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Article

Agro-Morphological Evaluation of Rice (*Oryza sativa* L.) for Seasonal Adaptation in the Sahelian Environment

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Abstract: In the Sahel zone of West Africa that extends from Senegal to Chad, temperatures can vary from less than 15 °C to 25 °C from November to February. These low temperatures affect the growth, development and yield of rice plants, and therefore constitute a major constraint to rice production in the Sahel. In order to identify rice varieties tolerant to cold stress at different developmental stages, a diverse set of 224 rice germplasm was evaluated for yield and yield-related traits in Ndiaye, Senegal, using three different sowing dates. The first sowing date (October 2010), was chosen so as to expose the rice plants to cold stress at the reproductive stage while the rice crop planted at the second sowing date (January 2011) experienced cold stress at the vegetative stage. The third sowing date (July 2011) was the normal planting date for irrigated rice in the Sahel and it served as the control date when the crop does not experience any cold stress throughout its growth cycle. Among the data collected, significant genetic variation was detected and genotype-by-environment interaction was also significant for the traits. At the vegetative stage, cold stress reduced tillering and plant vigor and delayed flowering but increased yield, whereas at the reproductive stage, aside from delaying flowering, cold stress also inhibited panicle exsertion and reduced panicle length, spikelet fertility, grain filling and strongly reduced yields. Principal Component Analysis and correlation analysis using agro-morphological traits helped to identify genotypes that were tolerant to cold stress at either the vegetative or the reproductive stage and the traits associated with high yield under cold stress at each of these stages. Our results can be used to develop cold tolerant rice varieties adapted to double cropping in the Sahelian zone of West Africa.

Keywords: *Oryza sativa*; rice; Sahelian environment; low temperature; cold season; agro-morphological characters

1. Introduction

Rice, considered the basic food of Asian countries, has become one of the most consumed food crops in Africa. Statistics show a consumption growth of around 6% per annum in West Africa while production increased by an average of 2% to 3% [1]. With the domestic supply being far less than the rice demand in Africa, the shortfall is met through imports, which impose a severe strain on meager foreign exchange reserves. In fact, the import of rice by African governments is a real threat to their economies, food sovereignty and social stability. The adverse consequences of the global cereal crisis in 2008 on food security served as a warning to African governments. Consequently, several African governments such as Mali, Nigeria and Senegal have since launched national programs for sustainable intensification of rice production. A key component of these strategies is the double cropping of rice in irrigated perimeters especially in the Sahel zone of West Africa. However, in the Sahel zone of West Africa that extends from Senegal to Chad, there is strong seasonal variation in temperature, which dictates the optimum periods during which a good rice crop can be cultivated. From November to February (referred to locally as the Harmattan period), night temperatures can descend to 12 °C or less, which poses a constraint to rice growth and development. Thus cold stress is one of the major constraints to rice production in the Sahel zone of West Africa since the rice varieties presently being cultivated in the Sahel zone are sensitive to cold stress. Farmers are advised not to plant their rice crops during the cold Harmattan period. Cold stress affects the development of the rice plant at all stages of growth [2].

Generally, in the Sahel zone, the irrigated lowland rice crop is cultivated in the hot, wet season (July–November) and/or the hot, dry season (February–June). Any delay in sowing the dry season crop or prolonged low temperatures during the vegetative stage delays flowering and the dry season crop will be harvested at the beginning of the rains around July. This causes severe harvest and post-harvest problems for farmers leading to loss of production as well as reduced milling quality. A delay in the wet season sowing also leads to the crop maturing during the cold months of November/December and this also leads to high sterility in sensitive varieties. Thus development of rice varieties tolerant to low temperatures at the vegetative stage will allow farmers to plant their dry season crop in December or January and harvest in April or May and hence avoid the overlap with the wet season. Varieties with reproductive stage tolerance will enable farmers realize good harvests even when they plant late during the wet season.

Intraspecific genetic variability for cold tolerance has been reported in rice and according to reports *japonica* cultivars are more tolerant to low temperatures than *indica* cultivars [3]. Mackill and Lei [2] also reported that genetic variation for cold tolerance is the result of local adaptation due to the fact that in the temperate and high altitude zones cold tolerance trait is under strong selection pressure because low temperatures are consistently experienced in these zones. These results are consistent with previous studies showing that a single amino acid, Valine 99 change in Zeta class GSTs (glutathione transferases) corresponding to an SNP (single nucleotide polymorphism), was associated to cold sensitivity in rice [4].

Genetic variability for cold tolerance has been evaluated by researchers at germination, seedling and reproductive stages in rice breeding programs. Cold stress in rice leads to poor germination and stunted growth of seedlings, lengthens the growth cycle, reduces tillering [5] and increases plant mortality [2]. Physiologically, low temperatures induce several abnormalities in the reproductive organs including enlargement of anther cell walls and tapetal cells, reduction in the numbers of mature pollen and increased male sterility [6,7]. In fact, low temperature-induced infertility in rice is reported to be due to the inhibition of microsporogenesis, which leads to degeneration of pollen microspores [8]. Recently, Sakata *et al.* [8] also showed that microsporogenesis is disrupted by low temperature due to a reduction of bioactive gibberellins, GA4 and GA7. Nonetheless, a high variability for cold tolerance in rice has been reported in several studies. For instance, in a study on more than 700 *japonica* cultivars collected from Japan, Europe, China, Russia and other regions, Kotaka and Abe (1988) found a high genetic variability for seedling germinability [9]. In addition, an evaluation of 20 Chilean rice genotypes and 192 Japanese accessions at 13 °C, led to the identification of cold tolerance genotypes, based on coleoptile length reduction, coleoptile length after cold treatment, coleoptile length recovery and coleoptile regrowth [10,11]. Similar studies were carried out in 477 landraces from five cropping regions in Yunnan, China, which is considered the center of genetic diversity and cold tolerance [12]. This investigation showed that genetic variation for cold tolerance existed within accessions collected from this region and Northwest Yunnan housed the strongest cold tolerant landraces in China while South Yunnan had the most sensitive [13]. According to Dingkuhn and Miézan [14], temperatures below 18 °C can induce up to 50% sterility and this can reach up 100% at 10 °C in very sensitive varieties. In a study of reproductive stage cold tolerance conducted on 23 elite rice cultivars from eight countries, only accessions from Uzbekistan (Avangard and Mustaqillik) and Korea (Jinbu) showed high cold tolerance under cold-water and greenhouse conditions showing 71% to 79% spikelet fertility [15,16].

Based on the hypothesis that the sowing date does not have any effect on the varieties, the objectives of this research were to identify cold tolerant rice genotypes that are adapted to the Sahel zone of West Africa and the agro-morphological traits associated with this adaptation.

2. Results

2.1. Air and Water Temperature in the Experiment Field

During the course of the three trials, water temperature ranged from as low as 15 °C in January to a maximum of 40 °C in August (Figure 1). The coolest month, January, coincided with the reproductive stage of the first sowing date (Date 1) and the vegetative stage of the second sowing date (Date 2). Low water and air temperatures were registered during the early part of the drying season (November to March) and the hottest temperatures were experienced in May–June. The relative humidity was low during the dry season varying between 40% to 60% and high in the rainy season (July–October) ranging from 70% to 90%. Minimum temperatures during the rainy season ranged from 18 °C to 25 °C while maximum temperatures ranged from 30 °C to 40 °C.

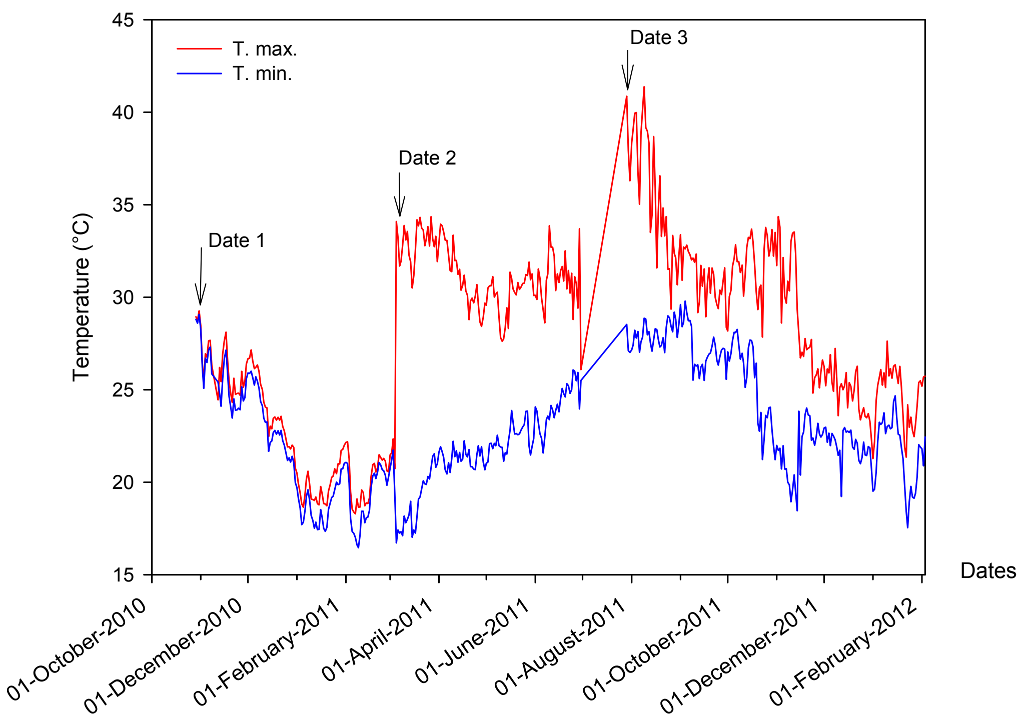

Figure 1. Trend of temperature of standing water in the trials. "T. max." and "T. min." mean, respectively, maximum temperature and minimum temperature.

2.2. Effect of Sowing Date on Yield and Morpho-Physiological Traits

For all parameters measured, the interaction between sowing date and genotype was highly significant ($p < 0.001$), with the exception of tiller number, panicle length and panicle exsertion, as was revealed from the ANOVA results (Table 1). However, since the interaction for grain yield was significant, and it being the main breeding target, means for the measured traits were computed for each sowing date separately.

Table 1. ANOVA results showing level of significance for the genotype x sowing date interaction for morpho-physiological traits measured during course of field trials conducted at Ndiaye, Senegal (n = 224).

Source	df	Sum Square	Mean Square	F Value
Sterility (%)				
Date (D)	2	210,311	105,155	349.52 ***
Genotype (G)	223	118,178	530	5.31 ***
GXD	418	189,714	454	4.55 ***
Panicle length				
Date (D)	2	2,354.9	1,177.44	165.63 ***
Genotype (G)	223	6,436.2	28.86	2.87 ***
GXD	418	5,474.3	13.1	1.30
Panicle exsertion				
Date (D)	2	2,888.8	1,444.38	174.70 ***
Genotype (G)	223	4,269.2	19.14	2.15 **
GXD	418	3,740.8	8.95	1.00
Tiller number				
Date (D)	2	15,294.3	7,647	115.21 ***
Genotype (G)	223	21,584.2	96.8	4.24 ***
GXD	418	14,093.4	33.7	1.48
Days to mature				
Date (D)	2	167,171	83,585	147.30 ***
Genotype (G)	223	180,984	812	16.091 ***
GXD	418	98,802	236	4.69 ***
Plant height (cm)				
Date (D)	2	113,738	56,869	46.85 ***
Genotype (G)	223	339,532	1,523	8.21 ***
GXD	418	164,196	393	2.12 **
Harvest index				
Date (D)	2	4.3328	2.1664	83.65 ***
Genotype (G)	223	5.8066	0.02604	5.67 ***
GXD	418	5.1519	0.01233	2.69 ***
Yield				
Date (D)	2	2,303	1,151.5	215.92 ***
Genotype (G)	223	1,219.6	5.47	5.43 ***
GXD	418	2,056.13	4.92	4.88 ***
Biomass				
Date (D)	2	149,199	74,599	8.95 **
Genotype (G)	223	1,246,177	5,588	3.00 ***
GXD	418	1,426,052	3,412	1.83 *

*, **, ***—Significant at 5%, 1% and 0.1% respectively.

2.3. *Effect of Low Temperatures on the Morpho-Physiological Traits of Rice*

2.3.1. Spikelet Sterility

At the first sowing date when cold stress coincided with the reproductive stage, spikelet sterility was very high with many entries including the sensitive check IR36 showing 100% sterility (Figure 2Ai). Silewah, the tolerant check, had less than 40% sterility. Several lines were identified that had very low spikelet sterilities at this date with some having less than 10% sterility. At the second and third sowing dates, however, most entries, including the cold tolerant and sensitive checks had less than 40% sterility (Figure 2Aii,Aiii).

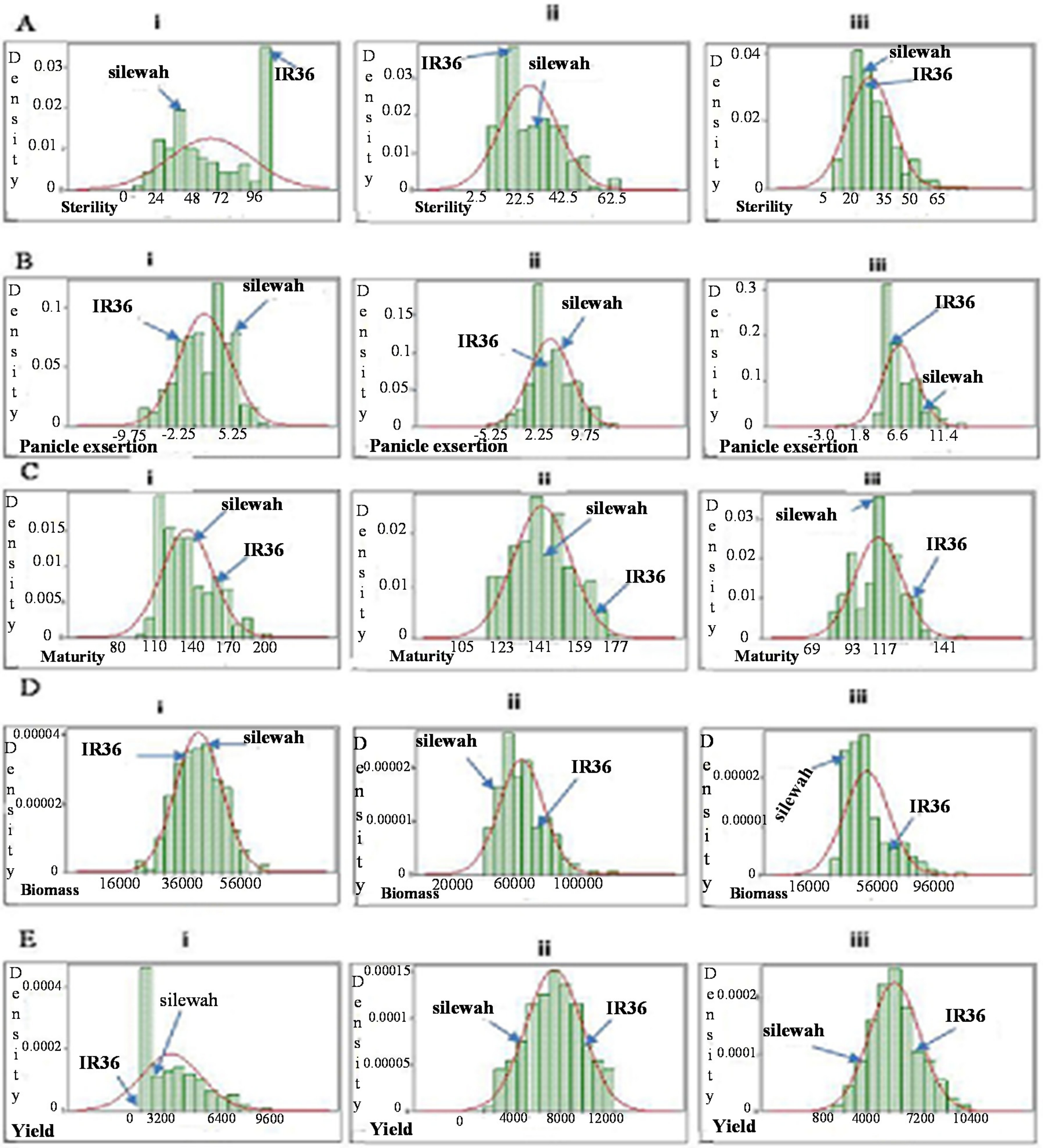

Figure 2. Frequency distribution of a set of lines for (**A**) spikelet sterility (%); (**B**) panicle exsertion (cm); (**C**) duration (days); (**D**) biomass (kg/ha) and (**E**) grain yield (kg/ha) during three different sowing dates at Ndiaye (Senegal).

2.3.2. Panicle Exsertion

More than 40% of entries including IR 36, the sensitive check, had negative panicle exsertion at the first sowing date (Figure 2Bi), indicating that the whole of the peduncle and part of the lower panicle branches were hidden in the flag leaf sheath at maturity. During the second and third sowing dates, however, panicle exsertion was better for most entries compared to the first date, as more than 90% of entries had positive panicle exsertion. When cold stress coincided with the vegetative stage (Date 2), panicle exsertion was reduced relative to the third date with more than 40% of entries having 4 cm or less panicle exsertion during Date 2 (Figure 2Bii) while more than 90% of entries had 4 cm or more panicle exsertion at Date 3 (Figure 2Biii).

2.3.3. Crop Maturity

Cold stress delayed crop maturity by lengthening the duration. Plants matured much later in the trials of Date 1 and Date 2 with more than 40% of entries maturing at 140 days or more after sowing for both sowing dates. However, this delay in maturity was more severe in Date 2 where less than 20% of entries matured at less than 120 days after sowing (Figure 2Cii) while for Date 1 almost 40% matured at 120 days after sowing (Figure 2Ci). For Date 3, which did not experience any cold stress, more than 90% of entries matured at less than 120 days after sowing (Figure 2B). Silewah matured at less than 140 days for all three sowing dates while IR 36 matured at less than 140 days only at Date 3 (Figure 2Ciii). At Dates 1 and 2, IR 36 matured at more than 160 days after sowing (Figure 2Ci,Cii).

2.3.4. Biomass

At Date 1, biomass production was lower than for Dates 2 and 3 (Figure 2Dii,Diii). At Date 1 (Figure 2Di), less than 20% of entries produced biomass greater than 56,000 kg/ha while at Dates 2 and 3, more than 40% of entries produced biomass above 56,000 kg/ha. Silewah produced more biomass than IR 36 only at Date 1 but at Dates 2 and 3, IR 36 had a higher biomass production than Silewah.

2.3.5. Grain Yield

Grain yields were severely reduced at Date 1 when cold stress coincided with the reproductive stage compared to Dates 2 and 3. At Date 1, more than 30% of entries did not produce any yield (Figure 2Ei). Generally, yields were highest at Date 2 with more than 50% of entries yielding above 6000 kg/ha and several entries yielding above 10,000 kg/ha (Figure 2Eii). At Date 1, less than 10% of entries yielded above 6000 kg/ha and at Date 3 (Figure 2Eiii) less than 30% of entries yielded above 6000 kg/ha.

2.3.6. Grain Filling

Analysis of grain filling patterns of the tolerant check Silewah and Hwanghaezo, which showed good cold tolerant traits (earliness, good panicle exsertion, moderate sterility for Date 1) revealed that when grain filling occurred under optimal conditions (Sowing Dates 2 and 3), both cold tolerant and sensitive varieties had 60% or more medium-sized and heavy grains (Figure 3). However, when grain filling coincided with low temperatures (Sowing Date 1), tolerant varieties such as Silewah and Hwanghaezo, still had more grains in the medium-sized to heavy grain classes while sensitive varieties Sahel 108 and IR36 had more than 60% of grains in the light grain classes.

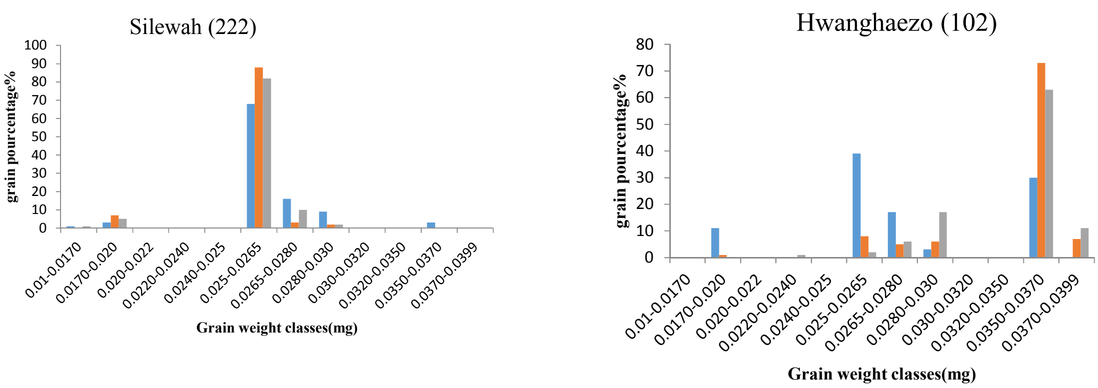

Figure 3. *Cont.*

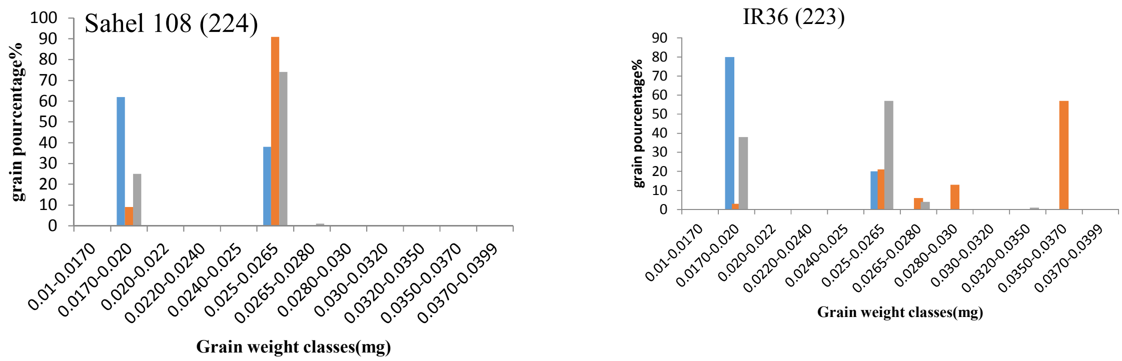

Figure 3. Frequency distribution of classes of grains by weight range during different sowing dates (blue = Date 1, red = Date 2, green = Date 3).

2.3.7. Correlation between Agro-Morphological-Physiological Parameters at Different Sowing Dates

Concerning the association of yield and morpho-physiological traits during the different sowing dates, the strongest correlations ($p < 0.001$) were found between yield and spikelet sterility, harvest index, panicle exsertion, and maturity duration (Table 2). Strong negative correlations were detected between yield and spikelet sterility, harvest index and spikelet sterility, and harvest index and maturity duration. Yield was positively correlated with biomass and harvest index at all sowing dates as well as with tiller number during the second and third plant sowing dates. For the first sowing date, during which the reproductive phase of the rice plants coincided with cold stress, strong positive correlations were found between yield and panicle exsertion and yield and harvest index. Biomass and plant height were negatively correlated, spikelet sterility and maturity duration were, however, strongly and negatively correlated ($p < 0.001$) with yield during the same sowing date. This implies that earliness, low sterility and high harvest index are important traits for cold tolerance when the stress occurs at the reproductive phase of the rice plant. However, when cold stress occurred at the vegetative stage (sowing Date 2), yield was positively correlated with biomass, harvest index and tiller number and negatively correlated to spikelet sterility (Table 3). Maturity duration had strong positive correlations with biomass, sterility and tiller number during all three sowing dates and with panicle length during sowing Dates 2 and 3 (Table 4). Harvest index, however, was negatively correlated with maturity duration at all three sowing dates.

Table 2. Pearson correlation coefficients between morpho-physiological parameters at the first sowing date ($n = 224$). Plants were exposed to cold stress at reproductive stage.

Trait	Panicle Length (cm)	Panicle Exsertion (cm)	Tiller Number	Days to Mature	Plant Height (cm)	Yield (kg/ha)	Biomass (kg/ha)	Biological Harvest Index (%)
Sterility (%)	−0.136 *	−0.298 **	0.163 *	0.452 **	−0.297 **	−0.815 **	−0.087	−0.675 **
Panicle length (cm)		−0.152 *	−0.271 **	−0.312 **	0.538 **	0.051	0.009	0.187 *
Panicle exsertion (cm)			−0.218 *	−0.082	0.168 *	0.270 **	0.118	0.209 *
Tiller number/m^2				0.316 **	−0.451 **	−0.121	0.12	−0.232 *
Days to mature (days)					−0.495 **	−0.303 **	0.284 **	−0.464 **
Plant height (cm)						0.139 *	−0.055	0.210 *
Yield (kg/ha)							0.285 **	0.680 **
Biomass (kg/ha)								0.068

*, **—Significant at 5% and 1% respectively.

Table 3. Pearson correlation coefficients between morpho-physiological parameters at the second sowing date (n = 224). Plants were exposed to cold stress at vegetative stage.

	Panicle Length (cm)	Panicle Exsertion (cm)	Tiller Number	Days to Mature (Days)	Plant Height (cm)	Yield (kg/ha)	Biomass (kg/ha)	Biological Harvest Index (%)
Sterility (%)	0.31 **	0.009	0.287 **	0.531 **	0.082	−0.385 **	0.246 *	−0.438 **
Panicle length (cm)		0.261 *	0.122	0.325 **	0.285 **	−0.051	0.343 **	−0.296 **
Panicle exsertion (cm)			0.003	0.059	0.572 **	−0.128	0.171 *	−0.368 **
Tiller number/m^2				0.415 **	−0.039	0.229 *	0.479 **	−0.161 *
Days to mature (days)					−0.038	−0.049	0.442 **	−0.424 **
Plant height (cm)						−0.17 *	0.193 *	−0.491 **
Yield (kg/ha)							0.435 **	0.481 **
Biomass (kg/ha)								−0.145 *

*, **—Significant at 5% and 1% respectively.

Table 4. Pearson correlation coefficients between morpho-physiological parameters at the third sowing date (n = 224). Normal planting date, plants were not exposed to cold stress at any stage.

	Panicle Length (cm)	Panicle Exsertion (cm)	Tiller Number	Days to Mature	Plant Height (cm)	Yield (kg/ha)	Biomass (kg/ha)	Biological Harvest Index (%)
Sterility (%)	0.206	0.008	0.178	0.379	0.126	−0.388	0.246	−0.463
Panicle length (cm)		−0.05	0.247	0.482	0.229	0.066	0.532	−0.527
Panicle exsertion (cm)			−0.111	−0.142	0.084	−0.003	-0.003	0.055
Tiller number/m^2				0.47	−0.082	0.198	0.343	−0.192
Days to mature (days)					−0.001	0.006	0.588	−0.635
Plant height (cm)						−0.074	0.218	−0.33
Yield (kg/ha)							0.308	0.313
Biomass (kg/ha)								−0.58

2.4. Principal Component Analysis (PCA) of Yield and Related Traits during the Different Sowing Dates

2.4.1. First Sowing Date

The projection of agronomic parameters in the PCA plot (Figure 4) shows that Axis 1 is constituted by yield, harvest index, sterility, panicle exsertion and crop duration. This axis opposes varieties with good yield and a relatively high harvest index, a short maturity cycle and low fertility (cold tolerant) against those with high sterility, late maturing, and low yields (cold sensitive). Axis 1 is characterized by varieties that are moderately tall (110–130 cm) or tall (more than 130 cm). They were all early maturing (101–125 days) with yields of 3 to 9 t/ha, low spikelet sterility (6%–35%) and a relatively high harvest index (0.41 to 0.57). Axis 2 is determined by the biomass, panicle length and plant height. To the right of the axis are short varieties (less than 90 cm). They have a long duration (over 140 days), a very strong tillering (30–52), a very high sterility (100%), low harvest index (0.05 to 0.2) and zero yields. Axis 2 is composed of varieties that are characterized by high biomass production (60 to 80 g/hill), a high yield (4–7 t/ha), good tillering (25–50), long panicles (12–19 cm), short stature, and very long duration (133–200 days). At the bottom of Axis 2 are the varieties with low biomass production (less than 50 g/hill), a very low yield (less than 1 t/ha), long panicles (more than 20 cm long), medium duration and are tall (more than 130 cm).

2.4.2. Second Sowing Date

In the PCA plot for the second sowing date (Figure 5), Axis 1 is characterized by harvest index (HI), sterility, panicle length and growth duration (Maturity). Axis 2 is characterized by the yield, tillering, height and biomass (5B). The projection of the individuals on these axes (Figure 5B) allows us to characterize them. Thus, the left side of Axis 1 there are genotypes characterized by a harvest index greater than 0.50, low fertility (5%–18%), long panicles (10–20 cm) and early maturity (100 to 125 days).

To the right of Axis 1 are those with low harvest indices (less than 0.20), a high rate of sterility (more than 50%) and a low yield (less than 2 t/ha).

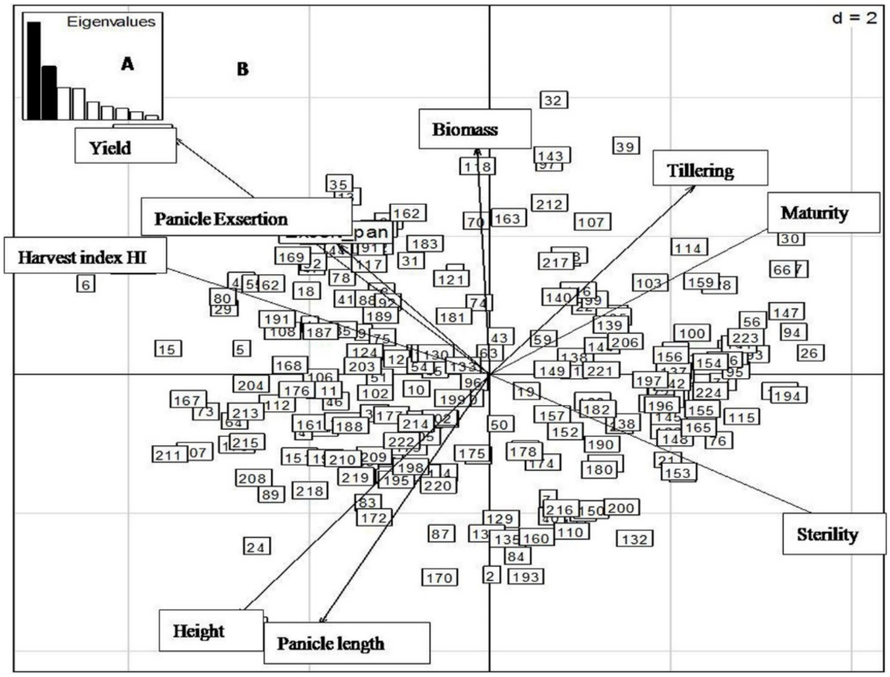

Figure 4. Projection of agronomic parameters and rice varieties on the first factorial plane (Axis 1 and Axis 2), for the first sowing date.

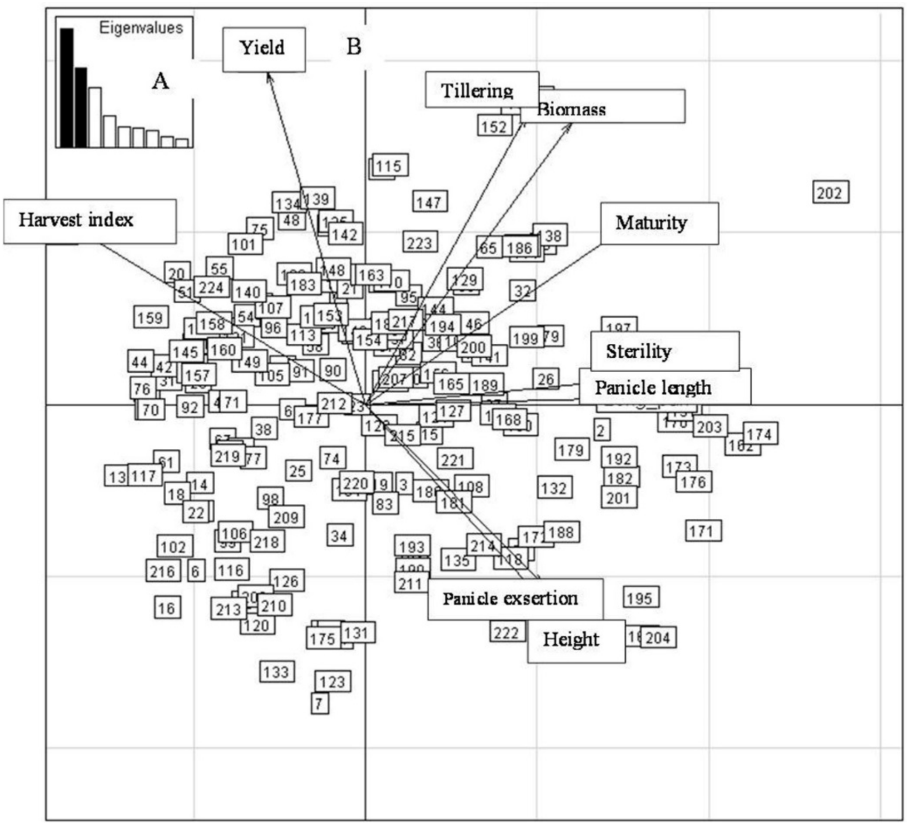

Figure 5. Projection of agronomic parameters and rice varieties (2B) on the first factorial plane (Axis 1 and Axis 2), Date 2 based on Principal Component Analyses.

They were late maturing (140 to 174 days) and had relatively long panicles (20–30 cm). The most critical at the top of the Axis 1 are genotypes with good yields (6–11 t/ha), relatively large biomass (73 to 100 g/hill), high tillering (26–37) and short stature (61 to 97 cm). At the bottom of Axis 2, we find varieties that give a low biomass production (less than 40 g per hill), low yield (2–4 t/ha) and medium tillering (10–19).

2.4.3. Third Sowing Date

For the normal sowing date (July) for irrigated rice in the Sahel, the PCA plot showed that Axis 1 is defined by biomass, harvest index, panicle length and crop duration while Axis 2 is defined by sterility, yield and tillering (Figure 6).

The projection of the individuals on these axes allowed us to distinguish two groups of genotypes. To the left side of Axis 1 are genotypes that are characterized by low biomass, high harvest index (above 0.50) and panicles of medium size (13–22 cm). These genotypes are short (less than 100 cm) and early maturing (less than 100 days). In the right part of Axis 1 are varieties that produce high biomass (92–137 g/hill) and are short and have medium duration, low harvest indices and long panicles (25 to 30 cm). In the group located in the positive part of Axis 2 are those characterized by a high tillering (20 to 47), a good yield (6–9 t/ha) and low sterility (9%–35%). The group located in the negative part of the Axis 2 has the low-yielding varieties (1 to 2 t/ha), high rates of sterility (34%–72%) and low tillering (7–20).

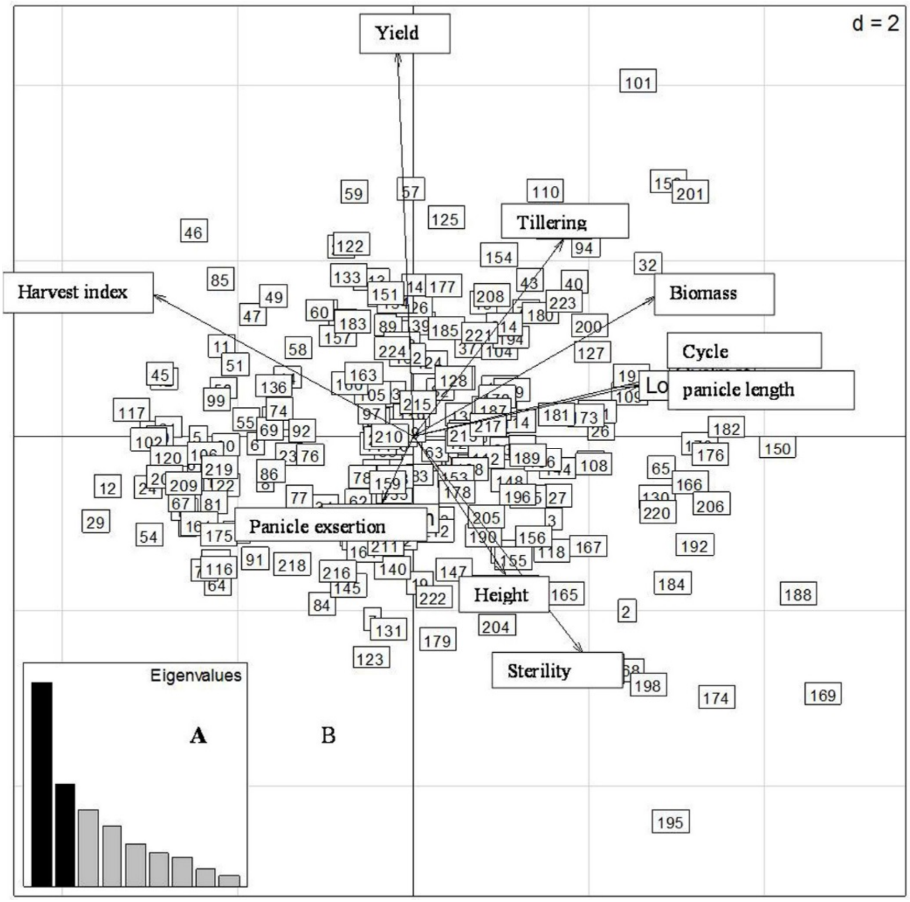

Figure 6. Projection of agronomic parameters and rice varieties on the first factorial plane (Axis 1 and Axis 2) Date 3 based on ACP analyses.

3. Discussion

Due to its tropical and subtropical origin, rice is sensitive to low temperatures during all stages of development affecting grain quality and yield [6]. Molecular and cellular responses to this abiotic stress have been intensively studied over the past 20 years at morphological, physiological and biochemical level [2,8,17]. This led to improve our understanding of the effect of this abiotic stress on rice development and to develop new strategies in the breeding programs. Therefore, the identification of rice accession tolerant to cold stress is relevant for improving *indica* production in the Sahelian region.

3.1. Genotype-by-Environment Interaction for Grain Yield and Agro-Morphological Traits

Seasonal temperature variation in the Sahelian zone of West Africa requires use of rice varieties that are adapted to cold stress experienced during the Harmattan period. In these studies, analysis of variance for grain yield and related traits for the 224 rice genotypes showed that performance of genotypes varied significantly between sowing dates. These findings were in agreement with previous works, which allowed to discriminate cold tolerant from the sensitive genotypes [8,10,11]. The implication is that separate breeding for adaptation to dry or wet season cultivation should be undertaken. Generally, yields were highest for the January sowing date when cold stress coincided with the vegetative stage, followed by the normal July sowing date. Yields were lowest for the October sowing date, which mimicked late sowing during the wet season.

3.2. Impact of Cold Stress on Grain Yield and Agro-Morphological Traits

The analyses performed on the nine agronomic parameters taking into account the three sowing dates revealed the negative impact of low temperatures, based on the development phase, on the parameters directly related to performance of rice. Relative to the normal July sowing date for irrigated rice in the Sahel zone, cold stress at the reproductive stage severely reduced grain yield, harvest index, panicle exsertion and panicle length while increasing crop duration and spikelet sterility. The large number of varieties that had a negative panicle exsertion indicated an inhibition of the release of the panicle out of the sheath by cold stress. In this case, the spikelets covered by the flag leaf are not pollinated. Cold stress has a more direct effect on spikelet fertility because when it occurs during microsporogenesis, it causes degeneration of microspores, resulting in sterility cold stress [18]. Included in the group of cold tolerant rice genotypes for sowing Date 1 were V5 (87041-TR 990-11-2-1), V6 (88021-TR 1046-2-1-2-1), V13 (89014-TR 1134-2-2-3), V15 (89018-TR 1138-4-2-1), V18 (83025-TR 643-1-1-1-1), V35 (IR 57257-34-1-2-1), V69 (NONG 56), V162 (FANDRAPOTSY 104) and V169 (BOTRYKELY) that had high yields and good cold tolerance traits even after experiencing cold stress at the reproductive stage. In the PCA plots, IR 36 (V223) and Sahel 108 (V224), the cold sensitive checks, lay with a group of sensitive genotypes including V21 (China 1039), V26 (HEXI 24), V56 (IR 80098-38-3-1-2), V76 (PSB RC 94), V77 (PSB RC 96), V94 (SU 98), V100 (HAMNAM 14), V115 (PAVLOVSKY), V145 (RASSI), V146 (ITA 212), V147 (JAYA), V148 (NERICA-L19), V153 (WAS62-B-B-14-1), V154 (WAS62-B-B-17-1-1-3), V155 (WAS55-B-B-2-1-2-5), V194 (SONA), V196 (THAPACHINIYA) and V197 (TOKAMBANY 663). This group had no yields, was 100% sterile and had poor panicle exsertion for the first sowing date. Thus, late sowing in the Sahel during the rainy season which exposes the reproductive stage of rice to cold stress will lead to severe yield loss in sensitive varieties such as Sahel 108, the most widely cultivated variety in Senegal. These effects of cold stress on rice are similar to those reported earlier by Kaneda *et al.* [19] and Zenna *et al.* [20]. Besides reducing grain yield, cold stress at the reproductive stage especially in the sensitive genotypes, also increased the proportion of partially-filled grains which are more prone to breakage during milling compared to well-filled grains. It will reduce the quality of milled rice leading to low milling recovery.

On the other hand, when cold stress occurred at the vegetative stage, the rice crop on average produced more biomass, had a higher yield and matured later than during the normal July sowing date. Thus cold stress at the vegetative stage could be exploited to increase rice yields although varieties should be selected that combine high yields with little delay in maturity. Delayed maturity, which is induced in sensitive varieties by cold stress, leads to increased production costs due to the higher consumption of irrigation water and other inputs such as fertilizers and herbicides. A group of cold tolerant genotypes was identified for the second sowing date when cold stress occurred at the vegetative stage, which had good yields, short duration and high biomass such as V8 (88024-TR 1049-6-1-2-1), V9 (88076-TR 1101-9-2-1), V10 (88088-TR 1113-4-1-1), V11 (88090-TR 1115-4-1-6), V20 (BR28), V21 (China 1039), V46 (IR73944-1-2-2), V78 (RCPL -3-2), V51 (IR73689-31-1), V62 (Jarrah), V88 (Skau 382), V207 (Suparica 1) and V220 (Fofifa3737). This group also has great potential for double cropping in the Sahel.

The importance of choice of cold tolerant donor parent can be seen in some of the genotypes such as V21 (China 1039) and V115 (PAVLOVSKY), which had good yields and early or medium durations and high spikelet fertility with vegetative stage cold tolerance but were 100% sterile, gave no yields and had poor panicle exsertion with reproductive stage cold stress. Such lines should only be used in situations where cold stress coincides with the seedling stage such as December and January in the Sahel for the dry season rice crop.

3.3. Association of Morpho-Physiological Traits with Grain Yield under Cold Tolerance Stress

Cold tolerance at the reproductive stage was associated with earliness, high harvest index, low sterility, good panicle exsertion and high biomass production. This assertion confirms the results of Sharifi [20] suggesting that the early maturing variety was more tolerant to cold stress. Cold stress also delays maturity, which could enable the rice crop to accumulate more biomass needed for high yield. This explains the positive correlation between biomass and crop duration. In our study, high yields under reproductive stage cold stress were associated with earliness which implies that despite the cold stress, tolerant genotypes were able to produce high biomass and mature earlier than sensitive genotypes. When cold stress occurred at the vegetative stage only, grain yield was more strongly associated with biomass, tillering ability and sterility than with maturity. Even though such associations were manifested during the first sowing date, such cold tolerant genotypes will be useful for farmers who cultivate dry season rice. This will enable farmers to plant during the cold December or January months in the Sahel and harvest in May thus enabling farmers to complete post-harvest operations and prepare their fields before the main cropping season, which begins in July. Panicle exsertion was also affected by cold stress which also results in further yield loss because poorly exserted panicles have high sterilities since the rice grains covered by flag leaf sheaths at maturity are often sterile.

4. Materials and Methods

4.1. Plant Material and Site Characteristics

The plant material studied consisted of 224 rice (Table A1) varieties with different origins. Silewah, a traditional variety from Indonesia, was used as the international cold tolerant check and IR36 was used as the international cold sensitive check. Sahel 108, which is the most widely cultivated irrigated lowland rice variety in Senegal, was used as the local sensitive check.

The trials were conducted at the research station of the Africa Rice Center located at Ndiaye (Latitude 16°32.141 N, Longitude 15°11.545 W), (Senegal), using three different sowing dates: 18 October 2010 (Date 1); 17 January 2011 (Date 2); and 6 July 2011 (Date 3). The soil at the site is hydromorphous with a pH ranging from 4.2 to 6.5.

The first sowing date (Date 1) was chosen so that the reproductive phase of the rice crop coincided with low temperatures while the second sowing date (Date 2) exposed the vegetative stage of the rice crop to cold stress. The third sowing date (Date 3) was the normal sowing date during which the rice crop grows from seedling to maturity stage without experiencing any cold stress.

4.2. Experimental Design

An augmented design was used for the three sowing dates using five incomplete blocks with 48 entries per block including 4 repeated checks (Sahel 108, Silewah and IR36). The plot size was 1 m^2 with 20 cm between hills, within and between rows.

4.3. Cultural Practices

All three trials were direct seeded. Pre-germinated seeds were used on wet puddled soil with 20 cm × 20 cm spacing at a rate of 3 seeds per hill which was later thinned to one seedling per hill after germination. Fertilizer application was performed in accordance with local recommendations as follows—150 Kg/ha N, 60 Kg/ha P_2O_5 and 60 Kg/ha K_2O. The first application was made 20 days after sowing using urea (46% N), Di-Ammonium Phosphate (DAP) (18% N, 46% P) and KCl (60% K). Nitrogen was given in three split applications with 40% as basal application, 40% at tillering and 20% at panicle initiation. Weeds were controlled with the herbicide Bensulfuron methyl (Londax) at 100 g/ha and by hand weeding.

4.4. Parameters Measured

4.4.1. Air and Water Temperature

The air and water temperatures during the trial were measured using digital thermometers (TGP-4520, Gemini Data Loggers, 2011, Chihester, England) and a nearby weather station. Average maximum (max) and minimum (min) water temperatures were recorded daily with digital thermometers, which were placed in two different blocks.

4.4.2. Crop Measurements

Agro-morphological traits were measured following the Standard Evaluation System of IRRI (IRRI, 2002). Traits measured included plant height and tiller number at maturity, number of days to attain physiological maturity, panicle length and panicle exsertion. Total plant biomass, yield and yield components were estimated from three hill samples collected from the interior of the plots. Four yield components were measured, namely, number of panicles per m^2, number of grains per panicle, thousand grain weight and percent sterility. Yield was computed on an individual plant basis, which was later converted to kg/ha using the formula:

$$\text{Grain yield (kg/ha)} = (\text{grain weight per plant (g)} \times (25\,\text{plants/m}^2 \times 10)$$

which was derived from (grain weight per plant (g)/1000) × (25 plants/m^2 × 10,000 m^2).

4.5. Statistical Analysis of Data

For all traits measured, data from the three sowing dates were combined and analysis of variance (ANOVA) and correlation analysis were conducted using R software, (R Development Core Team, 2011, version 3.1.1). In order to determine the traits most closely associated with grain yield for the different sowing dates, a standardized Principal Component Analysis (sPCA) was performed with R software [21] using the adjusted means of the measured traits separately for each sowing date.

5. Conclusions

Our data showed that low temperature stresses at the reproductive stage leads to a lengthening of the crop duration, and reduced panicle exsertion, spikelet fertility and grain filling. The consequence of all these phenomena is the drastic decrease in the yield of susceptible varieties. However, cold stress at the vegetative stage delayed maturity and increased biomass and yield compared to the normal July sowing date for irrigated rice in the Sahel. Cold stress at either the vegetative or reproductive stages delayed maturity. Thus in cold tolerance breeding programs, earliness should be a priority trait in both parental and breeding line selection. Cold tolerant lines identified in our study can be used either as parents or proposed directly to farmers after validation trials and consumer acceptability tests. The variable associations between grain yield and agro-morphological traits under vegetative or reproductive cold stress can be exploited in cold tolerant rice breeding programs aimed at developing rice varieties adapted to double cropping in the Sahelian zone of West Africa.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix

Table A1. List of genotypes used in this study.

Number	Genotype	Origin	Subspecies	Number	Genotype	Origin	Subspecies
1	82079-TR489-3-1-1	TURKEY	*Japonica*	29	HR 17570-21-5-2-5-2-2-1-5	PHILIPPINES	*Japonica*
2	86011-TR 888-2-1-2-1	TURKEY	*Japonica*	30	IR 776-74-3B-1-1-12-5	IRRI	*Indica*
3	87020-TR 968-1-1-1	TURKEY	*Japonica*	31	HWASEONGBYEO	KOREA	*Japonica*
4	87024-TR 972-6-3-1	TURKEY	*Japonica*	32	IR 49830-7-1-2-3	IRRI	*Indica*
5	87041-TR 990-11-2-1	TURKEY	*Japonica*	33	IR57107-2B-12-2-2-2	IRRI	*Indica*
6	88021-TR 1046-2-1-2-1	TURKEY	*Japonica*	34	IR 59471-2B-20-2-1	IRRI	*Indica*
7	CHANDANATH-3	TURKEY	*Japonica*	35	IR 57257-34-1-2-1	IRRI	*Indica*
8	88024-TR 1049-6-1-2-1	TURKEY	*Japonica*	36	IR 60059-4B-4B-4-1-1-2-1	IRRI	*Indica*
9	88076-TR 1101-9-2-1	TURKEY	*Japonica*	37	IR 64	IRRI	*Indica*
10	88088-TR 1113-4-1-1	TURKEY	*Japonica*	38	IR 64629-6-2-2-2	IRRI	*Indica*
11	88090-TR 1115-4-1-6	TURKEY	*Japonica*	39	IR 65469-161-2-2-3-2-2	IRRI	*Indica*
12	89010-TR1130-8-1-1-2	TURKEY	*Japonica*	40	IR 66097-8-1-1-1	IRRI	*Indica*
13	89014-TR 1134-2-2-3	TURKEY	*Japonica*	41	IR 68333-R-R-B-22	IRRI	*Indica*
14	69016-TR 1136-1-1-1	TURKEY	*Japonica*	42	IR 68349-131-2-2-3	IRRI	*Indica*
15	89018-TR 1138-4-2-1	TURKEY	*Japonica*	43	IR68399-78-2-3-3-1	IRRI	*Indica*
16	90040-TR 1232-4-1-1	TURKEY	*Japonica*	44	IR 71121-35-1-1-1-2	IRRI	*Indica*
17	90051-TR 1243-2-2-1	TURKEY	*Japonica*	45	IR 71131-BF 4-B-30-5	IRRI	*Indica*
18	83025-TR 643-1-1-1-1	TURKEY	*Japonica*	46	IR 73944-1-2-2	IRRI	*Indica*
19	BR1543-9-2-1	BANGLADESH	*Indica*	47	IR 73305-14-2-2	IRRI	*Indica*
20	BR 28	BANGLADESH	*indica*	48	IR73688-82-2-3-2-2	IRRI	*Indica*
21	CHINA 1039	INDIA	*Indica*	49	IR 73688-82-3	IRRI	*Indica*
22	CT6744-F2-CA-8	CHILE	*Japonica*	50	IR 84645-305-61-1-B	IRRI	*Indica*
23	GAUTAM	INDIA	*Japonica*	51	IR 73689-31-1	IRRI	*Indica*
24	GWANSAN 2	DPR KOREA	*Japonica*	52	IR 73690-7-2-1-1-3-2-2-1	IRRI	*Indica*
25	H231-59-3-1	ARGENTINA	*Japonica*	53	IR 74506-28-4-3-2-1-3-2-2	IRRI	*Indica*
26	HEXI 24	CHINA	*Japonica*	54	IR 74520-29-4-2-2-2-4-1-1	IRRI	*Indica*
27	HEXI 25	CHINA	*Japonica*	55	IR 76687-22-1-3-2-5	IRRI	*Indica*
28	HR17512-11-2-3-1-4-2-3	PILIPPINES	*Japonica*	56	IR 80098-38-3-1-2	IRRI	*Indica*
57	PSB RC28	IRRI	*Indica*	92	SR22746-68-2-3-4-2-4	KOREA	*Japonica*

Table A1. *Cont.*

Number	Genotype	Origin	Subspecies	Number	Genotype	Origin	Subspecies
58	PSB RC 44	IRRI	*Indica*	93	STEJAREE 45	RUSSIA	*Japonica*
59	PSB RC 64	IRRI	*Indica*	94	SU 98	DPR KOREA	*Indica*
62	JARRAH	AUSTRALIA	*Japonica*	95	SUREX 95	TURKEY	*Japonica*
63	K-39-96-1-1-1-2	INDIA	*Indica*	96	WON 124	DPR KOREA	*Japonica*
64	CHANDANATH-1	NEPAL	*Japonica*	97	YR1076-8-4-1-2-3-1-2	KOREA	*Japonica*
65	MAHSURI	MALAYSIA	*Indica*	98	ZHI 20-5	CHINA	*Japonica*
66	MANAW THUKHA	MALAYSIA	*Indica*	99	CHOJANG	KOREA	*Japonica*
67	MILYANG15	KOREA	*Japonica*	100	HAMNAM 14	DPR KOREA	*Japonica*
68	MILYANG 55	KOREA	*Japonica*	101	HAMNAM15	DPR KOREA	*Japonica*
69	NONG 56	DPR KOREA	*Japonica*	102	HWANGHAEZO	DPR KOREA	*Japonica*
70	NR 11	VIETNAM	*indica*	103	NONG 57	DPR KOREA	*Indica*
71	OLBYEO1	KOREA	*Japonica*	104	RYONGSONG12	KOREA	*Indica*
72	OM 987-1	VIETNAM	*Indica*	105	YUNLEN 4	CHINA	*Japonica*
73	PANDA	ITALY	*Indica*	106	X-JIGNA	ETHIOPIA	*Japonica*
74	PSB RC 4	IRRI	*Indica*	107	WD1 DALAN KAOUYE	NIGER	*Indica*
75	PSB RC 92	IRRI	*Indica*	108	TY 53	NIGER	*Indica*
76	PSB RC 94	IRRI	*Indica*	109	DS 10	NIGER	*Indica*
77	PSB RC 96	IRRI	*Indica*	110	TY 47	NIGER	*Indica*
78	RCPL-3-2	INDIA	*Indica*	111	TY 32	NIGER	*Indica*
79	RCPL-3-6	INDIA	*Indica*	112	DS 4	NIGER	*Indica*
80	ROJOFOTSY 653	MADAGASCAR	*Japonica*	113	IR 84649-97-2-B-B	IRRI	*Indica*
81	SANT ANDEREA	ITALY	*Japonica*	114	IR 8	IRRI	*Indica*
82	SIM 2 SUMADEL	PHILIPPINES	*Indica*	115	PAVLOVSKY	RUSSIA	*Japonica*
83	SKAU 105	INDIA	*Japonica*	116	PLOVDIV 22	RUSSIA	*Japonica*
84	PALUNG-2		*Japonica*	117	CALORO	USA	*Japonica*
85	SKAU 27	INDIA	*Japonica*	118	SAHEL 177	AFRICARICE	*Indica*
86	SKAU337	INDIA	*Japonica*	119	IR84649-50-1-B	IRRI	*Indica*
87	SKAU 339	INDIA	*Japonica*	120	KOSHIHIKARI	JAPAN	*Japonica*
88	SKAU 382	INDIA	*Japonica*	121	L 202	USA	*Japonica*
89	SKUAT 27	INDIA	*Japonica*	122	IR 50	IRRI	*Indica*
90	SR 13349-59-1	KOREA	*Japonica*	123	JUMALI MARSHI	NEPAL	*Japonica*
91	SR18518-BF-4-B-12-1-2	KOREA	*Japonica*	124	PADI LABOU ALUMBIS	MALAYSIA	*Japonica*
125	KANTO	MADAGASCAR	*Japonica*	155	WAS55-B-B-2-1-2-5	AFRICARICE	*Indica*
126	DOONGARA	AUSTRALIA	*Japonica*	157	WAS200-B-B-1-1-1	AFRICARICE	*Indica*
127	C21	PHILIPPINES	*Indica*	158	WAS122-IDSA-11-WAS-6-3	AFRICARICE	*Indica*
128	WAS187-7-WASB-1-WAS1	AFRICARICE	*Indica*	159	WAS194-B-1	AFRICARICE	*Indica*
129	LENG KWANG	CHINA	*Japonica*	160	WAS114-B-IDSA-B-WAS-1-5-FKR-1	AFRICARICE	*Indica*
130	KUNMINGXIAOBEIGU	CHINA	*Japonica*	161	87025-TR 973-3-1-1	TURKEY	*Japonica*
131	DOURADO AGUILHA	BRAZIL	*Japonica*	162	FANDRAPOTSY 104	MADAGASCAR	*Japonica*
132	THANGONE	LAOS	*Japonica*	163	93-11	BHUTAN	*Indica*
133	JUMALI	NEPAL	*Japonica*	164	AL CHIAO HONG	CHINA	*Japonica*
134	PADI SASAHAL	MALAYSIA	*Indica*	165	BETSILAIZIAN	MADAGASCAR	*Japonica*
135	MITAK	INDONESIA	*Japonica*	166	BODOMANO	MADAGASCAR	*Japonica*
136	SASANISHIKI	JAPAN	*Japonica*	167	BOTOHAVANA	MADAGASCAR	*Japonica*
137	SAHEL 201	AFRICARICE	*Indica*	168	BOTRA MAITSO	MADAGASCAR	*Japonica*
138	SAHEL 202	AFRICARICE	*Indica*	169	BOTRYKELY	MADAGASCAR	*Japonica*
139	SAHEL 134	AFRICARICE	*Indica*	170	BOTRY 731	MADAGASCAR	*Japonica*
140	SAHEL 159	AFRICARICE	*Indica*	171	GAJPATI	NEPAL	*Indica*

Table A1. *Cont.*

Number	Genotype	Origin	Subspecies	Number	Genotype	Origin	Subspecies
141	SAHEL 208	AFRICARICE	*Indica*	172	GOPAL	NEPAL	*Indica*
142	SAHEL 209	AFRICARICE	*Indica*	173	JENJAR	NEPAL	*Indica*
143	SAHEL 210	AFRICARICE	*Indica*	174	KATI	BHUTAN	*Indica*
144	IR 84649-231-1-1-B	IRRI	*Indica*	175	MACHA PUCHRE-3	NEPAL	*Japonica*
145	RASSI	INDIA	*Indica*	176	KITRANA 508	MADAGASCAR	*Japonica*
146	ITA 212	AFRICARICE	*Indica*	177	LAL AMAN	MADAGASCAR	*Japonica*
147	JAYA	INDIA	*Indica*	178	LATSIBOZAKA-112-1	MADAGASCAR	*Japonica*
148	NERICA-L19	AFRICARICE	*Indica*	179	LOHAMBITRO 224	MADAGASCAR	*Japonica*
149	NERICA-L40	AFRICARICE	*Indica*	180	MACAN BINUNDOK	MADAGASCAR	*Japonica*
150	GAMBIAKA KOKUM	MALI	*Indica*	181	MADINIKA 1329	MADAGASCAR	*Japonica*
151	WAS127-12-1-2-1	AFRICARICE	*Indica*	182	MAKALIOKA 34	MADAGASCAR	*Japonica*
152	WAS21-B-B-20-4-3-3	AFRICARICE	*Indica*	183	MALADY	MADAGASCAR	*Japonica*
153	WAS62-B-B-14-1	AFRICARICE	*Indica*	184	MAMORIAKA	MADAGASCAR	*Japonica*
154	WAS62-B-B-17-1-1-3	AFRICARICE	*Indica*	185	MENAHODITRA 1234	MADAGASCAR	*Japonica*
186	NGAJA	BHUTAN	*Indica*	206	VARY VATO 154	MADAGASCAR	*Japonica*
187	PA TOU HUNG	CHINA	*Indica*	207	SUPARICA 1	ETHIOPIA	*Japonica*
188	PURBIA	NEPAL	*Indica*	208	AD01	ETHIOPIA	*Japonica*
189	RAY JAZAYKAYZ	BHUTAN	*Indica*	209	DEMWOZE	ETHIOPIA	*Japonica*
190	RAY NABJA	BHUTAN	*Indica*	210	NERICA 6	AFRICARICE	*Japonica*
191	ROJOFOTSY 693	MADAGASCAR	*Japonica*	211	AD 012	ETHIOPIA	*Japonica*
192	ROJOMENA 1034	MADAGASCAR	*Japonica*	212	MTU-1001	ETHIOPIA	*Indica*
193	SHORT GRAIN	THAILAND	*Japonica*	213	AURAT-7	ETHIOPIA	*Indica*
194	SONA	IRAN		214	AD 048	ETHIOPIA	*Japonica*
195	TELOVOLANA 177	MADAGASCAR	*Japonica*	215	GUMARA (IAC 164)	ETHIOPIA	*Japonica*
196	THAPACHINIYA	NEPAL	*Indica*	216	FOFIFA 3730	MADAGASCAR	*Japonica*
197	TOKAMBANY 663	MADAGASCAR	*Japonica*	217	BG 90-2	ETHIOPIA	*Indica*
198	TOKAMBANY 669	MADAGASCAR	*Japonica*	218	WAB 189-B-B-B-HB	ETHIOPIA	*Japonica*
199	TSAKA	MADAGASCAR	*Japonica*	219	KOKIT (IRAT-209)	ETHIOPIA	*Japonica*
200	TSIPALA 1231	MADAGASCAR	*Japonica*	220	FOFIFA 3737	ETHIOPIA	*Japonica*
201	TSIPALA B160	MADAGASCAR	*Japonica*	221	DIAMANTE	CHILE	*Japonica*
202	TSIPALAFOTSY 1883	MADAGASCAR	*Japonica*	222	SILEWAH	INDONESIA	*Japonica*
203	TSIPALA MENA 626	MADAGASCAR	*Japonica*	223	IR 36	IRRI	*Indica*
204	VARY LAVA DE MAROVATO	MADAGASCAR	*Japonica*	224	SAHEL 108	AFRICARICE	*Indica*
205	VARY MADINIKA 3494	MADAGASCAR	*Japonica*				

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