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# Resistance of pearl millet hybrids to four isolates of downy mildew (*Sclerospora graminicola* (Sacc.) Shroet.) collected in Niger

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## Abstract

Pearl millet is the only reliable source of food for many of the people living in the arid zones of India and Africa. Downy mildew is the most calamitous disease of pearl millet. Resistance to the pathogen is required to guarantee a stable harvest. Eight OPVs were used to generate 16 variety-cross hybrids during the 2012's dry season in the INRAN station of Kollo. Pot-grown seedlings of parents, hybrids and checks were inoculated with 4 isolates of *Sclerospora graminicola* under glass house conditions at ICRISAT Centre in Sadoré. The isolates collected from different fields in Niger were not different from each other. The isolate from Maradi showed the highest level of pathogenicity. Twelve hybrids and six parents were resistant over the 4 isolates with a mean disease incidence less than 10%. Souna-3 and HKB showed the lowest GCA for the disease incidence. The hybrids Ankoutess x Souna-3 and Moro x HKP-GMS had the best SCA for disease resistance. There was a prevalence of additive gene action in terms of the inheritance of the resistance, also affected by epistatic effects. The resistant parents will be used for further pearl millet breeding activities.

**Keywords:** Pearl millet, hybrid, downy mildew, isolate, disease resistance, GCA, SCA.

**Abbreviations:** OPV: open-pollinated variety, GCA: general combining ability, SCA: specific combining ability, ANOVA: analysis of variance, ICRISAT: International Crops Research Institute for the Semi-Arid Tropics, NARS: National Agricultural Research System, BP: Before Present, DM: downy mildew.

## INTRODUCTION

Pearl millet, *Pennisetum glaucum* (L.) R. Brown or *Cenchrus americanus* (L.) Moronne, constitutes the primary staple food for millions of people living in the arid tropical zones. This highly variable cross-pollinated crop was domesticated over

4,500 years BP in the southern margins of the Sahara (Manning *et al.* 2010). The cereal is particularly adapted to the unpredictable conditions encountered in these areas where no other cereal crop can give consistent harvests. In West Africa, five countries generate 84% of the continent's total millet harvest. In order of mass production they are: Nigeria, Niger, Mali, Burkina Faso, and Senegal. In all these countries, the crop constitutes the bedrock for food security. For instance in

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Niger, pearl millet provides for 75% of the total cereal production and when its harvest fails, the population faces severe threats of food security (Kaka, 2002; République du Niger, 2020; FAOSTAT, 2022). Apart from its primary role in food security by providing good nutritional supplies, pearl millet contributes to livelihood systems as income sources to small-scale farmers who represent 83% of the total population. Though its better adaptation compared to the other cereals to the changing environment under the tropics and despite its primary role in food security, the productivity of the crop is limited by many factors. Drought and sandy soil of low fertility combined with numerous biotic stresses like low yield potential of the traditional varieties planted by farmers are the main constraints. With a population growth (3,8%) faster than food progress (2%) and limitation of favorable lands, increased grain yield and its stability are vital in breeding programs to provide food security (Grebmer *et al.*, 2019, République du Niger, 2020). Large breeding efforts in Niger have been deployed to produce only OPVs mostly by mass selection which is inappropriate for yield enhancement (Niangado and Ouendeba, 1987; Falconer and Mackay, 1996). Other approaches such as hybridization should be explored to address the yield limitation barrier. Hybrid variety is distinguished from OPV by the fact that hybrid seed must be produced by controlled pollination between 2 distinct parents. Several types of hybrid varieties exist depending on the structure of the parental groups. They are (i) single cross hybrid (inbred line x inbred line), (ii) three way-cross hybrid ( $F_1$  of two inbred lines x inbred line), (iii) double cross hybrid ( $F_1$  of two inbred lines x  $F_1$  of two inbred lines) and, (iv) population hybrid (population x arbitrary). As yield potential and plant homogeneity are increased from the variety-cross to the single hybrid, both durability and stability of resistance tend to decrease. Variety-cross and top-cross hybrid breeding are some of the considered method for maximizing the potential of the crop in the unstable environments of arid zones particularly of Sahel Africa (Wricke and Weber, 1986; Andrews and Bramel-Cox, 1993; Talukdar *et al.*, 1999; Vetriventhan *et al.*, 2008, Sattler *et al.*, 2019). Nonetheless, pearl millet varieties of any kind are subjected to attack at different levels from the obligate biotrophic oomycete parasite, *Sclerospora graminicola* (Sacc.) Schroet. The fungus reproduces both by sexual and asexual means and constrains heavily the production potential of the crop varieties mainly the single hybrids (Taunk *et al.*, 2018). The disease is very serious because the initial infection of a compatible host rapidly becomes systemic leading to release of large numbers of wind-borne asexual zoospores. Severely infested plants die outright or produce no grain as panicles are replaced by leafy "green ears". Later the obligate pathogen undergoes sexual reproduction to form its soil-borne resting oospores, which generate pathogenic variability. Disease incidence of 80 to 90% in some farmers' fields were reported in India and annual yield losses were estimated at \$134 million across Niger, India, Burkina Faso, Chad, Mali, Nigeria, Senegal, Sudan,

Tanzania, and Zambia (Gilijamse and Jeger, 2002; Yadav and Rai, 2013; Shivhare and Lata, 2017; Bollam *et al.*, 2018). Although there are some disease management options available including cultural and chemical control measures, the most appropriate way to manage the disease is through host-plant resistance as it is an eco-friendly and a highly cost-effective method for the resource poor farmers (Zarafi *et al.*, 2004; Yadav and Rai, 2013; Taunk *et al.*, 2018). Stability of yield is a major objective in breeding cultivars because in pearl millet producing areas of Asia and Africa, farmers are largely dependent on the crop as their main source of staple food grain (Bollam *et al.*, 2018; République du Niger, 2020). Furthermore, populations of the downy mildew causal agent from Southern Niger, Burkina, Mali and Northern Nigeria were reported to be the most virulent in the world (Andrews *et al.*, 1985; Niangado and Ouendeba 1987; Singh *et al.*, 1993; Jones *et al.*, 1995; Prakash *et al.*, 2014). In West Africa, the leading worldwide producer of pearl millet, the above countries are moving to hybrid production to capitalize on higher yields available through heterosis. Hence, care should be taken to control the disease in the region. For the reasons, breeding new pearl millet cultivars shall be coupled with screening for resistance to the downy mildew disease. The present study screened new developed variety-cross hybrids and their parents for acceptable levels of resistance to 4 isolates of the pathogen collected throughout the country.

## MATERIAL AND METHODS

Genetic material studied included three landraces: Ankoutess, Gamoji, Moro, and five improved OPVs: Ex-Borno, HKB, HKP-GMS, H80-10Gr, and Souna-3 from which 16 inter-population hybrids were generated. The genetic material also included 7 checks. Among the checks, SOSAT C-88 and ICMV-IS 90311 were selected for their high resistance to the disease, while 7042 and PE 08407 were highly susceptible. The other 3 checks or elite controls were the landrace Batoukouché and two improved cultivars: ICMV-IS 89305 and Zatib. The two latter varieties and HKP-GMS correspond to the most widely and continuously grown varieties in the country. Isolates originated from Gaya and Maradi INRAN stations, ICRISAT research station in Sadoré and from farmers' fields in Liboré. There consisted of leaves harvested from diseased plants that showed symptoms including stunting, leaf chlorosis, and transformed ears. The trials were run in a glass house at ICRISAT Sahelian Centre in Sadoré in a completely randomized block design in three replications using three sowing dates for each pathogen isolate. Each isolate was used to evaluate disease reaction in separate tests run in the same glass house during successive sowing dates over 2 months. There were 32 pots (one pot per entry) per replication for a total of 96 pots for each sowing date or 96 x 3 or 288 pots per isolate. Seeds were sown in a mixture of manure and sand in a 1:1 ratio. Thirty two (32) grains were sown per pot and were grown in a glasshouse at approximately 35°C. The

number of seedlings in each pot was recorded before inoculation. The four isolates were maintained in different glass houses on the same highly susceptible host variety (7042). To prepare the inoculums, fresh leaves showing good sporulation on the abaxial leaf surface were harvested from the susceptible control 7042 plants. Leaves were washed with moist cotton to remove old sporangiophores. Leaf tops and bottoms were removed by cutting. The remnant segment was cut into short sections. These sections were deposited in trays abaxial side up. To get new sporulation, trays were introduced into a dark, moist chamber and incubated at 20°C, with the temperature dropping to 0°C after 8 h to prevent spore release. The next morning cuttings carrying sporangia were washed in distilled ice water to harvest these asexual spores, and the suspension filtered to remove debris. A haemocytometer was used to obtain an average of three to four estimates on the number of spores/ml. The concentration was finally adjusted to  $1.9 \times 10^5$  spores  $\text{ml}^{-1}$ . The chilled inoculums were sprayed onto pots of seedlings very quickly to avoid any damages to spores due to temperature change. The young seedlings were inoculated the 4<sup>th</sup> day after sowing. Inoculation was carried out in a growth room maintained at 20°C in the dark for one night. The material lying on staggered benches was covered with a white polyethylene sheet to maintain high humidity. The whole material was transferred thereafter to a glasshouse equipped with fogging system used to maintain a high relative humidity for an optimum disease symptom development. Diseased plants characterized by leaf chlorosis were counted approximately two weeks after the inoculation. The analysis of data was carried out using GenStat to sort out the difference between plant genotypes and isolates. Genotypes were classified according to Ball in Ati and Ikpe (2020) as (i) Highly Resistant (HR) for 0-5% disease incidence, (ii) Resistant (R) for 5-10% disease incidence, (iii) Moderately Resistant (MR) for 10-25% disease incidence, (iv) Moderately Susceptible (MS) for 25-50% disease incidence, (v) Susceptible (S) for 50-80% disease incidence, and (vi) Highly Susceptible (HS) for > 80% disease incidence.

## RESULTS

The mean disease incidence of the parents was similar with the 4 isolates (table 1). Gamoji, one of the central varieties in our breeding program showed its highest disease incidence with the isolate from Maradi. In sum, HKP-GMS and Souna-3 both pollinators were the most susceptible parental varieties, the latter originating from Senegal.

Concerning the hybrids, the isolate of Maradi showed a mean disease incidence almost two times greater than that induced by the leftovers (table 2). Crosses involving HKB and Souna-3 showed good level of disease resistance, while Souna-3 is the second most susceptible parent. Those involving the 2 remnant male parents, I mean HKP-GMS and H80-10Gr, were more susceptible.

The two checks SOSAT-C88 and ICMV-IS 90311, confirmed their high resistance to the disease. They showed a similar disease incidence of 3.2% and 3.1%, respectively (Table 3). SOSAT C-88 was highly resistant to the isolates of Liboré, Maradi, and Tara. Its level of resistance decreased when treated with the isolate of Sadoré. The second resistant control showed similar behavior, but here the susceptibility was recorded with the Maradi's isolate. The two susceptible checks PE 08407 and 7042 were highly infected, showing a similar disease incidence of 93.6 and 93.7%, respectively. Batoukouché and the 2 popular improved varieties ICMV-IS 89305 and Zatib were moderately resistant over the 4 isolates. The mean disease incidence for the parents was close to that of the hybrid populations (Table 4).

The overall mean disease incidence with the isolate from Maradi was the highest. Nevertheless, the difference between the isolates was not significant from the combined analysis (Table 5). Thus, the isolates had similar degrees of pathogenicity that is; they incited similar levels of mean disease incidence in the panel of the 31 host entries.

We used HKP-GMS, the most susceptible entry among those involved in the development of the hybrid populations to compare its crosses with resistant parents for each of the 4 isolates. For the isolate from Liboré, crosses involving HKP-GMS (with a disease incidence of 17.2%) to the 4 highly resistant varieties (Ankoutess (2.4%), Gamoji (3.2%), Ex-Borno (3.4%), and Moro (4.3%)) gave the following offspring incidence rates: 5.7%; 7.4%; 8.7%; and 12.1%; for Gamoji x HKP-GMS, Ex-Borno x HKP-GMS, Moro x HKP-GMS, and Ankoutess x HKP-GMS, respectively. All the offspring showed disease incidence between that of its two parents. Gamoji x HKP-GMS showed a disease incidence closer to that of its resistant parent. The three other hybrids showed each a disease incidence rather closer to the mean of its parents (10.3%; 10.7%; and 9.8% for mean of parents of Ex-Borno x HKP-GMS, Moro x HKP-GMS, and Ankoutess x HKP-GMS, respectively). Two resistant varieties were identified for the Maradi isolate. Ex-Borno, the most resistant (5.1%), crossed to HKP-GMS (12.3%) produced a hybrid slightly more susceptible (13.5%) than its susceptible parent, but the deviation from the susceptible parent was not significant ( $\text{l.s.d.5\%} = 7.5$ ). HKP-GMS, crossed to the second resistant parent Moro (7.0%), gave a hybrid (11.5%) also similar to the susceptible parent. Among parental varieties, Gamoji (2.2%) and Ankoutess (6.6%) were resistant to the Sadoré isolate. The cross between Gamoji and HKP-GMS (12.0%) was highly resistant (2.9%) with a disease incidence similar to its resistant parent (2.2%). The hybrid Ankoutess x HKP-GMS had a disease incidence (12%) equal to its susceptible parent (12.0%). Ex-Borno was resistant to the Sadoré isolate with a disease incidence of 8.7%. Ex-Borno x HKP-GMS hybrid was more resistant (6.3%) than the resistant parent (8.7%), but the deviation from the resistant parent was not significant ( $\text{l.s.d.5\%} = 7.9$ ). For the Tara isolate, Ex-Borno (2.4%) was highly resistant. Its hybrid with HKP-GMS (21.7%) was slightly

**Table 1:** Mean disease incidence (%) caused by 4 isolates of *S. graminicola* on a set of 8 pearl millet OPVs taken as hybrids' parents.

Parents	Isolates				Mean	Sensibility
	Liboré	Maradi	Sadoré	Tara		
Ankoutess	2.4	13.1	6.6	13.5	8.9 ab	R
Ex-Borno	3.4	5.1	8.7	2.4	4.9 a	HR
Gamoji	3.2	15.7	2.2	6.6	6.9 ab	R
Moro	4.3	7.0	11.3	15.7	9.6 ab	R
HKB	4.2	9.9	9.5	7.6	7.8 ab	R
HKP-GMS	17.2	12.3	11.9	21.7	15.8 b	MR
H80-10Gr	12.6	9.1	7.0	3.4	8.0 ab	R
Souna-3	13.8	7.2	11.8	8.0	10.2 ab	MR
<b>Mean</b>	<b>7.6</b>	<b>9.9</b>	<b>8.6</b>	<b>9.9</b>	<b>9.0</b>	<b>R</b>

**Table 2:** Mean disease incidence (%) caused by 4 isolates of *S. graminicola* on a set of 16 pearl millet cross-OPV hybrids.

Hybrids	Isolates				Mean	Sensibility
	Liboré	Maradi	Sadoré	Tara		
Ankoutess x HKB	5.6	9.9	5.9	5.0	6.6 a	R
Ankoutess x HKP-GMS	12.1	29.8	12.0	13.3	16.8 b	MR
Ankoutess x H80-10Gr	0.9	25.3	0.9	13.5	10.1 ab	MR
Ankoutess x Souna-3	3.4	7.1	4.4	1.5	4.1 a	HR
Ex-Borno x HKB	3.7	9.7	6.9	6.9	6.8 a	R
Ex-Borno x HKP-GMS	7.4	13.5	6.3	9.5	9.2 ab	R
Ex-Borno x H80-10Gr	6.2	21.5	11.3	6.4	11.3 ab	MR
Ex-Borno x Souna-3	6.6	8.9	3.3	1.7	5.1 a	R
Gamoji x HKB	6.1	13.4	1.6	3.9	6.2 a	R
Gamoji x HKP-GMS	5.7	15.4	2.9	11.5	8.9 ab	R
Gamoji x H80-10Gr	3.8	14.4	3.2	13.0	8.6 ab	R
Gamoji x Souna-3	6.2	9.6	6.0	2.6	6.1 a	R
Moro x HKB	2.5	7.0	7.6	5.6	5.7 a	R
Moro x HKP-GMS	8.7	11.5	9.1	7.1	9.1 ab	R
Moro x H80-10Gr	8.9	12.1	11.9	19.3	13.0 ab	MR
Moro x Souna-3	5.1	10.3	5.5	5.0	6.5 a	R
<b>Mean</b>	<b>5.8</b>	<b>13.7</b>	<b>6.2</b>	<b>7.9</b>	<b>8.4</b>	<b>R</b>

resistant (9.5%). This hybrid was significantly different from each of its two parents (l.s.d. 5% = 5.1). It had a disease incidence closer to the mean of the two parents (12%). Gamoji, which was resistant (6.6%), crossed to HKP-GMS gave progeny with a disease incidence of 11.5%. This hybrid was also different from its parents and had a disease incidence closer to the mean of the two parents (14.1%).

To know more about the inheritance of the resistance and the particular breeding value of some genotypes, we estimated

the combining ability of the parents and the hybrids. Two male parents, Souna-3 followed by HKB had the lowest GCA for disease incidence that is, the highest GCA for resistance to the fungus. The other pollinators HKP-GMS and H80-10Gr had the highest GCA for the disease susceptibility (Table 6). The hybrids Ankoutess x Souna-3 and Moro x HKP-GMS showed the lowest SCA for disease incidence that is, the highest SCA for disease resistance. Yet, HKP-GMS and Moro showed each a negative GCA for the matter. So, parents with

**Table 3:** Mean disease incidence (%) caused by 4 isolates of *S. graminicola* on a set of 7 pearl millet checks

Checks	Isolates				Mean	Sensibility
	Liboré	Maradi	Sadoré	Tara		
Batakouché	14.8	20.8	7.8	12.8	14.0 c	MR
ICMV-IS 89305	10.5	15.9	22.3	13.9	15.6 c	MR
Zatib	7.0	15.0	9.4	13.2	11.1 ac	MR
SOSAT C-88	2.9	0.0	8.9	3.0	3.2 b	HR
ICMV-IS 90311	0.0	9.7	1.0	1.6	3.1 a	HR
PE 08407	83.4	97.0	96.0	98.2	93.6 d	HS
7042	84.8	98.6	91.4	100	93.7 d	HS
<b>Mean</b>	<b>29.0</b>	<b>36.7</b>	<b>33.6</b>	<b>34.7</b>	<b>33.5</b>	<b>MS</b>

**Table 4:** Mean disease incidence (%) caused by 4 isolates of *S. graminicola* on a set of 31 pearl millet entries.

	Isolates				Mean
	Liboré	Maradi	Sadoré	Tara	
Mean parents	7.6	9.9	8.6	9.9	9.0
Mean hybrids	5.8	13.7	6.2	7.9	8.4*
Mean checks	29.0	36.7	33.6	34.7	33.5
Overall Mean	11.5**	18.0**	13.0**	14.4**	14.2
I.s.d. 5%	5.9	7.6	7.9	5.2	7.1

\*\* , \* Significant at  $P < 0.001$  and at  $P < 0.01$ ; respectively.

negative GCA can produce hybrids with a positive SCA. Gamoji and HKB showed positive GCA for the disease resistance. The resulting hybrid also showed a positive SCA. In contrast, Ex-Borno had a positive GCA but the hybrid developed from Ex-Borno and HKB showed a negative SCA.

## DISCUSSION

Downy mildew was known to be very severe in West Africa, indicating that pearl millet germplasm originating from this part of the continent should be a good source of resistance to the pathogen. The fact was underlined by the overall disease incidence of 9% meaning the parental landraces involved in the current research were resistant under the classification established by Ball in 1983 (Andrews and Bramel-Cox, 1993). For example HKB, H80-10Gr, Ex-Borno, and HKP-GMS confirmed even high resistance to the disease in agreement with the findings of Zarafi (2007). Still the variety HKP-GMS has been reported as only resistant by Wilson *et al.* (2008) and even susceptible by ICRISAT (2008). According to Ouendeba *et al.* (1993), Ex-Borno with negative GCA for disease incidence would be a good source of downy mildew

tolerance under Niger growing conditions. As reminder, Ex-Borno was developed from a mixture of early landraces through seven generations of gridded mass selection at humid location in Nigeria under high disease pressure (Andrews and Bramel-Cox, 1993). This concept of resistance of West African germplasm to downy mildew was supported by Wilson *et al.* (2008) whom found from trials conducted during two years in Burkina, Ghana, Mali, Niger, Nigeria, Senegal, and Zambia that United States' entries were among the most susceptible to downy mildew. They concluded that such result was expected, since *S. graminicola* is not found in the western hemisphere and the entries were developed in the absence of selection pressure from the pathogen. Otherwise, the 4 isolates in contact with the parental OPVs as well as with the inter-population hybrids incited similar mean disease incidence. The reason may come up from the related composition of both kind of genetic formula. An out crossing pearl millet variety is a mixture of genetically different and heterozygous individual plants. Inter-population hybrid is also heterozygous and heterogeneous since it consists only of crosses between 2 genetically different OPVs. It is considered as retaining a range of genes of interest, such as downy mildew

**Table 5:** Combined ANOVA of glass house data for downy mildew incidence caused by 4 isolates of *S. graminicola* on 31 pearl millet entries.

Source of variation	d.f	Mean squares
Isolates	3	235.4
Entry	31	1848.03***
<b>Parents</b>	7	40.47
Female parents	3	17.85
Male parents	3	54.91*
<b>Hybrids</b>	15	42.77
GCA	7	15.941**
SCA	15	13.632*
<b>Checks</b>	7	6822.49***
Total error	123	469.88

\*\*\*, \*\*, \* Significant at  $P < 0.001$ , at  $P < 0.01$ , and at  $P = 0.05$ ; respectively

**Table 6:** Mean GCA & SCA for disease incidence caused by 4 isolates of *S. graminicola* on a set of 8 parents and 16 hybrids, respectively.

Parents	GCA	Hybrids	SCA	Hybrids	SCA
Ankoutess	1.0	Ankoutess x HKB	-0.7	Gamoji x HKB	0.9
Ex-Borno	-0.3	Ankoutess x HKP-GMS	4.7	Gamoji x HKP-GMS	-1.2
Gamoji	-0.9	Ankoutess x H80-10Gr	-1.7	Gamoji x H80-10Gr	-1.3
Moro	0.2	Ankoutess x Souna-3	-2.3	Gamoji x Souna-3	1.6
HKB	-2.1	Ex-Borno x HKB	0.7	Moro x HKB	-0.8
HKP-GMS	2.6	Ex-Borno x HKP-GMS	-1.4	Moro x HKP-GMS	-2.1
H80-10Gr	2.4	Ex-Borno x H80-10Gr	0.9	Moro x H80-10Gr	2.0
Souna-3	-3.0	Ex-Borno x Souna-3	-0.1	Moro x Souna-3	0.9
	**		*		*

\*\* , \* Significant at  $P < 0.01$  and at  $P = 0.05$ ; respectively.

resistance genes, presumed to occur in both parents (Andrews *et al.*, 1997). According to Haussmann *et al.* (2012) such genotypes with high degrees of heterozygosity and genetic heterogeneity for adaptation traits help achieving better individual and population buffering capacity, synonymous of yield stability in variable environments. Even though the 4 isolates provoked comparable disease incidence regardless the nature of the genetic material, the isolate of Maradi in particular induced upon the  $F_1$  progeny a disease incidence twofold higher than that stimulated by the 3 remnant isolates. It should be noted in controlled crosses, all of the offspring plants have their genome each half coming from different parent. So, even being composed of heterozygous individuals a variety-cross hybrid has advanced level of uniformity than OPV, due to the analogous genome of these components. Uniformity is subsequent to increased susceptibility (Wricke and Weber, 1986; Andrews and Bramel-Cox, 1993; Talukdar *et al.*, 1999), reason why the resistance

turned to moderate from OPVs to hybrids under the influence of the most aggressive isolate that is from Maradi. This isolate presents 2 challenges. First and specifically the parent Gamoji while having a good general combining ability for grain yield beyond its capacity to be easily converted into CMS carrier, also dropped to moderate resistance under this isolate. The second threat comes from the important fact that the department of Maradi is known to be the top pearl millet producing area in Niger (FEWS NET, 2017). Sharma *et al.* (2010) found among 46 different isolates collected in seven states in India, those assembled from Rajasthan and Gujarat, the major pearl millet growing states, were the most virulent. The Tara isolate was the second most virulent for which another variety for interest, Moro slumped as well to moderate resistance. Maradi and Tara, are located both in Southern Niger, part of the country where pearl millet coevolved with the most virulent populations of downy mildew in the world (Singh *et al.*, 1993; Jones *et al.*, 1995; Prakash *et al.*, 2014).

The check ICMV-IS 90311 illustrated its high resistance to downy mildew in agreement with the findings of Wilson *et al.* (2008) and Zoclanclounon *et al.* (2018). SOSAT-C88 was highly resistant in Senegal according to the latter author and Zarafi (2007) but resistant according to Wilson *et al.* (2008). An ICRISAT-NARS bred pearl millet synthetic variety; it was developed by recombining 19 S<sub>1</sub> progenies selected at Cinzana in 1988 from a cross between Souna and Sanio landraces. Tested in Nigeria over 15 environments from 1996 to 1998 SOSAT-C88 showed less than half downy mildew incidence compared to the most current resistant parent Ex-Borno (Gupta, <https://www.icrisat.org/what-we-do/crops/PigeonPea/archives/sosat88.html>). Nonetheless, the highly resistant cultivar SOSAT-C88 proved resistance breakdown in contact with the Sadoré isolate, though the most avirulent pathotype. This inoculum was originally collected from ICRISAT research station field where SOSAT C-88 has been extensively grown for more than 25 years. The reduced resistance may therefore be due to the frequent multiplication of these improved varieties in the Sadoré research station and around by ICRISAT for long. For instance, Wilson *et al.* (2008) observed that Zatib developed in and more widely grown in Niger had intermediate levels of susceptibility in Niger and Nigeria, but was relatively resistant elsewhere. Sosat-C88 is widely grown in Mali, it had intermediate levels of susceptibility in Mali, but was comparatively more resistant in most of the other West African locations. The cultivation of a resistant genotype in the same area for a long time exposes the cultivar to the attack of the downy mildew capable of adaptive genetic change as noted by Thakur *et al.* (2008) and Sharma *et al.* (2010). Downy mildew is an out breeder having high genetic variability enhanced by its heterothallism. Furthermore, *S. graminicola* contains dormant genetic material capable of expression to breakdown the resistance of even the most tolerant host genotype (Michelmore *et al.*, 1982). Once the susceptibility of a cultivar is exploited, disease incidence increases with time due to the accumulation of spores in the soil. The wide and continuous cultivation of HKP-GMS and ICMV-IS 89305 like Zatib may be also the reason why these cultivars showed increased disease incidence. According to Bassirou *et al.* (2023) the pearl millet most widely cultivated variety HKP-GMS in Niger, accounted alone for 90% of improved seeds available for sale in 2019, followed by SOSAT-C88 (6%), whereas other improved varieties accounted for only 4%. Whist all of the three entries were reported as resistant by Wilson *et al.* (2008), HKP-GMS was differently stated as highly resistant by Zarafi (2007) and susceptible by ICRISAT (2008). Even though research activities on downy mildew in West Africa have been run in different localities and years and even using different screening methods, the level of incidence did not change much. It remained within resistance class for almost all the improved OPVs we assessed. According to Zarafi (2007) the effect of downy mildew on yield is influenced by pearl millet genotype, aggressiveness of the pathogen, and environmental

conditions. But for Ball (1983) the most important conclusion was that variation is determined by host and pathogen genotypes. Pathotypes can vary from a location to another and even within location (Ball, 1983; Thakur *et al.*, 2004a; Bollam *et al.*, 2018), but even so these cultivars maintained their capacity to withstand the disease; an additional support about the good resistance of the West African germplasm to the oomycete.

In terms of inheritance of resistance to the disease, most of the hybrid progenies had a phenotype intermediate between their parents for the Liboré isolate. All the offspring showed disease incidence between those of its two parents. However, one hybrid showed disease incidence closer to that of its resistant parent suggesting dominant inheritance of resistance. Three hybrids showed a disease incidence rather closer to the mean of its parents suggesting resistance is additively inherited. Therefore, the inheritance of the resistance for Liboré isolate was dominant to additive. Shetty *et al.* (2001) reported similar results in India. For the Sadoré isolate the inheritance of resistance was dominant to recessive. For the Tara isolate, all the offspring expressed a disease incidence closer to the mean of its parents indicating additive gene effects. The inheritance of the resistance was controlled by recessive gene for the Maradi isolate. Moreover the inheritance of the resistance besides being dominant, additive or recessive is under control of non-allelic effects, because all the hybrids showed an SCA for resistance different from zero. In sum for the 11 cases studied, 5 presented an additive inheritance of the resistance, 3 were dominant, and the other 3 were recessive. There was a prevalence of additive inheritance of resistance. This was supported by the similarity between the mean disease incidence of the parents and that of the hybrids. According to Prakash *et al.* (2014), the inheritance of downy mildew resistance in pearl millet is highly variable and inconsistent. This could be attributed to lack of homozygous resistant/susceptible genotypes, a lack of genetically pure pathogen isolate, and variable environmental conditions. According to Raj *et al.* (2018), the complexity in the inheritance of the disease resistance may be due to the out-crossing nature of both the pathogen and the cereal host. The presence of heterothallism and different sexual compatibility types in *S. graminicola* further enhances the difficulty by generating new variability in the pathogen. The resistance mechanism is governed either by additive or dominant gene effects, or both, while in others, both additive and non-additive gene effects with epistatic interactions were important. However Jogaiah *et al.* (2014) found the DM disease screening data of F<sub>1</sub> plants remained highly resistant corresponding to one of its parental line indicating dominance of resistance. Similarly, Raj *et al.* (2018) revealed that resistance to DM is controlled by one to two dominant genes according to the treatment. The hybrid populations, although they are probably more highly heterozygous than their parental varieties, do not show an improvement in disease

resistance compared to their parents; that is, resistance is largely additive. Therefore the most resistant hybrids would be expected to be based on genetically diverse parental populations that are individually resistant to the target pathogen isolate (s). According to Yadav and Rai (2013), DM damages are highly important on those hybrids having DM-susceptible lines in their parentage or when specific hybrid is cultivated continuously in large area. For the isolate of Maradi, the most virulent one, the completely recessive inheritance of the resistance would suggest that development of inbred lines using conventional pedigree breeding would be effective. As reminder, the resistance check SOSAT C-88 was developed by recombining 19 lines selected from the third generation of selfing. The hybrids developed in this study were more phenotypically uniform than their parents. Uniformity was a drawback of hybrids in Asia because of susceptibility to downy mildew. Monitoring of the disease resistance in West Africa should be of concern.

HKP-GMS was susceptible to all the isolates but it was involved in 2 crosses with high heterosis for grain yield. Indeed in terms of yield potential, 8 of the 16 hybrids outyielded the check Zatib which in turn produced more than the best parent Souna-3 (Issaka *et al.*, unpublished). One of the 2 hybrids, Ankoutess x HKP-GMS had the highest disease incidence (16.3%) over the 4 isolates. This result is in agreement with the classification established by Ball (1983) and Thakur *et al.* (2004b) according to which genetic material still moderately resistant for a disease incidence between 16 to 25%; while it falls into the category of susceptible one according to Shetty *et al.* (2016). However, the resistance of hybrids could be enhanced through inbreeding followed by crossing within the parental material. The residual variability for DM resistance could be exploited to improve the resistance levels of susceptible material. Selection for residual variability for DM resistance in a susceptible landrace population 7042S led to the development of a resistant line which is the most commonly utilized source in DM resistance breeding (Yadav and Rai, 2013). Furthermore almost all the hybrids had average disease incidence over the 4 isolates of less than 12 %. Hybrids Ankoutess x HKB, Ankoutess x Souna-3, Ex-Borno x Souna-3, Gamoji x HKB, Gamoji x Souna-3, and Moro x Souna-3 were more like their resistant parent in terms of resistance with disease incidences near 5% when averaged across 4 isolates. In summary, parents and hybrids with lower mean disease incidence compared to 94% for each of the 2 susceptible checks may represent a privileged germplasm for an effective disease resistance breeding program.

HKP-GMS and Moro showed each a negative GCA both for grain yield and for disease resistance. The variety-cross issued showed high heterosis both for grain yield and disease resistance. HKB and Gamoji had each a positive GCA both for grain yield and disease resistance. The cross Gamoji x HKB had high SCA both for grain yield and disease resistance. Ex-Borno had negative GCA for grain yield and positive GCA for disease resistance. Ex-Borno x HKB had negative SCA for grain yield and for disease resistance. We could draw the

conclusion that hybrid with high yield may have good resistance to downy mildew irrespective to the GCA nature of its parents.

Indeed heterosis is not the stimulation effect of only the yield component but the superiority of the hybrid with one or more characters in comparison with the corresponding parents (Wricke and Weber, 1986). And cross between positive GCA parents does not necessarily produce positive SCA hybrids (Talukdar *et al.*, 1999). In fact GCA is primarily a function of additive gene action which can be fixed through selection and inbreeding while SCA depends on non-additive gene action that can only exist in heterozygote. SCA is largely dependent on genes with dominance or epistatic effects (Sprague and Tatum, 1942; Andrews *et al.*, 1997). Souna-3 being the second most susceptible entry among the parental material had the highest GCA for disease resistance. This showed again the importance of the Senegal material vs Niger one in breeding program to provide for high and stable grain yield. Selfing will uncover and allow discarding deleterious genes while fixing the favorable ones. HKB known for its long spike desired by farmers in Niger and Senegal showed the second best GCA for the disease resistance. Moreover it had also a good GCA for grain yield and plant height. The two best combiners among the parents, Souna-3 and HKB, crossed to each of the four female parents gave always hybrids with a disease incidence less than one l.s.d. at 5%. Souna-3 and HKB could be recombined to develop superior lines to be crossed to Ankoutess, Ex-Borno, Gamoji, or Moro or any combination from the 4 female parents for an enhanced disease resistance. All the hybrids showed a SCA different from null confirming the existence of epistatic effects within the hybrids.

The results of the present study showed breeding hybrid populations may be a reasonable way to improve the resistance of pearl millet cultivars to downy mildew. Besides being resistant as well as the OPVs parents, most of the new hybrids developed out yielded their parents. But for a large scale production of hybrid seed, male sterility genes should be introgressed in the background of the seed parent. Backcrossing the male sterility system into a whole OPV to develop variety-cross hybrid is a big issue. Whilst exploiting the new generated genotypes with high and stable yield, another type of hybrid could be explored. Top-cross hybrids appear more feasible and most suitable to sustainably increase pearl millet yields and give maximal benefits to West African farmers. Fortunately, almost all the starting material showed a low disease incidence indicating its good value as source to derive lines of interest. Lines development from good combiners may make easier to study in clearer manner the inheritance of the disease. Because pearl millet constitutes the most important staple food particularly in Niger, screening for the disease resistance should continue to identify valuable new germplasm, inbred lines or else. As the downy mildew incidence caused by a pathotype mixture is similar to the one obtained by the more virulent pathotype, screening for resistance to the disease in the future could be carried out only in Maradi



in contact with the most aggressive isolate.

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