

# Seeking for resilient and nicking parents among advanced lines developed from West African pearl millet germplasm for future hybrid production

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**ABSTRACT:** The identification of parental genetic material that will provide crosses with high heterosis for grain yield and its stability is the most important factor in hybrid millet development in the arid zones of Asia and Africa. Self-pollination method was used to develop advanced lines. A total of 91 progenies of  $S_5$  to  $S_9$  generations were screened under natural downy mildew infestation in Niger. The screening activities occurred in the course of rainy seasons 2021 at Bengou and Tara, 2022 at Bengou, Tara, and Kala-paté, and lastly in 2023 only at Bengou. Entries were laid regardless the year and the location in RCBD in 3 replicates. Tested lines were significantly different for the numbers of days to blooming despite their genetic level guarantying hence a selection of good nicking hybrid parents. Some of the genetic material yielded as high as the checks while twelve accessions amongst 3 Gam-B-37-30-2, 3 Gam-B-11-1-14, 2 HKP-GMS-R-128-1, and 4 Moro-R-86-17-2 of the diverse inbreeding levels and all different each other revealed a null downy mildew disease incidence. Already improved material like Moro-R and HKP-GMS-R got almost to homozygous stage while Gam-B offspring developed from a landrace remained highly heterozygous even after numbers of self-fertilization cycles.

**KEYWORDS:** Pearl millet, advanced lines, cluster, inbreeding, genetic admixture, downy mildew, disease resistance, variability

**Abbreviations:** OPV: open-pollinated variety, CMS: cytoplasm male sterile, GCA: general combining ability, SCA: specific combining ability, ANOVA: analysis of variance, RCBD: randomized complete block design.

## I. INTRODUCTION

Pearl millet [Pennisetum glaucum (L.) R. Br] is the next-generation crop holding the potential of nutritional richness and the climate resilience. Being a climate-resilient crop is important to minimize the adverse effects of climate change and it has the potential to increase income and food security of farming communities in arid regions (Serba et al., 2017; Satyavathi et al., 2021; Yadav et al., 2021; Gaoh et al., 2023). Yet, according to De Rouw (2004), the Sahel is currently facing an increasing food deficit problem because of insignificant yield increases of pearl millet. Indeed, breeding effort in Sahel Africa was focused on developing OPVs mostly using mass selection, a method which is inappropriate for yield enhancement (Niangado and Ouendeba, 1987; Falconer and Mackay, 1996, Rattunde et al., 1997; Kanfany et al., 2020). It is thus crucial that productivity of this key crop is increased to ensure at least the minimal food security. Therefore, other approaches to address the yield limitation barrier should be explored. In fact, one challenge to developing the commercial potential of pearl millet is the capacity of growers to provide a consistent product that meets market standards. Pearl millet varieties with high yield and disease resistance are an integral part of meeting this challenge. Hybrid breeding is one of the most effective approaches for maximizing the potential of the crop and the development of high yielding hybrids is the major target of pearl millet researchers globally (Devos et al., 2006; Kapila et al., 2008; Wilson et al., 2008; Ambawat et al., 2020). The aim of the breeder of cross-pollinated crops is to increase the amount of heterosis over the level already found in random mating populations. This can be achieved by crossing two selected genetically different populations. In this regard, INRAN in charge of pearl millet improvement in Niger has developed variety-cross hybrids from West African germplasm belonging to two putative heterotic groups. The crosses Gamoji x HKB and Moro x HKP-GMS gave the highest SCA for grain yield of 163% and 155%, respectively (Issaka et al., 2023a). The effect of heterosis can be increased further if specific inbred lines are developed and crossed.

Thus, a hybrid breeding program starts with the production of inbred lines. Inbred lines provide maximum heterosis effects when they are fixed at loci involved in the hybrid reaction. Still, pearl millet varieties of any kind are subjected to attack at different levels from the obligate biotrophic oomycete parasite, Sclerospora graminicola (Sacc.) Schroet. Whereas, 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). For the reasons dominant genes for resistance in the female parent are desirable because all hybrids produced using such parent will express the resistance (Talukdar et al., 1999; Hanna and Rai, 1999). Besides high yield and resistance to downy mildew, the maintainer line should have good GCA for yield and head exertion (Andrews et al., 1997; Talukdar et al., 1999). Breeding female (A) lines is necessarily followed by the development of restorer (R) ones which restore the fertility in the hybrid. Dominant nuclear genes capable of restoring pollen fertility of a CMS line must be identified into agronomically superior varieties considered as the pollen parent (Talukdar et al., 1999; Havey, 2004). Specific character that is usually sought in pollinators in addition to high level of specific combinability with the female is high tillering capacity which contributes to abundant and continued pollen shed (Andrews et al., 1997; Talukdar et al., 1999). Pollinator should flower 2 to 4 days before to nick with the female parent (Bidinger and Hash, 2003; Serba et al., 2017; Khair and Bhanvadia, 2019). The aim of the current research is to evaluate advanced lines under downy mildew natural disease infestation, and to identify those with synchronous flowering for hybrid production.

## II. MATERIAL AND METHODS

Advanced lines developed from 4 open pollinated varieties (Table 1) belonging to 2 putative heterotic groups were used in this study. The eastern Niger group is characterized by ecotypes with short and large panicle's diameter and includes Gamoji and Moro. Yet, it should be noted that Moro is of thin panicle and has further the highest content in iron and zinc among the whole germplasm. HKB and HKP-GMS belong to the western Niger heterotic group. This cluster covers genotypes with long and thin panicles. Long spike is a high desirable trait for pearl millet growers in the central and south zones of the country. HKP-GMS is the most widely grown variety in Niger due to its large adaptation, acceptable grain yield, and short vegetative cycle, but it is afterward the most susceptible to downy mildew attack. In terms of head compactness, Moro is naturally the best throughout the country and may belong also to the  $2^{nd}$  heterotic group due to its thin panicle. On the opposite side Gamoji and HKB had the loosest spikes, and HKP-GMS is of intermediate caliber. All the genetic material except the landrace Gamoji was developed through mass selection from local landraces. Gamoji was successfully converted into A<sub>4</sub> CMS carrier while Moro and HKP-GMS proved to restore fertility over the same system.

Parents	DMI	SPL	SPD	SPN	GCA <sub>GY</sub>	Hybrids	SCA <sub>GY</sub>
Gamoji	6.9	43	14.8	60	37	Comoii y UVD	162
HKB	7.8	62	12.1	59	40.4	Сатојі х нкв	105
Moro	9.6	43	10.7	66	-72.1	Mana a HKD CMC	155
HKP-GMS	15.8	54	11.8	70	-3.5	MOTO X HKP-GMS	155

 Table 1: List of lines' parents and some of their interesting characteristics

**N.B:** DMI stands for downy mildew incidence, SPL for spike length, SPD for spike diameter, SPN for spike number per plot, and GY for grain yield. Source: Issaka *et al.*, 2023 a & b

The line breeding process began really in the off season of October to January 2014 and continuously run so far. Sometimes we advanced lines twice in the long off season and once during screening operation in the normal campaign. To produce inbred lines, main shoots of concerned genotypes were covered with glassine bags after exertion of the tip apex. During the evaluation in the normal season, only plants free from mildew attack are each time selected to constitute the next generation of self-fertilization. The selection of promising lines was also based on other phenotypic traits such as the tillering propensity, the plant height, and especially the characteristics of the ear. Say, the 2 methods of screening for downy mildew resistance are complementary; the field trial allows the selection of morphologically desired genotypes while the laboratory technique can screen field escaping genotypes. Diseased plants which were rated as healthy before were rejected at maturity. At harvest time spikes were labeled and threshed individually thereafter, and grains were dried under natural ventilation in a laboratory for the cycle to start again. Additional selection traits encompassed the grain characteristics such as its size, color, uniformity, and the quantity of seed available to cover the necessary number of trials. Once reaching the fifth generation of inbreeding, 22 lines were selected among hundreds developed from the 4 parents for evaluation and data field record.

Three checks were added to the screening test for downy mildew resistance under natural infestation. Trials were implemented in Bengou (11° 58' 44,4 N; 3° 33' 25,6'' E) and Tara (11° 90' 357'' N; 3° 30' 25,6'' E), both experimental stations located in the rainiest (rainfall in 2021 was 796.9 mm in 61 days and 962.4 mm in 51 days in Bengou and Tara, respectively) part of the country. Knowing the most favorable period reaction of inoculums, field assessment was conducted on July. The reason for planting at this period was seedling stage to coincide with the most regular and highest moisture time for optimal disease growth. In the following year a larger number of lines were screened and the locations of Kala-paté (13° 12' N & 2° 56' E) and N'Dounga (11° 22' N & 2° 14' E) in Sahelian agrozone were added to the list of testing environments. In the 4 sites, 2 sets of 25 lines at eighth to ninth generation of selfing were screened in neighboring but separated plots. One set consisted only of progenies derived from the cross Moro x HKP-GMS and the second a mixture of offspring issued from the 2 former varieties added to those from Gamoji. Finally, 19 lines developed from Gamoji, considered as female lines were evaluated in the downy mildew international nursery in Bengou, in 2023. Variety SOSAT-C88 well known for its high grain yield and good resistance to the oomycete was used as check during the 2<sup>nd</sup> year. It was added with Gamoji, the original parent of all the Gam-lines, in the last experiment. All the evaluation tests were run following the same protocol. Indeed, accessions without any seed treatment were planted in a randomized complete block design in three replications. Each plot was comprised of 20 hills arranged in 2 rows of 7.2 m length. Spacing between rows as well as between hills was 0.8 m. In sum the plot was 12.8 m<sup>2</sup>. Fertilizers were applied at the INRAN recommended rate (50 kg/ha of NPK 15-15-15, and 100 kg/ha of urea in 2 fractions). The trials were hand weeded, and thinned 16 to 18 days after sowing to leave one plant per hill. Data were recorded on (i) days to flowering (DFL): number of days from sowing to the time at which 50% of plants within a plot flowered; (ii) plant height (PLH): height of the plant from the ground level to the spike tip in centimeter; (iii) main shoot diameter (MSD): main stem girth at half height in millimeter; (iv) spike length (SPL): interval between the tip of the spike and its bottom in centimeter; (v) spike diameter (SPD): spike girth at its middle distance in millimeter; (vi) spike number (SPN): number of spikes per plot; (vii) grain yield (GY): grain weight per plot. Grain yield was measured as kg per plot, and then converted to kg per hectare; (viii) number of hills (NHH): number of hills harvested per plot (ix) downy mildew incidence (DMI): number of diseased plants by the total one within the plot in percentage. Data (ii), (iii), (iv), and (v) on entries were collected on three plants taken at random within each plot. Analysis of variance was carried out using GenStat to sort out the difference and correlation between variables.

## III. RESULTS

ANOVA of variables : For the first trial run in 2021, the combined ANOVA showed HKB-R-lines group had the highest DMI of 24%, which is more than the double of the overall mean (table 2). Consequently it illustrated one of the lowest grain yields in the middle of varietal bunches, even confirming its longest spike. Yet, three lines proved good resistance with a null disease incidence amongst Gam-B-37-30-2-5-2 presenting the top grain yield amid the whole entries. It had also together with the local variety that is the best check in the matter, the best tillering capacity. In general the female Gam-cluster recorded the peak tillering ability and the maximum grain yield. A sub-cluster of HKP-GMS-R formula registered a low amount of hills harvested together with a bad tillering capability and the poorest grain yield afterward. From the 3 sentences above we could already imagine the relationship linking the grain yield to the tillering propensity. Otherwise, the difference relating lines is significant in matter of DFL between and even inside offspring groups giving thus a large choice in selecting hybrid parental material. The distance between the 2 most distant lines or the standard deviation was of (63-58) = 5, (63-61) = 2, (70-61) = 9, and (64-63) = 1 point for the Gam-B, HKB-R, HKP-GMS-R, and Moro-R in that order. It was of (64-61) = 3 points between the 2 more distant groups which are the Gam-B and the HKP-GMS-R clusters. Nonetheless, in sum, the female Gam-group was of earlier cycle compared to all remaining genotypes' clusters. Talking about PLH, Gam-females are shorter than possible males of any genotypes' group. For spike length and girth the difference among entries was highly significant, Moro confirmed its thinnest spike diameter.

Table 2: Mean performance of 25 pearl mille	et genotypes for 9 traits across 2 sites over 202
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GERMPLASM	DMI	DFL	PLH	SPL	SPD	NHH	NSH	GY	MSD
Gam-B-37-30-1-2-1	7	63	184	45	34	15	40	943	13
Gam-B-37-30-1-2-10	25	58	215	49	24	15	45	792	12
Gam-B-37-30-2-5-2	0	61	190	43	33	16	61	1188	13

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Gam-B-37-30-2-5-4	2	62	160	45	33	15	36	504	13
Gam-B-37-30-2-5-6	2	61	164	45	34	16	35	467	15
Variables' mean for Gam-B group	7.2	61	183	45	32	15	43	779	13
HKB-R-1-7-6-4-3	25	63	199	51	30	17	45	598	14
HKB-R-1-7-9-1-2	21	61	209	58	23	10	24	332	13
HKB-R-1-7-9-1-4	42	63	228	58	24	12	25	319	14
HKB-R-1-7-9-1-5	10	62	213	57	26	14	36	498	14
Variables' mean for HKB-R group	24	62	212	56	26	13	32	437	14
HKP-GMS-R-128-1-14-2-1	5	63	222	44	24	16	49	680	15
HKP-GMS-R-128-1-196-1-1	12	61	240	59	24	15	51	1018	14
HKP-GMS-R-128-1-196-1-5	11	70	202	50	26	13	29	376	14
HKP-GMS-R-128-1-196-4-15	3	63	237	53	27	10	18	274	14
HKP-GMS-R-128-1-196-4-19	7	65	211	43	26	12	18	348	14
HKP-GMS-R-128-1-196-4-25	9	65	214	51	26	10	14	284	13
HKP-GMS-R-128-1-196-4-27	0	62	224	54	25	7	13	202	15
HKP-GMS-R-128-1-22-1-2	4	64	220	47	26	11	30	387	14
HKP-GMS-R-128-1-22-1-3	2	64	205	41	25	8	17	262	13
HKP-GMS-R-128-1-31-1-1	4	63	192	49	25	12	32	369	13
HKP-GMS-R-128-1-31-1-3	9	69	179	41	27	6	10	122	14
Variables' mean for HKP-GMS group	6	64	213	48	25	11	25	393	14
Moro-R-68-17-2-1-4	0	64	226	49	23	11	31	547	12
Moro-R-68-17-2-1-5	7	63	234	49	22	14	49	746	13
Variables' mean for Moro-R group	3	63	230	49	22	12	40	646	12
НКР	13	60	230	54	25	17	56	1103	14
Zatib	17	62	225	52	25	17	47	1096	13
Local	10	60	246	57	25	18	62	1172	13
Mean	10	63	211	<b>50</b>	27	13	35	<b>585</b>	14
l.s.d	16	4.1	31.8	7.2	3.3	3.5	17.6	334	1.5
<b>CV%</b>	<b>99.7</b>	3.2	7.3	7	6.1	12.7	24.2	27.7	5.5
F.pr	**	***	****	****	****	****	****	****	ns

ns = non significant; \*\* significant at 0.01; \*\*\* significant at 0.005; \*\*\*\* significant at <.001

In 2022, a set of 2 trials were implemented in 4 locations during the normal cropping season. Germination was bad in N'Dounga hence data were not collected. Yet, we bagged what germinated and harvested advanced seed. Furthermore the experiment consisting of the mixed lines was damaged by flooding in Tara and then discarded. Finally data were recorded only in Bengou and Kala-paté for the mixed set and in these localities plus Tara for the Moro-R and HKP-GMS-R progenies' set. The HKB-R cluster was useless because of its lack of performance in 2021, so not considered for the period of 2022. The Gam-group was enlarged with a different sub-group while the HKP-GMS-R faction going towards inbreeding depression was also excluded. The Gamlines kept the advantage of better tillering capacity and accordingly that of grain yield (table 3). Entries were significantly different about the variable DMI which mean value was similar to that of the check SOSAT-C88. One female line Gam-B-37-30-2-5-8-1-2 was added to the list of genotypes with null downy mildew incidence. There was significant difference between maintainer lines and those of the leftover groups in matter of SPD and DFL. In general, the difference appeared even between lines inside the same original parent regardless to the groups. Many Moro-R lines could be used to cross with the Gam-female germplasm and vice-versa due to the difference in time of blooming of 2 to 5 days between individual lines. Here also, the variability intra-cluster was higher than that between clusters concerning the DFL parameter. Also, crosses become naturally possible between Moro-R and HKP-GMS-R in which the latter group will take part as female component. Male parental material was taller than the female one. There was no significant difference for the variables SPN and GY, may

be due to the fact that counting and threshing operations were made manually. Indeed the related coefficients of variation were high.

GERMPLASM	DMI	DFL	PLH	SPL	SPD	NHH	NSH	GY	MSD
Gam-B-11-1-14-5-2-4-2-1	6.4	66	211	48	25	16	42	617	15
Gam-B-11-1-14-32-2-2-1-2	1.0	65	214	37	26	14	42	713	13
Gam-B-11-1-14-32-13-3-2-2	6.6	67	211	46	23	14	55	820	14
Gam-B-37-30-1-2-1-1-1	6.6	66	207	44	24	18	51	859	11
Gam-B-37-30-1-2-1-5-4	1.9	67	217	37	27	16	51	678	12
Gam-B-37-30-2-5-3-1-1	1.8	64	357	48	27	16	45	973	14
Gam-B-37-30-2-5-3-1-2	1.7	67	232	44	23	17	45	699	13
Gam-B-37-30-2-5-3-1-4	8.6	64	232	49	27	14	34	657	16
Gam-B-37-30-2-5-3-1-5	4.6	63	209	44	22	16	42	851	12
Gam-B-37-30-2-5-3-1-6	8.6	68	211	44	23	16	55	737	14
Gam-B-37-30-2-5-7-1-1	4.3	62	199	40	21	14	57	691	14
Gam-B-37-30-2-5-8-1-1	0.0	65	198	35	24	19	51	788	14
Gam-B-37-30-2-5-8-1-2	1.8	65	225	42	23	16	45	749	14
Gam-B-37-30-2-5-8-2-1	2.6	71	216	46	27	13	29	910	16
Gam-B-37-30-2-5-8-2-2	0.8	68	214	46	25	15	42	705	14
Variables' mean for Gam-B group	4	66	223	43	24	16	46	763	14
Moro-R-68-17-2-1-4-4-3	2.8	67	237	56	22	17	44	705	14
Moro-R-68-17-2-1-5-1-2	1.7	61	230	49	22	17	38	884	14
Moro-R-68-17-2-1-5-1-3	19.9	65	220	45	26	16	43	838	13
Moro-R-68-17-2-1-5-1-4	12.2	66	226	43	26	18	49	872	13
Moro-R-68-17-2-1-5-3-4	3.8	69	229	47	25	14	41	490	17
Moro-R-68-17-2-1-4-3-2	0.8	65	235	45	26	15	32	536	13
Moro-R-68-17-2-9-8-3-1	1.0	66	222	53	21	16	43	837	15
Moro-R-68-17-2-9-12-1-1	3.9	64	221	45	24	16	47	720	16
Variables' mean for Moro-R group	6	65	227	48	24	16	42	735	14
HKP-GMS-R-128-1-14-2-1-5-1	3.5	70	203	38	24	15	38	626	13
HKP-GMS-R-128-1-14-2-1-3-1	8.8	66	215	39	25	16	45	598	14
Variables' mean for HKP-GMS group	6	68	209	38	24	15	41	612	13
SOSAT C-88	4.6	59	252	40	27	17	50	1058	14
Mean	4.3	66	225	44	24	16	44	754	14
l.s.d	8.6	4.2	90.5	5.2	2.8	5.1	26.6	350	2.6
CV%	174	5.6	24.6	7.0	6.9	19.5	36.4	<b>40.7</b>	11.5
F.pr	**	****	ns	****	****	ns	ns	ns	*

**Table 3:** Mean performance of 26 mixed millet genotypes for 9 traits across 2 sites over 2022

ns = non significant; \* significant at 0.05; \*\* significant at 0.01; \*\*\*\* significant at <.001

Four lines: Moro-R-68-17-2-9-3-1, Moro-R-68-17-2-9-1-23-2, Moro-R-68-17-2-9-1-23-4, and HKP-GMS-R-128-1-22-1-1-2 were evidence for good grain yield and null disease incidence (table 4). In sum the mean disease incidence was smaller than that of the resistant check. Lines were significantly different from each other within and between varieties according to the DFL. The standard deviation for DFL inside lines-groups was superior to that between groups. Moro-R group besides being earlier was taller than HKP-GMS-R, thus crosses could be carried out between the 2 accessions, with Moro-R playing now again the role of male parent. Entries were also significantly different in view of the most important trait in breeding staple cereals that is the grain yield. Its

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mean average was not different from that of the check SOSAT-C88. Nonetheless, entries were not different for 7 among the 9 variables measured.

GERMPLASM	DMI	DFL	PLH	SPL	SPD	NHH	NSH	GY	MSD
Moro-R-68-17-2-9-1-23-1	4.2	66	223	45	26	16	37	705	14
Moro-R-68-17-2-9-28-7-2	1.2	66	208	41	23	12	34	559	14
Moro-R-68-17-2-1-8-1	2.2	70	214	48	25	16	31	579	15
Moro-R-68-17-2-1-8-2	3.5	63	218	45	28	16	47	888	16
Moro-R-68-17-2-9-3-1	0.0	63	237	46	25	15	43	786	14
Moro-R-68-17-2-9-1-23-2	0.0	64	221	45	23	16	37	716	15
Moro-R-68-17-2-9-6-3	1.1	64	210	42	24	18	46	819	12
Moro-R-68-17-2-9-1-23-3	3.5	66	220	40	24	16	47	856	13
Moro-R-68-17-2-9-1-23-4	0.0	63	210	40	24	14	41	833	13
Moro-R-68-4-2-3-1-3-3	0.6	64	223	45	25	18	50	836	14
Moro-R-68-17-2-9-28-7-1	0.6	64	218	49	21	16	35	593	14
Moro-R-68-17-2-9-28-7-3	4.1	65	382	47	24	14	41	568	14
Moro-R-68-17-2-9-28-7-4	3.5	61	232	43	24	17	47	786	13
Variables' mean for Moro-R group	1.9	64	232	44	24	16	41	732	14
HKP-GMS-R-128-1-22-1-1-2	0.0	65	214	37	26	16	45	851	13
HKP-GMS-R-128-1-31-2-3-1	2.3	67	224	44	25	16	39	728	15
HKP-GMS-R-128-1-31-2-3-4	2.7	70	217	46	25	15	38	810	15
HKP-GMS-R-128-1-31-2-3-6	2.4	64	225	46	26	16	42	910	16
HKP-GMS-R-128-1-196-1-1-1	0.6	65	213	44	24	15	45	880	12
HKP-GMS-R-128-1-196-1-1-3	0.6	67	214	44	26	16	30	554	14
HKP-GMS-R-128-1-196-1-1-4	1.7	69	225	42	27	16	45	718	15
HKP-GMS-R-128-1-196-1-1-6	5.6	66	220	47	23	14	36	514	13
HKP-GMS-R-128-1-196-1-1-7	3.1	69	220	48	24	16	35	576	14
HKP-GMS-R-128-1-196-1-1-9	5.7	69	224	46	26	17	39	754	14
HKP-GMS-R-128-1-196-2-1-2	0.7	65	227	49	23	17	38	786	12
HKP-GMS-R-128-1-196-4-27-1	0.6	63	200	39	23	13	35	423	14
Variables' mean for HKP-GMS-R group	2.2	66	218	44	25	16	39	709	14
SOSAT C-88	6.2	62	205	41	22	15	44	856	14
Mean	2.2	65	225	44	24	16	40	726	14
l.s.d	4.8	4.2	85.9	8.9	3.5	3.6	17.7	<b>298</b>	3.0
CV%	239	5.6	23.3	12.2	8.8	19.9	38.5	44.2	13.2
F.pr	ns	****	ns	ns	ns	ns	ns	*	ns

Table 4: Mean performance of 26 pearl millet genotypes for 9 traits across 3 sites over 2022

ns = non significant; \* significant at 0.05; \*\*\*\* significant at <.001

The last trial was implemented in 2023 mainly to improve the disease resistance of the female parent cluster and also to check possible differences between the 2 Gam-B sub-groups. Lines were highly different in regard to 7 traits amid the 9 recorded. Four genotypes: Gam-B-11-1-14-32-4-1-1-3, Gam-B-11-1-14-32-4-1-1-5, Gam-B-37-30-2-5-8-1-3-3, and Gam-B-37-30-2-5-8-1-3-3 showed a downy mildew null incidence and 3 from this ensemble were not significantly different from the known high yielding SoSat-C88 in matter of GY. The fourth

was dwarf with larger and shorter spikes. Although the latter variety has good tillering capacity compared to the 3 formers, its grain yield was lesser than the half of that of its relatives. Again this demonstrated the positive correlation connecting grain yield and spike length observed in almost all experiment cases. All told, at comparable values of DMI, DFL, NHH, and NSH the sub-group with the longer spike and thinner stem produce better even not significantly different grain yield. The group showed in general the lowest value in terms of plant height; a good confirmation of the female status that can play Gamoji' offspring. The DFL variable recorded the highest standard deviation of 23 and 15 for the sub-group Gam-B-11-1-14 and Gam-B-37-30 correspondingly, compared to only 2 points linking the 2 progenies' sub-groups.

GERMPLASM	DMI	DFL	PLH	SPL	SPD	NHH	NSH	GY	MSD
Gam-B-11-1-14-32-4-1-1-1	8.3	81	173	43	31	19	67	1032	14
Gam-B-11-1-14-32-4-1-1-2	6.0	58	206	56	21	16	44	847	12
Gam-B-11-1-14-32-4-1-1-3	0.0	65	209	54	27	20	67	1388	13
Gam-B-11-1-14-32-4-1-1-4	16.7	74	197	46	25	18	47	935	15
Gam-B-11-1-14-32-4-1-1-5	0.0	68	235	56	29	19	67	1470	14
Gam-B-11-1-14-32-4-1-1-6	3.3	59	190	50	25	19	70	1299	15
Gam-B-11-1-14-32-4-1-1-7	3.3	68	211	49	29	19	73	1207	15
Gam-B-11-1-14-32-12-1-2-4	0.0	63	233	58	31	11	34	1335	15
Variables' mean for Gam-B-11-1-14 sub-group	5	67	207	52	27	18	59	1189	14
Gam-B-37-30-2-5-3-1-1-1	6.8	63	218	46	30	18	66	1301	15
Gam-B-37-30-2-5-3-1-1-2	5.0	65	165	36	36	20	58	941	15
Gam-B-37-30-2-5-3-1-6-1	10.1	75	143	40	34	15	30	383	18
Gam-B-37-30-2-5-3-1-6-2	5.5	77	176	44	33	17	48	586	16
Gam-B-37-30-2-5-7-1-4-2	1.7	72	155	50	31	19	56	702	14
Gam-B-37-30-2-5-8-1-1-1	1.8	62	172	47	30	14	58	945	15
Gam-B-37-30-2-5-8-1-3-3	0.0	62	148	44	33	19	53	546	12
Gam-B-37-30-2-5-8-1-3-5	1.8	74	178	41	33	19	54	785	15
Gam-B-37-30-2-5-8-2-1-1-1	1.7	70	192	45	37	20	65	938	16
Gam-B-37-30-2-5-8-2-1-1-2	3.3	66	175	43	37	19	55	1191	15
Gam-B-37-30-2-5-8-2-2-1	3.5	69	220	41	35	18	46	816	14
Variables' mean for Gam-B-37-30 sub-group	4	69	177	43	34	18	54	830	15
Gamoji	2.0	56	225	43	30	16	67	1227	12
SoSat C-88	0.3	62	239	29	29	20	83	1854	13
Mean	3.9	<b>67</b>	193	<b>46</b>	31	18	57	1035	15
l.s.d	8.7	5.1	28.4	6.1	0.4	4.0	17.8	<b>588</b>	0.3
CV%	137	4.6	8.9	8.1	7.9	14	18.8	34.4	13
F.pr	*	****	****	****	****	***	****	***	ns

Table 5: Mean performance of 21 pearl millet genotypes for 9 traits across one site over 2023

ns = non significant; \* significant at 0.05; \*\*\* significant at 0.005; \*\*\*\* significant at <.001

#### CORRELATION between variables

DMI	-		Table 6:	Correlation b	etween varial	bles for the set of	of 22 entries i	n 2021
GY	0.05	-						
MSD	-0.07	-0.27	-					
NHH	0.19	0.82****	-0.06	-				
NSH	0.08	0.93****	-0.18	0.90****	-			
PLH	0.23	0.25	-0.03	0.04	0.19	-		

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SPD	-0.27	0.12	0.13	0.28	0.11	-0.76****	_		
SPL	0.50**	0.16	0.04	0.15	0.17	0.62****	-0.44*	-	
DFL	-0.16	-0.56***	0.19	0.52**	-0.58***	-0.22	-0.03	-0.37	-
	DMI	GY	MSD	NHH	NSH	PLH	SPD	SPL	DFL
DMI	-		Table 7: C	orrelation b	etween variab	oles for the mix	ked set of 20	6 entries in	n 2022
GY	0.10	-							
MSD	-0.09	-0.20	-						
NHH	0.06	0.33	-0.44*	-					
NSH	0.16	0.20	-0.30	0.38*	-				
PLH	-0.12	0.36	0.10	0.06	-0.11	-			
SPD	0.20	0.06	0.07	-0.17	-0.32	0.36	-		
SPL	0.07	0.04	0.16	-0.38*	0.34	-0.06	-0.43*	-	
DFL	-0.02	-0.41*	0.14	-0.30	-0.23	-0.24	0.09	-0.07	-
	DMI	GY	MSD	NHH	NSH	PLH	SPD	SPL	DFL
DFL	-		Table 8: Co	orrelation be	etween variab	les for the set	of 26 entrie	s in 2022	
DMI	0.17	-							
GY	-0.28	-0.02	-						
MSD	0.23	0.15	-0.06	-					
NHH	0.04	0.00	0.47**	-0.07	-				
NSH	-0.46	0.05	0.77****	-0.17	0.38*	-			
PLH	-0.02	0.22	-0.16	0.04	-0.11	0.09	-		
SPD	0.31	0.06	0.33	0.44*	0.30	0.25	0.02	-	
SPL	0.30	0.18	-0.20	0.21	0.25	-0.35	0.30	-0.07	-
	DFL	DMI	GY	MSD	NHH	NSH	PLH	SPD	SPL
DFL	-		Table 9: C	Correlation b	etween varial	bles for the set	of 21 entrie	es in 2023	
DMI	0.45*	-							
GY	-0.44	-0.35	-						
MSD	0.54**	0.38	-0.32	-					
NHH	0.18	-0.06	0.16	-0.17	-				
NSH	-0.24	-0.35	0.68****	-0.35	0.64***	-			
PLH	-0.41	-0.24	0.78****	-0.35	-0.10	0.35	-		
SPD	0.36	-0.17	-0.34	0.45*	0.11	-0.18	-0.38	-	
SPL	-0.18	-0.15	0.07	-0.13	-0.36	-0.21	0.22	-0.47	-
	DFL	DMI	GY	MSD	NHH	NSH	PLH	SPD	SPL

Seeking for resilient and nicking parents among...

From table 6 to 9, there was a positive correlation between NHH and NSH on one side and between MSD and SPD on another side. This latter association became more significant with increased cycles of inbreeding. Similarly, PLH was positively linked to SPL and to GY. The nature of the link changed from negative to positive and even significant between DMI and DFL through advanced generations. In sum GY was positively and highly correlated with NSH whereas its association was slightly positive and none significant with the SPD. It turned down from negative and significant to none with advanced generations for DFL, meaning that high grain yield is related to early flowering.

## IV. DISCUSSIONS

HKB which complete designation is Haini Kirey of Bengou is a local variety constantly cultivated in the area. Even though, it was reported as resistant cultivar and this even from trials' results conducted at Bengou (Wilson

et al., 2008; Issaka et al., 2023a). Nonetheless, its offspring showed a high disease incidence out of loose spikes, after 5 generations of selfing each coupled with selection of just resistant genotypes. Determinism of the resistance to the oomycete may be controlled by additive gene effect. This is the reason why isolated lines became so susceptible compared to their original parent. Thus, a performing variety does not necessary yield good offspring, and therefore shall not be considered based on its performance per se. Still, among the genetic material tested, HKB-R cluster had being the tallest and one of the earliest in terms of number of days to flowering among the male parental complex could be considered as pollinator in future breeding work. This role was reinforced by the fact that it had the best spike length too. Indeed, the correlation between spike length and GY was positive from our study. Long spikes were preferred by Niger pearl millet growers wherever the commerce is based on bundles (Bezancon et al., 2001; Buerkert et al., 2001; Omanya et al., 2007; Drabo et al., 2019). Therefore, HKB-R derivatives could be used in breeding program whenever the spike length constitutes a desirable trait. Furthermore, the wild variety registered good GCA a heritable trait for 6 agronomic parameters over 7 measured (Issaka et al., 2023a). Although, the genetic treasure HKB-R may be, it should be noted resistance to downy mildew is a prerequisite in breeding pearl millet hybrid parents. Yet, one option to get benefit from the HKB-R genotypes in the future would be to test the material in more environments as the disease could vary from one locality to another (Issaka et al., 2023b). HKB was developed by mass selection, a method expected to be more effective for qualitative traits such as plant height, panicle length, and seed mass. However, mass selection technique is less efficient for low heritable traits and another option is to improve the original population, for instance via recurrent selection through full-sib progenies. This method has proven very effective as a result of concentrating favorable genes ending up in increased mean of the population and superior performance of the best families for the target attribute. It could by the way boost the valuable use of non-elite source materials, where the greater opportunities for recombination could break linkages between genes for the desired trait and unfavorable agronomic characteristics.

Moreover, through its tandem cycling of selection and recombination, recurrent selection is a means particularly useful for improvement of polygenic traits at once of several qualitative others like disease resistance (Rattunde and Witcomb, 1993; Weltzien et al., 1995; Rattunde et al., 1997; Weltzien et al., 2020). It would be therefore a proper approach of upgrading varieties like HKB that already showed good GCA for quantitative trait akin to grain yield. For breeding programs emphasizing the development of inbred lines and hybrids from populations derived from distinct heterotic groups, reciprocal recurrent selection for a few cycles is suggested to help derive superior heterotic inbred lines. To do so, the population from one heterotic pool will serve as a tester for the population from the other heterotic pool to improve the GCA and SCA of both the populations in different heterotic pools (Patil et al., 2021). Otherwise, the HKP-GMS-R-128-1-196-4 bloc showed a general reduction of NHH and consequently of NSH which outcome was a lesser grain production. But 2 factions that are HKP-GMS-R-128-1-22 and HKP-GMS-R-128-1-31, pertaining to the same HKP-GMS-R cluster had similar decreased NHH per plot; still the GY was here better. It was not a matter of poor germination, but lines extracted from the same parent would reach inbreeding depression at different generations of selfing. Indeed, in out crossing cereals like pearl millet, stigmata inside one spike are usually pollinated by different males of diverse genetic level. If not, NSH had the highest positive and significant correlation with grain yield in the current study, that is the number of tillers per ha. Because high grain yield and its stability is the top priority in breeding pearl millet in the arid zones of Asia and Africa, the HKP-GMS-R-128-1-196-4 faction revealed then useless (Bidinger and Hash, 2003; Ambawat et al., 2020; Weltzien et al., 2020). Indeed, having by now reached homozygosity status it played lesser grain production sustained by the susceptibility to the main disease. As opposed the Gam-B group being a good general combiner, with the highest grain yield, the largest panicle girth, and the shortest plant height may play a good role of female parent. Nonetheless, the Gam-B had the earliest vegetative cycle what hinders its role of female since in normal hybrid production the female parent should be the late flowering component indoor the couple.

With progress in generation via self-pollination, results showed an encouraging modification concerning the DFL variable. Besides being taller, Moro-R was and became the earlier flowering edifice than HKP-GMS-R and Gam-B, respectively. The original variety Moro was of early maturing cycle compared to both the improved HKP-GMS and the landrace Gamoji according to Issaka *et al.* (2023a). Yet, when only small samples of 2 and 5  $S_5$  lines of Moro and Gamoji in that order, were considered for testing; the Moro cluster revealed to be the late maturing group. Still, the same sample of Moro lines confirmed their early maturing status against HKP-GMS-R. Nonetheless, with few more advanced generations of selfing, Moro-R became again of short duration put side by side to Gamoji-B. Should this behavior be either directed by the sample size or the genetic nature of the landrace? Really, HKP-GMS or "Haini Kirey Précoce" in French, was developed by mass selection for earliness "Précoce" following recurrent droughts occurred in the sixties in Niger. It was moreover improved by GMS

method, an abbreviation to design "Gridded Mass Selection". It then contained genotypes of similar blooming time. But, Gamoji revealed a high genetic mixture difficult to be fully uncovered even after numbers of inbreeding generations. We observed without any doubt in the course of inbreeding process that Gamoji, an unimproved ecotype, split into many biological types including those with different number of days to flowering. Definitively lines were significantly dissimilar in matter of DFL between and even inside offspring's groups in spite of the self-fertilization cycle. Except for the Moro-R and HKB-R factions, the standard deviation intra-cluster was always higher to the inter-cluster one. In fact the 2 factions consisted of small samples. But similar sample of no more than 2 HKP-GMS-R lines illustrated a standard deviation for DFL greater to that observed between the corresponding clusters. May be this difference due to the genetic background? The most advanced generation of Gam-B's group scored the premier standard deviation, perhaps due to its huge genetic admixture as noted before. Anyway, except for this complex most of the standard deviation value stuck between 1 to 4 days inside clusters, the same span time for all stigmata of a given ear to come out day by day and to be available for cross-fertilization (Bidinger and Hash, 2003; Serba et al., 2017; Khair and Bhanvadia, 2019). For standard deviation up to this value, one plant's offspring may come from different tillers of same or diverse stages. In reality, in pearl millet, the first tiller generally appears in the axil of the third leaf nearly 12 days after seedling emergence. Subsequent primary tillers continue to develop alternatively at approximately every 3 days at a mean temperature of 25°C. Secondary tillers are produced from primaries at a similar rates, resulting in a potentially very large number of tillers, all at different phases of development, in a very short period (Bidinger and Hash, 2003; De Rouw, 2004).

Such circumstances are very convenient for OPVs of same maturing group to cross-fertilize each other because of synchronous flowering. For a large scale production of hybrid seed efficient pollination method will be necessary. This method will be facilitated by using synchronous flowering parents, that is, as said earlier the pollinator should preferably flower before the female parent. Thus, Moro-R could play the role of pollinator against both HKP-GMS-R and Gam-B. Indeed, in addition to earliness, it revealed being taller than both female progenies. Plant height is another important factor in the choice of hybrid parents as the height of the male parent should be greater than that of the female parent. Taller pollinator allows for free flow of pollen across the rows and thus cuts down the seed production cost by giving better seed set, and consequently higher hybrid seed yield per unit area. This allows as well planting greater female: male ratio, which also leads to higher hybrid seed yield (Prem et al., 2006). Being the earliest flowering group and the tallest genotypes mean also that Moro-R has the speediest plant growth rate. Therefore, it could be involved in developing early flowering and high yielding hybrids which could escape terminal drought very frequent in Sahel. According to Bidinger and Hash (2003), early genotypes that flowered 20 days before the onset of a terminal drought stress had four-fold lower vield reduction than later-flowering genotypes that flowered only 10 days before the onset of the same stress. Although, the mean grain yield recorded by the 3 different clusters was similar, the Gam-B families were slightly better in general. In seed production it is economically important to use the higher yielding parent as the female to increase the production per unit area (Andrews et al., 1997; Talukdar et al., 1999).

Recall Gamoji and Moro pertained to 2 heterotic groups according to their panicle girth. It was not the general case in the current results, but it was evident when considering various individual lines. The role of Gam-B as female parent is further strengthened by the high GCA for grain yield and an acceptable GCA for resistance to downy mildew of the original landrace. In fact GCA is heritable from one progeny to another and A-lines with high GCA for grain yield confer stability of hybrid production over more diverse environments (Hanna and Rai, 1999). Else, the direction of the variety-cross hybrid Moro x HKP-GMS made manually should change with regard to the exploitation of CMS hybrids. Even though the 2 parents showed similar grain yield with minor advantage for Moro-R, this adjustment would be of worth value because the conversion of Moro edifice into stable A4 CMS carrier was not successful. But this is at population level and things could change when dealing with advanced lines. Coming again to the variable DFL, it increased with recurrent inbreeding in the present study. Conversely DFL is negatively correlated with grain yield, the most significant breeding objective for pearl millet. Really, in the course of selfing process lines become weaker. Shull (1908) observed in maize as result of self-fertilization, a reduction or a deterioration of plant vigor succeeding a physiological infirmity, competitive weakness, and susceptibility to disease. However, early establishment of a leaf area sufficient to intercept a majority of the incoming radiation is essential for producing a large crop biomass. Total biomass productivity in pearl millet, as in any crop, is a product of growth rate and growth duration (Bidinger and Hash, 2003; Srivastava et al., 2020). Yet, the plant vigor is restored by crossing and heterosis can manifest by virtue of improvement of several traits during plant growth and development. In pearl millet, considerable growth differences between hybrids and their parents was observed during different stages of growth and development (Srivastava et al., 2020). Similarly, DFL was positively correlated with the downy mildew disease incidence. Downy mildew is the most important disease of pearl millet in Africa and Asia. This requires the use of sources of resistance in the breeding material. The pathogen is an obligate parasite that reproduces both by sexual and asexual means. In contact with water, the inoculums germinate and the attacked plant develops chlorosis, starting at the abaxial base of a leaf and spreading until the entire organ is covered. Diseased plants die or become stunted or develop malformed heads called green ears (Michelmore et al., 1982; Ball, 1983; Singh et al., 1987; Andrews and Bramel-Cox, 1993; Singh, 1995; Gilijamse and Jeger, 2002; Thakur et al., 2008). All at all, the attack of the oomycete is extended as longer as the host vegetative cycle. For instance, since a female parent should flower later than a male one it will be exposed to the disease for longer period. Still, the determinism of genetic control of the resistance to the oomycete should vary. In case of dominant inheritance of the resistance, cross involving one resistant parent will bear the resistance, while two resistant parents will be necessary whenever the inheritance is controlled by additive genes. This would represent a guide for selecting parents to be used in the development of resistant hybrids. The most resistant hybrid may be produced by crossing two lines with the highest general combining ability for resistance. According to Issaka et al. (2023b), among the existing material, HKB had the lowest GCA for DMI followed by Gamoji. Gamoji offspring out of the inherited GCA for resistance is auxiliary supported by some of its elements which showed null disease incidence and favorable grain yield.

Results from table 4 explained that entries at the sixth generation of selfing for HKP-GMS-R and a mixture of  $S_6$  and  $S_7$  for Moro-R were different only about the variables DFL and GY over the 9 traits measured. Even though pearl millet is a highly cross pollinated crop, the amount of heterozygosity observed among lines, even being pulled out of 2 different cultivars was very less, which could be due to homogeneous and homozygous nature of qualitative lines' traits obtained from several generations of directional selection and selfing. As opposed, the presence of significant phenotypic differences particularly for DFL and secondary for grain yield between the tested entries suggested the existence of sufficient phenotypic and genetic variation in the experimental material for selection. Note that DFL revealed a highly significant difference throughout all the experiments, what is constructive for easy identification of good nicking parents for hybrid production. Nevertheless, in view of results displayed by table 5, Gam-B lines while being at higher level of self-fertilization  $(S_8 \text{ to } S_9)$  were very much different in regard to 7 traits amid the 9 recorded. This result demonstrated again the existing genetic load within pearl millet African landraces. Pucher et al. (2016) and Sattler et al. (2019) reported that high levels of genetic admixture in West African naturally occurring populations or released OPVs as the cause for failure to identify heterotic pools. The lack of differentiation among such populations was probably due to the high out crossing nature of pearl millet leading to the concomitant high rate of pollen-mediated gene flow within the region. Still 3 Gam-B lines, all belonging to the sub-cluster displaying longer ear, recorded a lofty GY similar to that of SoSat-C88 at the side of a null downy mildew incidence. Again this demonstrated the positive correlation between grain yield and spike length highlighted in almost all experiment cases. According to De Rouw (2004) grain yield in pearl millet is primarily a function of grain or panicle number per unit area. Long panicle containing as many as fertile florets ending up in large seed numbers, were preferred by farmers in the Sahel zone of Africa (Bidinger and Hash, 2003; Omanya et al., 2007; Drabo et al., 2019; Kanfani et al., 2020; Bassirou et al., 2023).

# V. CONCLUSIONS AND PERSPECTIVES

Self-fertilization followed by selection is truly one of the best ways to discover previously hidden genetic combinations and rid genotypes of those that are undesirable. One of the great results is the obtaining of lines completely free of mildew during this research work. Indeed, the absence of resistant parents was the first blocking point in the development of millet hybrids in Niger. A step has therefore been taken with the advent of these new genotypes. Another key result is that the method also allowed a segregation of populations into lines that were sometimes very distant in terms of flowering date. This makes the choice of synchronous parents for the production of hybrids more flexible, unlike with OPVs where the flowering date is fixed. Working with several varieties also increases the chance in the qualitative choice of future parents who will produce good hybrid vigor. One of the most significant breeding objectives for pearl millet is to develop lines with higher grain yield. Certain lines showed a grain yield close to that of the control SOSAT-C88, yet one of the best varieties if not the best in terms of productivity on the continent. It is therefore the fundamental trait and any genotype is only really valid in view of the contribution it can make in building this yield. Thus, in terms of the relationships between the variables analyzed, the tillering capacity, the number of hills harvested, the length and diameter of the ear present a positive correlation with the productivity of the crop. The correlation is even greater with the tillering capacity and the number of hills harvested. This last trait would mean that crop productivity could be improved by adjusting population density. The length and diameter of the ear come secondarily and respectively complete the list of parameters favorable to increasing yield. On the other hand, the date of 50% flowering and the incidence of downy mildew illustrated the highest values in terms of negative correlation with yield. The Moro-R and HKP-GMS-R lines that are closed of homozygosity with trait of interest can be utilized in breeding program straightaway as parents for production of hybrids by crossing with other divergent lines or may be used in the line development programs. But remember that resistance to downy mildew is sometimes controlled by additive genes which require both parents to be resistant, so the path to follow is cautious for the moment. Other lines of interest and showing a disease incidence close to null should be pushed to next generation of inbreeding. Meanwhile, testing should be carried out using more entries in more environments for several years.

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