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Evaluation of Yield Indices and Identification of Yield Responsive Genes (Gn1a and Gs3) of Selected Rice Genotypes

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ABSTRACT

This research aimed at evaluation of yield Indices and identification of yield responsive Genes (Gn1a and Gs3) of selected rice Genotypes. A total of ten rice genotypes including four improved rice (high yielding) genotypes and six local rice (landraces) genotypes obtained from Maslaha seeds limited Gusau, Zamfara State were collected. The Data was collected for plant height, tiller number, number of panicles, panicle length, panicle weight and days to 50% flowering were recorded, grain length and seed dimension was measured. The genomic DNA of the Rice accession was extracted using a standard DNA extraction Kit (Plant/Seed Miniprep kit). The PCR reaction was set using a total of 40 ul reaction mixture containing 4ul 10x dream taq green buffer, 1.0 ul of dNTPs, 2.0 ul of each primer, 0.4ul of dream taq total nucleic acid polymerase, 2ul total nucleic acid template and 28.6 ul of molecular grade water. The Data collected for grain yield attribute and seed germination was subjected to one way analysis of variance (ANOVA) using statistical analysis system (SAS Version 9.4). The results were expressed as mean + Standard Deviation (SD) of three replicates and the differences between the means were separated using Duncan's new multiple range test (DNMRT). The result showed that significant differences exist amongst entries for plant height at maturity. Panicle length observed in this study was significantly different in all the entries with FARO67 (25.92cm) recorded the highest compared to genotype Bvfort (20.37cm) which had the lowest. PCR products revealed the presence of Gn1a and Gs3 genes in the ten rice cultivars screened. Eight rice cultivars (FARO44, FARO59, FARO67, Bvfort, Fanjim, Kamrun, Maikwalli and Jamila) have Gn1a and Gs3 gene and shows during PCR Analysis while the remaining two cultivars (FARO60 and Danruwa) have the gene but do not show during the PCR analysis. This investigation therefore, recommends that accessions like Danruwa, kamrun and FARO60 which showed desirable yield, quantitative and qualitative traits in this study should be used for further rice improvement programmes.

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Introduction

Rice (*Oryza*) belongs to the family Graminae (formerly Poceae), sub family Oryzoidae and is consumed by more than fifty percent of the world's population especially in developing countries. The third highest cereal produced is rice after wheat and maize [1]. It can grow for more than a year under favourable conditions and are well adapted to aquatic habitats. Rice leaves are flattened and elongated with inflorescence made up of spikelets bearing flowers which are largely self- pollinating and produce monocotyledonous seeds. *Oryza sativa*, one of the two most widely grown species of rice, is believed to have been domesticated from wild grass *Oryza rufipogon* about 12,000 years ago in China while *Oryza glaberrima* was domesticated from *Oryza barthii* later in West Africa over 3500 years ago [2]. Improvement of the rice grain yield per unit area is the only potential way to achieve increased rice production because of the reduction in area devoted to rice production [3]. Thus, to increase the yield potential of rice, many strategies have been developed including new plant type and exploiting hybrid vigour through hybrid rice varieties. Identification of genes contributing to higher yield, disease and pest resistance, salinity, temperature and drought tolerance from wild weedy and land races in rice are possible if the selection strategy of the parental lines shifts from looking for the phenotype to looking for the genes with the aid of genetic linkage maps and molecular markers. Rice grain yield is a quantitative polygenic trait and highly influenced by environment [4].

For rice breeders, identification of suitable genotypes containing these agronomic traits for grain yield determination should be the first critical step toward breeding high yield hybrid rice. In the last few decades, many QTLS related to rice grain yield were identified. Amongst which include grain number 1a (gn1a), the first gene to be isolated that controls rice grain number reported by Ashikari et al. Grain size 3 (Gs3) was the first major gene to be isolated that determine rice grain size, then grain weight and width2 (Gw2) that influences rice grain width and weight and grain incomplete filling 1 (Gif1) that regulates rice grain filling [5,6]. To date, a number of other grains yield related genes have been reported in rice genotypes such as dense and erect protein 1 (Dep1) that determines rice panicle architecture. [7-9]. The Osspl14 that promotes panicle branching and higher grain yield in rice [10]. The Gs2, Gs5 and Gs6 that regulates grain size, DST and Osspl13/Glw7 that regulates length of grains [11-15].

Increasing rice production can be fully exploited with various gene pool resources and its suitable environment to obtain desirable high yields and some stress tolerance. The development of new rice lines

with the improvement of the yield of required information related to the combination of parental abilities and gene action involved in the expression of maturity and morphological characteristics of plants [16]. The selection of assorted parents for hybridization must therefore be based on the ability of the different parental lines to combine. In preparing the program efficiently and effectively, the analysis of genetic variance and the mode of inheritance of quantitative and qualitative traits are of primary importance. Genetic properties of the breeding material and the environmental conditions under which experiments are conducted determine the heritability of any traits [17]. Thus, a greater range of genetic diversity, high heritability and high genetic advance play a key role to enhance rice yield.

Globally, improving quantity and quality of rice grain has been approached to solve several problems among the world population such as decreasing the number of hidden hunger and malnutrition [18].

Morphological traits have been used to assess the genetic variation and relationships among populations of rice [19,20]. It is considered as the primary step in the classification and evaluation of germplasm [21]. These qualitative characters are crucial for plant description and influenced by consumer preference, socio-economic scenario and natural selection. Several morphological characters are the major determining factors of rice grain yield including number of panicles per hill, the number of filled grains per panicle, and weight of filled grain per hill [22,23]. This research aimed at evaluating the yield attributes and identification of GN1a and GS3 genes in selected rice genotypes with a view to determine high yielding genotypes. The objectives are to; determine grain yield attributes (panicle weight, panicle type and grain number) of the selected rice genotypes and amplify the yield responsive genes (GN1a and GS3) of the selected rice genotypes using polymerase chain reaction (PCR).

Materials and Methods

Study Area

This research work was carried out in plant physiology laboratory and biological garden of Usmanu Danfodiyo University, Sokoto located within the latitude 13°129853N, longitude 5°203644E and altitude 302m above the sea level. Sokoto is located in the North West of Nigeria, between latitudes 13° 4' 07''North and longitudes 05° 14' 49''East and above 265m the sea level. The State accounts for 2.3% of Nigeria's total population. Situated in the North Western corner of Nigeria, Sokoto State territory occupies 25,973 square kilometres. Sokoto shares its borders with Niger Republic to the North, Zamfara State to the East, Kebbi state to the South-West [24]. The State has an estimated population of about 4,742,459 people as of 2015 with 95.9 persons per square kilometre, and 3% growth rate annually based on 2006 population census [25]. Occupation of city inhabitants includes farming, trading, commerce, with a reasonable proportion of the population working in private and public sectors.

Plant Materials and Origin of Rice Genotypes

A total of ten rice genotypes including four improved (high yielding) varieties and six landraces obtained from Maslaha seeds limited Gusau, Zamfara State were collected. The improved rice genotype samples included: FARO44, FARO59, FARO60, and FARO67. The landraces are Jamila, Dan-Ruwa, Bvfort, Fanjim, Kamrun and Mai Kwalli. The seeds were stored in 9cm ×4cm brown envelops that allowed for aeration and stored in refrigerator (4°C) before used.

Table 1: Rice Accessions and their Place of Collections

S/N	Genotypes	Ecotypes	Place of collection
1	Bvfort	Landrace	Kamrun Bajju
2	Dan ruwa	Landrace	Kamrun Bajju
3	Fanjim	Landrace	Kamrun Bajju
4	Jamila	Landrace	Maslaha, Gusau
5	Kamrun	Landrace	Kamrun Bajju
6	Maikwalli	Landrace	Maslaha, Gusau
7	FARO44	Released	Maslaha, Gusau
8	FARO59	Released	Maslaha, Gusau
9	FARO60	Released	Maslaha, Gusau
10	FARO67	Released	Maslaha, Gusau

Seed Multiplication

The seeds of the selected rice genotypes (10 entries) were sown on February 3rd, 2023 for seed multiplication using 1m x 1m plastic trays in the Biological Garden of UDUS, Sokoto using completely randomized design (CRD). Soils of the experimental site were sampled at 15cm depth and analyzed for Physico-chemical characteristics to reveal nutrients availability. At maturity, panicles were hand harvested and manually thrashed to obtain paddy. The crop was to be harvested when the grains are hard and are turning yellow/ brownish at 30 - 45 days after flowering or a month after 50 % flowering.

Data Collection

The Plant height (cm) was measured from soil surface to tip of the plant at reproductive stage using the meter rule, tiller number was obtained by counting the number of tillers per plant randomly per plot manually with hand and averaged across replications for each genotypes during the maturity stage, the total number of panicles was counted and recorded at the maturity stage before harvest, the extent to which the panicle is exerted above the flag leaf sheath and it was score using the scale of 1-9 as described by IRRI (2013). 1-Enclosed (panicle is partly or completely enclosed within the leaf sheath of the flag leaf blade), 3-Partly exerted (panicle base is seen slightly beneath the collar of the flag leaf blade), 5-Just exerted (panicle base coincides with the collar of the flag leaf blade), 7-Moderately well exerted (panicle base is seen above the collar of the flag leaf blade) and 9-Well exerted (panicle base is seen well above the collar of the flag leaf blade). The panicle length was measured from the base of the panicle to the tip. A total of five panicles were measured from each entry, Panicles weight was obtained from harvested samples of genotypes and weighed on a balanced scale to determine the weight in grams and Days to 50% flowering were recorded when half of the plant population started flowering.

Seed Germination for DNA Assays

Ten seeds of each accession were germinated in sterilized beakers and paper towel in laboratory conditions. Prior to germination, laboratory bench was surface clean with 70% ethanol to avoid risk of contamination. After germination, 10 days old seedlings were used for DNA and RNA isolation.

Extraction of Genomic DNA

The genomic DNA of the Rice accession was extracted using a standard DNA extraction Kit (Plant/Seed Miniprep kit) according to manufacturer's instruction. Beta-mercaptoethanol was added to the genomic lysis buffer to a dilution of 0.5% (v/v) i.e., 250 µl per 50ml or 500 µl per 100 ml for optimal performance. The

finely cut rice samples (~150 mg) was transferred to the Lysis Tube (2.0 mm) containing 750 µl BashingBead Buffer and process at maximum speed for ≥ 5 minutes.800µl was transfer to a Column in a collection Tube and centrifuge at 10,000 x g for 1 minute. 200 µl DNA pre wash buffer was added to the column in a new collection tube and centrifuge at 10,000 x g for 1 minute.500µl g-DNA Wash Buffer was added to the column and centrifuge at 10,000 x g for 1 minute, Column was transfer to a clean 1.5 ml micro centrifuge tube and add 100 µl (50 µl minimum) DNA Elution buffer directly to the column matrix centrifuge at 10,000 x g for 30 seconds to elute the DNA.Place a Filter in a clean collection tube and add 600 µl prep solutioncentrifuge at 8000 x g for 3 minutes. The elute DNA transfer to a prepared filter in a clean 1.5 ml microcentrifuge Tube and centrifuge at exactly 16000 x g for 3 minutes.

Primers Design

The forward and reverse primers of the selected genes were designed manually using VECTOR NTI (Version 11.5 Advanced) Software and the thermodynamic properties of each primer were checked using the default parameter settings of the software (Table 2).

Table 2: Yield responsive gene, respective primers with %GC Content, Tm and expected ban sizes

Gene	Forward primer, 5-3	Lgth	GC %	Tm	Reverse Primer, 5-3	Lgth	GC %	Tm	Expected Amplicon size, bp
Gn1a	GCCTTCCATCG TCAGCAC	18	61.1	51.5	GCAGTTGAGCA TGAGGAG	18	57.9	50.5	185
Gs3	CAAGTGCGTG CTGCCTCA	18	61.1	53.9	AGCGGCACGA GCATCAGC	18	64.7	52.5	390

Amplification of Gene using PCR

The PCR reaction was set using a total of 40 ul reaction mixture containing 4ul 10X dream taq green buffer, 1.0 ul of dNTPs, 2.0 ul of each primer, 0.4ul of dream taq total nucleic acid polymerase, 2ul total nucleic acid template and 28.6 ul of molecular grade water. Thermal-cycling was performed in a supercycler programmed as follows: 95°C for 3 min initial denaturation, 30 cycles of 95°C for 20 sec, 58°C for 30 sec annealing (for GS3 and GN1a) and 72°C for1 min extension and final extension cycle of 72°C for 5 min.

1% agarose gel stained with 5µlethidium-bromide in a horizontal electrophoresis tank system containing 1x TAE buffer. About 6ul of 100bp ladder were used at both edges. The electrophoresis run was programmed as follows: 35 min, current/100 milliamps (mA) and voltage/120 V. the gels were visualized and their snap shots taken in a gel documentation system (desktop gel imager, scope 21).

Data Analysis

The Data collected for grain yield attribute and seed germination was subjected to one way analysis of variance (ANOVA) using statistical analysis system (SAS Version 9.4). The results were expressed as mean + Standard Deviation (SD) of three replicates and the differences between the means were separated using Duncan's new multiple range test (DNMRT).

Results

The results on the evaluation of yield and identification of yield responsive genes (GN1a and GS3) in selected rice genotype were presented in this chapter.

Seed Percentage (%) Germination

The results of the percentage germination of both land races and released genotype were taking fourteen days after sowing. The seed germination percentage of the selected genotype varied from 73.4% to 100%. The accession Danruwa has the highest germination percentage of 100%, while the "FARO59" accession had the lowest percentage of 73.4%. The accessions, FARO44, Fanjim, Maikwalli, Jamila, FARO67, FARO60, Bvfort, and Kamrun had 80%, 80%, 80%, 86.6%, 93.4%, 93.4% and 93.4 respectively. However, the results differ significantly ($p<0.05$) as shown in Figure 1.

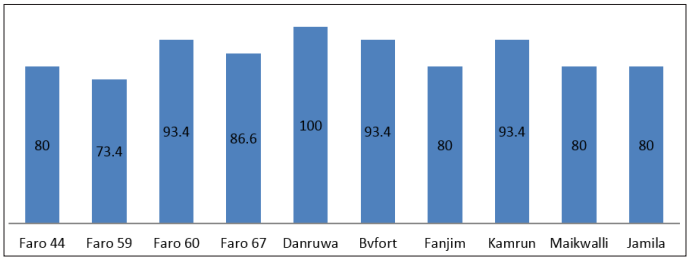

Figure 1: Seed Percentage (%) Germination

Morpho-Agronomic Traits

Significant differences exist amongst entries for plant height at maturity with Maikwalli (92.47cm) recorded the tallest plants while Danruwa (73.87cm) had the shortest plant height. Number of tillers counted were significantly different at ($p<0.05$) among the genotypes such that FARO60 (37.00) produced the highest number of tillers compared to Maikwalli (18.33) which produced the lowest tillers among all the entries. Panicle length observed in this study was significantly different in all the entries with FARO67 (25.92cm) recorded the highest compared to genotype Bvfort (20.37cm) which had the lowest as shown in Table 1.

There was significant difference in the entries for panicle number such that Kamrun (28.33) had the highest panicle number among the entries studied; Whereas Jamila (14.00) recorded the least panicle number. Grain number per panicle observed among the entries was not statistically different with FARO44 (172.69) recorded the highest number compared to Danruwa (141.33) which recorded the lowest among all the genotypes. Panicle weight was statistically different among all the genotypes such that Bvfort (3.03g) recorded the highest weight compared to FARO59 (1.47g) which recorded the lowest among all the genotypes as shown in Table 2.

Days to 50% flowering were significantly ($p<0.05$) different for all the entries such that Fanjim (126), Jamila (130) and Maikwalli (131) recorded the longest days to flowering compared to Danruwa (85) which was the shortest duration to 50% flowering amongst the entries. The individual performance in terms of grain yield of some rice genotypes is significantly different ($p<0.05$) for all the entries such that Kamrun (1771.70) recorded the highest yield

compared to FARO59 (637.20) which recorded the lowest yield per plant as shown in Table 3.

Panicle exertion was significantly ($p<0.05$) different among the genotypes with the following FARO59, FARO67, Danruwa, Fanjim and Jamila recorded the highest score (9) compared to FARO44, FARO60, Bvfort, Kamrun and Maikwalli which recorded the lowest score (3) as shown in Table 10. Panicle base was significantly ($p<0.05$) different among the genotypes with the following FARO59, FARO67, Danruwa, Fanjim and Jamila are well exerted at panicle base while FARO44, FARO60, Bvfort, Kamrun, and Maikwalli are partly exerted at panicle base and both of the varieties possess 2 clefts at the ligules as shown in Table 4.

Table 1: Mean Values for Plant Height (PH) Number of Tillers (NT) and Panicle Length (PL)

S/N	Genotype	PH	NT	PL
1	FARO44	78.57±7.24^{bc}	32.00±1.41^{ab}	22.28±1.30^{ab}
2	FARO59	77.83±1.63^{c}	28.00±0.71^{bc}	21.17±0.18^{ab}
3	FARO60	84.43±7.13^{abc}	37.00±1.41^{a}	26.38±2.11^{a}
4	FARO67	81.10±10.95^{bc}	24.33±1.47^{cd}	25.92±2.76^{a}
5	Dan ruwa	73.87±15.77^{d}	23.67±1.08^{cde}	21.64±0.39^{ab}
6	Bvfort	86.20±3.96^{abc}	24.00±1.41^{cd}	20.37±0.39^{b}
7	Fanjim	84.83±2.22^{abc}	20.67±2.19^{de}	20.50±0.31^{b}
8	Kamrun	82.47±20.24^{abc}	20.33±1.08^{de}	22.10±0.64^{ab}
9	Maikwalli	92.47±8.51^{a}	18.33±1.08^{e}	20.56±0.32^{b}
10	Jamila	90.10±2.72^{ab}	21.00±1.41^{de}	20.43±0.47^{b}

Values are Mean ± SD of biological triplicate, means with the same letters are not significantly different (P<0.05)

Table 2: Mean Values for Panicle Numbers (PN) Number of Grain per Panicle (NGPP) and Panicle Weight (PW)

S/N	Genotype	PN	NGPP	PW
1	FARO44	22.67±2.19^{abc}	172.69±19.86^{a}	2.40±0.10^{b}
2	FARO59	20.00±0.17^{bcde}	150.67±18.58^{a}	1.47±0.05^{c}
3	FARO60	26.00±2.83^{ab}	151.33±17.01^{a}	2.40±0.21^{b}
4	FARO67	17.67±1.78^{cde}	163.67±8.62^{a}	2.60±0.26^{ab}
5	Dan ruwa	20.33±1.08^{bcd}	141.33±9.50^{a}	2.83±0.21^{ab}
6	Bvfort	17.00±1.41^{cde}	166.33±6.66^{a}	3.03±0.15^{a}
7	Fanjim	14.33±1.08^{de}	159.33±22.90^{a}	2.70±0.10^{ab}
8	Kamrun	28.33±1.08^{a}	175.00±10.54^{a}	2.83±0.11^{ab}
9	Maikwalli	16.00±0.71^{de}	160.33±19.66^{a}	2.97±0.12^{a}
10	Jamila	14.00±0.71^{e}	157.00±13.00^{a}	2.67±0.25^{ab}

Values are Mean ± SD of biological triplicate, means with the same letters are not significantly different (P<0.05)

Table 3: Mean Values for Days to Fifty Percent Flowering (DF) and Grain Yield (GY)

S/N	Genotype	DF	GY
1	FARO44	104.00±6.00^{cde}	1204.80±73.20^{bc}
2	FARO59	91.00±4.73^{de}	637.20±41.30^{d}
3	FARO60	103.00±5.57^{cde}	1689.10±335.10^{a}
4	FARO67	107.00±9.64^{bcd}	1259.06±233.10^{b}
5	Dan ruwa	85.00±4.36^{e}	1204.10±64.30^{bc}
6	Bvfort	114.00±5.29^{abc}	1049.40±121.40^{bcd}
7	Fanjim	126.00±5.29^{ab}	795.20±112.20^{cd}
8	Kamrun	93.00±6.43^{de}	1771.70±83.30^{bcd}
9	Maikwalli	131.00±5.51^{a}	975.10±60.70^{a}
10	Jamila	130.00±12.72^{a}	759.70±39.20^{d}

Values are Mean ± SD of biological triplicate, means with the same letters are not significantly different (P<0.05)

Table 4: Panicle exertion of Ligule, Panicle Base and Scale

S/N	Genotype	Ligule Panicle base	Scale
1	FARO44	2 Cleft Partly exerted	3
2	FARO59	2 Cleft Well exerted	9
3	FARO60	2 Cleft Partly exerted	3
4	FARO67	2 Cleft Well exerted	9
5	Dan ruwa	2 Cleft Well exerted	9
6	Bvfort	2 Cleft Partly exerted	3
7	Fanjim	2 Cleft Well exerted	9
8	Kamrun	2 Cleft Partly exerted	3
9	Maikwalli	2 Cleft Partly exerted	3
10	Jamila	2 Cleft Well exerted	9

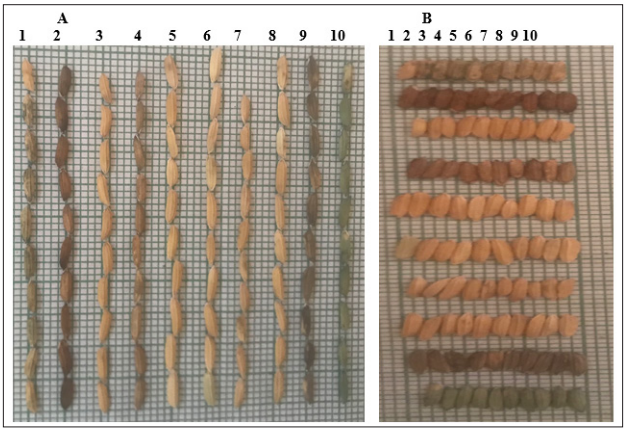

Plate 1: Grain characterization of selected rice genotypes (A) Grain length Analysis of rice genotype lane 1 = FARO44; lane 2 = FARO59; lane 3 = FARO60; lane 4 = FARO67; lane 5 = Danruwa; lane 6 = Bvfort; lane 7 = Fanjim; lane 8 = Kamrun; lane 9 = Maikwalli; lane 10 = Jamila (B) Grain width Analysis of rice genotype lane 1 = FARO44; lane 2 = FARO59; lane 3 = FARO60; lane 4 = FARO67; lane 5 = Danruwa; lane 6 = Bvfort; lane 7 = Fanjim; lane 8 = Kamrun; lane 9 = Maikwalli; lane 10 = Jamila

Gene Amplification

The amplification of selected yield responsive genes (GN1a and GS3) using Multiplex polymerase chain reaction (PCR) revealed that FARO59, FARO67, Bvfort, Fanjim, Kamrun, Maikwalli and Jamila on the gels shows double and clear bands using band size of 185 bp and 390 bp and FARO44 show only one band which is Gn1a.

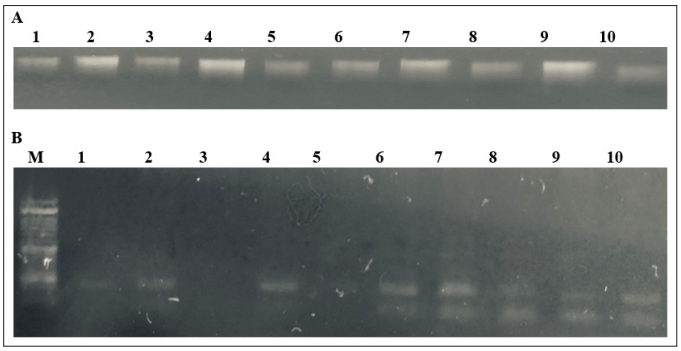

Figure 4: Molecular identification of Gene (A) Isolated genomic DNA of the selected rice genotypes Lane M = 100bp ladder; lane 1 = FARO44; lane 2 = FARO59; lane 3 = FARO60; lane 4 = FARO67; lane 5 = Danruwa; lane 6 = Bvfort; lane 7 = Fanjim; lane 8 = Kamrun; lane 9 = Maikwalli; lane 10 = Jamila (B) Amplified gene in both landraces and released verities using Multiplex PCR Analysis Lane M = 100 bp total nucleic acid ladder; lane 1 = FARO44; lane 2 = FARO59; lane 3 = FARO60; lane 4 = FARO67; lane 5 = Danruwa; lane 6 = Bvfort; lane 7 = Fanjim; lane 8 = Kamrun; lane 9 = Maikwalli; lane 10 = Jamila.

Discussion

The significant differences ($P<0.05$) among the accessions for days to 50% flowering in this study is similar to those previously reported by Weiya et al., who observed variation in days to flowering of several genotypes and identified a regulatory gene responsible for variation in this physiological trait among rice genotypes [26]. Variation among genotypes for this quantitative trait might be due to the genetic makeup of the genotype or interaction with the environment. The availability of early flowering and maturing genotypes are important for the avoidance of drought condition. The significant differences ($P<0.05$) among the accessions for tiller number in this finding is supported by that of Rahman et al. [27]. So also, highly significant ($P<0.05$) Variability among the accessions for this quantitative trait based on the data analysis was in agreement with earlier findings of Zahid et al., they studied twelve (12) genotypes of coarse rice and reported highly significant variation for various morphological traits including number of panicles per plant. Similar findings by Hassan et al. reported that genetic variation was responsible for the significant differences [28,29]. Analysis of variance for plant height that was not found to be significant ($P>0.05$) among the various accessions studied this is in conformity with findings of Kole and Hasib [30]. Hussain et al. reported that planting and sowing methods, transplanting date and soil condition affect plant height in rice [31].

The significant ($P<0.05$) difference observed among the genotypes for panicle exertion was in conformity with that of Sarawagi et al. who reported similar variation in rice genotypes for panicle exertion [32]. Panicles enclosed in leaf sheath consume time in harvesting or processing as the panicles have to be removed from the sheath. In the present study, it can be deduced that days to 50 % flowering, panicle weight, panicle number, tiller number, plant height, grain yield, were the most important traits which accounted for much of the variability among the rice genotypes, these findings agree with Caldo et al. [33]. This result corroborates with the findings of Rasheed et al. and Girish et al. also reported the positive association of yield per with plant height at genotypic level [34,35. This implies that increase in plant height may increase grain yield [36].

In the present study, the extraction method was based on standard DNA extraction Kit (Plant/Seed Miniprep kit). The band size of 185 bp and 390 bp Multiplex PCR products for Gn1a and Gs3 genes respectively on the gels shows double and clear bands for all the rice genotypes screened except lane 3 and 5 (FARO60 and Danruwa). This indicates that all the genotypes have both Gn1a and Gs3 genes. This is supported by the observed band sizes of 185 and 390 bp for Gn1a and Gs3 PCR positive results for the genotype while FARO60 showed no bands may belongs to the interspecific rice (NERICA) and as such may have different gene alleles.

The PCR assay did work because only two total nucleic acid-template lanes showed no band as expected. In the present study, lane 1 (FARO44) showed single band and 1 single weak band. The weak bands may be due to small copy numbers of the targeted DNA region within the extracted genomic total nucleic acid. Lorenz (2012) pointed out that it is not the concentration of the extracted total nucleic acid that actually counts rather the number of copies of the region targeted. He posited that copies of 104 to 107 of the targeted total nucleic acid region are required for PCR with total reaction volume of 50µl.

The two other sample genotypes (FARO60 and Danruwa) show no bands at all. This indicates that the two genotypes have no Gn1a and Gs3 gene or have a different variant of the gene resulting in primer mis-match with the complementary sequences. The puzzles can be solved by using degenerate primer sets which have the ability to amplify different variants of a gene instead of the sequence specific primers used in this research [37]. Furthermore, primer sets used to screen for Gn1a and Gs3 (not shown) require further optimization because none of the genotypes screened was amplified. These indicate that both the primer sets may be having regions of preference on the genomic total nucleic acid different from the targeted sequence on the positive control genotype. Moreover, success of PCR is critically dependent on the design of an effective primer pair [38]. PCR failures could be as result of polymorphisms such as SNP, indels and copy number variations [38]. Unexpected SNP in a designed primer, in particular in the 3‟ end (SNP-in primer), primers designed within the intron/exon boundaries or within repetitive DNA elements are possible reasons for PCR failures [39].

Conclusions

In this study, all the ten (10) genotypes evaluated showed better grain characteristics and germination. FARO60, Maikwalli, Bvfort and Kamrun accessions proved to be the best in terms of grain characteristics and FARO44, FARO60 and Kamrun proved to be the best better yield and as such its adoption could increase rice production and thus help restore Nation's hope of food security in the near future. The positive correlation that existed between grain yield and plant height, grain yield and panicle weight, grain yield and days to 50 % flowering should be used as basis for improvement of the rice varieties cultivated in the lowland environment.

Recommendations

Based on the results of the study, it is recommended that

- FARO44 and Maikwali which out yielded the standard check could be subjected to further trial to ascertain their level of performance
- Accessions like kamrun and FARO60 which showed desirable yield, quantitative and qualitative traits in this study should be used for further rice improvement programmes.
- Quantitative RT-PCR should be carried out to know the level of expression of the genes in those cultivars found to be positive of the genes screened and those found to be robustly expressed be transferred to other accessions for their yield improvement.

References

1. FAO (2012) (Food and Agricultural Organization of the United Nations) Rome, Italy, Statistical Data Base. http://faostat.fao.org.
2. Sweeney MS, McCouch (2007) The complex history of the rice domestication. Annual Botany 100: 951-957.
3. Cassman KG, Dobermann A, Walters DT, Yang H (2003) Meeting cereal demand while protecting natural resources and improving environmental quality. Annual Review of Environmental Resources 28: 315-358.
4. Ashfaq M, Khan AS, Khan SHU, Ahmed R (2012) Association of various morphological traits with yield and genetic divergence in rice (Oryza sativa). International Journal of Agriculture and Biology 14: 55-62.
5. Song XJ, Huang W, Shi M, Zhum z, Lin HX (2007) A QTL for rice grain width and weight encodes a previously unknown ring-type E3 ubiquitin ligase. Nature Genetics 39: 623-630.
6. Wang E, Wang J, Zhu X, Hao W, Wang L, et al. (2008) Control of rice grain-filling and yield by a gene with a potential signature of domestication. Nature genetics 40: 1370-1374.
7. Zhou Y, Zhu J, Li Z, Yi C, Liu J, et al. (2009) Deletion in a quantitative trait gene QPE9-1 associated with panicle erectness improves plant architecture during rice domestication. Genetics 183: 315-324.
8. Huang X, Qian Q, Liu Z, Sun H, He S, et al. (2009) Natural variation at the Dep1 locus enhances grain yield in rice. Nature genetics 41: 494-497.
9. Wang J, Nakazaki T, Chen S, Chen W, Saito H, et al. (2009) Identification and characterization of the erect-pose panicle gene Ep conferring high grain yield in rice. (Oryza sativa L) Theoretical and applied genetics 119: 85-91.
10. Miura K, Ikeda M, Matsubara A, Song XJ, Ito M, et al. (2010) Osspl14 promotes branching and higher grain productivity in rice. Nature genetics 42: 545-549.
11. Li Y, Fan C, Xing Y, Jiang Y, Luo L, et al (2011) Natural Variation in Gs5 Plays an Important Role in Regulating Grain Size and Yield in Rice. Nature genetics 43: 1266-1269.
12. Sun L, Li X, Fu Y, Zhu Z, Tan L, et al. (2013) Gs6, a member of the grass gene family, negatively regulates grain size in rice. Journal of Integrative Plant Biology 55: 938-949.
13. Hu J, Wang Y, Fang Y, Zeng L, Xu J, et al. (2015) A rare allele of Gs2 enhances grain size and grain yield in rice. Molecular Plant 8: 1455-1465.
14. Liu Z, Feng YQ, Yuan L, Li C (2013) Rice zinc finger protein DST enhances grain production through controlling Gn1a/Osckx2 expression. Proceedings of the national academy of science 110: 3167-3172.
15. Si L, Chen J, Huang X, Gong H, Luo J, et al. (2016) Osspl13 controls grain size in cultivated rice. Nature genetics 48: 447-456.
16. Karim KY, Ifie B, Dzidzienyo D (2020) Genetic characterization of cassava (Manihot esculenta crantz) genotypes using agro-morphological and single nucleotide poly- morphism markers. Physiology and Molecular Biology of Plants 26: 317-330.
17. Sabri RS, Rafii MY, Ismail MR (2020) Assessment of agro-morphologic performance genetic parameters and clustering pattern of newly developed blast resistant rice lines tested in four environments. Agronomy 10: 1098-1115.
18. Burchi F, Fanzo J. Frison E (2011) The role of food and nutrition system approaches in tackling hidden hunger. International Journal of Environmental Research and Public Health 8: 358-373.
19. Malek MA, Rafii MY, Afroz SS (2014) Morphological characterization and assessment of genetic variability, character association, and divergence in soybean mutants. Science world Journals 2014: 968796.
20. Pandey P, Irulappan V, Bagavathiannan MV (2017) Impact of combined abiotic and biotic stresses on plant growth and avenues for crop improvement by exploiting physio-morphological traits. Front Plant Science 8: 537.
21. Hien Nl, Sarhadi WA, Oikawa Y (2007) Genetic diversity of morphological responses and the relationships among Asia aromatic rice (Oryza sativa L.) cultivars. Tropics 16: 343-355.
22. Xing YZ, Zhang qF (2010) Genetic and molecular bases of rice yield. Annual Review Plant Biology 61: 421-422.
23. Zhou Y, Tao Y, Yuan Y (2018) Characterization of a novel quantitative trait locus, GN4-1, for grain number and yield in rice (Oryza sativa L) Theory of Apply Genetics 131: 637-648.
24. MOCIT (2002) Guide to Sokoto state's economic potentials. Commerce department, ministry of commerce, industry and tourism. Sokoto state pp: 4-18.
25. NPC Report of Nigeria (2006) National Population Commission on Census. Population Development Review 33: 206-210.
26. Weiya X, Yongzhong X, Xiaoyu W, Yu Z, Weijiang T, et al. (2008) Natural variation in Ghd7 an Important Regulator of Heading Date and Yield Potential in Rice. Nature Genetics 40: 761-767.
27. Rahman MM, Hussain A, Sayed MA, Ansari A, Mahmud MAA (2011) Comparison among clustering in multivariate analysis of rice using morphological traits, physiological traits and simple sequence repeat markers. Journal of Agricultural and Environmental Sciences 11: 876-882.
28. Zahid AM, Akhtar M, Anwar M, Adil J (2005) Genotypic and Phenotypic, Correlation and Path Analysis in Coarse Grain Rice. Proceeding of the International Seminar on Rice Crop. Rice Research Institute, 2-3 October, Kala Shah Kau, Pakistan.
29. Hassan G, Khan NU, Khan QN (2003) Effect of transplanting date on the yield and yield components of different rice cultivars under high temperature of DI Khan Science. Khy 16: 129-137.
30. Kole PC, Hasib KM (2008) Correlation and Regression Analysis in Scented Rice. Madras Agriculture Journal 95: 178-182.
31. Hussain S, Ramzan M, Aslam M, Zaheen M, Ehsan SM (2005) Effect of Various Stand Establishment Method on Yield and Yield Components of Rice. Proceeding of the International Seminar on Rice Crop. Rice Research Institute, 2-3 October, Kala Shah Kau, Pakistan.
32. Sarawgi AK, Subba-Rao LV, Parikh M, Sharm B, Ojha GC (2013) Assessment of the variability of rice (Oryza sativa L) germplasm using agro-morphological characterization.

Journal of Rice Research 6: 152-178.

33. Caldo RA, Sebastian LS, Hernandez JE (1996) Morphology-based Genetic Diversity Analysis of Ancestral lines of Rice in Philippine Rice Cultivars. Philippines Journal of Crop Science 21: 86-92.
34. Rasheed MS, Sadaqat HA, Babar M (2002) Correlation and path co-efficient analysis for yield and its components in rice. Asian Journal of Plant Sciences 1: 241-244.
35. Girish TN, Gireesha TM, Vaishali MG, Hanamareddy BG, Hittalmani S (2006) Response of New IR/Moroberekan Recombinant Inbred Population of Rice (Oryza sativa L) from Indica x japonica Cross for Growth and Yield Traits Under Aerobic Conditions. Journal of Euphytica 152: 149-161.
36. Hallauer AR, Miranda JB (1988) Quantitative Genetics in Maize Breeding. 2nd edition, Iowa State University Press.
37. Gahoi S, Arya L, Anil SS (2013) Dpprimer – a degenerate PCR primer design tool. Bioinformation 9: 937-940.
38. Kalendar R, Lee D, Schulman AH (2009) Fastpcr software for pcr primer and probe design and repeat search. Genes, genomes and genomics 3: 1-14.
39. Piriyaponga J, Ngamphiw C, Assawamakin A, Wangkumhang P, Suwannasri P, Ruangrit, et al. (2009) Rexprimer: an integrated primer designing tool increases PCR effectiveness by avoiding 3‟ SNP-in-primer and mis-priming from structural variation. BMC genomics 10: S4.