)/dx$

Where dy =plot weight and dx=plot area.

Experimental design

In both locations, in all growing season, trials were laid out under incomplete block design with two replications. The experimental plot size was 4 rows, 3.2 m long and 50 cm apart and 20 cm within a row. The harvested net plot size was 3.2 m^2 of the centered two rows of each plot. Other practices were carried out as recommended by National *Phaseolus* Bean Research Program in Tanzania (Binagwa, 2017).

Statistical analysis

The collected data were subjected to GenStat 16th Edition with the following linear model:

$Yijk = \mu + Gi + \gamma j + Gi*\gamma j + \partial k + Gi*\gamma j* \partial k + eijk$

Where Yijk = Response variable (Yield) for variety i, environment j and season k; μ = Overall mean for all the observed response; Gi = Fixed effect of variety; γj = random environmental effect of the observed response; Gi*γj = Interaction effects between variety and environment; ∂k = Random effect of replication within a season; Gi*γj*∂k = Interaction effect of variety, environment and season; eijk = Random term error which is assumed to be normally distributed with 0 mean and variance δ^2 which were summarized in a given results. Data were tested using Analysis of Variance (ANOVA) for single and multiple treatment interactions. The protected Least Significant Differences (LSD) of (p=0.05) were used to test for treatment comparison (Clewer and Scarisbrick, 2001; Yu 2008).

RESULTS

Classification of dry bean genotypes used

Through seed morphological description process, the

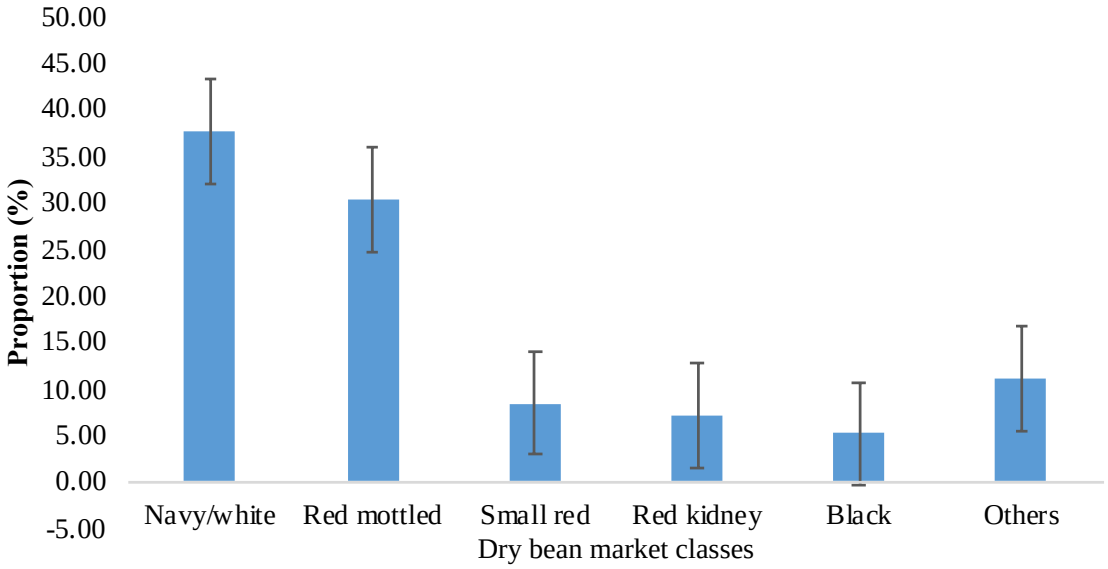

Figure 1. Proportional of dry bean seed market classes.

genotypes utilized were classified as 63.50% from Mesoamerican and 36.50% from Andean gene pools of origin. Based on market class, navy/white is dominant by 32.70% followed by red mottled, 30.33%. The remaining market classes were small red (8.53%), red kidney (7.10%), black, carioca, kablanket and cream (Figure 1). Seed size was large, medium and small, proportional to 36.02, 9.95 and 54.03%, respectively.

Effects of fungal disease-causing pathogens

The ANOVA analysis showed that the effects of genotype, environment, season and the interactions of genotype and environment (G*E) were significantly different at p<0.001; while that of genotype and seasons where significant at p=0.002 for ALS infections. The effects of ALS disease caused by *P. griseola* were high at Lyamungo site with the severity of up to 5.00 scale; while 4.20 at SARI. The G*E effects were observed between the range of 2.00 to 3.50 scale (Figure 2A). In relation to season, infection was high in the 2017/18 season ALS, with severity score >5.00; while that of 2016/17 reached ~2.80 and most of the genotypes expressed none pathogen infection (Figure 2B). Overall, 38 genotypes had higher severity scores above the grand mean (Supplement 2). For PM infection caused by *E. polygoni*, disease severity was high at Lyamungo site with score >6.00; while ~4.50 at SARI (Figure 2C and D). The effects of genotypes, environment, season, G*E, G*S and G*E*S were also significant (p<0.001) for PM infection. The PM infection was high at Lyamungo, followed bywith SARI site during the 2017/2018 growing season, with severity scores between 2.00 to 7.00 and 1.00 to 5 .00 (Figure 2E) (Supplement 2).

Effect of bean bacterial blight pathogen

Two bean growing season results showed significant difference (p<0.001) between genotype, season, G*E, G*S and G*E*S for CBB reaction, and about 82 genotypes showed their disease severity scores were above the grand mean of >3.00 scores. The effect was high at Lyamungo, ~4.70; while at SARI, severity scores was high up to 4.20 (Figure 2F). The growing season of 2017/2018 had more infections of CBB than that of 2016/2017 growing season, with more scatter points above 3.50 disease scores (Figure 3A and Supplement 2).

Infection of bean common mosaic virus

There was significant difference (p<0.001) between G*E and G*E*S (p=0.03) for dry bean common mosaic virus. A few genotypes, namely CC 906, 222/1, Flor De Mayo, DOR 755, MLB48-89A and A 686, had severity scores within the ranges of 3.25 to 4.50. Narrow variation observed showed resistance scores across the tested sites due to most genotypes (Figure 3B and Supplement 2).

Grain yield production across the environment and season

Genotype, environment, G*E and G*E*S showed

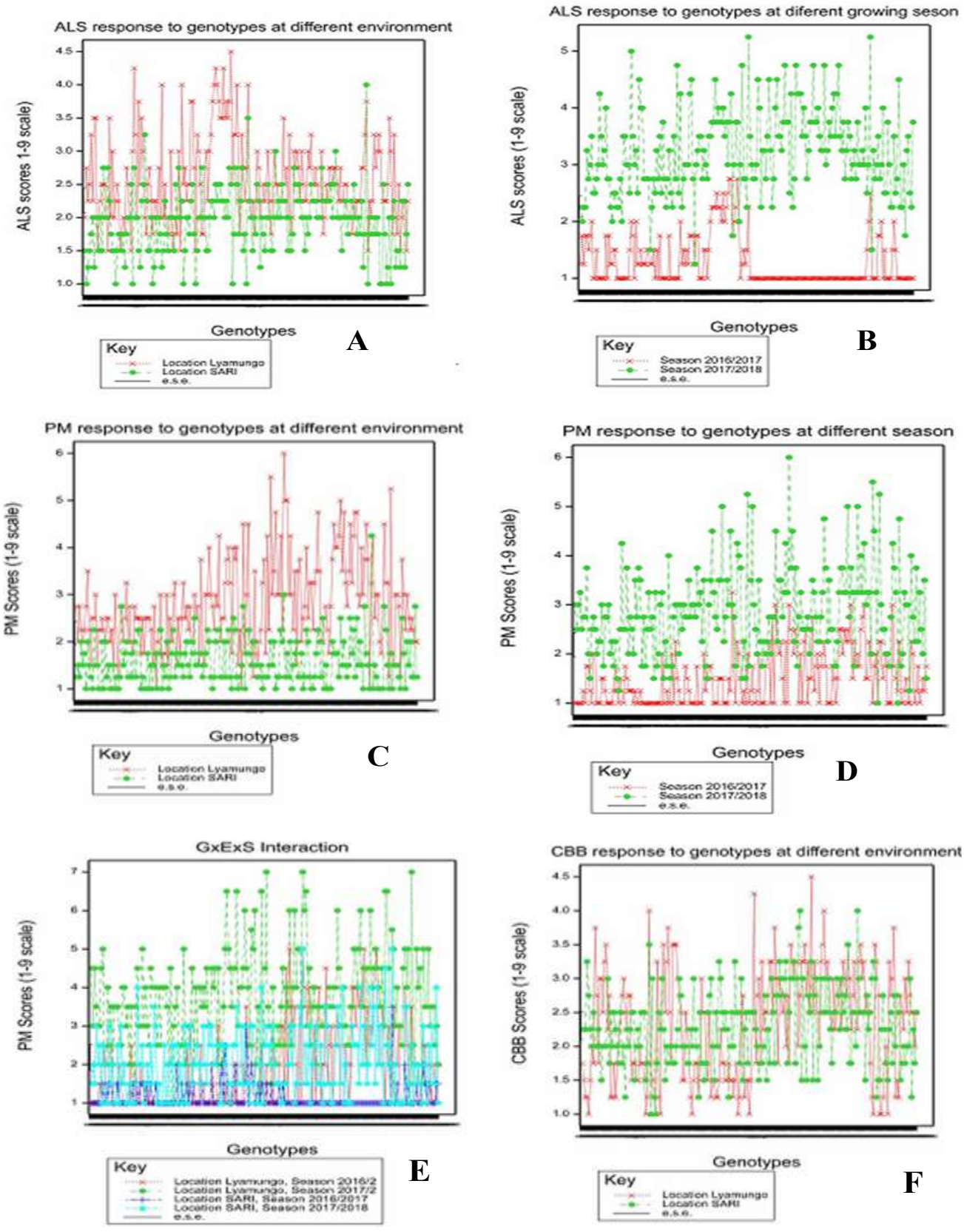

Figure 2. Response of disease to dry bean genotypes (A) genotype and environment interaction for ALS; (B) Genotype and season interaction for ALS; (C) Genotype and environment interaction for PM; (D) Genotype and season interaction for PM; (E) Genotype, environment and season interaction for PM; (F) Genotype and environment interaction for CBB.

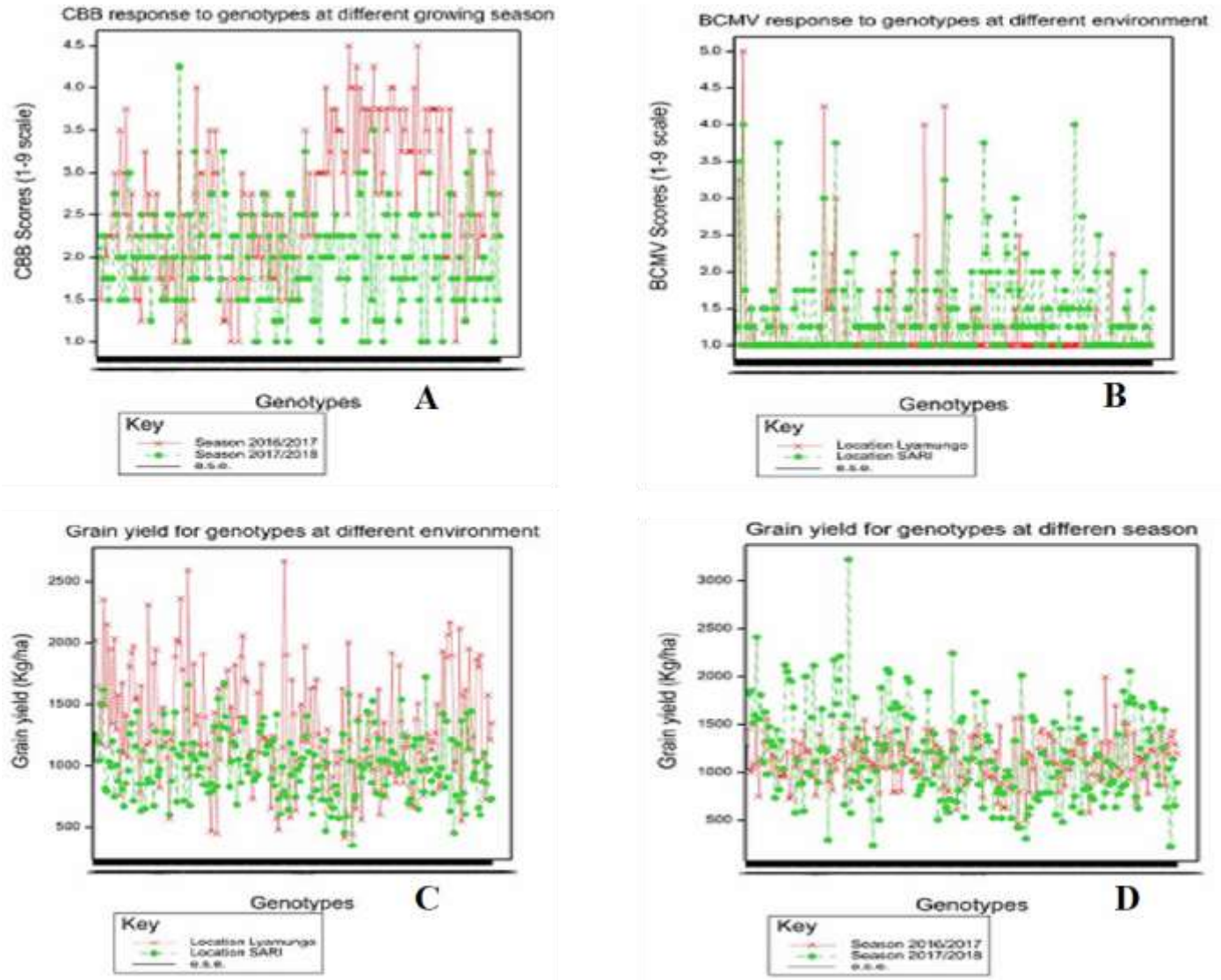

Figure 3. Response of disease to dry bean genotypes and grain yield; (A) Genotype and season interaction for CBB; (B) Genotype and environment interaction for BCMV; (C) Genotype and environment interaction for grain yield; (D) Genotype and season interaction for grain yield.

significant difference (<0.001) in season (p=0.004). Grain yield of all genotypes at the Lyamungo site was higher than that of SARI site across the testing seasons. For instance, maximum yield at Lyamungo was >2500 kg/ha; while the highest yield at SARI was ~1800 kg/ha and the lowest yield <500 kg/ha, with the grand mean of 1151.54 kg/ha for both locations (Figure 3C). The 2017/2018 growing season resulted in higher yield of >3000kg/ha; while that of 2016/2017 gave rise to approximately 2100 kg/ha (Figure 3D). Generally, Lyamungo site during 2017/2018 season performed better in this study and the best top five bean genotypes, FEB 189, A774, NUA 16, KG 71-4 and DOR 766, produced yield of 2127, 1982, 1793, 1725 and 1715 kg/ha, respectively (Figure 2C and D and Supplement 2).

Climate and diseases occurrence during this study period

Annual rainfall (mm), maximum temperature (°C) and mean temperature for consecutive four cropping seasons (2015-2018) were collected as well as annual relative humidity (%) for two cropping seasons (2017-2018). High annual rainfall was observed at Lyamungo site than that of SARI, across the cropping seasons. For instance, in 2018, annual rainfall was 2156.40 mm at Lyamungo but 1169.20 at SARI (Figure 4A). Mean and maximum temperature was higher at SARI compared to Lyamungo (Figure 4B and C); while relative humidity was reverse to temperature (Figure 4D). The negative correlation in both sites indicated that the higher the rainfall, the lower the

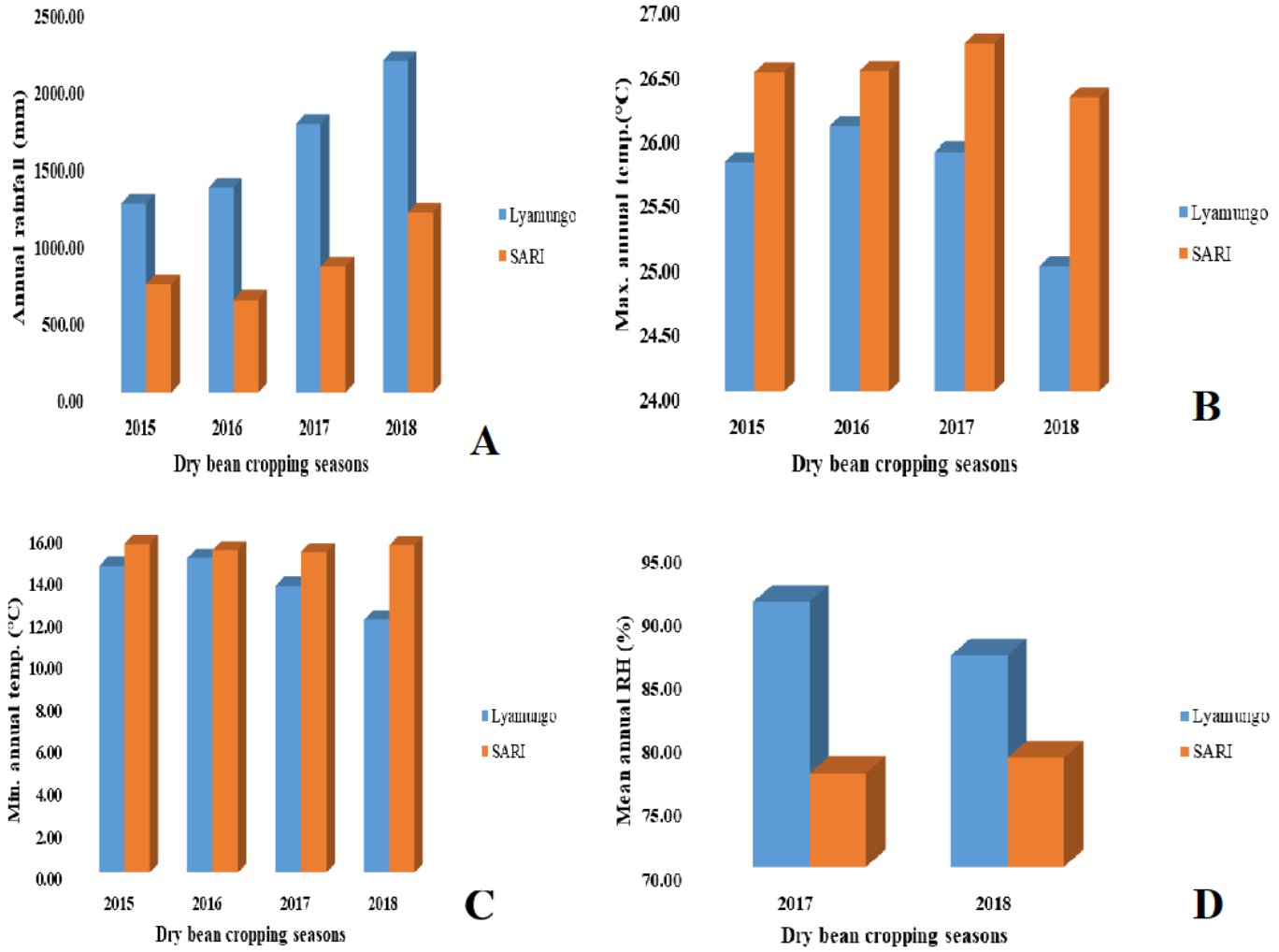

Figure 4. Annual climate statistics for experimental sites; (A) Annual rainfall, (B) Maximum average annual temperature; (C) Minimum average annual temperature; (D) Mean annual relative humidity.

temperature and the higher the relative humidity in these cropping seasons. Through comparative analysis, the high rainfall and relative humidity resulted in high severity of fungal, bacterial and viral diseases at Lyamungo; while at SARI, though disease severity was lower, productivity was also low, which may be attributed to terminal drought and abortion of flowers due to high temperature.

Dry bean market class variations with respect to diseases effects and grain yield

Small red genotypes dominated the higher grain yield (>1500 kg/ha) compared to all accessions; while navy and red mottled genotypes produced yield range between 1000 and 1490 kg/ha. Red mottled genotypes with large to medium size were more susceptible to ALS, CBB and PM, with lower yield compared to other market classes. Small seeded genotypes, especially black, red and khaki striped such as A 686, MLB 48-89A, DOR 755, FLOR DE MAYO, 222/1 and CC 906, were more susceptible to BCMV disease and their yield was lower (Supplement 2). Although some genotypes were not infected by pathogens, their yields were lower (<1000 kg/ha), which may be due to poor germination and adaptability (Table 1). From the combined analysis that expressed the effects of GxE across the two bean growing seasons, ten resistant genotypes were identified for each diseases and were listed in Table 1. From this identification, small white and small red expressed trait of resistance under this field conditions than other market classes.

DISCUSSION

The responses of genotypes to diseases through genotype and environmental interactions led to yield variations. Infection caused by ALS disease-causing

Table 1. Identified top 10 genotypes with trait of resistance per specific disease, market type, severity and grain yield.

Economic disease	Genotypes	Market type	Score (1-9)	Yield (kg/ha)
Angular Leaf Spot (ALS)	KG 30-29	White	1.00	1257.00
	Ranjonomby	White	1.00	1679.00
	DOR 755	Small red	1.00	1545.00
	ZABR 16575-51F22	White	1.00	962.00
	CN Bunsi (64)	White	2.00	897.00
	CC814	Carioca	2.00	1345.00
	CN Bunsi (60)	White	2.00	815.00
	CN Bunsi (62)	White	2.00	1145.00
	G 31	White	2.00	1169.00
	G 78	White	2.00	1239.00
Powdery mildew (PM)	PAN 72	White	1.00	1219.00
	CN Bunsi (64)	White	1.00	897.00
	SM 133	White	1.00	1412.00
	ZABR 16573-78F22	White	1.00	1070.00
	F1Population	White	1.00	916.00
	G 30	White	1.00	1190.00
	G 60	White	1.00	1378.00
	MEX 54	Cream	1.00	1725.00
	MICHETTE	White	1.00	645.00
	Awash-1	White	1.00	1270.00
Common Bacterial Blight (CBB)	DOR 771	Small red	1.00	1481.00
	DOR 662	Carioca	1.00	1042.00
	DOR 766	Small red	1.00	1715.00
	KG 4-20	Small red	1.00	1223.00
	RRN 47	Small red	1.00	1485.00
	Selian 05	Khaki	1.00	1171.00
	DOR 711	Small red	1.00	1368.00
	MAZ 41	Medium red	2.00	927.00
	DOR 708	Red kidney	2.00	1199.00
	KG 114-185	Small red	2.00	1542.00
Bean Common Mosaic Virus (BCMV)	296/6	Carioca	1.00	1278.00
	ALS 3	Black	1.00	1472.00
	BAT 332	Cream	1.00	1391.00
	C.2014/Hu/11	White	1.00	1149.00
	C.2017/Hu/11	White	1.00	1224.00
	C.2019/Hu/11	White	1.00	1244.00
	CANPSULA	White	1.00	1014.00
	CIM 9313-1	Khaki	1.00	1500.00
	CN Bunsi (62)	White	1.00	1145.00
	CN Bunsi (64)	White	1.00	897.00

pathogen was observed during this study. Extreme rainfall and relative humidity in the field created an environment for diseases occurrence. Each bean genotype responded differently to pathogen infection in different environments, though phenotypic expression was strongly influenced by this pathogen. Some reports also stated that this pathogen caused serious infection because of high level of moisture in dry bean production areas, such as excess moisture in the field in Kenya (Mwang`ombe et al., 2007) and great lake regions in Tanzania, Uganda and Ethiopia (Pastor-Corrales et al., 1998; Nkalubo et al., 2007). Some genotypes which

tolerated excess moisture identified in this study could be selected for production in these specific areas. Also, other effects of ALS to yield loss in dry bean genotypes was observed in a three-year field experiment conducted by Jesus et al. (2001) in the 1997 and 1999 experiments, whereby ALS reached higher disease levels than rust under rust and ALS inoculations pathogens. Same results were gotten by Cichy et al. (2015), Liebenberg et al. (1997) on Andean diversity study, which was attributed to G*E and inappropriate good agricultural practices.

Powdery mildew severity was less considered as economic disease in Tanzania, but these experiments showed its economic impact, in that those plants attacked failed to produce even a single seed. The infection was high, up to 6.00 severity scores at Lyamungo site for most large seeded genotypes including NUA 137, NUA 145, NUA 15, SWAP 09 and NUA 244 which leads to poor grain yield of 1016.87, 758.28, 1056.09, 549.89 and 857.27kg/ha, respectively. Same infection was caused by *E. polygoni* pathogen of PM causes extensive damage with significant losses of up to 69% in Columbia, US; the infection occurred during flowering time (Steadman et al., 2005) and other losses of about 40 to 50% at Mexico's farmers' field. Effects of CBB range from leaves, pods to seeds and that's why it is regarded as seed borne disease (Lopez et al., 2006). The effects occurred both in the field through natural infection, which occurs under normal environmental conditions, and in greenhouse through inoculation procedures. High severity occurs under high rainfall and relative humidity as well as warm temperature conditions, between 25 and 35°C (Chaube et al., 1992). However, in the green house, high relative humidity does not influence the CBB, causing organisms to infect plants like that under field conditions (Akhavan et al., 2009).

This study shows the environmental condition for the diseases to occur across the tested locations. *Bean common mosaic virus* showed less variation in its occurrence in the testing sites, while few genotypes were affected by this virus. Despite not expressing its economic importance, but for those few genotypes affected, it really hit across the tested environments. The losses caused by BCMV and BCMNV impacted severely not only on commercial scale cultivation of this high-value crop but also on production by smallholder farmers in the developing world, where bean serves as a key source of dietary protein and mineral nutrition (Worrall et al., 2015). The tested resource materials in this study reflects a better source of Mesoamerican gene pool to improve the common bean growing cultivars, especially those succumbed by ALS, PM, CBB and BCMV diseases (Table 1). Even the identified Andean with moderate resistant for the above diseases could be improved via available resistant bean varieties with the aim to develop the preferred market classes based on region preferences. Additionally, the study shows that extreme rainfall and temperature affects bean grown with disease occurrence and flower abortion respectively, which can cause poor yield. The genetic and environmental differences contribute to the phenotype and sometimes it is used to confirm the GxE interactions among treatment effects (Tabery and Griffiths, 2010). The future approach on these materials will be to carry genotypic sequencing for better correlation between field and laboratory data.

Conclusion

From this research study, different genotypes expressed trait of resistance under field conditions for the four foliar economic diseases of ALS, CBB, PM and BCMV. Most of the small seeded genotypes dominated the identified genotypes for each trait of focus on the target diseases. This reflects the opportunities to improve the Mesoamerican gene pool across the dry bean research networks. Despite this fact, some of the genotypes expressed trait of resistance under the field but more work needs to be done under the controlled environment with focus placed on the identified genotypes. Apart from disease, other environmental factors like ambient temperature, rainfall variations and relative humidity affected the yield variations.

CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The authors are grateful for the support rendered by Tuskegee University through the Integrative Biosciences (IBS) Ph.D. Program under the synergism of the College of Agriculture, Environment and Nutrition Sciences (CAENS), College of Arts and Sciences (CAS) and College of Veterinary Medicine (CVM). The authors also appreciate the Government of Tanzania (GoT) for the assistance rendered through Tanzania Agricultural Research Institute – Selian Centre and the International Center for Tropical Agriculture (CIAT) as well as Pan-Africa Bean Research Alliance (PABRA).

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Supplement 1. Seed characterization.

S/N	Genotype	Gene pool	Origin	Market class	Seed color	Seed size
1	MAZ 37	Andean	CIAT breeding line	Red kidney	Red	Large
2	MAZ 41	Mesoamerican	CIAT breeding line	Red mottled	Red	Medium
3	MAZ 42	Mesoamerican	CIAT breeding line	Red mottled	Red mottled	Medium
4	MAZ 44	Andean	CIAT breeding line	Red mottled	Red mottled	Large
5	MAZ 46	Andean	CIAT breeding line	Red mottled	Red mottled	Large
6	MAZ 47	Andean	CIAT breeding line	Red mottled	Red mottled	Large
7	MAZ 48	Andean	CIAT breeding line	Red mottled	Red mottled	Large
8	MAZ 50	Mesoamerican	CIAT breeding line	Red mottled	Red mottled	Medium
9	MAZ 52	Mesoamerican	CIAT breeding line	Red mottled	Red mottled	Medium
10	MAZ 59	Mesoamerican	CIAT breeding line	Red mottled	Red	Medium
11	MAZ 56	Andean	CIAT breeding line	Red mottled	Red mottled	Large
12	MAZ 57	Andean	CIAT breeding line	Red mottled	Red mottled	Large
13	MAZ 49	Andean	CIAT breeding line	Red mottled	Red mottled	Large
14	MAZ 70	Mesoamerican	CIAT breeding line	Carioca	Brown striped	Small
15	MAZ 72	Andean	CIAT breeding line	Red kidney	Red	Large
16	MAZ 74	Andean	CIAT breeding line	Red kidney	Red	Large
17	MAZ 84	Andean	CIAT breeding line	Red mottled	Red mottled	Large
18	MAZ 255	Andean	CIAT breeding line	Red mottled	Red mottled	Large
19	DOR 662	Mesoamerican	CIAT breeding line	Carioca	Brown striped	Small
20	DOR 708	Mesoamerican	CIAT breeding line	Red kidney	Purple	Medium
21	DOR 710	Mesoamerican	CIAT breeding line	Small red	Red	Small
22	DOR 711	Mesoamerican	CIAT breeding line	Small red	Red	Small
23	DOR 755	Mesoamerican	CIAT breeding line	Small red	Red	Small
24	DOR 766	Mesoamerican	CIAT breeding line	Small red	Red	Small
25	CN Bunsi (60)	Mesoamerican	CIAT breeding line	Navy	White	Small
26	CN Bunsi (62)	Mesoamerican	CIAT breeding line	Navy	White	Small
27	CN Bunsi (63)	Mesoamerican	CIAT breeding line	Navy	White	Small
28	CN Bunsi (64)	Mesoamerican	CIAT breeding line	Navy	White	Small
29	CN Bunsi (65)	Mesoamerican	CIAT breeding line	Navy	White	Small
30	CN Bunsi (66)	Mesoamerican	CIAT breeding line	Navy	White	Small
31	CN Bunsi (67)	Mesoamerican	CIAT breeding line	Navy	White	Small
32	CN Bunsi (68)	Mesoamerican	CIAT breeding line	Navy	White	Small
33	NUA 209	Andean	CIAT breeding line	Red mottled	Red mottled	Large
34	NUA 210	Andean	CIAT breeding line	Red mottled	Red mottled	Large
35	NUA 272	Mesoamerican	CIAT breeding line	Yellow	Cream	Medium
36	NUA 231	Andean	CIAT breeding line	Red mottled	Red mottled	Large
37	NUA 244	Andean	CIAT breeding line	Red mottled	Red mottled	Large
38	NUA 40	Andean	CIAT breeding line	Red mottled	Red mottled	Large

Supplement 1. Contd.

39	NUA 48	Andean	CIAT breeding line	Red mottled	Red mottled	Large
40	NUA 57	Andean	CIAT breeding line	Red mottled	Red mottled	Large
41	NUA 9	Andean	CIAT breeding line	Red mottled	Red mottled	Large
42	NUA 64	Andean	CIAT breeding line	Red mottled	Red mottled	Large
43	NUA 110	Andean	CIAT breeding line	Red kidney	Red	Large
44	NUA 117	Mesoamerican	CIAT breeding line	_	_	Medium
45	NUA 212	Andean	CIAT breeding line	Red mottled	Red mottled	Medium
46	NUA 213	Andean	CIAT breeding line	Red mottled	Red mottled	Large
47	NUA 125	Mesoamerican	CIAT breeding line	_	Red	Medium
48	NUA 232	Andean	CIAT breeding line	Red mottled	Red mottled	Large
49	NUA 130	Andean	CIAT breeding line	Red mottled	Red mottled	Large
50	NUA 137	Andean	CIAT breeding line	Red kidney	Red	Large
51	NUA 156	Mesoamerican	CIAT breeding line	_	Red	Medium
52	NUA 23	Andean	CIAT breeding line	Red mottled	Red mottled	Large
53	NUA 11	Andean	CIAT breeding line	Red mottled	Red mottled	Large
54	NUA 13	Andean	CIAT breeding line	Red mottled	Red mottled	Large
55	NUA 15	Andean	CIAT breeding line	Red mottled	Red mottled	Large
56	NUA 16	Mesoamerican	CIAT breeding line	Small red	Red	Small
57	NUA 17	Andean	CIAT breeding line	Red mottled	Red mottled	Large
58	NUA 18	Andean	CIAT breeding line	Red mottled	Red mottled	Large
59	NUA 19	Andean	CIAT breeding line	Red mottled	Red mottled	Large
60	NUA 256	Andean	CIAT breeding line	Speckled sugar	Purple striped	Large
61	NUA 30	Andean	CIAT breeding line	Red mottled	Red mottled	Large
62	NUA 31	Andean	CIAT breeding line	Red mottled	Red mottled	Large
63	NUA 134	Andean	CIAT breeding line	Red kidney	Red	Large
64	NUA 115	Andean	CIAT breeding line	Red mottled	Red mottled	Large
65	NUA 116	Andean	CIAT breeding line	Red mottled	Red mottled	Large
66	NUA 129	Mesoamerican	CIAT breeding line	_	Red	Medium
67	NUA 152	Andean	CIAT breeding line	Red kidney	Red	Large
68	NUA 158	Mesoamerican	CIAT breeding line	_	Black stripe	Medium
69	NUA 160	Mesoamerican	CIAT breeding line	Small red	Red	Small
70	NUA 161	Andean	CIAT breeding line	Red kidney	Red	Large
71	NUA 163	Mesoamerican	CIAT breeding line	Small red	Red	Small
72	NUA 165	Mesoamerican	CIAT breeding line	Red mottled	Red mottled	Medium
73	NUA 39	Andean	CIAT breeding line	Red mottled	Red mottled	Large
74	NUA 59	Andean	CIAT breeding line	Red mottled	Red mottled	Large
75	NUA 66	Mesoamerican	CIAT breeding line	Red mottled	Red mottled	Medium
76	NUA 67	Andean	CIAT breeding line	Speckled sugar	Purple striped	Large
77	NUA 79	Andean	CIAT breeding line	Red kidney	Red	Large

Supplement 1. Contd.

78	NUA 145	Andean	CIAT breeding line	_	Purple	Large
79	NUA 200	Andean	CIAT breeding line	Red mottled	Red mottled	Large
80	NUA 204	Mesoamerican	CIAT breeding line	Red mottled	Red mottled	Medium
81	NUA 207	Mesoamerican	CIAT breeding line	Speckled sugar	Purple striped	Medium
82	NUA 209	Andean	CIAT breeding line	Red mottled	Red mottled	Large
83	NUA 211	Andean	CIAT breeding line	Speckled sugar	Purple striped	Large
84	NUA 224	Andean	CIAT breeding line	Red mottled	Red mottled	Large
85	NUA 225	Andean	CIAT breeding line	Red mottled	Red mottled	Large
86	NUA 226	Andean	CIAT breeding line	Red mottled	Red mottled	Large
87	NUA 229	Andean	CIAT breeding line	Red mottled	Red mottled	Large
88	NUA 233	Andean	CIAT breeding line	Red mottled	Red mottled	Large
89	NUA 235	Andean	CIAT breeding line	Red mottled	Red mottled	Large
90	NUA 236	Andean	CIAT breeding line	Red mottled	Red mottled	Large
91	NUA 238	Andean	CIAT breeding line	Red mottled	Red mottled	Large
92	NUA 239	Andean	CIAT breeding line	Red mottled	Red mottled	Large
93	NUA 240	Andean	CIAT breeding line	Red mottled	Red mottled	Large
94	NUA 245	Andean	CIAT breeding line	Red mottled	Red mottled	Large
95	NUA 257	Andean	CIAT breeding line	Red mottled	Red mottled	Large
96	NUA 273	Andean	CIAT breeding line	Speckled sugar	Purple striped	Large
97	G 5	Mesoamerican	Ethiopia	Navy	White	Small
98	G 23	Mesoamerican	Ethiopia	Navy	White	Small
99	G 30	Mesoamerican	Ethiopia	Navy	White	Small
100	G 31	Mesoamerican	Ethiopia	Navy	White	Small
101	G 60	Mesoamerican	Ethiopia	Navy	White	Small
102	G 78	Mesoamerican	Ethiopia	Navy	White	Small
103	G 79	Mesoamerican	Ethiopia	Navy	White	Small
104	G 87	Mesoamerican	Ethiopia	Navy	White	Small
105	G 90	Mesoamerican	Ethiopia	Navy	White	Small
106	G 100	Mesoamerican	Ethiopia	Navy	White	Small
107	CZ 114-8	Mesoamerican	Kenya	Small red	Red	Small
108	CZ 102-24	Andean	Kenya	Red kidney	Red	Large
109	CZ 102-29	Andean	Kenya	Red kidney	Red	Large
110	CZ 108-27	Mesoamerican	Kenya	Navy	White	Small
111	CZ 114-46	Mesoamerican	Kenya	Small red	Red	Small
112	CZ 114-50	Mesoamerican	Kenya	Small red	Red	Small
113	CZ 114-51	Mesoamerican	Kenya	Small red	Red	Small
114	KG 114-177	Mesoamerican	CIAT breeding line	_	Purple	Small
115	KG 114-178	Mesoamerican	CIAT breeding line	_	Purple	Small
116	KG 114-179	Mesoamerican	CIAT breeding line	Black	Black	Small

Supplement 1. Contd.

117	KG 114-182	Mesoamerican	CIAT breeding line	Black	Black	Small
118	KG 114-185	Mesoamerican	CIAT breeding line	Small red	Red	Small
119	KG 4-3	Mesoamerican	CIAT breeding line	_	Khak	Small
120	KG 4-20	Mesoamerican	CIAT breeding line	Small red	Red	Small
121	KG 15-6	Andean	CIAT breeding line	Kablanket	Dotted purple	Large
122	KG 30-29	Mesoamerican	CIAT breeding line	Navy	White	Small
123	KG 24-43	Mesoamerican	CIAT breeding line	Navy	White	Small
124	KG 65-5	Mesoamerican	CIAT breeding line	Navy	White	Small
125	KG 67-10	Mesoamerican	CIAT breeding line	Navy	White	Small
126	KG 67-11	Mesoamerican	CIAT breeding line	Navy	White	Small
127	KG 71-4	Mesoamerican	CIAT breeding line	_	Purple	Small
128	KG 71-5	Mesoamerican	CIAT breeding line	_	Purple	Small
129	KG 97-11	Andean	CIAT breeding line	Speckled sugar	Brown striped	Large
130	ZABR 16575-17F22	Mesoamerican	CIAT breeding line	Navy	White	Small
131	ZABR 16575-24F22	Mesoamerican	CIAT breeding line	Navy	White	Small
132	ZABR 16575-39F22	Mesoamerican	CIAT breeding line	Navy	White	Small
133	ZABR 16575-51F22	Mesoamerican	CIAT breeding line	Navy	White	Small
134	ZABR 16575-60F22	Mesoamerican	CIAT breeding line	Navy	White	Small
135	ZABR 16575-86F22	Mesoamerican	CIAT breeding line	Navy	White	Small
136	ZABR 16573-78F22	Mesoamerican	CIAT breeding line	Navy	White	Small
137	ZABR 16574-46F22	Mesoamerican	CIAT breeding line	Navy	White	Small
138	ZABR 16576-11F22	Mesoamerican	CIAT breeding line	Navy	White	Small
139	ZABR 16577-51F22	Mesoamerican	CIAT breeding line	Navy	White	Small
140	RANJONOMBY	Mesoamerican	CIAT breeding line	Navy	White	Small
141	Navy line 5	Mesoamerican	CIAT breeding line	Navy	White	Small
142	Navy line 19	Mesoamerican	CIAT breeding line	Navy	White	Small
143	Navy line 15	Mesoamerican	CIAT breeding line	Navy	White	Small
144	Navy line 22	Mesoamerican	CIAT breeding line	Navy	White	Small
145	Navy line 25	Mesoamerican	CIAT breeding line	Navy	White	Small
146	Navy line 38	Mesoamerican	CIAT breeding line	Navy	White	Small
147	Navy line 40	Mesoamerican	CIAT breeding line	Navy	White	Small
148	Navy line 48	Mesoamerican	CIAT breeding line	Navy	White	Small
149	Navy line 43	Mesoamerican	CIAT breeding line	Navy	White	Small
150	Navy line 51	Mesoamerican	CIAT breeding line	Navy	White	Small
151	Navy line 52	Mesoamerican	CIAT breeding line	Navy	White	Small
152	Nany line 54	Mesoamerican	CIAT breeding line	Navy	White	Small
153	RWR 2075	Andean	Rwanda	Red kidney	Red	Large
154	RWR 1059	Mesoamerican	Rwanda	Red mottled	Red mottled	Medium
155	KABABALA	Mesoamerican	CIAT breeding line	Navy	White	Small

Supplement 1. Contd.

156	SWP 12	Mesoamerican	Kenya	Navy	White	Small
157	SWP 09	Andean	Kenya	White kidney	White	Large
158	SWP 10	Mesoamerican	Kenya	Navy	White	Small
159	MEXICO 54	Mesoamerican	CIAT breeding line	Carioca	Cream	Medium
160	PAN 72	Mesoamerican	CIAT breeding line	Navy	White	Small
161	JESCA	Andean	Tanzania	Kablanket	Dotted purple	Large
162	Selian 05	Mesoamerican	Tanzania	_	Khak	Small
163	CC 13	Mesoamerican	CIAT breeding line	Small red	Red	Small
164	CC 547	Mesoamerican	CIAT breeding line	Black	Black	Small
165	CC 814	Mesoamerican	CIAT breeding line	Carioca	Brown striped	Small
166	CC 960	Mesoamerican	CIAT breeding line	Black	Black	Small
167	RRN 47	Mesoamerican	CIAT breeding line	Small red	Red	Small
168	RRN 48	Andean	CIAT breeding line	Red mottled	Red mottled	Large
169	217/7	Mesoamerican	CIAT breeding line	_	Khak	Small
170	217/2	Mesoamerican	CIAT breeding line	_	Khak	Small
171	222/1	Mesoamerican	CIAT breeding line	Black	Black	Small
172	296/6	Mesoamerican	CIAT breeding line	Carioca	Brown striped	Small
173	RAZ 36	Mesoamerican	CIAT breeding line	Navy	White	Small
174	RAZ 44	Mesoamerican	CIAT breeding line	Navy	White	Small
175	CIM 9313-1	Mesoamerican	CIAT breeding line	_	Khak	Small
176	Lyamungo 90	Andean	Tanzania	Red mottled	Red mottled	Large
177	ALS 3	Mesoamerican	CIAT breeding line	Black	Black	Small
178	BAT 332	Mesoamerican	CIAT breeding line	Cream	Cream	Small
179	IBC 2	Mesoamerican	CIAT breeding line	Navy	White	Small
180	Awash-1	Mesoamerican	Ethiopia	Navy	White	Small
181	Awash Meka	Mesoamerican	Ethiopia	Navy	White	Small
182	PI 207262	Mesoamerican	CIAT breeding line	_	Khak	Small
183	A 686	Mesoamerican	CIAT breeding line	Black	Black	Small
184	A 774	Mesoamerican	CIAT breeding line	Cream	Cream	Small
185	A 797	Andean	CIAT breeding line	Red mottled	Red mottled	Medium
186	G 5686	Mesoamerican	CIAT breeding line	Cream	Cream	Large
187	CANPSULA	Mesoamerican	CIAT breeding line	Navy	White	Small
188	TU	Mesoamerican	CIAT breeding line	Black	Black	Small
189	CAL 113	Andean	CIAT breeding line	Red mottled	Red mottled	Large
190	C.202/Hu/3	Mesoamerican	CIAT breeding line	Navy	White	Small
191	C.202/Hu/11	Mesoamerican	CIAT breeding line	Navy	White	Small
192	C.2014/Hu/11	Mesoamerican	CIAT breeding line	Navy	White	Small
193	C.2019/Hu/11	Mesoamerican	CIAT breeding line	Navy	White	Small
194	C.2017/Hu/11	Mesoamerican	CIAT breeding line	Navy	White	Small

Supplement 1. Contd.

195	C.2018/Hu/11	Mesoamerican	CIAT breeding line	Navy	White	Small
196	MLB 17-89A	Andean	CIAT breeding line	Red mottled	Red mottled	Large
197	MLB 40-89A	Mesoamerican	CIAT breeding line	_	Khak	Small
198	MLB 48-89A	Mesoamerican	CIAT breeding line	_	Purple	Small
199	Amendon	Mesoamerican	CIAT breeding line	_	Dotted purple	Medium
200	Vax 1	Mesoamerican	CIAT breeding line	Carioca	Brown striped	Small
201	Vax 2	Mesoamerican	CIAT breeding line	Carioca	Brown striped	Small
202	FLORDEMAYO	Mesoamerican	CIAT breeding line	_	Dotted purple	Small
203	FEB 181	Mesoamerican	CIAT breeding line	Small red	Red	Small
204	FEB 189	Mesoamerican	CIAT breeding line	Small red	Red	Small
205	SAB 662	Mesoamerican	CIAT breeding line	Navy	White	Small
206	CORNELL 49822	Mesoamerican	CIAT breeding line	black	Black	Small
207	SM 133	Mesoamerican	CIAT breeding line	Navy	White	Small
208	MONT-CALM	Andean	CIAT breeding line	Red kidney	Red	Large
209	MICHETTE	Mesoamerican	CIAT breeding line	Navy	White	Small
210	MEXICAN 142	Mesoamerican	CIAT breeding line	Navy	White	Small
211	R.K. MICHIGA	Andean	CIAT breeding line	Red kidney	Red	Large
212	DONTIMOTEA	Mesoamerican	CIAT breeding line	small red	Red	Small
213	F1 Population	Mesoamerican	CIAT breeding line	Navy	White	Small

Supplement 2. Combined data analysis.

S/N	Accessions	DFF days		DM days		ALS score		PM score		CBB score		BCMV score		Yield (kg/ha)	
		LYA	*SARI*	*LYA*	*SARI*	*LYA*	*SARI*	*LYA*	*SARI*	*LYA*	*SARI*	*LYA*	*SARI*	*LYA*	*SARI*
1	217/2	49.25	41.50	99.75	97.75	2.00	1.50	2.75	1.25	1.75	2.25	1.00	1.25	2021.48	1244.30
2	222/1	49.00	43.00	103.50	102.50	2.75	1.00	2.00	1.50	1.50	2.25	3.25	3.50	1644.77	1201.25
3	296/6	48.25	43.50	99.75	97.25	2.25	1.25	2.75	1.25	1.25	2.25	1.00	1.00	1513.12	1042.66
4	A 686	48.25	43.00	101.75	102.50	2.50	1.50	2.25	2.00	1.25	3.25	5.00	4.00	1175.31	1502.73
5	A 774	45.25	40.00	99.25	95.00	3.25	1.75	2.00	1.50	1.00	2.75	1.00	1.75	2350.23	1613.05
6	A 797	47.75	40.50	101.75	100.00	2.25	2.00	2.25	1.75	1.50	2.25	1.00	1.25	1488.75	813.20
7	ALS 3	46.25	38.50	100.50	98.75	3.50	1.25	2.75	1.00	2.00	2.00	1.00	1.00	2152.27	790.97
8	AMENDON	44.75	39.75	100.00	98.50	3.50	2.00	3.50	2.00	1.75	2.50	1.50	1.50	1158.44	1030.86
9	Awash Meka	49.25	44.25	102.50	100.50	1.50	2.00	2.25	1.50	3.75	2.00	1.00	1.25	1952.03	992.27
10	Awash-1	47.75	41.00	101.50	99.75	2.00	1.50	1.50	1.25	2.75	2.00	1.25	1.00	1339.06	1201.70
11	BAT 332	49.75	43.25	101.50	99.75	2.25	2.00	2.50	1.00	1.75	2.25	1.00	1.00	2033.44	749.06
12	C.2014/Hu/11	44.50	37.75	99.75	101.50	2.50	1.50	2.50	2.25	3.00	2.00	1.00	1.00	1292.58	1005.00

Supplement 2. Contd.

13	C.2017/Hu/11	49.00	43.00	103.25	102.75	2.50	2.75	3.00	1.50	3.00	1.50	1.00	1.00	1576.25	872.73
14	C.2018/Hu/11	45.00	39.50	101.00	101.75	2.50	2.00	1.50	1.50	2.50	2.00	1.00	1.50	759.06	1117.89
15	C.2019/Hu/11	47.25	42.50	101.75	105.50	2.00	2.00	2.25	1.00	3.25	2.00	1.00	1.00	1678.75	808.52
16	C.202/Hu/3	45.00	38.75	99.00	101.00	1.50	2.75	2.25	1.00	3.50	2.00	1.00	1.50	1093.28	672.03
17	CAL 113	42.25	39.00	97.00	96.00	3.50	2.25	2.50	2.00	2.25	3.00	1.00	1.25	1418.37	856.68
18	CANPSULA	46.00	38.75	102.25	100.75	2.00	1.75	2.25	1.00	2.75	1.75	1.00	1.00	1065.31	961.72
19	CC 13	49.25	43.00	103.00	98.50	3.00	1.50	2.00	2.00	1.25	2.50	1.75	1.75	1806.56	1259.92
20	CC 547	49.50	43.00	102.00	98.50	2.00	1.50	2.50	1.25	1.25	2.00	1.25	1.00	1918.91	1342.34
21	CC 814	47.50	42.00	97.75	98.75	2.25	1.00	2.50	1.50	1.75	2.50	1.00	1.50	1974.53	715.78
22	CC 906	47.50	44.50	104.00	97.50	2.50	1.50	2.00	1.00	1.25	2.25	2.75	3.75	1535.39	1160.86
23	CIM 9313-1	49.75	44.25	100.50	96.75	2.25	1.75	2.25	1.00	1.75	2.00	1.00	1.00	1557.81	1442.42
24	CN Bunsi (60)	49.00	42.00	103.00	100.25	1.50	1.75	1.50	1.50	2.25	1.75	1.00	1.25	776.87	852.66
25	CN Bunsi (62)	49.25	42.75	104.50	100.75	1.75	1.50	3.00	1.00	2.50	2.50	1.00	1.00	1653.05	636.41
26	CN Bunsi (63)	44.50	39.50	98.00	99.75	2.00	1.75	1.50	1.25	2.75	1.75	1.25	1.00	1040.94	1121.56
27	CN Bunsi (64)	46.75	40.25	98.75	99.25	1.75	1.25	1.25	1.00	3.00	2.00	1.00	1.00	1105.08	653.75
28	CN Bunsi (65)	45.00	42.75	97.25	99.25	2.75	1.75	2.75	1.25	2.50	1.25	1.00	1.00	1174.30	855.94
29	CN Bunsi (66)	48.50	41.00	99.75	95.00	2.50	2.00	2.75	2.75	2.25	2.25	1.00	1.00	2309.30	895.56
30	CN Bunsi (67)	44.75	39.50	98.25	95.25	2.00	2.00	2.00	1.50	2.75	2.00	1.00	1.50	1192.03	1037.69
31	CN Bunsi (68)	50.25	42.25	104.50	100.00	1.50	2.50	2.50	1.75	2.75	2.25	1.00	1.75	1330.00	862.97
32	CORNELL 49242	48.25	43.00	103.75	102.75	3.00	1.00	3.25	2.00	1.50	2.50	1.00	1.00	1833.12	675.70
33	CZ 102-24	39.75	34.50	95.75	94.75	4.25	2.75	2.00	1.25	1.75	2.50	1.00	1.00	1950.43	1281.78
34	CZ 102-29	42.25	36.00	97.25	99.50	3.25	2.00	2.75	1.25	1.75	1.50	1.00	1.25	931.73	922.55
35	CZ 108-27	47.00	39.25	100.00	98.00	2.00	2.00	2.00	1.75	1.50	2.00	1.00	1.75	817.60	971.16
36	CZ 114-46	40.25	35.25	93.50	98.75	3.75	1.50	1.75	1.25	1.50	2.25	1.00	1.00	1462.84	1158.22
37	CZ 114-50	40.25	34.75	94.75	99.00	2.50	1.25	2.75	2.00	2.00	2.00	1.00	1.00	1474.09	1425.10
38	CZ 114-51	41.00	36.75	95.75	72.25	3.50	2.25	2.50	1.25	1.75	2.50	1.00	1.25	1194.47	1268.41
39	CZ 114-8	43.00	38.25	97.25	98.25	3.00	2.50	2.50	2.25	1.75	2.50	1.00	1.75	1057.69	1271.36
40	DONTIMOTEO	47.25	38.00	103.50	99.50	2.00	3.25	2.50	2.25	1.50	2.50	1.00	2.25	568.61	604.92
41	DOR 662	47.00	41.75	100.75	98.50	1.75	2.25	1.75	1.00	1.00	1.50	1.00	1.00	1209.06	875.70
42	DOR 708	47.50	42.00	100.25	102.25	2.50	1.50	2.50	1.25	1.25	1.75	1.00	1.00	1257.27	1141.02
43	DOR 710	45.75	40.50	101.00	98.25	2.75	1.50	1.75	1.00	4.00	3.50	1.25	1.25	1888.98	1437.03
44	DOR 711	49.00	41.00	101.25	103.00	2.25	2.00	2.50	1.00	1.75	1.00	1.00	1.00	2027.27	707.89
45	DOR 755	49.50	44.00	105.25	103.25	1.75	1.00	2.50	1.75	1.50	3.00	4.25	3.00	2001.87	1088.67
46	DOR 766	45.75	39.75	100.25	99.75	2.25	1.25	2.50	1.00	1.25	1.25	1.50	1.00	2359.37	1070.47
47	DOR 771	48.25	41.75	101.25	101.25	2.25	2.00	2.25	1.50	1.00	1.00	1.75	1.00	1780.78	1180.47
48	F1POPULATION	44.75	41.50	100.00	99.00	2.00	1.50	1.50	1.00	3.25	1.75	1.50	1.00	1117.73	714.87
49	FEB 181	47.50	41.75	101.25	100.00	2.75	2.00	2.75	1.50	1.25	2.50	2.25	1.50	1459.30	977.95

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50	FEB 189	46.50	39.00	100.25	98.25	2.00	1.75	1.00	1.75	1.00	2.25	1.00	1.75	2590.31	1662.94
51	FLOR DE MAYO	46.00	39.25	103.75	103.75	4.00	1.25	2.00	1.00	3.00	3.00	3.00	3.75	915.87	678.75
52	G 100	44.25	39.50	96.75	93.75	2.00	1.50	2.25	1.75	3.50	2.25	1.00	1.00	1103.05	1084.05
53	G 23	48.50	40.50	101.50	98.75	2.50	2.25	3.00	2.25	2.50	1.50	1.00	1.00	1830.78	1185.62
54	G 30	44.75	40.25	96.75	100.25	2.00	1.75	1.50	1.00	3.25	2.00	1.00	1.00	1334.45	1045.55
55	G 31	46.25	44.00	105.50	101.50	1.75	1.50	1.75	1.50	3.75	1.75	1.00	1.75	1412.50	925.23
56	G 5	46.00	39.75	100.00	100.25	1.75	2.00	1.75	1.25	2.50	2.00	1.50	1.00	1174.61	981.09
57	G 5686	41.00	37.00	98.00	97.75	3.00	2.00	3.00	2.00	1.75	2.00	1.00	2.00	1095.23	1092.78
58	G 60	46.00	39.50	99.50	101.00	1.50	2.00	1.50	1.00	3.50	1.75	1.00	1.00	1910.31	846.33
59	G 78	49.00	43.00	103.25	99.25	1.75	1.50	2.50	1.25	3.50	1.75	1.00	1.00	1394.84	1084.06
60	G 79	46.50	42.00	101.75	102.00	2.00	2.50	2.00	1.75	3.50	2.00	1.00	2.25	1178.44	842.75
61	G 87	48.00	41.50	102.75	99.00	1.75	2.00	3.00	2.25	3.00	2.25	1.00	1.25	978.44	778.91
62	G 90	42.75	35.75	99.00	99.50	3.00	2.75	3.25	1.25	2.75	3.00	1.00	1.25	467.97	857.97
63	IBC 2	45.00	42.00	104.25	98.50	1.50	2.00	1.50	1.75	2.50	2.75	1.25	1.75	1277.19	793.81
64	JESCA	35.00	34.50	93.50	92.25	4.00	2.25	2.50	1.50	1.50	2.00	1.00	1.25	1435.00	1319.42
65	KABALABALA	47.75	40.00	101.00	97.00	2.50	1.00	1.75	1.75	1.50	2.00	1.00	1.00	448.94	830.00
66	KG 114-177	42.75	37.25	97.25	95.75	2.75	1.50	2.50	1.50	2.50	2.00	1.00	1.00	1628.46	1545.24
67	KG 114-178	44.75	40.00	97.25	96.00	2.25	2.00	3.25	1.25	2.25	1.75	1.00	1.25	1047.98	1214.76
68	KG 114-179	43.25	38.00	98.50	97.00	2.50	2.00	2.50	1.75	2.00	1.75	1.00	1.25	1341.87	1318.12
69	KG 114-182	42.25	37.25	97.25	72.75	2.50	1.75	2.75	2.00	1.25	2.25	1.25	1.00	1639.62	1673.37
70	KG 114-185	43.50	38.25	95.75	97.25	3.75	2.25	2.25	1.50	1.00	2.00	1.25	1.00	1683.41	1400.67
71	KG 15-6	43.00	38.00	99.25	98.00	3.75	2.50	2.75	1.25	1.50	3.00	1.00	1.25	1777.93	1298.85
72	KG 24-43	46.00	43.00	103.75	102.00	2.75	1.75	2.00	2.00	1.75	2.25	1.00	1.50	1020.29	826.88
73	KG 30-29	49.00	43.00	103.25	103.00	1.50	1.00	2.00	1.75	1.75	2.00	1.75	1.25	1477.69	1035.77
74	KG 4-20	42.25	37.25	96.50	100.50	3.25	1.50	3.00	2.50	1.25	1.25	1.00	1.25	1250.38	1194.90
75	KG 4-3	44.75	39.75	97.75	99.50	3.00	2.00	2.50	1.50	1.50	2.50	1.00	1.00	1820.14	1166.20
76	KG 67-10	48.00	39.50	101.25	101.75	2.50	2.00	2.75	1.50	3.00	1.75	1.00	1.00	1394.69	686.14
77	KG 67-11	45.25	38.75	98.50	100.75	1.75	2.75	1.50	1.75	2.50	1.75	1.00	1.50	1278.67	1049.61
78	KG 67-5	44.25	41.50	99.50	98.75	1.75	2.00	3.75	1.75	2.50	1.50	1.00	1.75	1887.89	861.48
79	KG 71-4	42.50	37.25	96.25	95.25	2.75	2.00	1.75	1.25	1.75	1.75	1.00	1.00	2056.49	1392.88
80	KG 71-5	42.75	37.25	95.75	96.50	3.00	1.50	1.50	1.50	1.50	2.50	2.00	1.00	1709.81	1351.73
81	KG 97-11	47.50	42.75	103.00	101.00	2.25	1.50	3.00	1.75	2.50	2.75	1.00	2.25	1686.72	1001.41
82	LYAMUNGU 90	38.00	33.50	94.50	93.00	3.00	2.00	3.00	2.25	1.25	3.00	1.00	1.00	1078.27	947.21
83	MAZ 37	39.50	37.00	96.50	97.75	3.25	2.25	4.00	2.00	1.50	2.00	1.00	1.00	1100.23	1057.27
84	MAZ 41	40.25	36.25	97.00	99.50	3.75	2.25	3.00	1.25	1.50	1.50	1.00	1.25	732.50	1122.19
85	MAZ 42	42.00	37.00	99.25	98.25	4.00	2.00	2.75	1.00	1.25	2.50	1.00	1.00	884.14	885.94
86	MAZ 44	40.00	33.25	99.00	96.50	4.25	2.50	2.25	1.25	1.25	2.00	1.25	1.00	960.08	911.72

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87	MAZ 46	43.25	37.00	98.50	99.25	4.00	2.25	3.00	2.00	1.50	3.25	1.50	1.50	1596.09	929.92
88	MAZ 47	43.25	38.50	98.25	96.25	3.75	2.50	2.75	2.25	2.25	3.00	1.50	1.50	1102.19	1275.86
89	MAZ 48	39.50	33.75	98.75	98.25	3.50	2.50	4.25	2.25	1.75	2.50	1.00	1.75	1829.61	1330.86
90	MAZ 49	41.25	35.00	99.50	97.50	3.50	2.25	2.00	1.25	1.75	2.25	1.00	1.25	1229.69	1392.50
91	MAZ 50	41.25	35.00	98.75	99.75	4.25	2.25	2.50	1.25	1.50	2.50	1.00	1.25	1227.42	1095.00
92	MAZ 52	41.50	38.25	100.00	101.00	3.50	2.00	2.50	2.00	1.50	2.25	2.50	2.00	1201.17	1160.16
93	MAZ 55	40.25	33.25	95.25	97.50	3.75	2.25	3.25	1.25	1.75	1.25	1.25	1.25	1166.87	1173.94
94	MAZ 56	42.00	40.75	98.25	99.50	3.50	2.00	3.75	2.00	1.50	2.00	1.00	1.00	655.70	897.19
95	MAZ 57	41.00	35.25	97.00	100.75	3.75	2.75	4.00	2.25	1.50	2.50	1.00	1.00	1242.58	1203.98
96	MAZ 59	40.00	33.50	95.50	94.00	4.50	2.50	3.25	1.25	1.25	2.00	4.00	1.75	971.87	836.48
97	MAZ 70	45.75	40.75	102.25	101.75	2.50	1.00	1.75	1.00	1.50	3.25	1.00	1.00	572.42	1421.95
98	MAZ 72	41.50	35.50	97.75	96.50	3.25	1.75	4.00	2.50	1.75	2.50	1.00	1.00	479.61	731.02
99	MAZ 74	41.25	37.00	97.25	96.75	3.25	2.75	3.75	2.25	1.00	2.50	1.00	1.00	771.17	607.11
100	MAZ 84	40.50	35.00	95.50	95.50	4.00	2.50	4.00	1.25	1.25	1.75	1.00	1.25	1151.33	991.33
101	MEX 54	46.00	41.50	101.50	99.75	2.25	1.75	1.50	1.00	2.50	3.00	1.25	1.25	2660.62	789.61
102	MEXICAN 142	48.50	41.50	104.50	103.50	1.75	2.25	2.25	1.25	2.75	1.50	1.00	1.75	1901.64	750.70
103	MICHETTE	45.00	38.25	99.25	97.50	3.25	2.75	1.00	1.50	1.25	2.25	1.50	2.00	644.53	645.94
104	MLB 17-89A	46.50	40.25	100.25	97.75	2.75	1.50	4.50	2.75	1.50	2.50	1.75	1.00	576.80	1186.80
105	MLB 40-89A	49.00	45.75	100.75	96.75	2.25	1.75	1.75	1.00	1.50	1.75	1.00	1.00	1702.81	926.80
106	MLB 48-89A	45.75	42.00	102.25	101.75	2.75	1.00	3.00	1.75	1.00	2.50	4.25	3.25	1423.36	1096.95
107	MONT-CALM	39.00	34.00	95.00	94.00	4.00	3.50	4.50	2.25	1.50	2.50	1.00	1.25	638.83	744.77
108	Navy line 15	42.25	41.00	102.00	104.50	2.25	2.25	2.25	1.75	2.50	2.50	1.50	2.75	1043.59	969.53
109	Navy line 19	45.50	41.75	100.25	100.75	2.00	2.00	2.00	1.50	4.25	2.50	1.00	1.50	1054.61	872.86
110	Navy line 22	44.50	40.25	96.75	96.00	2.25	1.50	1.25	2.00	1.75	2.25	1.00	1.00	1498.67	1005.48
111	Navy line 25	50.50	43.25	104.25	99.75	1.75	2.00	3.50	2.00	2.75	2.00	1.00	1.75	1202.19	1058.31
112	Navy line 38	42.00	38.25	100.25	98.25	2.25	1.75	1.75	1.50	3.00	1.50	1.00	1.50	1973.75	1241.09
113	Navy line 40	40.00	34.00	95.75	85.50	3.00	2.50	1.50	1.25	1.75	1.75	1.00	1.00	1204.61	1406.87
114	Navy line 43	44.75	41.50	97.00	96.75	2.75	2.25	2.00	1.25	3.25	1.75	1.00	1.25	1248.44	1115.00
115	Navy line 48	46.50	42.50	101.75	103.00	2.00	1.25	2.25	1.50	2.25	2.00	1.00	1.00	1629.53	743.98
116	Navy line 5	45.00	39.00	104.50	104.50	2.25	2.00	3.25	1.00	2.50	2.75	1.00	1.25	748.91	605.62
117	Navy line 51	45.50	42.50	101.75	98.25	2.50	1.75	3.75	1.25	2.50	1.50	1.00	1.25	1639.37	1086.95
118	Navy line 52	48.00	44.50	102.25	101.75	2.25	1.50	1.75	1.25	2.75	2.25	1.00	1.00	1703.52	798.41
119	Navy line 54	45.00	37.00	100.00	97.75	2.50	2.00	4.25	1.25	3.25	1.75	1.00	1.00	1257.03	996.84
120	NUA 11	42.50	40.00	98.75	100.75	3.00	2.50	2.25	1.50	3.25	3.00	1.50	2.00	1155.62	914.87
121	NUA 110	39.00	35.75	96.00	98.00	2.25	2.00	5.50	2.00	2.50	2.50	1.00	1.00	727.50	724.06
122	NUA 116	45.00	40.50	100.75	101.25	1.75	1.50	3.00	1.50	3.75	1.75	1.50	1.25	1051.02	1206.56
123	NUA 117	46.00	39.00	102.00	100.25	2.25	1.50	3.50	2.00	3.00	2.75	1.00	1.50	976.09	469.30

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124	NUA 125	40.75	34.25	99.00	100.00	3.00	2.00	4.75	1.00	1.75	2.75	1.00	1.25	1294.61	1020.78
125	NUA 129	38.75	35.25	95.75	96.25	2.50	3.00	3.00	1.25	3.00	3.25	1.00	2.00	677.89	723.58
126	NUA 13	44.25	35.75	101.50	94.00	2.50	2.00	3.00	1.25	2.75	3.00	1.00	3.75	819.45	580.94
127	NUA 130	41.50	35.25	95.50	100.75	2.25	2.25	4.25	2.25	2.50	2.75	2.00	2.25	915.08	658.05
128	NUA 134	42.25	37.00	100.00	102.75	2.25	2.00	2.25	2.00	2.75	3.00	1.25	2.75	1121.72	840.94
129	NUA 137	36.50	37.75	97.25	97.25	2.25	2.50	6.00	3.00	2.00	3.25	1.00	2.00	1016.87	772.16
130	NUA 145	43.25	40.00	99.50	96.50	3.50	2.25	5.00	1.25	2.75	1.75	1.00	1.75	758.28	569.22
131	NUA 15	35.75	34.75	96.75	95.50	2.50	2.25	5.00	1.75	1.75	2.50	1.00	1.00	1056.09	1111.72
132	NUA 152	43.75	38.25	100.25	99.00	2.75	2.00	3.00	1.25	3.50	3.00	1.00	1.25	1629.69	1257.89
133	NUA 156	43.25	40.50	99.25	100.50	2.00	1.50	4.25	2.00	3.25	3.00	1.00	2.00	416.80	455.70
134	NUA 158	46.50	41.00	98.25	100.00	3.25	2.00	3.00	1.00	3.00	3.25	1.00	1.25	616.95	584.83
135	NUA 16	42.75	40.00	98.25	99.25	1.75	1.50	2.25	1.50	3.25	1.75	1.00	1.00	2003.67	1581.41
136	NUA 160	43.75	38.00	98.00	100.50	2.00	1.75	3.50	1.75	3.25	3.00	1.00	1.00	1210.70	1036.09
137	NUA 161	43.25	38.50	102.00	99.00	3.00	2.75	1.50	2.00	2.00	3.75	1.25	2.50	444.22	352.66
138	NUA 163	44.00	38.25	99.50	101.25	2.75	2.50	3.50	1.25	2.75	4.00	1.00	2.25	1042.03	723.83
139	NUA 165	42.75	36.50	97.50	96.75	3.00	2.25	3.75	1.25	3.25	1.50	1.00	1.75	651.64	758.12
140	NUA 17	43.50	38.50	98.75	97.75	2.75	2.50	2.00	1.75	3.25	3.00	1.00	2.00	1392.19	1360.39
141	NUA 18	43.25	35.50	97.25	97.50	2.25	2.25	2.50	1.00	3.50	2.00	1.00	1.25	1574.37	1002.50
142	NUA 19	44.75	38.00	101.00	97.00	3.00	2.00	2.75	1.25	2.75	1.50	1.50	3.00	559.30	907.73
143	NUA 200	43.00	36.75	97.75	95.50	2.50	2.00	4.00	2.25	3.25	2.75	1.00	1.25	967.81	978.12
144	NUA 204	42.00	34.25	100.00	102.50	2.25	1.50	2.50	2.00	2.50	2.50	2.50	1.75	1048.28	1131.87
145	NUA 207	44.25	36.25	101.25	100.25	2.25	2.00	3.25	1.25	4.50	3.25	1.00	1.25	1207.81	1442.03
146	NUA 209	46.50	39.00	100.00	99.00	3.00	1.75	3.25	1.00	2.50	1.50	1.00	1.25	1424.53	1012.66
147	NUA 210	43.00	40.50	99.50	97.50	2.75	2.00	2.25	1.00	3.25	2.75	1.00	2.25	1074.37	861.09
148	NUA 211	40.25	35.00	98.25	100.25	3.25	2.50	3.25	1.25	2.75	3.00	1.00	1.50	972.34	1527.66
149	NUA 212	42.50	36.25	96.00	97.25	2.50	2.25	3.50	1.75	2.50	1.50	1.00	1.25	795.47	1351.09
150	NUA 213	40.75	34.50	98.25	76.75	2.50	2.25	4.75	2.25	2.00	2.00	1.00	1.50	1264.45	771.80
151	NUA 224	42.75	38.00	101.25	100.50	2.75	2.25	3.50	2.00	3.25	3.00	1.00	2.00	1622.50	1219.92
152	NUA 225	43.00	39.50	97.50	99.50	2.50	2.00	2.00	1.25	3.50	2.50	1.00	1.25	601.41	1040.78
153	NUA 226	46.25	40.25	100.75	97.50	2.25	2.00	2.00	2.50	4.00	2.50	1.00	1.00	1025.00	759.45
154	NUA 229	46.00	40.00	101.25	101.00	2.25	2.00	2.50	1.00	3.00	3.00	1.00	1.25	1349.14	1047.27
155	NUA 23	41.25	37.00	98.00	97.25	3.00	2.75	2.75	1.50	3.25	2.50	1.00	2.00	744.84	840.94
156	NUA 231	47.00	41.75	100.75	101.25	1.75	2.25	2.25	1.00	3.00	3.00	1.50	1.25	1033.36	1037.81
157	NUA 232	46.25	38.50	102.25	100.25	2.00	2.25	1.75	1.00	2.50	2.25	1.00	1.00	937.42	1203.59
158	NUA 233	44.25	39.00	101.25	103.25	2.50	2.25	2.75	1.00	2.25	1.75	1.00	1.50	1917.66	1122.42
159	NUA 235	40.75	34.75	96.50	94.75	2.25	2.25	3.75	2.00	3.00	2.50	1.00	1.50	1020.62	1098.98
160	NUA 236	42.75	35.50	98.00	101.00	2.75	2.25	4.50	1.75	2.25	2.50	1.00	1.25	856.56	1007.03

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161	NUA 238	42.00	36.50	96.75	98.25	2.75	2.50	4.00	1.75	2.50	3.00	1.00	1.25	927.19	983.20
162	NUA 239	41.75	39.25	97.50	93.75	2.50	2.00	4.00	1.75	2.75	2.75	1.00	2.00	1817.11	945.23
163	NUA 240	45.00	37.25	98.25	97.50	2.75	2.50	4.25	1.25	3.00	2.25	1.00	2.00	1368.98	1537.89
164	NUA 244	45.25	40.25	98.75	97.25	2.75	3.00	5.00	2.50	2.25	3.00	1.00	1.00	857.27	1018.59
165	NUA 245	41.50	37.25	97.50	96.50	2.50	2.00	3.50	1.50	3.25	2.50	1.00	1.50	1267.11	1239.30
166	NUA 256	43.00	39.00	101.50	100.00	2.25	2.00	4.75	2.00	3.00	2.75	1.00	1.50	1150.78	975.08
167	NUA 257	40.75	35.75	98.25	97.75	2.75	2.00	3.25	1.50	2.75	2.25	1.00	1.75	1244.53	696.72
168	NUA 272	48.00	41.25	98.25	99.25	2.25	1.50	2.75	1.25	3.50	3.50	1.00	1.50	674.14	731.56
169	NUA 273	42.75	37.50	96.25	98.25	2.25	2.00	3.50	1.50	3.00	1.75	1.00	1.25	855.70	867.97
170	NUA 30	39.75	36.50	97.75	99.50	2.50	2.25	4.50	2.50	2.25	1.75	1.00	1.50	670.62	1165.31
171	NUA 31	42.50	36.00	96.50	98.50	2.50	2.00	3.25	1.75	2.75	2.50	1.00	1.50	1379.69	1217.89
172	NUA 39	45.25	39.75	97.25	98.00	2.00	1.75	4.25	2.25	3.25	2.50	1.00	4.00	1507.66	649.84
173	NUA 40	42.00	38.75	102.00	96.75	2.00	2.00	4.75	1.25	2.50	2.00	1.00	2.00	1108.36	960.62
174	NUA 48	46.00	41.75	99.50	95.00	2.00	2.25	4.75	2.50	2.25	4.00	1.00	1.25	1036.64	1223.73
175	NUA 57	46.75	40.25	98.00	96.25	1.75	2.00	3.00	1.50	3.25	3.00	1.25	1.50	758.44	788.05
176	NUA 59	45.75	40.75	102.75	103.75	2.25	2.50	2.75	2.00	3.50	2.50	1.25	2.75	1053.28	1722.67
177	NUA 64	43.75	39.25	98.75	98.25	2.00	2.00	4.00	1.50	3.00	2.50	1.00	1.25	1262.19	964.14
178	NUA 66	42.75	38.25	97.75	100.75	2.25	1.75	3.00	1.00	3.25	2.50	1.00	1.00	1205.23	975.86
179	NUA 67	46.75	41.25	98.50	98.00	2.25	2.00	3.75	2.75	2.50	1.50	1.00	1.50	1127.11	880.00
180	NUA 79	39.50	34.75	96.50	99.25	2.25	1.75	4.50	1.75	2.75	2.75	1.00	1.75	1195.00	773.28
181	NUA 9	43.50	38.50	99.25	99.25	2.75	2.25	3.75	1.50	2.50	2.25	1.00	1.00	1268.83	1055.23
182	PAN 72	45.25	40.75	99.00	98.00	2.75	1.25	1.00	1.00	1.75	2.50	1.00	1.00	1505.00	932.19
183	PI 207262	43.50	36.25	96.25	98.25	3.25	1.75	3.25	4.25	1.25	3.00	1.50	2.00	806.56	920.70
184	R.K. MICHIGA	35.50	35.00	95.25	95.50	3.75	4.00	4.25	1.00	1.00	3.25	1.00	2.50	855.16	982.62
185	RANJONOMBY	48.00	39.50	102.75	101.00	1.50	1.00	2.50	1.50	3.50	3.00	1.00	1.00	1933.36	1425.61
186	RAZ 36	47.25	39.00	100.25	100.00	2.25	2.00	3.00	1.25	3.25	1.25	1.00	1.00	1403.20	824.53
187	RAZ 44	49.50	44.25	103.00	98.25	2.00	1.75	3.00	1.00	2.75	1.50	1.00	1.00	1880.00	1359.45
188	RRN 47	47.50	42.50	99.00	99.75	2.25	1.75	2.00	1.00	1.00	1.50	1.00	1.00	2062.89	906.25
189	RRN 48	45.00	37.50	101.00	99.75	3.25	2.00	1.75	1.00	1.00	2.25	1.00	2.00	2168.36	633.05
190	RWR 1059	45.75	40.75	102.25	103.00	2.75	1.75	2.75	1.00	1.25	2.75	1.00	1.00	1899.37	1215.47
191	RWR 2075	41.50	38.25	99.25	100.50	3.00	2.00	4.50	2.75	1.75	2.25	2.25	1.25	961.17	453.28
192	SAB 662	47.25	42.00	98.00	98.75	3.25	1.75	3.25	2.25	2.00	1.75	1.00	1.00	1029.06	757.11
193	SELIAN 05	43.00	39.00	95.75	93.00	3.00	1.00	3.25	1.50	1.00	1.50	1.00	1.00	1160.67	1181.97
194	SM 133	44.00	41.25	99.50	96.75	2.50	1.00	1.25	1.00	3.25	2.25	1.00	1.25	2112.89	711.17
195	SWP 09	39.00	34.00	96.00	97.50	2.25	2.50	5.25	1.75	2.75	2.50	1.00	1.25	547.89	1367.11
196	SWP 10	46.75	40.25	100.75	100.50	2.25	1.75	1.75	1.00	3.00	1.75	1.00	1.25	1567.34	774.61
197	SWP 12	45.75	39.50	98.00	96.50	2.25	1.00	1.50	1.50	3.75	2.75	1.00	1.00	1616.72	605.16

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198	TU	45.00	39.50	98.50	100.75	2.50	1.25	3.00	1.50	1.25	2.00	1.00	1.50	1121.72	1432.42
199	VAX 1	43.00	36.00	100.00	100.50	3.50	2.00	3.00	1.50	1.50	2.75	1.50	1.75	1953.67	1139.45
200	VAX 2	47.50	39.50	100.50	99.50	3.00	1.00	2.75	1.25	1.25	2.50	1.25	1.25	1445.55	1446.64
201	ZABR 16573-78F22	46.75	40.50	100.50	99.75	1.75	2.00	1.25	1.00	2.75	1.50	1.00	1.00	1236.87	902.66
202	ZABR 16574-46F22	45.50	38.75	99.25	98.50	3.25	2.25	3.75	2.50	2.50	1.75	1.00	1.25	879.53	940.86
203	ZABR 16575-17F22	45.75	41.00	90.75	89.25	2.00	1.25	3.00	1.75	2.25	2.00	1.00	1.00	1862.11	1033.05
204	ZABR 16575-24F22	46.75	44.25	100.50	99.50	1.75	1.50	2.25	1.00	3.00	1.75	1.00	1.00	1802.73	659.22
205	ZABR 16575-39F22	47.75	41.75	99.50	99.50	2.50	1.50	3.00	2.00	2.50	2.00	1.00	1.00	1905.47	600.56
206	ZABR 16575-51F22	49.50	40.50	102.25	102.50	1.50	1.25	2.50	1.50	3.25	2.00	1.00	1.00	1050.23	873.67
207	ZABR 16575-52F22	45.50	38.25	101.25	101.00	2.00	2.25	2.25	2.00	2.75	3.00	1.00	2.00	1118.20	1100.48
208	ZABR 16575-60F22	42.25	36.25	96.75	101.25	2.25	2.25	2.50	1.25	2.50	1.25	1.00	1.25	727.42	856.58
209	ZABR 16575-86F22	48.00	42.25	100.50	101.00	2.25	1.25	2.25	1.25	2.50	2.25	1.00	1.00	1573.05	995.08
210	ZABR 16576-11F22	47.75	42.00	102.75	99.25	1.50	1.75	2.00	2.75	2.00	1.75	1.25	1.00	1214.69	724.77
211	ZABR 16577-51F22	45.00	42.50	99.25	98.25	2.25	2.50	2.00	1.25	2.50	2.50	1.00	1.50	1346.17	730.70
	Mean	44.50	39.16	99.45	98.53	2.75	1.95	2.80	1.58	2.33	2.28	1.19	1.45	1298.07	1005.00
	CV (%)		7.80		6.30		33.60		44.30		35.60		45.90		42.00
	SGE (5%)		ns		ns		<0.001		<0.001		<0.001		<0.001		<0.001

DFF: Days to 50% flowering; MD: days to maturity; ALS: Angular leaf spot; PM: powdery mildew; CBB: Common bacterial blight; BCMV: Bean common mosaic virus; CV: Coefficient variation; SGE: Significant effects; ns: Not significant.

Related Journals: