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Article

## Assessment of Rice Inbred Lines and Hybrids under Low Fertilizer Levels in Senegal

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**Abstract:** This research was conducted at the Africa Rice Sahel Regional Station (near Saint Louis, Senegal) during two wet seasons (*i.e.*, July to November) in 2010 and 2011 with the aim of assessing the performances of introduced hybrid cultivars along with an inbred check cultivar under low input fertilizer levels. The five treatments used in this study were (a) the control (without any fertilizer application), (b) 37.5–4.4–8.3 kg N–P–K ha<sup>-1</sup>, (c) half of recommend application in Senegal (75–8.75–16.5 kg N–P–K ha<sup>-1</sup>), (d) 112.5–13.3–24.8 kg N–P–K ha<sup>-1</sup>, and (e) the recommended application in the country (150–17.5–33 kg N–P–K ha<sup>-1</sup>). There were significant year and cultivar effects for all traits. The fertilizer levels affected significantly most traits except panicle length and 1000-grain weight. The year × fertilizer level and year × cultivar interactions were significant for most traits, but the fertilizer level × cultivar and year × fertilizer level × cultivar interactions were not significant. Days to maturity, plant height, panicle per m<sup>2</sup>, and grain yield increased with increasing fertilizer levels during the two wet seasons. The grain yield of rice hybrids (bred by the International Rice Research Institute) was not significantly higher than that of the check cultivar widely grown in Senegal. The assessment of other rice hybrid germplasm showing more adaptability to low fertilizer levels will facilitate further hybrid cultivar development in Africa.

**Keywords:** Africa; hybrid vigor; low input; N-P-K; nutrient-use-efficiency

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## 1. Introduction

Sub-Saharan African farmers considered fertilizer to be costly or unaffordable, particularly when fertilizer prices increased following the removal of subsidies. Fertilizers are more expensive in most of sub-Saharan Africa than in any other continent mainly due to the lack of efficient fertilizer market infrastructure and poor transport network [1]. Many countries were forced to reduce fertilizer imports or expansion plans. Senegal is currently one of the largest rice consumers in West Africa. Self-sufficiency rate of rice is as low as 20% of the total demand. Two main ecologies of rice exist in Senegal. The irrigated rice where the average yield is 6.5 t at farmers “level while under the upland ecologies farmers are getting less than 2 t per ha. Under irrigated conditions, the production cost is heavy due the use of pesticide, fertilizers, and non-adapted credit systems. The main abiotic constraints are problem soils like salinity, acidity, and iron toxicity for lowland and drought for upland ecology. Consumers’ preference varies from region to region and it is also based on the recipes. In the rice-growing environment, whole rice is preferred, while, in big towns, consumers prefer broken. Many farmers had to decrease their P and K inputs to offset production expenses, and some governments had to reduce fertilizer subsidies.

Fertilizers are very important inputs to intensify rice production elsewhere. The profitability of rice production systems depends on grain yield and amounts of inputs [2]. The appropriate fertilizer input allows the cultivars to achieve high grain yields, thereby, bringing profits to farmers.

Labor costs and the purchasing of synthetic fertilizers are often the largest investment made by rice farmer elsewhere. Enhanced fertilizer-use-efficiency can therefore benefit rice farmers. It can be improved by using cultivars with high nutrient-use-efficiency or nutrient management options that take into account the indigenous soil supply and an attainable grain yield based on climate, farmers’ knowledge regarding crop husbandry, and capital availability.

The aim of this experiment was to assess the efficiency of using synthetic fertilizers by high-yielding rice inbred and hybrid cultivars during the wet season in a location at Senegal’s Sahel. Such an assessment will allow identifying suitable rice hybrids for low-input agro-systems.

## 2. Materials and Methods

The field trials were at Africa Rice Sahel Regional Station, near Saint Louis (Senegal) during the season (*i.e.*, July–December) of 2010 and 2011. The rice germplasm included in both years were hybrids bred by the International Rice Research Institute [3], and the inbred cultivar Sahel 108 as check as it is widely grown by farmers in Senegal.

Five fertilizer treatments (F) were used in this study. They were F<sub>0</sub> (or control; *i.e.*, without any fertilizer application) F<sub>1</sub> (37.5–4.4–8.3 kg N–P–K ha<sup>-1</sup>) F<sub>2</sub> or half of recommend fertilizer application in Senegal (75–8.75–16.5 kg N–P–K ha<sup>-1</sup>), F<sub>3</sub> (112.5–13.3–24.8 kg N–P–K ha<sup>-1</sup>) and F<sub>4</sub> or the recommended application in the country (150–17.5–33 kg N–P–K ha<sup>-1</sup>).

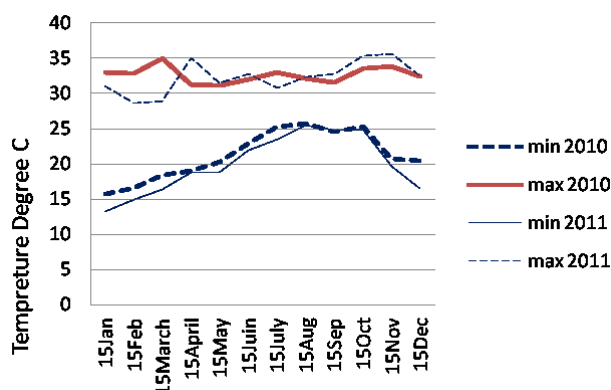
The hybrids and the inbred cultivar used as check were sown in a wet nursery. Seedlings were transplanted 25 days after sowing, using a single plant per hill and distancing each by 20 cm. The experimental layout was a randomized complete block design (RCBD) with three replications. Soil characteristics were measured before crop establishment and fertilizer application (Table 1). The minimum and maximum temperature of 2010 and 2011, in Saint Louis Senegal, are shown in Figure 1.

**Table 1.** Chemical soil analysis of plot fields in Saint Louis, Senegal during two wet seasons.

Year	Db (g cm <sup>-3</sup> )	pH	EC (dS m <sup>-1</sup> )	C (g kg <sup>-1</sup> )	N %	C/N	CEC (cmole /kg)	Soluble Cation (%)				Soil texture (%)			
								Al <sup>3+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	K <sup>+</sup>	Na <sup>+</sup>	Sand	Silt	Clay
2010	1.50	6.5	0.4	10	0.8	12.5	13	4.00	49.00	32.00	3	12	16	44	40
2011	1.59	6.6	0.45	9.8	0.75	13.0	14	7.00	45.00	31.00	4	13	16	30	54

Db = Bulk density; PH = alkalinity or acidity; Ec = electric conductivity; CEC = cation exchange capacity; C/N = means the ratio between carbon and nitrogen.

**Figure 1.** Minimum and maximum temperatures (°C) at Saint Louis (Senegal), in 2010 and 2011.



Days to 50% flowering, plant height (cm), panicle length (cm), spikelet sterility (%), 1000-grain weight, number of panicle per m<sup>2</sup>, and grain yield (t ha<sup>-1</sup>) were recorded according to the Standard Evaluation System for rice [3]. Grain quality traits such as total milling recovery, whole percentage, and alkali spreading value (ASV) were also recorded after harvesting F<sub>0</sub>, F<sub>2</sub> and F<sub>4</sub> plots in the 2010 and 2011 wet seasons. The method used for ASV involved the visual observation of the degree of dispersion of grains of the milled rice after their immersion in 1.7% KOH [4]. Alkali digestion determines indirectly the gelatinization temperature (GT). A low ASV corresponds to a high GT, and, conversely, a high ASV corresponds to a low GT. Rice with low amylose content has often a soft gel, low ASV, and high GT [5]. SAS version 9.2 [6] was used for analyzing the data. The main sources of variation were years, cultivars and fertilizer levels, as well as their 2- and 3-level factor interactions.

### 3. Results

The year affected significantly ( $p < 0.05$ ) most traits except spikelet sterility, while cultivars differed significantly ( $p < 0.01$ ) for all traits (Table 2). The effect of fertilizer levels was significant ( $p < 0.05$ ) for days to 50% flowering, plant height, panicle per m<sup>2</sup>, spikelet sterility, and grain yield. The year × fertilizer interaction was significant ( $p < 0.05$ ) for most traits except panicle per m<sup>2</sup> and

1000-grain weight, while there was a significant year  $\times$  cultivar interaction for most traits but panicle length (cm). The remaining 2- and 3-level interactions were non-significant ( $p > 0.05$ ).

**Table 2.** Analysis of Main square for the effect of year and fertilizer levels on traits assessed in 13 rice hybrids and inbred cultivar grown during the 2010 and 2011 wet seasons in Senegal.

Source of variation	DF <sup>†</sup>	Flowering (days after sowing)	Plant height (cm)	Panicle length (cm)	Number of panicles per m <sup>2</sup>	Spikelet sterility (%)	1000-grain weight (g)	Grain yield (t ha <sup>-1</sup> )
Year (Y)	1	25.7 *	46158.1 ***	2601.3 ***	1228719.3 ***	134.3	521.3 ***	6200.0 ***
Fertilizer (F)	4	69.1 ***	688.9 ***	1.2	170985.4 ***	152.6 *	1.9	67.6 ***
Cultivar (C)	13	325.1 ***	784.7 ***	22.5 ***	26087.8 ***	110.3 **	10.9 **	1.8 **
Y $\times$ F	4	82.0 ***	141.1 **	10.5 *	3181.1	162.9 **	4.8	49.0 **
Y $\times$ C	13	51.9 ***	140.5 ***	5.0	35649.6 ***	121.4 **	20.8 ***	2.1 **
F $\times$ C	52	4.4	24.0	2.0	2080.1	25.7	4.2	0.5
Y $\times$ F $\times$ C	52	3.5	10.8	1.6	3293.9	31.5	3.1	0.5
Error	280	6.57	28.33	3.32	479.7	38.8	4.41	0.65

<sup>†</sup> Degrees of Freedom \*, \*\* and \*\*\* indicate that the source of variation was significant at  $p \leq 0.05$ , 0.01 and 0.001, respectively.

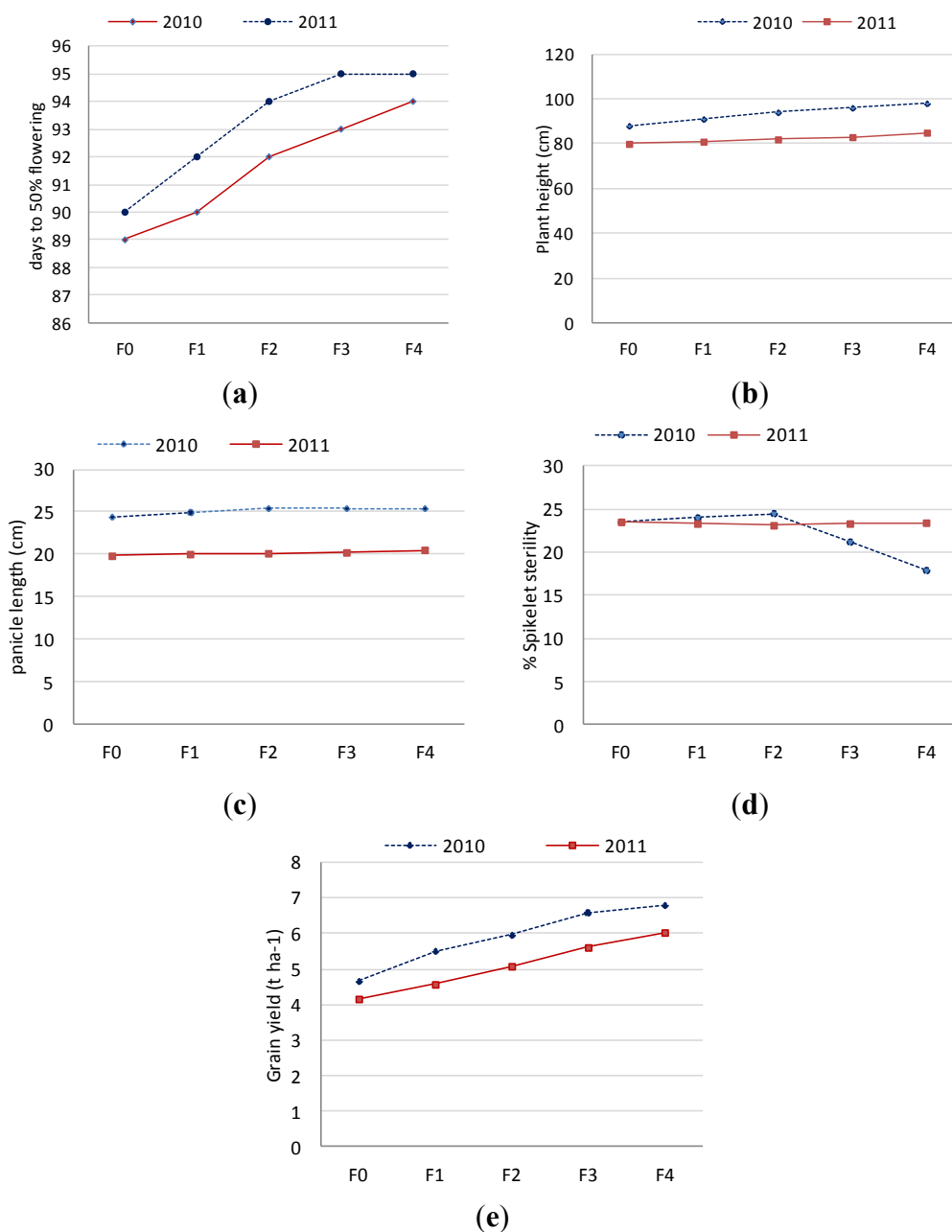
The wet season in 2010 was significantly ( $p < 0.05$ ) more benign than in 2011, as noted by the trait means (Table 3). Plant height, days to 50% flowering, panicles per m<sup>2</sup>, spikelet fertility, and grain yield increased significantly ( $p < 0.05$ ) when fertilizer levels went up (Table 3). Plots without fertilizer showed early days to 50% flowering (around 90 days) during both years while spikelet sterility decreased when using above half of the recommended fertilizer level for Senegal in 2010 (Figure 2).

**Table 3.** Mean performance across years and for each fertilizer levels across both years.

	Flowering (days after sowing)	Plant height (cm)	Panicle length (cm)	Panicles per m	Sterility (%)	1000-grain weight (g)	Grain yield (t/ha)
Year							
1	93.2 <sup>a</sup>	93.8 <sup>a</sup>	25 <sup>a</sup>	437 <sup>a</sup>	22.24	26.4 <sup>a</sup>	5.94 <sup>a</sup>
2	91.6 <sup>b</sup>	82.8 <sup>b</sup>	20 <sup>b</sup>	328 <sup>b</sup>	23.37	24.2 <sup>b</sup>	5.09 <sup>b</sup>
<i>p</i> -value	0.0486	<0.0001	NS <sup>‡</sup>	<0.0001	NS	<0.0001	<0.0001
Fertilizer levels							
F <sub>0</sub>	89.5 <sup>c</sup>	84.51 <sup>d</sup>	22.4	321 <sup>d</sup>	23.5 <sup>a</sup>	25.0	4.41 <sup>d</sup>
F <sub>1</sub>	91.0 <sup>b</sup>	86.63 <sup>c</sup>	22.5	355 <sup>c</sup>	23.7 <sup>a</sup>	25.2	5.04 <sup>c</sup>
F <sub>2</sub>	93.0 <sup>a</sup>	88.75 <sup>b</sup>	22.6	389 <sup>b</sup>	23.8 <sup>a</sup>	25.4	5.52 <sup>b</sup>
F <sub>3</sub>	94.0 <sup>a</sup>	89.5 <sup>a,b</sup>	22.7	416 <sup>a</sup>	22.2 <sup>a,b</sup>	25.3	6.10 <sup>a,b</sup>
F <sub>4</sub>	94.5 <sup>a</sup>	91.5 <sup>a</sup>	22.7	431 <sup>a</sup>	20.6 <sup>b</sup>	25.2	6.42 <sup>a</sup>
S.E.D. <sup>†</sup>	1.48	3.07	1.05	34.06	3.60	1.21	0.47
<i>p</i> -value	<0.0001	<0.0001	NS	<0.0001	0.0040	NS	<0.0001

<sup>†</sup> Standard error of differences; <sup>‡</sup> NS indicates non-significant differences ( $p > 0.05$ ); a, b, c, d means with same letter are not significantly different ( $p > 0.05$ ).

**Figure 2.** Response across rice cultivars to increasing fertilizer levels of days to flowering, (a) plant height, (b) panicle length, (c) spikelet sterility, (d) panicle m<sup>-2</sup>, and (e) grain yield in the 2010 and 2011 wet seasons in Saint Louis, Senegal.



There were significant differences ( $p < 0.05$ ) for most traits among the hybrids and between them and the check cultivar Sahel 108 (Table 4). IR86167H (97 days) had the longest period of vegetative growth, while the IRR1138 was the earliest for days to 50% flowering during the wet season of both years. The inbred cultivar Sahel 108 exhibited the shortest stature (74 cm), while the tallest (91.7 cm) were IR81954H and IR83212H. IR80228H had the largest panicle (24 cm) and the shortest panicle (21 cm) was noted in Sahel 108, which produced the highest number of panicles per m<sup>2</sup> (466). The highest and lowest percentages of spikelet sterility were found in hybrids IR83212H and IR 81954H, respectively. IR81950H had heavy grains (26.6 g for 1000 grains) that weighed significantly above those of Sahel 108 (24.7 for 1000 grains), while the lightest grains were found in IR81955H (24.4 g for

1000 grains). IR80228H showed the highest yielding hybrid (5.9 t ha<sup>-1</sup>), but its grain yield was not significantly different than that of Sahel 108 (5.9 t ha<sup>-1</sup>).

**Table 4.** Performance of 13 rice hybrids and inbred cultivar across fertilizer levels and wet seasons.

Cultivars	Days to 50 % flowering	Plant height (cm)	Panicle length (cm)	Panicle per m <sup>2</sup>	Sterility (%)	1000- grain weight (g)	Grain yield (t ha <sup>-1</sup> )
IR80228H	94 <sup>c,d,e</sup>	87.5 <sup>b</sup>	24.0 <sup>a</sup>	352 <sup>d,e</sup>	20.83 <sup>b,c</sup>	24.9 <sup>a,b</sup>	5.9 <sup>a</sup>
IR80814H	89 <sup>g,f</sup>	80.6 <sup>c</sup>	21.9 <sup>d,e,f</sup>	385 <sup>b,c,d,e</sup>	21.91 <sup>a,b,c</sup>	25.6 <sup>ab</sup>	5.1 <sup>b</sup>
IR81950H	96 <sup>a,b</sup>	83.9 <sup>b,c</sup>	23.5 <sup>a,b,c</sup>	393 <sup>b,c,d</sup>	20.86 <sup>b,c</sup>	26.6 <sup>a</sup>	5.2 <sup>b</sup>
IR81954H	95 <sup>a,b,c</sup>	91.7 <sup>a</sup>	22.7 <sup>a,b,c,d,e</sup>	372 <sup>c,d,e</sup>	19.01 <sup>c</sup>	25.9 <sup>a,b</sup>	5.7 <sup>a,b</sup>
IR81955H	93 <sup>e</sup>	80.7 <sup>c</sup>	23.1 <sup>a,b,c,d</sup>	423 <sup>a,b</sup>	24.26 <sup>a,b</sup>	24.4 <sup>b</sup>	5.7 <sup>a,b</sup>
IR82391H	90 <sup>f</sup>	83.9 <sup>b,c</sup>	22.2 <sup>c,d,e,f</sup>	385 <sup>b,c,d,e</sup>	24.50 <sup>a,b</sup>	25.3 <sup>a,b</sup>	5.3 <sup>a,b</sup>
IR83212H	94 <sup>c,d,e</sup>	91.8 <sup>a</sup>	22.9 <sup>a,b,c,d</sup>	405 <sup>b,c</sup>	26.35 <sup>a</sup>	24.5 <sup>b</sup>	5.2 <sup>b</sup>
IR84711H	95 <sup>b,c,d</sup>	81.4 <sup>c</sup>	22.6 <sup>a,b,c,d,e</sup>	385 <sup>b,c,d,e</sup>	24.40 <sup>a,b</sup>	25.3 <sup>a,b</sup>	5.5 <sup>a,b</sup>
IR84741H	89 <sup>g,f</sup>	81.4 <sup>c</sup>	22.5 <sup>b,c,d,e</sup>	369 <sup>c,d,e</sup>	23.36 <sup>a,b,c</sup>	25.3 <sup>a,b</sup>	5.7 <sup>a,b</sup>
IR85466H	96 <sup>a,b</sup>	85.8 <sup>b</sup>	22.4 <sup>c,d,e</sup>	358 <sup>c,d,e</sup>	21.18 <sup>a,b,c</sup>	24.8 <sup>b</sup>	5.5 <sup>a,b</sup>
IR85471H	87 <sup>h</sup>	76.3 <sup>d</sup>	21.4 <sup>e,f</sup>	392 <sup>b,c,d</sup>	22.91 <sup>a,b,c</sup>	25.6 <sup>a,b</sup>	5.6 <sup>a,b</sup>
IR86167H	97 <sup>a</sup>	87.1 <sup>b</sup>	22.8 <sup>a,b,c,d,e</sup>	340 <sup>e</sup>	23.53 <sup>a,b,c</sup>	25.7 <sup>a,b</sup>	5.6 <sup>a,b</sup>
IRR1138	88 <sup>g,h</sup>	81.1 <sup>c</sup>	23.9 <sup>a,b</sup>	348 <sup>d,e</sup>	22.31 <sup>a,b,c</sup>	25.6 <sup>a,b</sup>	5.4 <sup>a,b</sup>
Sahel 108	93 <sup>d,e</sup>	74.1 <sup>d</sup>	21.0 <sup>f</sup>	446 <sup>a</sup>	23.88 <sup>a,b,c</sup>	24.7 <sup>b</sup>	5.8 <sup>a,b</sup>
S.E.D. <sup>†</sup>	1.48	3.07	1.05	34.06	3.60	1.21	0.466
<i>p</i> -value	<0.0001	<0.0001	<0.0001	<0.0001	0.0007	0.0035	0.0011

<sup>†</sup> Standard error of differences; <sup>a-h</sup> means with same letter are not significantly different ( $p > 0.05$ ).

Average of grain yield for hybrid varieties under different fertilizer levels present in (Table 5). Generally grain yield increased with increasing of fertilizer levels for most hybrids and check variety. In the same time, no significant interactions observed between hybrid varieties different fertilizer levels. Grain quality traits did not follow any trend according to fertilizer levels (Table 6). The inbred check cultivar Sahel 108 had the best milling recovery and whole grain percentage, while IRR1138 exhibited the best ASV at zero and intermediate fertilizer levels.

**Table 5.** Grain yield average of 13 rice hybrids and inbred cultivar under different fertilizer levels.

Variety	Yield				
	N <sub>0</sub>	N <sub>1</sub>	N <sub>2</sub>	N <sub>3</sub>	N <sub>4</sub>
IR80228H	4.46	5.16	5.97	6.68	7.39
IR80814H	3.66	4.63	5.72	5.80	5.87
IR81950H	3.75	4.70	5.77	5.81	5.86
IR81954H	4.66	5.23	5.92	6.23	6.54
IR81955H	4.33	5.35	6.50	6.26	6.02
IR82391H	4.40	5.02	5.76	5.75	5.74
IR83212H	4.28	4.82	5.49	5.68	5.88

Table 5. Cont.

Variety	Yield				
	N <sub>0</sub>	N <sub>1</sub>	N <sub>2</sub>	N <sub>3</sub>	N <sub>4</sub>
IR84711H	4.09	4.99	6.01	6.09	6.17
IR84741H	4.20	5.08	6.08	6.43	6.77
IR85466H	4.62	5.18	5.86	5.84	5.82
IR85471H	3.96	4.94	6.04	6.36	6.68
IR86167H	4.26	4.94	5.75	6.22	6.68
IRR1138	4.08	4.99	6.03	6.04	6.05
Sahel 108	4.24	4.89	5.68	6.66	7.65

S.E.D. †: 0.466; *p*-value: 0.0011.

**Table 6.** Grain quality of 13 rice hybrids and inbred cultivars after using fertilizer levels: control without fertilizer application (F<sub>0</sub>), half-recommended application (F<sub>2</sub>) and recommended application (F<sub>4</sub>) after the 2010 and 2011 wet seasons in Saint Louis, Senegal.

	Total milling recovery (%)			Whole grain (%)			Alkali spreading value		
	F <sub>0</sub>	F <sub>2</sub>	F <sub>4</sub>	F <sub>0</sub>	F <sub>2</sub>	F <sub>4</sub>	F <sub>0</sub>	F <sub>2</sub>	F <sub>4</sub>
IR80228H	63.40	63.51	62.30	43.80	47.93	47.00	3.56	3.30	3.26
IR80814H	61.16	61.96	61.03	32.90	47.22	38.63	3.33	2.83	2.90
IR81950H	62.96	63.05	61.96	47.60	37.51	46.46	3.53	3.00	3.33
IR81954H	60.46	61.45	59.23	42.03	49.44	41.56	3.26	3.26	3.20
IR81955H	64.26	64.00	63.33	41.53	51.37	44.46	3.56	3.43	2.86
IR82391H	63.16	63.23	62.13	41.76	46.55	46.63	3.50	3.13	3.36
IR83212H	63.73	63.67	62.66	48.96	57.93	45.63	2.93	2.50	3.50
IR84711H	62.90	62.99	61.90	46.46	39.86	43.63	3.96	3.50	3.76
IR84741H	60.16	61.06	58.56	34.53	47.56	47.53	3.20	2.53	3.96
IR85466H	63.95	63.72	62.76	44.95	41.60	46.20	4.20	3.16	3.00
IR85471H	61.16	61.85	61.00	34.50	45.28	42.00	3.73	3.00	2.93
IR86167H	61.00	61.57	60.35	35.20	36.30	46.90	3.45	3.15	3.10
IRR1138	60.96	61.52	60.23	40.33	46.80	41.93	3.70	3.40	3.10
Sahel 108	64.40	64.08	63.63	54.30	54.43	49.00	2.33	2.50	3.16
S.E.D. †		1.436			4.89			0.291	
<i>p</i> -value		0.821			1.000			0.9739	

† Standard error of differences.

#### 4. Discussion

Grain yield decreased in most rice cultivars when lowering fertilizer use in the wet seasons of 2010 and 2011. An increase of fertilizer levels significantly improves growth, grain and straw yields of rice. This result also confirms that fertilizer (N, P and K) is essential for increasing rice grain yields [7–9]. It promotes rapid growth and increases leaf size, spikelet number per panicle, percentage of filled spikelet in each panicle and grain protein content [10–14] also indicated that the grain yield of most rice hybrids significantly increased when N-fertilizer went up to 180 kg ha<sup>-1</sup>. Lin *et al.* [15–22] found

that the system of rice intensification (SRI) can significantly reduce N-fertilizer use due to planting hybrid seed at low density.

High fertilizer levels also extended the vegetative growth. Plants were taller in the first year than in the second year, which suggest that climate and affect plant height. Although Yadav [16] indicated that nitrogen levels did not affect significantly plant height at all stages of rice growth, an increased use of fertilizers led to tall plants in this experiment, which supports previous findings by Van Hach [17].

Years affected significantly panicle length, which was bigger in 2010 than in 2011. This could be due to the wider range between day and night temperatures in 2011 (Figure 1). An increase on fertilizer levels led to having more panicles per m<sup>2</sup>, thereby suggesting that nitrogen seems to be effective for stimulating tillering. Increasing fertilizer levels did not lead to heavy grains because the sink capacity (*i.e.*, spikelet number) was high in the cultivars during both years. These findings agreed with previous research [12,18–20]. A high number of panicles per m<sup>2</sup> and the number of filled grains per panicle will increase grain yields.

The grain yield advantage of IRRI-bred hybrids was not significantly above than that of the inbred check cultivar in Senegal. Their grain yield was higher in 2010 than in 2011, when there was a three-week flood due to heavy rains. Newly bred hybrid rice cultivars have high physiological efficiency due to its vigorous root system, great sink size, large leaf area index during grain filling, and wide adaptability to various environments, including saline soils [21].

Testing new rice hybrids under low fertilizer levels may facilitate identify suitable germplasm showing adaptability to the low-input farming systems of Senegal. Nonetheless, plant breeders should keep in mind that the potential impacts of rice hybrids in sub-Saharan Africa will be influenced by other factors beyond the genotype, environment, and crop husbandry. They are related to conducive policy for adopting this seed technology, working input markets for seeds and fertilizers, institutional arrangements throughout the value chain (including extension systems), and social demographics influenced by end-users (*i.e.*, farmers, millers and consumers).

## 5. Conclusions

More studies need to be conducted to propose a specific formula of fertilizer per variety or group of varieties knowing that they respond differently. The same studies need to be conducted in areas with problem soils to combine tolerant varieties and fertilizer application to increase the average yield across ecologies.

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## Author Contributions

Ghislain Kanfany, Raafat El-Namaky, Kabirou Ndiaye and Karim Traore participated in the research and did data analysis, Raafat El-Namaky and Rodomiro Ortiz planed, analyzed results, and wrote this article.



## Conflicts of Interest

The authors declare no conflict of interest

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