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Determination of heterosis for downy mildew (*Sclerospora graminicola (Sacc.)* Schroet) tolerance in pearl millet (*Pennisetum glaucum L. R. Br.*)

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Abstract

Downy mildew (*Sclerospora graminicola* (Sacc.) Schroet) is the most important disease of pearl millet (*Pennisetum glaucum* L. R. Br.) in many countries of the world including Nigeria. The study was conducted at the Usmanu Danfodiyo University Dry land farm, Sokoto State and Bui village in Arewa local government area of Kebbi State both in Nigeria to determine heterosis for downy mildew in some pearl millet crosses. Twelve parents and thirty hybrids were evaluated for their reaction to the disease. The genotypes were sown in the field in randomized complete block design with two replications. They were inoculated by spraying them with sporangia suspension at seedling stage. Data were collected on downy mildew disease incidence, severity score and some agronomic traits. Analysis of variance for combine location depicts significant ($P < 0.05$) difference among the genotypes for all the characters except plant height (PHT), number of panicles harvested (NPH) and panicle weight (PWT). Heterosis over high parent and mid parent recorded positive and negative heterotic values in desired direction for grain yield (kg/ha), downy mildew incidence and severity across the locations.

Keywords: Heterosis; Hybrids; Downy mildew; Genotypes; Tolerance

1. Introduction

Pearl millet (*Pennisetum glaucum* L. R. Br.) is a diploid (2n = 2x = 14), warm-season C4 annual cereal crop grown in West Africa and on the Indian subcontinent for food and forage. It is a robust, quick growing cereal grass with large stems and leaves which are tall and vigorous, with exceptional grain and fodder yielding potentials. Pearl millet is the sixth most important cereal crop in the world [1]. Harvested from an area of 20 million ha in the semi-arid regions of Africa, pearl millet contributes 19% to the total area allocated to cereal production in the region [2]. The world pearl millet production is 28,357,451 tonnes with Nigeria contributing 5.25% as the fifth world top producer [3]. Downy mildew (*Sclerospora graminicola* (Sacc.) Schroet) is the most important disease of pearl millet in many countries of the world including Nigeria. Downy mildew infects the foliage and the panicles of the crop and causes 20-40% grain yield losses annually worldwide [4 and 5], and sometimes it could be much higher where a susceptible cultivar is frequently grown in the same field. On the basis of a few localized estimates in India the average annual yield losses can reach up to 40%, whereas 10-50% losses have been reported from Nigeria [6]. Breeding for tolerance to downy mildew therefore, has been accorded highest priority as reported by [5] and it is the integral part of the majority of the breeding program in Africa because, a large number of resistance sources (including elite breeding lines) have been identified in diverse genetic backgrounds and effective screening techniques have been developed [7]. According to [8], multi-location monitoring of downy mildew virulence is one of the efficient ways to assess genotypes. The aim of this research is to

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evaluate F_1 pearl millet hybrids for tolerance to downy mildew disease and determine heterotic relationship for downy mildew tolerance in pearl millet.

2. Material and methods

The field experiment was conducted at two locations, The first location Usmanu Danfodiyo University Sokoto (UDUS) dry land farm lies on latitude 13° 08′N and longitude 5° 13′E on an altitude of 278m above sea level about 11 km from Sokoto town. Sokoto falls under the Sudan Savanna ecological zone of Nigeria and it is characterized by a long dry season and a short wet season. Rainfall mainly starts in May and ends in early October with a peak in August. The annual rainfall for Sokoto ranges between 300 mm and 800 mm. The mean annual temperature is 34.5°C, although dry season temperatures in the region often exceed 40°C [9].The second experimental site Bui village in Arewa local government area of Kebbi State of Nigeria lies on an altitude of 244m above sea level on latitude 12° 11′E and longitude 4° 15′E. It receives a mean annual rainfall of 800mm between April to October with a peak period in August, while the remaining period of the year is dry [10]. The maximum and minimum temperature ranges from 40.3°C and 16.6°C respectively [11].

Table 1 List of Parents and Their Resistance Status

Parents	Designation	Resistance Status
AON 740	P1	Susceptible
BA-ANGOURE	P2	Susceptible
CAPARLGPMG20150141	P3	Susceptible
C-90-133	P4	Susceptible
Kiek	P5	Susceptible
Jirani	P6	Susceptible
CAPARLGPMG20150073	P7	Resistant
Dan Tchama 2	P8	Resistant
SOSAT C88	P9	Resistant
CAPARLGPMG20150042	P10	Resistant
AON 502	P11	Resistant
SuperSosat	P12	Resistant

Table 2 Format of Analysis of Variance (ANOVA) For Single Environment

Sources of variation	df	MS	EMS
Replication	$(r-1)$		
Genotype	$(g-1)$	M_2	$\sigma_e^2 + r\sigma_g^2$
Error	$(g-1)(r-1)$	M_1	σ_e^2

Where;

r = number of replicates; M_1 = error mean square

L = number of location; σ_e^2 = error variance

g = genotype; σ_g^2 = genotypic variance

d.f = degree of freedom; M_2 = genotype mean square

MS = mean square; EMS = expected mean square

The genetic materials consists of 42 entries, made up of 12 parents (Table 1) and 30 F_1 hybrids combined in randomized complete block design with 6 blocks per replication, 8 plots per block (7 test rows +1 infector row), 1 row per plot and 10 stands per row replicated 2 times. Each plot contained a row made up of 10 stands. Each plot has measured 5m long with intra and inter row spacing of 0.75m × 0.5m. The gross plot size is 10.5m × 37m. A highly downy mildew susceptible variety (Jirani) was sown as the infector row after 7 rows of the test lines. The test lines were sown 2 weeks after sowing the infector row and disease incidence level in these lines indicates the distribution of disease pressure in the field. N.P.K. (15:15:15) was broadcasted basally at the rate of 30kg/ha N, 30kg/ha P_2O_5 and 30kg/ha K_2O to all the plots while top dressing of Urea (46%) at the rate of 30kg of N was carried out at 6 weeks after planting. Thinning was done 2 weeks after sowing to maintain 2 plants per hill/stand and Weeding operations were carried out manually using hoe. The first weeding was carried out 2 weeks after sowing while the second weeding was carried out 5 weeks after sowing. Harvesting was done manually at physiological maturity 18 weeks after sowing. Each plot was manually threshed separately using a mortar and pestle to separate the seeds from the panicle. Data was collected on days to 50% flowering, plant height (cm), plant length (cm), number of harvested panicles, plant weight (g), panicle circumference (cm), panicle compactness, grain weight (kg/ha), 1000 grain weight (g), threshing percentage (%), downy mildew incidence (%) and downy mildew severity (%).The data collected were analyzed using GenStat 17th edition.

Table 3 Format of Analysis of Variance (ANOVA) For Combined Environment

Sources of variation	Df	MS	EMS
Environment	$(e-1)$	M_4	$\sigma_e^2 + r\sigma_g^2 + rg\sigma_e^2$
Replication	$(r-1)$		
Genotype	$(g-1)$	M_3	$\sigma_e^2 + r\sigma_g^2 + re\sigma_g^2$
G × E	$(g-1)(e-1)$	M_2	$\sigma_e^2 + r\sigma_g^2$
Error	$(g-1)(r-1)$	M_1	σ_e^2

Where:

r = number of replicates; $M_1 = \sigma_e^2$ = error mean square
L = number of location; d.f = degree of freedom
g = genotype; σ_g^2 = genotypic variance
M_3 = genotype mean square; M_2 = G × E mean square
M_4 = environment mean square; g = genotype
e = environment ; σ_{ge}^2 = G × E variance
MS = mean square; EMS = expected mean square

2.1. Estimates of Heterosis

Heterosis was estimated for Mid Parent Heterosis (MPH) and High Parent Heterosis (HPH) using the formula described by [12].

Mid-parent heterosis (MPH) is the average superiority of a hybrid over its parents and was calculated as:

$$\text{H (\%) Mp} = \frac{F_1 - Mp}{Mp} \times 100$$

Where; F_1 = the hybrid mean.

Mp = mean of mid-parent value.

High-parent heterosis (HPH) is the superiority of a hybrid over its best parent and was calculated as:

$$H\ (\%)\ Hp = \frac{F_1 - Hp}{Hp} \times 100$$

Where; F_1 = mean of F_1 generation

Hp = mean of superior parent.

3. Results and discussion

The result of the combined study across locations showed highly significant ($P < 0.01$) difference with regards to Genotype × Environment interaction for Grain weight per hectare (GW) and threshing percentage (THR%) and significant ($P < 0.05$) difference for days to 50% flowering (D50%F), Downy mildew severity (DMS) and 1000 grain weight (1000GW). There was no significant difference in the Genotype × Environment interaction for plant height (PHT), Downy mildew incidence (DMI), panicle length (PLEN), number of panicles harvested (NPH), panicle compactness (PCMP), panicle weight (PWT) and panicle circumference (PCIR) as shown in Table 2. The significant Genotype × Environment interaction for days to 50% flowering (D50%F), Downy mildew severity (DMS), Grain weight per hectare (GW), 1000 grain weight (1000GW) and threshing percentage (THR%) signifies that the genotypes respond differently across the two location. In UDUS Cross P12×P10 recorded the highest grain yield of 1416.8kg/ha followed by Cross P9×P7 with 723.5kg/ha. Cross P8×P5 recorded 2921kg/ha followed by Cross P12×P11 with 1279kg/ha in Bui. In the combined Cross P8×P5 recorded 1592.1kg/ha followed by Cross P12×P11with 1275.2kg/ha. This suggests that more than one test locations or environments are required to obtain reliable results. [13] also reported similar results that were significant for Genotype × Environment interaction in their studies of pearl millet parents and hybrids. This variation could be attributed to genetic and environmental effects as well as their interactions. Substantial variations in pearl millet genotypes have also been also reported in previous studies by [14] and [15]. While there was no significant difference in the interaction for Downy mildew incidence (DMI), plant height (PHT), panicle circumference (PCIR), panicle weight (PWT), panicle length (PLEN), panicle circumference (PCIR), panicle compactness (PCMP).

Across the two environments where the genotypes were evaluated, there was highly significant ($P < 0.01$) difference for days to 50% flowering (D50%F), Downy mildew incidence (DMI), Downy mildew severity (DMS), threshing percentage (THR%) and 1000 grain weight (1000GW) and significant ($P < 0.05$) difference for panicle compactness (PCMP) alone. There was no significant difference across the environments for number of panicles harvested (NPH), plant height (PHT), panicle circumference (PCIR), panicle weight (PWT), panicle length (PLEN) and Grain weight per hectare (GW). The environment source of variation was highly significant for days to 50% flowering (D50%F), Downy mildew incidence (DMI), Downy mildew severity (DMS), number of panicle harvested (NPH), panicle compactness (PCMP), 1000 grain weight (1000GW) and threshing percentage (THR%). This therefore indicates that difference exists in the environments of study with regards to the characters that were significant. Although both environments of Sokoto and Kebbi States fall under the Sudan Savannah ecological zone, they both differ in their soil type, altitude and rainfall duration. The soil in UDUS reveals that it is sandy with low silt and clay composition while the soil in Bui is less sandy with higher components of silt and clay. The result also revealed that there was no significant difference across the environments for panicles harvested (NPH), plant height (PHT), panicle circumference (PCIR), panicle weight (PWT), panicle length (PLEN) and Grain weight per hectare (GW). This indicates that results of these characters are the statistically similar for the two environments. This is because both environments fall under the same ecological zone.

Also there was highly significant ($P < 0.01$) difference among the genotypes evaluated for panicle length (PLEN), panicle circumference (PCIR), Grain weight per hectare (GW), threshing percentage (THR%) and significant ($P < 0.05$) difference among the genotypes for days to 50% flowering (D50%F), Downy mildew incidence (DMI), Downy mildew severity (DMS), panicle compactness (PCMP) and 1000 grain weight (1000GW). There was no significant difference among the genotypes for plant height (PHT), number of panicles harvested (NPH) and panicle weight (PWT). The genotype source of variation was significant in all the characters except for plant height (PHT), number of panicles harvested (NPH) and panicle weight (PWT). This implies that there is the presence of tremendous levels of variability among the parental lines and their hybrids evaluated in this study. [13] also reported similar results and found outstanding level of genetic variability among parental lines and their hybrids. The highly significant difference among the genotypes for most of the characters studied in the two environments indicates the presence of considerable amount of genetic variation among the parental lines and hybrids, suggesting progress that could be possible for further population improvement. This wide range of variation observed for the characters in both locations would offer scope of selection for development of desirable genotypes. These significant differences could also be attributed to the

composition of the population, which is made of parents whose accessions were collected from different pearl millet landraces across the pearl millet cultivating Northwest states in Nigeria with some states in Niger Republic, as well hybrids developed from the crossing of these selected accessions. This is in line with the studies of [16] in her studies of pearl millet parents and hybrids. Also [17] and [15] all reported similar results in their pearl millet studies.

Table 4 Combined analysis of variance mean squares for 12 characters evaluated at UDUS and Bui during 2018 raining season

Sources of Variation	Replication	Genotype	Environment	G× E	Error
d.f	1	46	1	46	42
D50%F	56.99	139.68*	834.47**	72.27*	38.19
PHT	1205	1433	24	734	1028
PLEN	15.99	275.64**	61.68	43.61	53.5
NHP	354.69	58.5	117.97	28.78	44.43
PWT	166354	34548	5582	21916	31684
PCIR	0.55	2.24**	1.2	0.8	0.48
PCMP	1.49	3.26*	15.45*	2.28	1.89
DMI %	4556.5	1323.1*	28825.8**	880.8	536.1
DMS %	536.5	840.0*	32761.8**	673.4*	375.2
GW	193341	394552**	17379	338784**	78168
THR%	317.29	248.62**	13368.58**	270.14**	91.1
1000GW	29.69	7.11*	102.78**	7.58*	3.99

D50%F =days to 50% flowering, PHT=plant height (cm), PLEN= panicle length (cm), NHP= number of harvested panicles, PWT= panicle weight (g), PCIR= panicle circumference (cm), PCMP= panicle compactness, DMI= downy mildew incidence, DMS= downy mildew severity, GW= grain weight (kg/ha), THR%= threshing percentage, 1000GW= 1000 grain weight (g) and d.f= degree of freedom. **=significant at 1% probability level, *=significant at 5% probability level.

Table 5 High parent and Mid parent heterosis for grain yield for crosses in UDUS and Bui evaluated during the 2018 raining season

		UDUS		BUI	
GENOTYPES	**CROSS**	**HPH**	**MPH**	**HPH**	**MPH**
Jirani×CAPARLGPMG20150141	P6×P3	-77.48	-74.89	-92.47	-91.02
Dan Tchama 2×C-90-133	P8×P4	-25.88	11.79	-20.95	23.31
SOSAT C88×Dan Tchama 2	P9×P8	116.81	210.36	74.03	92.52
SOSAT C88×BA-ANGOURE	P9×P2	33.35	96.44	40.45	101.55
CAPARLGPMG20150073×AON 740	P7×P1	-78.61	-74.75	-83.45	-72.57
Dan Tchama 2×Kiek	P8×P5	-14.63	3.07	1367.10	1611.69
Dan Tchama 2×CAPARLGPMG20150073	P8×P7	15.70	18.79	-1.76	5.51
C-90-133×AON 740	P4×P1	4.12	68.09	-3.50	80.79
CAPARLGPMG20150141×Kiek	P3×P5	-84.27	-81	293.31	325.50
Dan Tchama 2×CAPARLGPMG20150042	P8×P10	0.971	17.79	-6.53	40.25
SuperSosat×AON 502	P12×P11	0	38.48	979.32	1486.85
SuperSosat×AON 740	P12×P1	30.88	92.87	-45.39	-8.95

SuperSosat×CAPARLGPMG20150073	P12×P7	15.77	52.89	10.19	12.07
BA-ANGOURE×C-90-133	P2×P4	-1.17	52.79	256.71	351.92
AON 502×CAPARLGPMG20150073	P11×P7	-45.61	-41.63	687.44	1068.06
Jirani×Kiek	P6×P5	-65.44	-54.56	-48.19	-34.19
Jirani×SOSAT C88	P6×P9	-9.71	37.29	15.17	52.78
Kiek×AON 740	P5×P1	-28.40	-3.31	62.18	142.94
Kiek×C-90-133	P5×P4	-32.15	-9.32	57.96	162.95
SOSAT C88×AON 740	P9×P1	-27.31	12.61	-58.66	-36.22
SOSAT C88×C-90-133	P9×P4	-46.93	-41.72	96.42	219.94
SOSAT C88×Kiek	P9×P5	145.52	205.97	21.95	29.46
SuperSosat×Dan Tchama 2	P12×P8	-2.05	31.69	303.16	339.82
SuperSosat×Jirani	P12×P6	-43.12	-17.97	72.57	157.22
SuperSosat/Kiek	P12×P5	108.52	139.33	-8.99	14.11
CAPARLGPMG20150073×CAPARLGPMG20150042	P7×P10	-21.60	-6.51	34.19	108.55
Kiek×CAPARLGPMG20150042	P5×P10	-93.18	-90.72	100.55	27.51
SOSAT C88×CAPARLGPMG20150073	P9×P7	141.17	239.91	-58.13	-50.64
SOSAT C88×CAPARLGPMG20150141	P9×P3	19.05	70.42	-37.08	-28.11
SuperSosat×CAPARLGPMG20150042	P12×P10	219.36	373.73	16.50	82.41

Table 6 High parent and Mid parent heterosis for downy mildew incidence for crosses in UDUS and Bui evaluated during the 2018 raining season

		UDUS		**BUI**	
CROSS	**CROSS**	**HPH**	**MPH**	**HPH**	**MPH**
Jirani×CAPARLGPMG20150141	P6×P3	-10	57.32	-56	-54.99
Dan Tchama 2×C-90-133	P8×P4	-16.01	67.97	-15.63	-5.28
SOSAT C88×Dan Tchama 2	P9×P8	–	–	-92.04	-85.25
SOSAT C88×BA-ANGOURE	P9×P2	–	–	-37.14	17.08
CAPARLGPMG20150073×AON 740	P7×P1	56.11	212.23	-56.01	-15.39
Dan Tchama 2×Kiek	P8×P5	55.54	211.07	27.96	35.49
Dan Tchama 2×CAPARLGPMG20150073	P8×P7	–	–	7.96	100
C-90-133×AON 740	P4×P1	-83.99	-72.39	-26	-9.76
CAPARLGPMG20150141×Kiek	P3×P5	–	5.70	-9.44	11.18
Dan Tchama 2×CAPARLGPMG20150042	P8×P10	–	–	-22.24	44.05
SuperSosat×AON 502	P12×P11	–	–	-52.94	-48.39
SuperSosat×AON 740	P12×P1	–	100	-9.98	22.28
SuperSosat×CAPARLGPMG20150073	P12×P7	–	–	-28.14	32.54
BA-ANGOURE×C-90-133	P2×P4	-86.13	-72.26	31.26	42.39

AON 502×CAPARLGPMG20150073	P11×P7	–	–	-42.85	3.69
Jirani×Kiek	P6×P5	-100	-100	-36.36	-20.49
Jirani×SOSAT C88	P6×P9	-100	-100	-25.66	41.09
Kiek×AON 740	P5×P1	-100	-100	-36	-11.39
Kiek×C-90-133	P5×P4	-100	-100	-26.41	-13.15
SOSAT C88×AON 740	P9×P1	-100	-100	-66.06	-34.72
SOSAT C88×C-90-133	P9×P4	-100	-100	38.93	161.59
SOSAT C88×Kiek	P9×P5	-100	-100	33.98	145.93
SuperSosat×Dan Tchama 2	P12×P8	–	–	-5.56	-2.86
SuperSosat×Jirani	P12×P6	-100	-100	-40.55	-27.42
SuperSosat/Kiek	P12×P5	-100	-100	56.67	61.42
CAPARLGPMG20150073×CAPARLGPMG20150042	P7×P10	–	–	502.51	502.51
Kiek×CAPARLGPMG20150042	P5×P10	-100	-100	21.47	122.97
SOSAT C88×CAPARLGPMG20150073	P9×P7	–	–	876.88	876.88
SOSAT C88×CAPARLGPMG20150141	P9×P3	-100	-100	-15.73	59.56
SuperSosat×CAPARLGPMG20150042	P12×P10	–	–	14.32	110.86

4. High parent and mid-parent heterosis for yield

In terms of total grain yield positive heterotic values are desirable as an indication of the better performance of hybrids over both parents. The high parent heterosis for grain yield (kg/ha) ranged from -93.18% to 219.37% in UDUS and -92.47% to 1367.1% in Bui. Cross P12×P10 had the highest heterosis (HPH) of 219.37% followed by Cross P9×P5 with 145.52% in UDUS and Cross P7×P1 had the highest heterosis (HPH) of 1367.1% followed by Cross P12×P11 with 979.3% in Biu. On the other hand Cross P5×P10 recorded the lowest heterosis of -93.18% in UDUS and Cross P6×P3 with -92.47% in Biu as shown in Table 5. The mid- parent heterosis for grain yield (kg/ha) ranged from -81% to 239.91% in UDUS, and -91.02% to 1486.95% in Biu. Cross P9×P7 had the highest heterosis (MPH) of 239.91% followed by Cross P9×P8 with 210.36% in UDUS. Cross P12×P11 had the highest heterosis (MPH) of 1486.95% followed by Cross P8×P5 with 1611.69% in Bui. On the other hand Cross P3×P5 recorded the lowest heterosis of -81% in UDUS, Cross P6×P3 with -91.02% in Biu as shown in Table 5. Results of the estimates of mid parent and high parent heterosis in this study revealed significant positive heterosis for crosses evaluated for grain yield (kg/ha). Similar findings were reported by [18, 19 and 20] for grain yield in pearl millet. Crosses P8×P5, P12×P11, P12×P5, and P12×P10 had the highest positive heterosis across the two locations hence, could be excellent hybrid cultivars for grain yield in these locations. Negative heterosis observed for grain yield in some crosses in UDUS, Bui and across the locations could be due to sample variation, linkage, inadequate statistical and genetic models.

5. High parent and mid-parent heterosis for downy mildew incidence

In terms of downy mildew disease incidence negative heterotic values are desirable in breeding for downy mildew resistance. The high parent heterosis for downy mildew incidence ranged from -100% to 56.1% in UDUS, -66.06% to 876.88% in Biu. In UDUS most of the crosses recorded highest negative heterotic value (HPH) of -100% while Cross P9×P1 recorded -66.06% in Biu as shown in Table 6. The mid parent heterosis for downy mildew incidence ranged from -100% to 212.23% in UDUS, -85.25% to 876.88% in Bui. In UDUS most of the crosses recorded highest negative heterotic value (MPH) of -100%, while in Bui Cross P9×P8 highest negative mid parent heterosis of -85.25% as shown in Table 6. With regards to the downy mildew disease incidence and severity in UDUS most of the Crosses had highest negative heterosis while Crosses P9×P8, P12×P1, P12×P5 and P9×P1 had highest negative heterotic values in Bui and could be used as excellent cultivars for downy mildew resistance in Sokoto and Kebbi States. [16 and 21] also reported negative heterosis for downy mildew incidence and severity in their studies.

6. Conclusion

Based on the analysis of variance it could be concluded that there is significant difference among the genotypes for most of the characters studied in UDUS and Bui indicating that considerable amount of genetic variation existed among the parental lines and hybrids. The combined analysis of variance also indicated significant differences for environment, genotypes and genotype × environment interaction for most of the characters studied implying that the genotypes responded differently across the locations. Crosses P8×P5, P12×P11, P12×P5, and P12×P10 with positive and negative heterosis in desired direction for grain yield (kg/ha), downy mildew incidence and severity across the two locations could be excellent hybrids for grain yield and downy mildew tolerance in these locations.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest between the authors.

Statement of informed consent

There is no statement of informed consent because the study does not involve information about any individual.

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