

Yield Losses and Path Coefficient Analysis of Head Smut Disease (*Tolyposporium penicillariae*) in Pearl Millet Genotypes

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Authors' contributions

This work was carried out in collaboration between all authors. Author JL designed the study, wrote the protocol and wrote the first draft of the manuscript. Author KP reviewed the experimental design and all drafts of the manuscript. Authors JL, KP and OD managed the analyses of the study. Authors TB, OH and MM identified the genotypes. Authors JL and LN performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Head smut caused by *Tolyposporium penicillariae* Bref. is a devastating fungal disease that cause up to 30% yield losses in pearl Millet (*Pennisetum glaucum* (L.) R.Br.). An experiment was carried out in two sites (Koibatek and Marigat) in Kenya to estimate the losses in grain yield due to head smut at varying levels of susceptibility in 50 promising advanced pearl millet genotypes. The test germplasm were planted in a complete randomized block design (RCBD) in three replicates during the short rains (Sept -Dec 2011) and long rains (April-July 2012). To assess the yield loss, two experiments were set as sprayed with fungicide to control disease and unsprayed. Results showed that among the tested genotypes, KAT PM1 and ICMV 221 were resistant checks and showed minimum yield loss as compared to the susceptible genotypes, (SDMV 94001 and SDMV 94014)

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which had highest yield loss. The mean grain yield loss varied between 6.5 and 60.8% in different genotypes. Both incidence and severity of the disease were significantly and positively correlated with losses in grain yield but severity contributed more. The prediction models for estimating yield losses were derived from yield in protected plots compared to none protected plots. Results showed that yield and disease severity were highly significant among the genotypes tested ($F_{pr} < 0.001$) with yield ranging from 1172-4122 kg ha⁻¹. Overall mean yield for both the seasons in the two sites was 2650 kgs ha⁻¹ for the sprayed experiment and 2390 kgs ha⁻¹ in the diseased plots. The overall yield loss due to head smut was 18%. High yielding genotypes were SDMV 90031, IP 8783, SHIBE, ICMV 96603, ICMV221-1, IP6791 and ICMV 221 Bristled. These were recommended for further evaluation in multi-sites and be released as commercial varieties.

Keywords: *Pennisetum glaucum*; *Tolyposporium penicillariae*; disease severity; disease free; yield losses.

1. INTRODUCTION

Pearl millet is a drought tolerant cereal classified as the hardiest among all cereals. It is grown mainly in the Arid and Semi Arid tropics (ASALs) both for its grain and fodder and contributes to both nutritional and food security of the rural resource poor people in these areas [1]. It is also a very important cereal in the health and nutrition of young children and the elderly [2]. Besides the biotic and abiotic challenges, among the major cereals (maize, wheat, sorghum, finger millet etc), pearl millet has the highest adaptation to drought and heat [3]. In Kenya pearl millet yields remains low due to diseases and pests as well as growing of low yielding unimproved varieties among other challenges [4,5]. Head smut caused by *Tolyposporium penicillariae* Bref. [6] is a devastating fungal disease that cause up to 30% yield losses in pearl millet [7]. Head smut in pearl millet causes significant yield losses hence appropriate measures to control the disease should be put in place. In the ASAL pearl millet is mainly grown by small scale farmers making the use of fungicides to control the disease economically unlikely. Understanding the magnitude of yield losses due to this disease gives the best guidelines on the most appropriate control measures. The disease cause up to 30% yield loss besides causing 100% damage to individual panicles [8]. In Kenya, current pearl millet yields are very low (200-800 Kg ha⁻¹) as compared to its research potential of 1500-3000 Kg ha⁻¹ [4,9]. There is therefore need to identify genotypes that are resistant to major biotic stresses like head smut for increased yields and incomes to small scale farmers in ASALs.

2. MATERIALS AND METHODS

2.1 Site Description

The study was conducted at two sites in Kenya, Koibatek ATC (Agricultural Training Centre) and

Marigat (KARI –Perkerra). Amongst the two sites, Koibatek ATC is fairly wet and humid receiving more rainfall than Marigat. ATC-Koibatek lies at latitude 1°35' S, and longitude 36°66' E, altitude 1890 m above the sea level in agro-ecological zone 4 (Upper Midland 4). The average annual rainfall is 767 mm with a mean minimum temperature of 10.9°C and a maximum mean temperature of 28.8°C [10]. KARI Perkerra-Marigat lies at a latitude of 1°45' N and longitude 36°15' E with an altitude 1067 m above the sea level. The centre is situated in agro ecological zone 5 (Lower Midland 5). The mean annual rainfall at the Centre is 654 mm with an annual minimum temperature of 16.8°C and a maximum, temperature of 32.4°C [10].

2.2 Materials and Experimental Design

The genotypes evaluated were a collection of open pollinated varieties (OPVs) and commercially released varieties in East, Central and West Africa. These 50 genotypes were sourced from International Crops Research Institute for the Semi Arid Tropics (ICRISAT) in Kenya. Three (ICMV 221, KAT PM 1 and KAT PM 2) are commercial varieties in Kenya and served as checks. The test entries were planted in a Randomized Complete Block Design (RCBD), in three replicates. Two experiments were set (A&B), genotypes in Experiment A were not protected against the disease while those in B were protected using recommended rates of Ridomil fungicide to control and maintain the experiment disease free. In experiment B yield losses due to disease were estimated by calculating the percent yield loss in Experiment A. The experiments determined the grain yield performance and the level of yield losses due to head smut for each tested genotype. Disease development was allowed through natural infestation. The panicles were scored for smut severity as a percentage of florets that had smut

sori at the reproductive stage on a scale of 1-8 [11].

2.3 Disease Data Collection

Incidence and severity of head smut were recorded after every seven days from the booting stage up to harvesting. The incidence was determined by counting the number of plants infected per plot while severity was determined by the percentage infection of the individual plants using the disease rating scale on the florets [12] on a scale of 1-8 (Fig. 1). Where 1= highly resistant, 2 = resistant, 3-4 = moderately resistant, 5-6 = moderately susceptible 7= susceptible, and 8 highly susceptible. Any plants with <10% of florets infected were considered highly resistant, between 11-30% florets resistant, 31-40% florets infected moderately resistant, between 40-50% moderately susceptible, 51-70% florets infected susceptible and 71-100% floret infected as highly susceptible [11,13].

2.4 Yield Loss Estimation

The relative losses in yield for each variety were determined separately for each of the genotypes with different levels of disease. The yield losses from controlled experiment were estimated as percentage yield loss due to disease in the first experiment (A) compared to the calculated yield differences between the two experiments [14]. The experiment determined the grain yield performance and levels of resistance to head smut for the test genotypes considering their levels of resistance and disease severity

$$RL (\%) = \frac{Y_1 - Y_2}{Y_1} \times 100$$

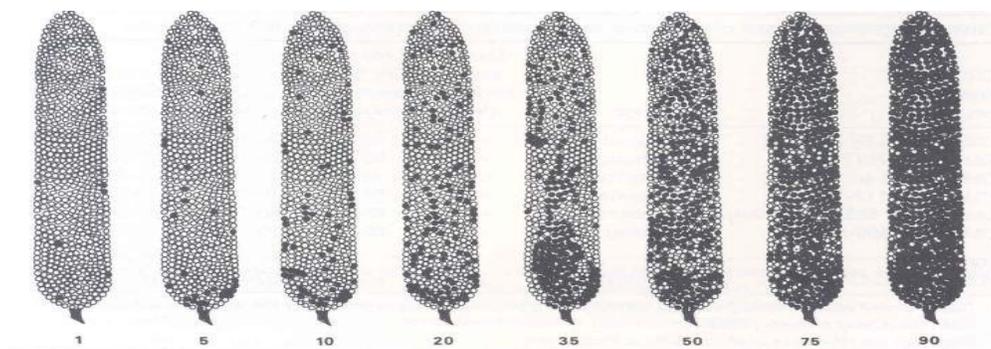


Fig. 1. Pearl millet panicles showing % area with smut *sori*

Where,

RL = relative loss (Reduction of the parameters grain yield)

Y1 = mean yield of respective genotype on protected plots (plots with maximum protection) and

Y2 = mean yield of the respective genotype in unprotected plots (i.e. unsprayed plots).

2.5 Statistical Analysis

Data was subjected to analysis of variance using Genstat release 14. Treatment means were separated using LSD at $P \leq 0.05$. Simple correlation coefficient (r) was carried out using Pearson's correlation. Homogeneity of error variance was carried out before pooling the data across environments using Bartlett's test for homogeneity and data transformation carried out by dividing mean response by respective root mean square error (MSE) for respective environments [15]. Simple Principal Analysis (PC) and Multivariate analysis was undertaken using JMP statistical software, version 10. Principal component analysis (PCA) was applied as a correlation tool in reduction and summary of standardized data from yield parameters.

Correlation was computed using mean values from each season and both seasons combined in the growing years. Relationships of grain yield and yield components were analyzed as seed per panicle, panicle height and 1000 seed weight as intermediary variables and other traits as independent variables. Similarly Principal component analysis was done in order to obtain an overview of the association between grain yield, yield components and other traits; this was done using eigen analysis of correlation matrix

where each eigenvalue corresponds to a proportion of the variance in the data set. The greatest amount of variance was assigned to the first principal component. The second principal component accounts for the second highest amount of variance and is orthogonal to the first and so on. The total sum of the principal components (eigenvalues) is equal to the sum of variances of the standardized variables [16].

3. RESULTS AND DISCUSSION

3.1 Yield Losses Due to Head Smut in Pearl Millet Genotypes in Koibatek and Marigat, Kenya

There was an average of 20% yield losses due to disease pressure for both the sites and the seasons. The results for combined ANOVA showed significant genotypic variation in grain yield loss due to head smut.

The highest yield loss of 28% was recorded in Koibatek during the long rains and 18% in the short rains hence an average yield loss of 23%. Slightly lower yield losses of 14% and 21% in Marigat were recorded with an average of 17.5%. More yield losses were observed in the long rains 23% as compared to 16% in the short rains in both sites combined. A consistent trend in response to the disease was noted and grain yield losses were consistent for tested genotypes across the sites and the seasons. In the protected experiment without disease pressure the yields were much higher as compared to the diseased experiment. Overall mean yield for both the seasons in the two sites was 2.65 ha⁻¹ tons for the protected plots as compared to 2.39 tons ha⁻¹ in the diseased plots a percent yield loss of 20.25% (Table 1). This confirms results by Phookan et al. [17] that smut cause yield losses of between 15-60 % in pearl millet productivity.

According to Rao et al. [11] pearl millet genotypes can be grouped into six groups of resistance and susceptibility in relation to their reactions to head smut disease. The most resistant genotypes had less than 10% infected florets with only less than 10% disease

incidence. This caused insignificant yield losses with SDMV 90031 having the minimum yield loss of 0.5%, and IP 6791, IP 8783, ICMV 93771, IP7390 with yield losses of 1.5%, 2.4%, 5.2% and 6% respectively (Fig. 2). The susceptible genotypes on the other hand had the greatest yield losses of 26%, 19%, and 15% for SDMV 94001, SDMV 94014, and SDMV 96063 respectively (Fig. 2). There was high humidity in Koibatek leading to more disease development and severity. The high disease pressure in this site caused high yield losses because high humidity favours spore development [11]. Yield losses ranging from 14-28% are close to those recorded by Jain et al. [18] who observed a yield loss of between 6%-40%. High yield loss in grain yield resulted from high incidence and severity of the disease. This was also evident from disease severity per genotype. Such results have been reported elsewhere by Salih et al. [19]. Highly susceptible genotypes (SDMV 94014 and SDMV 94001) had the highest severity of 65 and 78% and highest yield losses of 29% and 34% respectively (Figs. 2 and 3). Other genotypes that lost significant yield due to the disease were SDMV 96063 with 29% yield loss and disease severity of 72%, ICMV 94136, 19% yield loss and disease severity of 46% while IP 8856 lost 15% yield having a disease severity of 30% (Figs. 2 and 3). All these genotypes were classified as susceptible genotype to head smut.

3.2 Estimation of Yield Loss for Test Genotypes in Both Sites and Seasons Combined

Based on yield loss in unprotected plots as compared to protected plots the percent yield loss ranged from 2-34% (Fig. 2). The best performing genotypes: DMV 90031, ICMV221-1, IP 8783, IP6791 ICMV96603 and IP7390.

Results of disease severity are presented in Fig. 2. SDMV 90031 had minimal yield losses of 3% with disease severity of 10% while ICMV 221-1 had yield losses of 8% and disease severity of 45%. Genotype ICMV 96603 had only 4% yield loss with a disease severity of 10 %. IP8783 and IP 6791 were highly resistant recording yield

Table 1. Mean percentage yield loss for tested genotypes in sprayed and non sprayed experiments for both sites and seasons combined

	% yield loss short rains	% yield loss long rains	Overall % yield loss
ATC-Koibatek	18	28	23
KALRO Marigat	14	21	17.5
Overall % yield loss	16	24.5	20.25

loses of only 2% and 10% respectively both with disease pressure of 10%. The commercial resistant checks ICMV 221, KAT PM1, KATPM2 had <8% of yields losses all with an average disease severity of 10% thus maintaining resistance. The susceptible checks SDMV 94014, SDMV 94001 had the most yield losses of 34% and 29% respectively with disease severities of 78% and 65% (Figs. 2 & 3).

3.3 Correlation and Path Analysis of Yield, Yield Components, Disease Incidences and Severity of Genotypes in Both Seasons and Sites Combined

3.3.1 Correlation analysis

Significant ($P \leq 0.05$) and inverse correlation (r), was observed between grain yield and head smut incidence and severity ($r=-0.5^*$ and -0.76^*), respectively (Table 2). There was also a significant inverse correlation between the grain yield and the days to maturity ($r=-0.42^*$). Positive significant correlation was observed between the thousand seed weight and grain yield in both the protected experiment ($r=0.52^*$) and non protected experiment ($r=0.48^*$). Significant ($P \leq 0.05$) and positive relation was also observed between reproductive tillers ($r=0.04^*$), with grain yield. Correlation coefficient shows interrelationships between pairs of quantitative characters [20]. In plant breeding it is one of the guides facilitating interpretation of the obtained results and may form foundation for planning breeding programmes for increased genetic gains. Pearson coefficient of correlation (r) between two traits revealed that seed yield (tons ha^{-1}) was positively and significantly related to biomass ($r=0.79$), number of reproductive tillers ($r=0.72$).

3.4 Principal Component Analysis (PCA) for Yield and Yield Component in Test Genotypes Sites and Seasons Combined

Principal components analysis is a multivariate analysis used to study the kind of variation present in a selected population and multivariate polymorphism [21]. The first and the second principal components normally accounts for the first and second highest amount of variance [16]. Principal component analysis across the sites and seasons when data was pooled indicated that, only four principal components were significant. According to Hair et al. [22] Eigenvalues greater than 1 are considered

significant and component loadings greater than ± 0.3 were deemed meaningful. The sign indicates the direction of relationship between the components and the variables. Those with a positive sign indicate that the variables are positively related to the PC while those with a negative sign are negatively related to the PC [16]. The genetic diversity of 50 pearl millet genotypes was observed for their yield parameters as a requirement for the pre-selection of varieties for future breeding programs. The principal component analysis grouped the characteristics into grain yield in sprayed and non sprayed experiments, Days to maturity, plant height, resistance to bird damage, 1000 grain weight and the panicle characteristics. The combined analysis of data in both sites showed that four principal components explained 81% variation present within the genotypes (Table 3).

PCA 1 accounted for 27.7% of variation and was positively associated with grain yield in the sprayed and non sprayed experiment (0.31), reproductive tillers (0.28), and 1000-grain weight (0.19), and days to maturity (0.39). However, PCA 1 was negatively related to disease incidence (-0.32), disease severity (-0.31), and bird damage (-0.24). PCA 2 accounted for 19.1% of variation. It was positively associated with panicle length (0.16) and diameter (0.09), reproductive tillers (0.07, and plant height (0.32). PCA 2 was also negatively related to disease incidence (-0.037), disease severity (-0.39) and bird damage (-0.07). The 3rd and the 4th PCA accounted for 18.5% and 15.6% respectively (Table 3). The sign indicates the direction of relationship between the components and the variables.

As a result, only the first four principal components were considered in this study. The traits with loadings greater than ± 0.3 were taken to represent the corresponding principal axis. In this study 4 PCAs accounted for a total variation of 81% with PCA 1 accounting for 27.7% and PCA 2 accounting for 19.1%. PCA 3 accounted for 18.5% and the 4th PCA accounting for 15.6%. These results were similar to those achieved by [23] who found out that four principal components are significant with pearl millet productivity. The first PC was closely associated with days to maturity, days to 50% flowering and days to 50% maturity in 16 pearl millet genotypes [23]. In another study on analysis of 60 pearl millet genotypes for their biochemical composition four PC were considered [24].

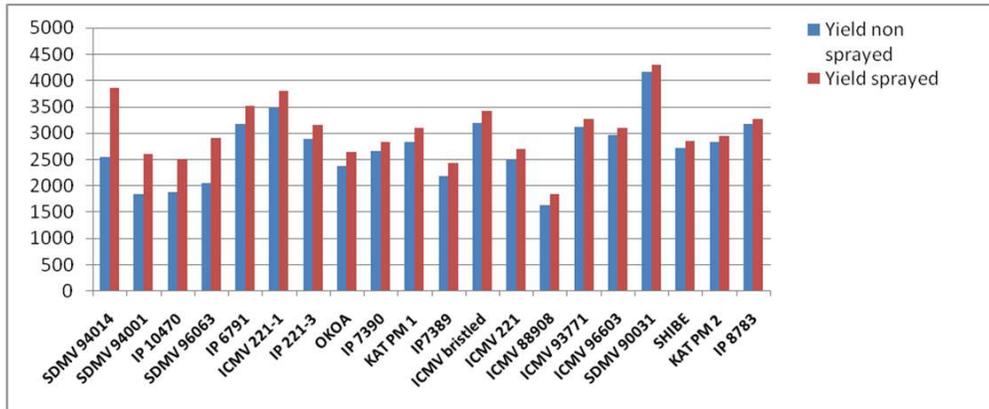


Fig. 2. Yield comparison (Tones/Ha) among genotypes in sprayed and non sprayed experiments

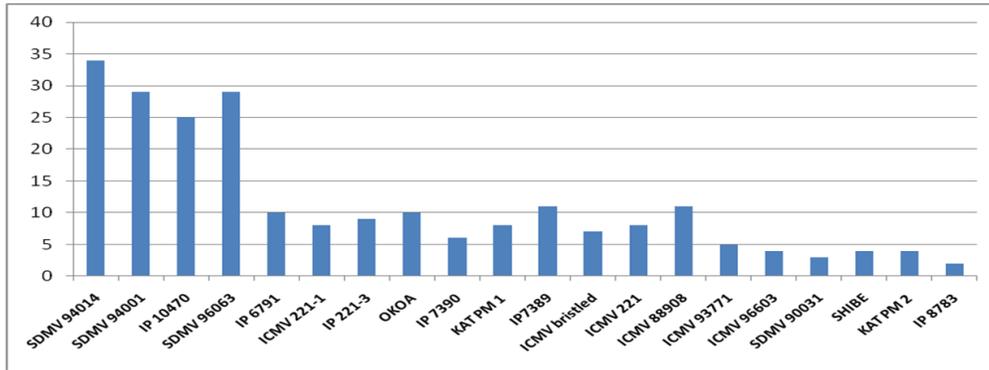


Fig. 3. Percent Yield loss amongst test genotypes in both Koibatek and Marigat sites

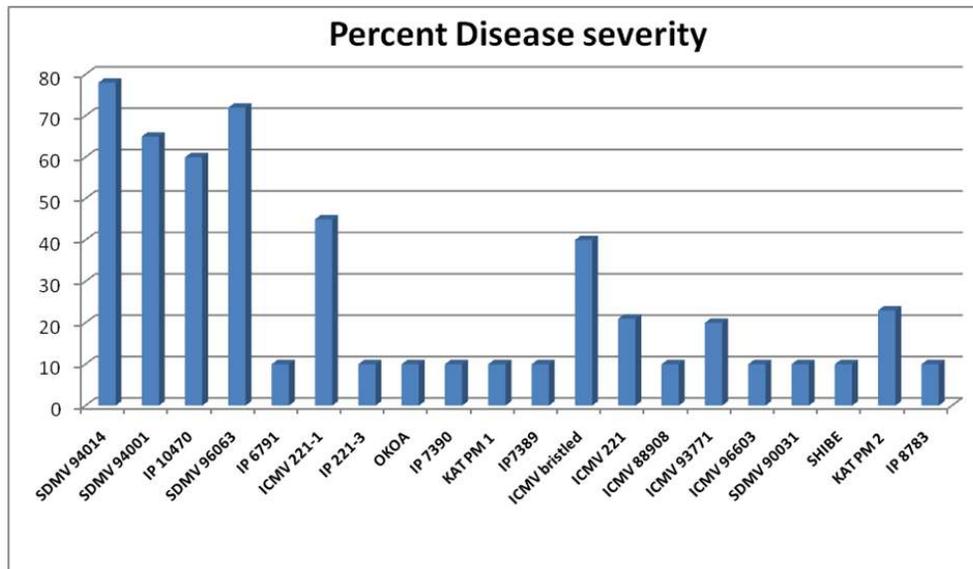


Fig. 4. The disease severity in percentage for all test genotypes in both sites

Table 2. Correlation coefficient for yield parameters and disease scores in both sites and seasons combined

	1000SW	BRD	BRSTLS	DAM	DSI	DSS	RT	VT	YLD N.S	YLD S
1000 SW	1									
BRD	-0.03*	1								
BRTLS	0.03*	0.8*	1							
DAM	-0.13*	-1*	-0.13*	1						
DSI	-0.17*	-0.2*	-0.14*	-0.7*	1					
DSS	-0.22*	-0.3*	-0.16*	-0.8*	0.8*	1				
RT	-0.39*	-0.6*	-0.22*	-0.1*	-0.2*	-0.46	1			
VT	-0.44*	-0.7*	-0.24*	-0.1*	-0.33*	-0.61*	0.05*	1		
YLD_N,S	0.48*	-0.8*	-0.25*	-0.1*	-0.42*	-0.76*	0.04*	-0.01*	1	
YLD S	0.52*	-0.9*	-0.27*	-0.1*	-0.5	-0.91*	0.03*	-0.02*	-0.13*	1

Key: 1000 WT= A thousand seed weight DAM = Days to maturity; BRD = Bird damage; BRSTLS = Presence or absence of Bristles DSI = Disease incidence DSS = Disease Severity Reproductive tillers VT = Vegetative Tillers YLD N.S = Yield in non sprayed experiment YLD S=Yield in the sprayed.* significant at ($P \leq 0.05$ ** significant at ($P \leq 0.001$)

Table 3. Principal component analysis (PCA) for yield and yield components in both sites and seasons combined

PC	EV	IND%	Cm%	1000 SWT	BRD	DAF	DAM	DSI	DSS	YNS	YL	PHT	VT	RT	PLT	PDM
1	3.59	27.7	28	0.19	-0.24	0.43	0.39	-0.32	-0.31	0.31	0.31	0.12	0.18	0.08	0.28	0.22
2	2.47	19.1	47	0.37	-0.07	-0.07	-0.02	-0.37	-0.39	0.46	0.45	0.32	0.09	0.07	0.16	0.09
3	2.05	18.5	65	0.09	-0.25	0.16	0.21	0.20	0.20	0.10	0.12	0.14	-0.54	-0.57	0.22	0.27
4	1.45	15.6	81	0.29	-0.19	-0.19	-0.10	0.28	0.26	-0.01	-0.05	-0.19	0.30	0.30	0.33	0.61

Key PC = Principal component, EV = Eigenvalue, IND = Individual %, CUM = Cumulative%, 1000 SWT = A thousand seed weight, BRD = Bird damage, DAF = Days to flowering, DAM = Days to Maturity, DSI = Disease incidence, DSS = Disease severity, YNS = Grain yield Non sprayed experiment, YS = Yield in sprayed experiment, PHT = Plant Height, VT = Vegetative tillers, RT = Reproductive tillers, PLT = Panicle Length, PDM = Panicle Diameter

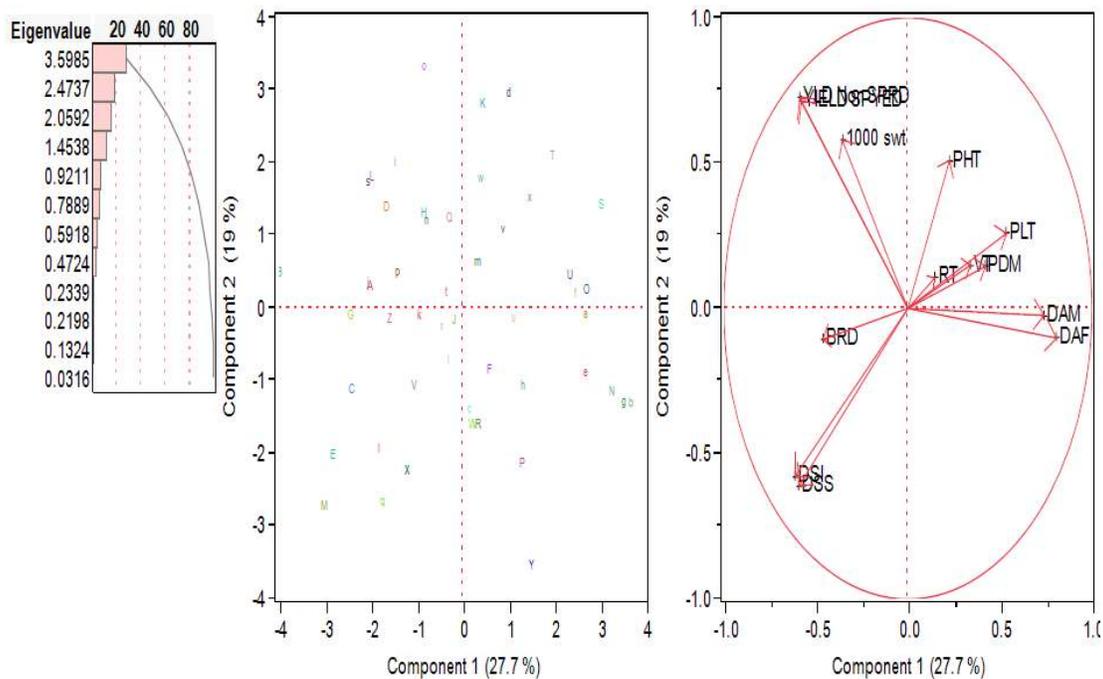


Fig. 5. Principal Component score plot of PC1, PC2, PC3 and PC4 describing the overall variation among Genotypes estimated using yield components data

The PCA for 50 pearl millet genotypes evaluated in this study, indicate that the number of reproductive tillers, 1000 seed weight, days to maturity and panicle characteristics are all important traits to be considered in breeding for grain yield in pearl millet [25]. All these traits accounted for the first and the most important PCA1. The results also indicate that disease incidence, severity, and bird damage all in the PCA1 are important traits that significantly affect grain yield in pearl millet productivity.

4. CONCLUSION

Development and selection of genotypes that produce better yields and resist abiotic and biotic stresses in ASALs is critical in maintaining pearl millet productivity [26] as well as enhancing food security. In the present study, genotypes SDMV 90031, IP7390, IP6791, ICMV93771, ICMV 221, ICMV221 Bristled, ICMV96603, SDMV 96063 and ICMV 91450 were resistant to head smut with minimal yield losses. These genotypes have a high potential of being developed into varieties hence should be considered for commercial production in Kenya. The most resistant genotypes were IP 8783, IP9946, ICMV 221-3, ICMV 91450, ICMV 88908, ICMV 94151. Genotype IP 8783 was not among the highest in yield but had the least losses due to disease, for

this reason it should be included in a breeding programme for genetic studies on its resistance to pearl millet head smut. The results suggested that there is adequate genetic variability present in the genotypes evaluated. The phenotype of a plant is the result of interaction of a large number of factors such that the final yield is sum total of effects of several component factors. Thus understanding the extent and nature of interrelationship between grain yield and its contributing characters and also among them is critical in general improvement of all crops. The findings from this study showed that, selection for disease resistance, yield (kg/ha), plant height, 1000-grain weight, days to maturity and number of reproductive tillers would be more effective traits in boosting grain yield performance and resistance to head smut. The results of PCA indicated that disease incidence, severity, and bird damage were important constraints affecting grain yield in pearl millet productivity, and breeding for their resistance is worthwhile investment. Unlike the other cereals: rice, wheat, barley and maize which are utilized both as food and industrial purposes, pearl millet has so far remained a traditional food crop for only subsistence production in Kenya and many other dry regions of Africa. Because of the numerous benefits from this crop it should be included in the national programs for food security.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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