Available online at www.sciencedirect.com

ScienceDirect

The Crop Journal

Alternate phenotype–genotype selection for developing superior high-yielding irrigated rice lines☆

Yonnelle Dea Moukoumbi[a,b,*], Raafat El-Namaky[a,c], Koffi Djaman[a], Daouda Mbodj[a], Baboucarr Manneh[a]

[a]Irrigated Rice Breeding Unit, Africa Rice Center (AfricaRice), Sahel Regional Station, BP 96 Saint Louis, Senegal
[b]National Institute of Agricultural Research, Gros bouquet, PMB 16169, Libreville, Gabon
[c]Rice Research & Training Center (RRTC), 33717 Sakha Kafr Sheikh, Egypt

ARTICLE INFO

Article history:
Received 6 March 2017
Received in revised form 13 August 2017
Accepted 12 October 2017
Available online 25 October 2017

Keywords:
Marker
Rice
Pedigree selection
Yield

ABSTRACT

Increase grain yield potential is one of the most important objectives of any cereal crop breeding program. To efficiently develop superior rice lines by the introgression of favorable alleles for yield and yield component traits, a strategy of alternate phenotype–genotype selection was used. The present study aimed to (i) investigate the allelic diversity of loci associated with major yield-component traits and (ii) phenotype and genotype advanced populations derived from crosses between NERICA-L-20 and Giza178 for yield component traits using agro-morphological descriptors and GRiSP polymorphic markers to select superior high-yielding rice lines. A total of 100 $F_{2:3}$ progeny were selected from 1000 F_2 plants and genotyped with 16 polymorphic markers linked to four major yield-component traits. Four promising $F_{2:3}$ lines (ARS 563–14, ARS 563–62, ARS 563–286, and ARS 563–41) bearing combinations of desirable alleles were selected. A selected set of 20 $F_{2:4}$ lines showed moderate to high heritability for all target traits. Fourteen $F_{2:5}$ lines derived from ARS 563–14 and 17 $F_{2:5}$ from ARS 563–286 families were evaluated in preliminary trials to estimate yield gain. The three top lines, ARS 563–286–16-1-1, ARS 563–286–5-1-1, and ARS 563–14–10-1-1, showed an increase of more than 10% grain yield over the best check, Sahel 108, which is widely cultivated in the Senegal River valley. The 16 markers linked to the target yield component traits can be used to fast-track breeding programs targeting rice productivity.

1. Introduction

Rice is the second most important cereal crop in the world after maize in terms of cultivated area, with 158.8 Mha under production in 2016 [1]. Global paddy rice production was 2.9 Mt. to a record of 749.7 Mt. (497.9 Mt. on a milled basis). In Africa, the expected 2016 production was 29.7 Mt. (19.4 Mt., milled basis), implying a 4% year-on-year expansion and a

☆ Peer review under responsibility of Crop Science Society of China and Institute of Crop Science, CAAS.

* *Corresponding author.*
E-mail address: moukoumbiyonnelle@outlook.com (Y.D. Moukoumbi).

https://doi.org/10.1016/j.cj.2017.08.010

new record [2]. At the Yield Potential International Workshop held by the Global Rice Science Partnership (GRiSP) in 2011, it was asserted that worldwide demand for rice is expected to rise by >25% by 2035 [3]. Since the 1960s, many high-yielding rice varieties and breeding lines have been developed by the International Rice Research Institute, including *Oryza sativa* L. IR8, IR36, IR64, and IR72 [4]. During the 1990s, Africa Rice Center (AfricaRice) scientists developed high-yielding upland and lowland New Rice for Africa (NERICA) and irrigated Sahel varieties [5,6] which have been distributed to farmers and breeders worldwide.

Yield potential is defined as the maximum achievable yield in the absence of biophysical, physiological, or economic constraints on production [7]. Increasing rice yield potential is one of the most important contributions for any rice breeding program aimed at developing high-yielding varieties. High-yielding technologies that have been developed include "new plant type", "hybrid rice", and "super hybrid rice" adapted to specific cropping conditions [8–10]. Rice research in Egypt during the past 15 years has increased the national average yield by >66%, from 5.71 to 9.84 t ha^{-1} [11]. This increase was achieved by growing modern inbred varieties, which cover almost 100% of the total rice area in the country. In West Africa, the current average yield potential of irrigated rice varieties such as the widely grown Sahel varieties developed by pedigree selection ranges from 10 to 12 t ha^{-1} [12]. Increasing yield potential requires continuous phenotypic selection of desirable lines from a large number of segregating populations until fixation of the desired trait [13,14]. The numbers of plants to select at each generation may be modified according to the species, the breeding objective, and the genetics of the traits of interest. This method is labor-intensive and time-consuming and requires a large nursery or field space for screening. In the last decade, different approaches including the use of wide crosses and gene pyramiding through molecular approaches [15] have been used to improve rice yield potential. Physiological approaches using simulation models predicted that an increase in rice yield potential of 25% is possible by changing the traits of the current plant type [16]. Molecular techniques are continuously being used to increase the number of genes discovered, with the aim of understanding the formation of grain yield. Eight quantitative trait loci (QTL) controlling spikelet number per panicle and 1000-GW were mapped by sequencing-based genotyping of 150 rice recombinant inbred lines [15]. The effects of four QTL from Nipponbare using chromosome segment substitution lines were validated and the QTL were pyramided in rice popular varieties in Asia [15]. Yield is a complex trait controlled by many genetic factors associated with yield-component traits [17]. Favorable alleles have been "mined" from natural cultivars and wild rice. These rice lines are IR24, Kasalath, Koshihikari, Menghui 63, and Nipponbare, in which functional genes have been identified by association analysis of target traits such as grain weight (*GW5*) [18], grain size (*GS3*) [19], grain number (*Gn1a*) [20], and strong stems and heavy panicles (*SCM2*/*APO1*), [21,22]. Reasonable combinations of favorable alleles are being used to increase rice yield potential, combining key traits such as excellent plant type, strong stems, and long and heavy panicles with well-filled kernels [16]. Alternative pedigree selection methods and use of markers associated with major QTL to target traits can be used by scientists to select high-priority lines for each generation [14].

The objectives of the present study were to (i) investigate the allelic diversity of loci associated with high-yielding parental lines in the varieties NERICA-L-20 and Giza178, with the aim of developing ARS 563 populations, (ii) phenotype and genotype F_2, $F_{2:3}$, $F_{2:4}$, and $F_{2:5}$ populations using agro-morphological quantitative and qualitative descriptors, yield and yield component traits, and GRiSP polymorphic markers to select new, superior, high-yielding rice lines.

2. Materials and methods

2.1. Agro-morphological measurement and statistical analyses

The experiments were conducted at the AfricaRice Regional Research Center in St Louis, Senegal, (16°14′ N, 16°14′ W, 9 m a.s.l.). An allelic diversity survey was conducted with 30 high-yielding rice varieties (Fig. 1) that were screened and selected from 300 high-yielding *indica* cultivars from West African countries during the 2012 dry and wet seasons in two locations. Markers polymorphic between NERICA-L-20 (AfricaRice) and Giza178 (Egypt Research Center) associated with grain weight (*GW5*, Marker_1 to Marker_3), grain size (*GS3*, Marker_4 to Marker 6), grain number (*Gn1a*, Marker_7 to Marker_10) and strong stems and heavy panicles (*SCM2*/*APO1*, Marker_11 to Marker_16) were used to show plant performance for yield component traits of each inbred line (Table 1). The F_1 (ARS 563) progeny derived from crosses between NERICA-L-20 and Giza178 were self-pollinated to generate large F_2, $F_{2:3}$, $F_{2:4}$, and $F_{2:5}$ populations. Field experiments were conducted twice a year from 2012 to 2014 and F_2 populations totaling 1000 plants were evaluated during the 2013 dry season. An augmented experimental design laid out in 40 blocks was used to evaluate yield potential. Each block contained two rows of each parent, two checks (Sahel 108 and Sahel 201, released by ISRA Senegal) and 29 F_2 lines. The parents and checks were replicated in each block. In contrast, a randomized complete block design with three replications was used to evaluate selected $F_{2:3}$, $F_{2:4}$, and $F_{2:5}$ lines. The transplanting density was 20 cm between plants within rows and 20 cm between rows. Fertilizers were applied at the rate of 150 kg ha^{-1} as follows: $NPK_{15-15-15}$ at vegetative stage and 60 kg ha^{-1} urea as top dressing at tillering and panicle initiation. Weeds were controlled manually throughout the growing season. The descriptors for rice [23] were used to record total biomass (TB), harvest index (HI), panicle number per square meter (PN/m^2), total grain number per panicle (GNP), 1000-weight grain (1000-GW), spikelet fertility (SF), and grain yield (GY) for selected $F_{2:3}$ and $F_{2:4}$. Tiller number at 60 days after planting (T60), plant height at 60 days after planting (H60), and days to heading at 50% flowering (DH50) were added as parameters for selected $F_{2:5}$ plants. Pedigree selection including the two parents and check varieties (Sahel 108 and Sahel 201) was conducted using a phenotypic acceptability parameter rate scaling that ranged from excellent (1) to unacceptable (9) with intermediate values of 3 (good), 5 (fair), and 7 (poor).

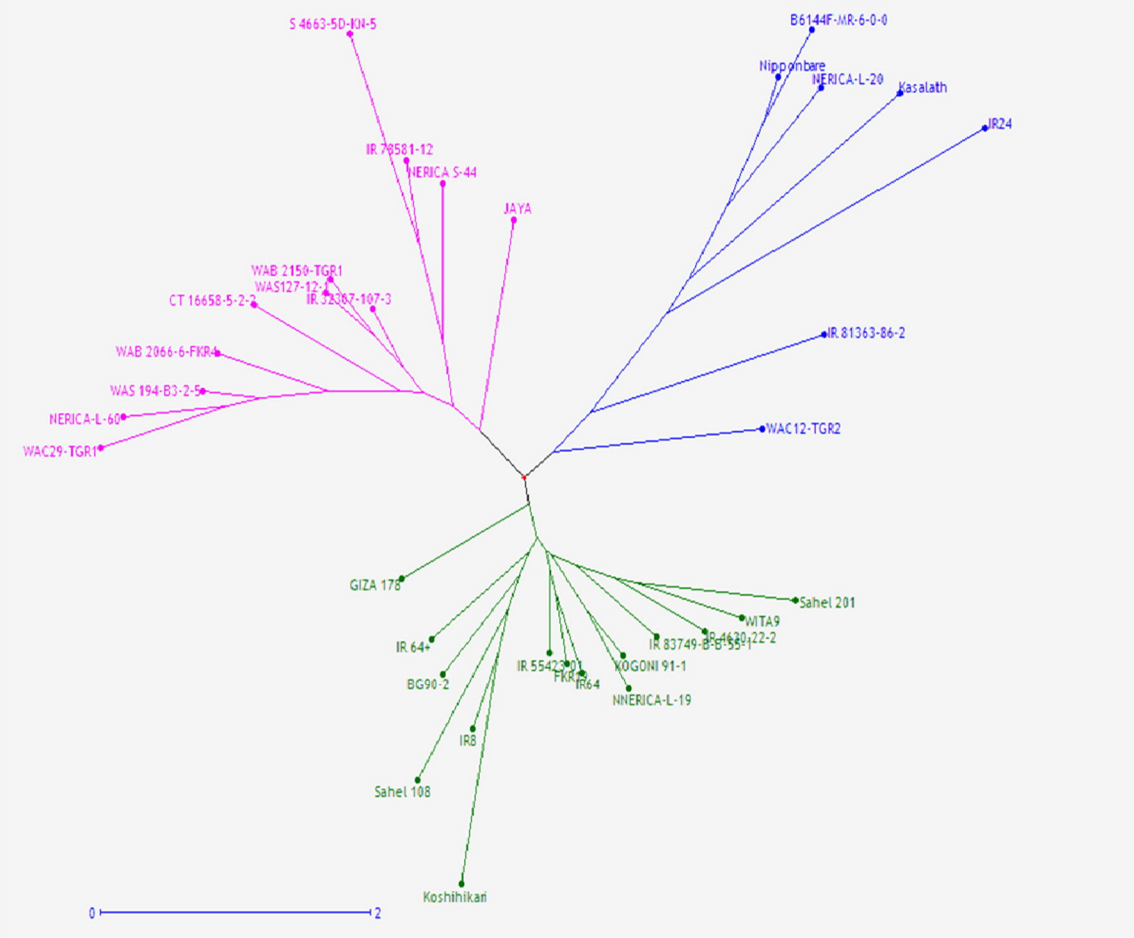

Fig. 1 – Allelic diversity survey of 30 high-yielding selected varieties and positive checks (IR24, Kasalath, Koshihikari, and Nipponbare) using weighted neighbor-joining clustering of genotype data from 11 polymorphic microsatellite markers associated with major yield-component traits.

ANOVA mixed models were fitted for 10 quantitative traits using XLSTAT software [24]. Broad-sense heritability (h^2) was calculated using the Breeding Management System\Workbench 3.09 software [25] according to the procedure described by [26,27].

$$h^2 = \frac{V_G}{V_P} = \left(\frac{V_G}{V_G + V_E}\right)$$

with V_G, genotype variance; V_P, phenotypic variance; V_E, environment variance.

Yield advantage (Y_{adv}) was estimated from grain yield over best parent (GY_{bp}), midparent (GY_{midp}), and standard check variety (GY_{sdc}) using the method described by [28]:

$$Y_{adv\_midp}(\%) = 100^{*}\left(\frac{GY - GY_{midp}}{GY_{midp}}\right)$$

$$Y_{adv\_sdc}(\%) = 100^{*}\left(\frac{GY - GY_{sdc}}{GY_{sdc}}\right)$$

$$Y_{adv\_bp}(\%) = 100^{*}\left(\frac{GY - GY_{bp}}{GY_{bp}}\right)$$

with Y_{adv_midp} (%), yield advantage over the mid-parent; Y_{adv_sdc} (%), yield advantage over the standard check variety; Y_{adv_bp} (%), yield advantage over the best parent; GY, promising line grain yield; GY_{bp}, best-parent grain yield; GY_{midp}, midparent grain yield; GY_{sdc}, standard check variety grain yield.

2.2. DNA extraction and favorable-allele tracking of 16 SSR and InDel markers associated with major QTL for yield and yield component traits

Genomic DNA was extracted from three-week-old leaves of all selected parental lines, F_2, $F_{2:3}$, and $F_{2:4}$ plants using the CTAB protocol [29] and genotyped with simple sequence repeat (SSR) and InDel GRiSP markers using PCR techniques. Sixteen primers associated with major QTL for yield component traits, as described in Table 1, were used according to the generation. Using the following program, 10 μL of each SSR-PCR mixture was amplified: initial denaturation (1 cycle of 94 °C for 4 min) followed by 35 amplification cycles including denaturation (94 °C for 1 min); hybridization of primers (55 °C for 1 min), elongation (72 °C for 2 min), and a final elongation (72 °C for 5 min). SSR/InDel-PCR products were separated on 8% polyacrylamide gel with 1x TBE buffer (40 mmol L^{-1} Trizma base-HCl, 40 mmol L^{-1} boric acid, and 1 mmol L^{-1}

Table 1 – Molecular markers associated with major QTL linked to yield and yield-component traits and their corresponding positive donors.

Yield component trait	Gene/QTL*	Markers	Marker abbreviations	Chromosome	Start position[1]	End position[2]	Gene function	Positive alleles (check varieties)	Sources
Grain weight	GW5	gw5–9311–5,724,000-5,730,000; Prdt 254 & 1465	Marker_1	5	Deleted	5,360,727	Regulate grain width and grain filling	Nipponbare	Miura et al. [18]
		gw5–9311–2225–2325; 3540–3640; Prdt 151 & 1362	Marker_2						
		gw5–9311–2225–2325; 3931–4031; Prdt 530 & 1741	Marker_3						
Grain size	GS3	SR17-InDel	Marker_4	3	16,729,715	16,735,077	Putative transmembrane protein	IR24	Xue et al. [19]
		RGS1-SSR1	Marker_5						
		RGS2-SSR2	Marker_6						
Grain number per panicle	*Gn1a*	Gn1a-SSR2–00	Marker_7	1	5,270,835	5,275,522	Oxydase/deshydrogenese 2	Koshihikari	Ashikari et al. [20]
		Gn1a-SSR2–01	Marker_8						
		Gn1a-SSR1–01	Marker_9						
		Gn1a-SSR1–03	Marker_10						
Strong stem and heavy panicles	SCM2/APO1	SCM2-RM20559	Marker_11	6	27,480,082	27,481,453	F-box protein containing	Koshihikari	Ookawa et al. [21]
		SCM2–3628-60	Marker_12						
		SCM2–3628–55-03	Marker_13						
		SCM2–3628–55-04	Marker_14						
		SCM2- SSR-gcggga-03	Marker_15						
		SCM2- SSR-gcggga-05	Marker_16						

EDTA), stained with 1 μg mL^{-1} bromophenol blue (3XSTR), and visualized with an ultraviolet transilluminator with the image captured by Syngen's G-Box gel imaging system. SSR/InDel (Table 1) profiles were scored and analyzed for allelic similarity (Fig. 1) in comparison with Nipponbare, Koshihikari, and IR24 as yield-component positive checks using Darwin software version 6 [30].

3. Results

3.1. Allelic polymorphic survey with 30 high-yielding varieties

Previously, an allelic polymorphism survey was conducted using the 30 selected high-yielding varieties (Fig. 1). Two varieties, NERICA-L-60 and WAB2066–6-FKR4-WAC1-TGR1-B-WATB12, combined three desirable alleles (*Gn1a*, *GS3*, and *GW5*) in their genetic backgrounds, whereas the remaining varieties carried only two favorable alleles, in several allele combinations (Table 2). NERICA-L-20 (*GS3* and *GW5*) and Giza178 (*Gn1a* and *SCM2/APO1*) were used as parental lines to develop ARS 563 populations. A polymorphism survey between the two parental lines was conducted using Nipponbare (*GW5*), IR24 (*GS3*), and Koshihikari (*Gn1a* and *SCM2/APO1*) as positive-allele check varieties to confirm the yield-component trait donor allele coming from each parent.

3.2. Forward breeding in the F_2, $F_{2:3}$, and $F_{2:4}$ generations

Marked segregation in the F_2 population was observed for all agronomic traits. A total of 1000 F_2 plants were phenotyped under field condition and 100 $F_{2:3}$ plants were selected based on their phenotypic acceptability, ranging from 1 (excellent) to 3 (good) under irrigated growth conditions. These $F_{2:3}$ plants were genotyped using highly polymorphic SSR/InDel markers.

Various numbers of introgressed QTL associated with yield-component traits were found. Forty-four $F_{2:3}$ plants showed two to three introgressions of favorable alleles such as GW5-GS3-SCM2/APO1, GW5-Gn1a-SCM2/APO1, GW5-GS3-Gn1a, Gn1a-GS3-SCM2/APO1, and GW5-GS3-Gn1a-SCM2/APO1 for three favorable allele combinations. However, 52 $F_{2:3}$ plants did not show any allele combinations. Four $F_{2:3}$ plants (ARS 563–14, ARS 563–62, ARS 563–286, and ARS 563–41) showed four segments found in chromosomes 1 (*Gn1a*), 3 (*GS3*), 5 (*GW5*), and 6 (*SCM2/APO1*) and were used for the next marker screening and advance (Fig. 2). Usually, the number of selected lines in the next screening could be increased. The stepwise screening method recommended by Sreewongchai et al. [14] was used to select superior, high-yielding new plant types. A total of three $F_{2:5}$ individual plants derived from F_2 ARS 563–14 and ARS 563–286 families were selected as ideotypes and identified as promising superior high-yielding lines. The alternate phenotype–genotype selection method used to advance progenies from F_2 to $F_{2:5}$ is described in Fig. 3.

3.3. Agro-morphological characterization of selected $F_{2:3}$ and $F_{2:4}$ pedigree selection

A total of 53 selected $F_{2:3}$ plants from ARS 563–14, ARS 563–62, ARS 563–286, and ARS 563–41 families were phenotyped and evaluated for high yield potential under field conditions (Table 3). The TB of the $F_{2:3}$ was lowest (1776 g m^{-2}), contrasting with those of the checks Sahel 108 (1950 g m^{-2}) and Sahel 201 (2106 g m^{-2}), and the two parents. HI was high (0.60) for the $F_{2:3}$ lines and ranged from 0.44 to 0.48 for the two parents. The PN/m^2 for the $F_{2:3}$ population was 566, exceeding those of both parents, NERICA-L-20 (427) and Giza178 (515). Moderate ($P < 0.01$) to high phenotypic variation ($P < 0.0001$) was observed for PN/m^2, GNP, and HI. GY showed significant ($P < 0.05$) differences, while TB, 1000-GW, and SF showed nonsignificant differences. $F_{2:3}$ 1000-GW was 25.70 g, in contrast to those of the two parents, 23.67 and 26.67 g; SF was higher than 75% for the $F_{2:3}$ population and their parents with an average of 76.47%. The average GY of $F_{2:3}$ population was 999 g m^{-2} while the parents showed GY values as follows: NERICA-L-20 (921 g m^{-2}) and Giza178 (1002 g m^{-2}). Broad-sense heritability (h^2) values were high for HI (0.6), PN/m^2 (0.78), and GNP (0.73) and ranged from moderate to low for other traits. A total of 31 $F_{2:4}$ plants were selected from the 53 selected $F_{2:3}$ plants showing superior high-yielding characteristics, using pedigree selection. The 31 $F_{2:5}$ plants derived from ARS563–14 and ARS563–286 families were used for preliminary yield performance trials.

3.4. Evaluation of selected $F_{2:5}$ ARS 563–14 and ARS 563–286 lines and preliminary yield performance estimation

The 31 selected plants of the two families ARS 563–14 (Table 4) and ARS 563–286 (Table 5) including the two parents and two checks were evaluated. Results from 14 $F_{2:5}$ (ARS 563–14) and 17 $F_{2:5}$ (ARS 563–286) showed high phenotypic variation ($P < 0.0001$) for DH50, total grain number per square meter (TGN/m^2), panicle length, GY, and SF. However, there were no significant differences for T60, H60, HI, PN/m^2, 1000-GW, and TB. The mean DH50 was <90 days for the $F_{2:5}$ lines, Sahel 108 and Giza178. The mean values of GNP ranged from 96 (ARS 563–286–12–1-4) to 151 (ARS 563–14–1-1-1). However, for TGN/m^2 the values were between 145 (ARS 563–14–1-1-1) and 503 (ARS 563–286–18-1-1). For 1000-GW, the values obtained were 23.07 g (ARS 563–286–16-1-1) and 28.73 g (ARS 563–286–14–1-1). In addition, the h^2 values obtained from ten quantitative traits ranged from low ($h^2 < 0.2$), to moderate ($0.2 < h^2 < 0.4$) and to high ($h^2 > 0.4$). GY ranged from 729.86 (ARS 563–14–7-7-1) to 1099.33 g m^{-2} (ARS 563–286–16-1-1). Yield values obtained with the two check varieties, Sahel 108 and Sahel 201, ranged from 700 to 870 g m^{-2}, while for the two parents the grain yield recorded was between 600 and 850 g m^{-2}.

The three top lines, ARS 563–286–16-1-1, ARS 563–286–5-1-1, and ARS 563–14–10-1-1, showed over 10% yield increase over the values obtained with the best parent, midparent, and standard check variety Sahel 108 (Table 6). The 11 best $F_{2:6}$ lines may be inferred to be homozygous for the QTL linked with the yield-component traits.

4. Discussion

The ARS563 populations developed from a cross between NERICA-L20 and Giza178 via alternate phenotype–genotype

Table 2 – Phenotypic values and allelic composition of selected yield component traits of 30 high-yielding varieties.

No.	Genotype	Phenotype					Allelic composition				
		Grain Yield (kg ha^{-1})	Number of panicles per plant	Grain number per panicle	1000-grain weight (g)	Phenotypic acceptability	*Gn1a*	*GS3*	*GW5*	*SCM2/ APO1*	Number of desirable alleles found per variety
1	IR32307–107–3-2-2	11,810	28	65	26.21	3	×			×	2
2	Giza178	11,321	22	152	19.89	1	×			×	2
3	Sahel 305	11,321	20	57	28.53	3	×			×	2
4	IR82574–643–1-2	11,277	19	139	24.78	3	×			×	2
5	NERICA –L-60	11,234	22	64	27.7	1	×	×	×		3
6	IR78581–12–3-2-2	11,123	20	136	33.07	3	×			×	2
7	WAS127–12–1-2-1	11,039	25	135	20.43	5	×			×	2
8	NERICA S-44	10,956	22	174	33.03	3	×			×	2
9	S4663-5D-KN-5-3-3	10,881	20	146	19.67	3		×	×		2
10	IR81363–86–2-3-2-2	10,878	19	136	27.1	3		×	×		2
11	CT 16658–5-2-2SR-2-3-6MP	10,409	15	72	27.21	3	×			×	2
12	WAS 62 B-B-14–1	10,386	19	66	21.15	3		×	×		2
13	Giza181	10,159	18	145	27.05	5	×			×	2
14	IET 2885	10,148	18	151	27.48	3	×			×	2
15	NERICA-L-20	10,039	25	130	20.43	1		×	×		2
16	IR1561–228–3-3	9980	27	170	24.83	3	×			×	2
17	FAROX 521–146-H1	9909	17	87	30.06	3	×			×	2
18	WAB 2066–6-FKR4-WAC1-TGR1-B-WAT-B12	9683	14	71	26.47	3	×	×	×		3
19	PCT 6\0\0\0 > 19–1–4-3-1-1-1-1-1-M	9558	20	144	35.47	3		×	×		2
20	CT 18148–10–4-2-3-4-1-M	9522	16	127	27.92	3		×	×		2
21	IR 06A150	9484	15	89	23.68	1	×			×	2
22	CT 18838–1–1-2-1SR-2P	9354	18	81	29.54	3		×	×		2
23	CT 17130-M-1-2-4-1-2-M	9322	13	120	33	3		×	×		2
24	IR 81358–98–1-3-2-3	9075	14	127	29.25	3	×			×	2
25	WAB 2150-TGR1-WAT3–1	8943	16	110	30.51	3	×			×	2
26	WAC12-TGR2	8805	20	160	34.1	3		×	×		2
27	WAC29-TGR1	8746	18	161	28.45	5	×			×	2
28	CT 18919–4–2-2-2SR-1P	8566	17	79	29.24	5	×			×	2
29	WAS 194-B3–2-5	8455	23	152	33.98	5	×			×	2
30	WAB 2128 – WAC B-1-TGR4-WAT B1	7330	18	152	29.98	3	×			×	2

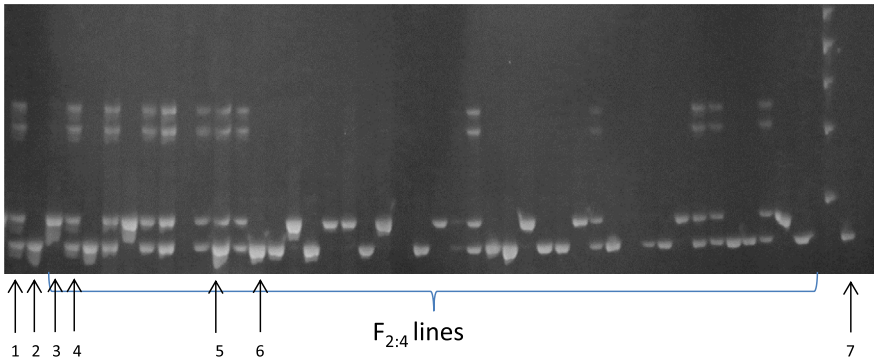

Fig. 2 – Forward breeding for grain size of selected $F_{2:4}$ lines using RGS1-SSR1 (Marker_5). 1: Giza178 (parent 1); 2: NERICA-L-20 (parent 2) = IR24 = positive check for yield component grain size (GS3); 3, 4, 5, 6: $F_{2:4}$ lines genotyped using Marker_5 for grain size (GS3); 7: ladder (100 pb).

selection combined with pedigree selection could contribute to identifying superior high-yielding rice lines compared with the parents and the standard check. As reported by Khush [8] and Sreewongchai et al. [14], this high yield was due to heterosis resulting from the use of different sources or different genetic backgrounds of the parents. The pedigree selection method is used for selection from segregating populations of crosses in self-pollinated crops and for combination or transgressive breeding. In fact, molecular characterization enabled the identification at an early stage of interesting recombinant lines with common region "introgressed" segments on chromosomes 1 (*Gn1a*), 3 (*GS3*), 5 (*GW5*), and 6 (*SMC2/APO1*). It also showed that the same segregating line is capable of accumulating varying combinations governing the expression of these different yield component traits [31]. The most important way, as reported by Fujita et al. [32], is to understand the enhancement of source size and translocation capacity as well as sink size

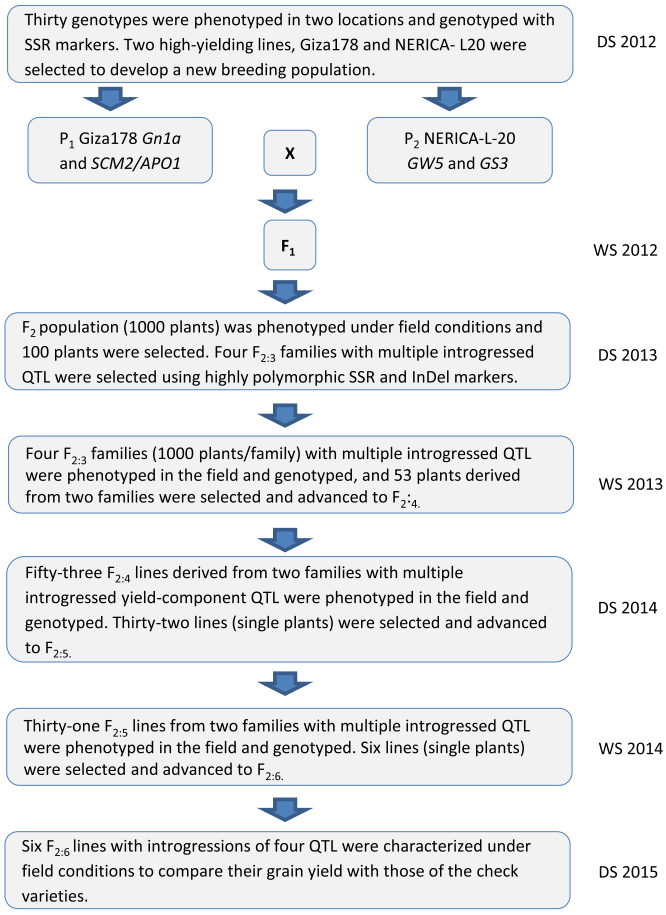

Fig. 3 – Procedural scheme for advancing selected lines through $F_{2:6}$ generation.

Table 3 – Average values of seven traits of the selected lines F_2, $F_{2:3}$ compared with parents and check variegties.

Variety/line [**]	TB (g m^{-2})	HI	PN/m^2	GNP	1000-GW (g)	SF (%)	GY (g m^{-2})
F_2	1600	0.64	650	300	27.23	80.00	1012
$F_{2:3}$	1776	0.60	566	263	25.70	75.70	999
NERICA-L-20	2106	0.44	427	211	26.67	80.43	921
Giza178	2075	0.48	515	300	23.67	78.81	1002
Sahel 108	1950	0.62	606	211	26.67	78.88	1075
Sahel 201	2015	0.54	450	200	26.00	75.00	900
Mean	1814	0.55	561.2	168.20	25.70	76.46	1001
Probability (α = 0.05)	0.290 [ns]	0.014 [**]	0.002 [***]	0.009 [***]	0.110 [ns]	0.290 [ns]	0.050 [*]
CV (%)	15.00	22.38	20.00	20.30	8.00	12.99	17.00

TB, total biomass (g m^{-2}); HI, harvest index; PN/m^2, panicle number per square meter; GNP, total grain number per panicle; 1000-GW, 1000-grain weight (g); SF, spikelet fertility (%); GY, grain yield (g m^{-2}).
[*] Significant at the 0.05 probability level.
[**] Significant at the 0.01 to 0.001 probability levels.
[***] Significant at the 0.0001 probability level.
[ns] Non-significant.

regarding the phenotypic characteristics of the population. That study showed that near-isogenic lines achieved 13%–36% yield increases with no negative effect on grain appearance. Expression analysis revealed that the gene was expressed in panicles, leaves, roots, and culms supporting the pleiotropic effects on plant architecture. Spikelet number (SPIKE) increased grain yield by 18% in the released *indica* cultivar *Oryza sativa* L. and increased the number of spikelets in the genetic background of other popular *indica* cultivars [32]. However, a negative correlation (−0.23) between grain weight and grain number, two major yield component traits, was reported by Venkateswarlu and Visperas [33], depending on lineage source.

Phenotypic variation was observed in $F_{2:3}$ and $F_{2:5}$ populations with good tillering ability and the semidwarf to intermediate plant height required in irrigated and rainfed lowland growth conditions. On the other hand, $F_{2:5}$ showed strong stems capable of supporting the heavy panicle weight conferred by Giza178 (*Gn1a* and *SMC2/APO1*). Plant height is one of the main descriptors often used to explain plant

Table 4 – Average values of 10 traits of 14 selected $F_{2:5}$ lines derived from ARS 563–14 compared with parents and check varieties.

Line/variety	T60 (day)	H60 (cm)	DH50 (day)	TB (g m^{-2})	HI	GNP	PN/m^2	1000-GW (g)	SF (%)	GY (g m^{-2})
ARS 563–14–6-1-1	11	96.50	73	981.25	0.50	116	270	25.17	94.84	1070.34
ARS 563–14–10-1-1	16	102.30	73	1243.75	0.61	121	374	25.69	82.32	1054.07
ARS 563–14–4-1-6	14	106.80	74	1393.75	0.52	128	307	29.04	91.34	1020.47
ARS 563–14–3-1-9	13	101.30	73	1087.50	0.58	132	301	28.90	93.86	998.88
ARS 563–14–1-1-1	16	104.00	76	1281.25	0.54	151	145	26.42	96.37	996.25
ARS 563–14–12–1-1	17	106.00	73	1137.50	0.57	124	393	25.02	89.39	978.34
ARS 563–14–5-1-3	19	104.30	75	1562.50	0.50	150	370	26.00	94.08	970.80
ARS 563–14–8-1-1	18	106.80	75	1475.00	0.51	135	387	27.85	92.64	952.10
ARS 563–14–14–1-1	14	98.00	75	1037.50	0.59	131	274	28.07	89.72	944.81
ARS 563–14–9-1-1	16	107.30	74	1262.50	0.58	150	330	23.56	85.54	941.23
ARS 563–14–2-1-1	12	103.80	75	981.25	0.53	130	270	27.02	96.32	923.90
ARS 563–14–11–1-1	18	98.75	73	1331.25	0.54	136	362	24.74	88.36	917.08
ARS 563–14–13–2-5	15	101.50	73	1093.75	0.44	132	324	24.75	87.81	869.65
ARS 563–14–7-7-1	16	99.50	75	1325.00	0.53	129	345	24.90	91.80	829.86
Giza178	16	91.38	82	1718.75	0.50	117	406	21.82	90.73	911.36
NERICA-L-20	14	83.13	110	1150.00	0.46	89	319	27.81	67.16	836.08
Sahel 108	13	90.38	76	1293.75	0.47	97	344	22.81	87.27	921.43
Sahel 201	13	93.25	87	1290.63	0.33	91	347	23.67	74.41	815.47
Mean	15	99.59	77	1259.83	0.51	125	330	25.58	0.88	861.98
Heritability	0.73	0.92	0.9	0.88	0.43	0.95	0.41	0.40	0.85	0.95
P-value	0.09 [ns]	0.01 [ns]	<0.0001 [***]	0.02 [*]	0.50 [ns]	<0.0001 [***]	0.31 [ns]	0.52 [ns]	0.03 [*]	<0.0001 [***]
CV (%)	13.9	7.42	13.41	16	15.9	17.5	18.7	9.9	10.04	13

T60, tiller number at 60 days after planting; H60, plant height at 60 days after planting; DH50, days to heading at 50% flowering; TB, total biomass (g m^{-2}); HI, harvest index; GNP, total grain number per panicle; PN/m^2, panicle number per square meter; 1000-GW, 1000-grain weight (g); SF, spikelet fertility (%); GY, grain yield (g m^{-2})..
[*] Significant at the 0.05 probability level.
[***] Significant at the 0.0001 probability level
[ns] Non-significant.

Table 5 – Average values of 10 traits of 14 selected $F_{2:5}$ lines derived from ARS 563–286 compared with parents and check varieties.

Line/variety	T60 (day)	H60 (cm)	DH50 (day)	TB (g m^{-2})	HI	GNP	PN/m^2	1000-GW (g)	SF (%)	GY (g m^{-2})
ARS 563–286–16-1-1	19	91.56	64	1430.96	0.55	121	315	23.07	92.49	1099.33
ARS 563–286–5-1-1	17	95.56	63	1118.46	0.57	119	234	27.73	93.09	1049.85
ARS 563–286–2-1-1	17	92.48	69	1371.23	0.60	116	463	25.19	92.97	994.11
ARS 563–286–9-1-1	18	95.96	69	935.31	0.68	107	397	23.21	87.42	975.78
ARS 563–286–3-1-1	19	96.48	63	1121.23	0.48	132	413	24.95	88.97	969.56
ARS 563–286–7-1-1	19	94.06	64	1368.46	0.55	97	259	25.53	91.62	950.09
ARS 563–286–4-1-3	18	96.46	67	1154.06	0.55	99	285	24.64	89.77	938.58
ARS 563–286–6-1-1	16	101.70	67	1260.31	0.54	100	410	26.53	88.93	926.88
ARS 563–286–1-1-5	20	99.96	63	1166.56	0.58	99	460	22.68	83.84	917.33
ARS 563–286–10-1-1	22	100.50	67	1027.48	0.51	108	325	22.96	87.68	912.16
ARS 563–286–17–1-1	18	98.23	65	1333.73	0.61	108	388	24.95	77.56	909.65
ARS 563–286–11–2-8	17	96.56	66	1237.21	0.54	115	403	25.51	89.77	905.25
ARS 563–286–14–1-1	17	96.06	62	1193.46	0.59	81	334	28.73	87.76	893.42
ARS 563–286–8-1-1	23	99.23	67	1296.23	0.46	119	407	24.53	93.72	843.27
ARS 563–286–12–1-4	18	92.31	66	987.21	0.58	112	334	22.93	90.94	813.84
ARS 563–286–13–1-1	21	95.73	64	1008.73	0.57	76	269	24.06	86.03	808.28
ARS 563–286–18-1-1	22	97.46	63	1422.81	0.65	107	503	26.12	94.20	803.00
Giza178	14	92.50	77	1516.67	0.47	115	404	23.86	95.60	843.04
NERICA-L-20	16	83.58	102	1160.42	0.47	90	317	28.14	67.69	691.52
Sahel 201	14	95.58	82	1333.33	0.40	93	292	25.53	84.05	833.06
Sahel108	13.8	93.81	71	1680.96	0.46	137	484	26.73	84.78	871.07
Mean	17.71	95.63	68.68	1245.22	0.54	106.32	361.92	25.31	88.46	924.14
Heritability	0.61	0.75	0.96	0.61	0.64	0.79	0.66	0.67	0.93	0.85
P-value	0.11 ns	0.02 *	<0.0001 ***	0.09 ns	0.08 ns	0.02 **	0.06 ns	0.07 ns	<0.0001 ***	0.01 **
CV (%)	9.59	16.86	5.04	21.9	15	14.79	16	17.6	7.91	15.7

T60, tiller number at 60 days after planting; H60, plant height at 60 days after planting; DH50, days to heading at 50% flowering; TB, total biomass (g m^{-2}); HI, harvest index; GNP, total grain number per panicle; PN/m^2, panicle number per square meter; 1000-GW, 1000-grain weight (g); SF, spikelet fertility (%); GY, grain yield (g m^{-2}).

* Significant at the 0.05 probability level.

** Significant at the 0.01 to 0.001 probability levels.

*** Significant at the 0.0001 probability level.

ns Non- significant.

architecture that supports heavy panicles [34]. The selected $F_{2:5}$ lines showed moderate to high heritability for all traits, revealing good to excellent performance of these lines.

The three top selected $F_{2:5}$ lines, ARS 563–286–16-1-1, ARS 563–286–5-1-1, and ARS 563–14–10-1-1, showed an increase of more than 10% grain yield following standard heterosis in comparison with the best check, Sahel 108.

Marker identification of QTL associated with target traits in different crops has contributed to developing methods that combine conventional and molecular breeding to make

Table 6 – Preliminary yield performance from best selected $F_{2:6}$ lines derived from ARS 563–286 and ARS 563–14 families.

Lines	GY (g m^{-2})	GY_{sdc} (g m^{-2})	GY_{bp} (g m^{-2})	GY_{midp} (g m^{-2})	Performance over best parent (%)	Performance over midparents (%)	Performance over standard check variety (%)	P-value	LSD
ARS 563–286–16-1-1	1099.33	971.07	943.04	917.28	13.21	16.57	19.85	0.012 **	3.02
ARS 563–286–5-1-1	1049.85				8.11	11.33	14.45		
ARS 563–286–2-1-1	994.11				2.37	5.42	8.38		
ARS 563–286–9-1-1	975.78				0.49	3.47	6.38		
ARS 563–14–10-1-1	1054.07	921.43	911.36	928.72	14.40	15.66	13.50	0.035 *	16.4
ARS 563–14–4-1-6	1020.47				10.75	11.97	9.88		
ARS 563–14–3-1-9	998.88				8.41	9.60	7.55		
ARS 563–14–1-1-1	996.25				8.12	9.31	7.27		
ARS 563–14–12–1-1	978.34				6.18	7.35	5.34		
ARS 563–14–5-1-3	970.8				5.36	6.52	4.53		
ARS 563–14–8-1-1	952.1				3.33	4.47	2.52		

GY, promising lines grain yield; GY_{sdc}, standard check variety grain yield; GY_{bp}, best parent grain yield; GY_{midp}, mid-parent grain yield.

* Significant at the 0.05 probability level.

** Significant at the 0.01 to 0.001 probability levels.

progress in marker-assisted breeding [35]. Selection may be applied at any plant growth stage and in small populations. In that case, phenotyping and genotyping by the so-called alternate phenotype–genotype selection method and marker-assisted selection may be used to reduce field trial size by excluding unfavorable genotypes before planting the population in the field [14]. Genotype and phenotype are still used to refer to the individual's DNA and traits. The use of markers linked to QTL associated with target traits is contributing to improving the efficiency and precision of conventional plant breeding via marker-assisted selection [36].

In conclusion, alternate phenotype-genotype selection may prove useful for accelerating rice breeding programs.

Acknowledgments

We acknowledge funding to the GRiSP New Frontiers Project (DRPC2012-025). We also thank the irrigated breeding unit and the Biotechnology Laboratory at Africa Rice Saint Louis for assistance provided by the technicians.

REFERENCES

[1] Statista, Statistics on "Rice": world rice acreage from 2008/2009 to 2015/2016 (in million hectares), https://www.statista.com/statistics/271969/world-rice-acreage-since-2008/ 2017.
[2] FAO, Rice Market Monitor (RMM), volume XIX, Issue No. 3, October 2016 (Rome, Italy, 2016).
[3] N. Palmer, Rice roadmap provides an alternative to the quest for "mega-varieties"CIAT Blog http://www.ciatnews.cgiar.org/2011/09/08/rice-roadmap-provides-an-alternative-to-the-quest-for-mega-varieties/ 2011.
[4] IRRI, Annual Report of the Director General, 2005–2006, Volume 16, IRRI, Makati City, Philippines, 2006 (www.irri.org).
[5] Africa Rice Center (WARDA), Africa Rice Trends: Overview of Recent Developments in the Sub-Saharan Africa Rice Sector, Africa Rice Center Brief, Cotonou, Benin, 2007.
[6] Africa Rice Center (AfricaRice), New Breeding Directions at AfricaRice: Beyond NERICA, Africa Rice Center, Cotonou, Benin, 2010.
[7] V.O. Sadras, K.G. Cassman, P. Grassini, A.J. Hall, W.G.M. Bastiaanssen, A.G. Laborte, A.E. Milne, G. Sileshi, P. Steduto, Yield Gap Analysis of Field Crops: Methods and Case Studies, FAO Water Reports No. 41, Food and Agriculture Organization of the United Nations (FAO) and the Robert B, Daugherty Water for Food Institute at the University of Nebraska (DWFI), Rome, Italy, 2015.
[8] G.S. Khush, Breaking the yield frontier of rice, Georgetown Dent. J. 35 (1995) 329–332.
[9] S.B. Peng, G.S. Khush, P. Virk, Q.Y. Tang, Y.B. Zou, Progress in ideotype breeding to increase rice yield potential, Field Crop Res. 108 (2008) 32–38.
[10] P.S. Virk, G.S. Khush, S.B. Peng, Breeding to enhance yield potential of rice at IRRI: the ideotype approach, Int. Rice Res. Notes 29 (2004) 5–9.
[11] F.M. Xie, B. Hardy, Accelerating Hybrid Rice Development, IRRI, Los Baños, The Philippines, 2009.
[12] K. Traore, V.B. Bado, M.K. N'Diaye, Manuel Pratique sur les Normes de Production et de Certification de Semences de Riz, AfricaRice Sahel Station, Saint Louis, Senegal, 2012 (in French).
[13] T.R. Hargrove, V.L. Cabanilla, W.R. Coffman, Twenty years of rice breeding, Biomed. Sci. 38 (1988) 675–681.
[14] T. Sreewongchai, P. Rattanapol, V. Vichukit, Alternate phenotype-genotype selection method for developing photoperiod intensive, good cooking quality and potential high-yielding rice lines, Kasetsart, J. (Nat. Sci.) 48 (2014) 851–859.
[15] G. Zong, A.H. Wang, L. Wang, G.H. Liang, M.H. Gu, T. Sang, B. Han, A pyramid breeding of eight grain-yield related quantitative trait loci based on marker-assistant and phenotype selection in Rice (*Oryza sativa* L.), J. Genet. Genomics 39 (2012) 335–350.
[16] M. Dingkuhn, M.P. Jones, D.E. Johnson, A. Sow, Growth and yield potential of *Oryza sativa* and *O. glaberrima* upland rice cultivars and their interspecific progenies, Field Crop Res. 57 (1998) 57–69.
[17] J. Demol, J.P. Baudoin, P.B. Louant, R. Maréchal, G. Mergeai, E. Otoul, L'amélioration des plantes. Application aux principales espèces cultivées en régions tropicales, Les Presses Agronomiques de Gembloux, Gembloux, Belgique, 2002 (in French).
[18] K. Miura, M. Ikeda, A. Matsubara, X.J. Song, M. Ito, K. Asano, M. Matsuoka, H. Kitano, M. Ashikari, OsSPL14 promotes panicle branching and higher grain productivity in rice, Nat. Genet. 42 (2010) 545–549.
[19] D.W. Xue, Q. Qian, S. Teng, Identification and Utilization of Elite Genes from Elite Germplasms for Yield Improvement, in: W.G. Yan, J.S. Bao (Eds.), Rice-Germplasm, Genetics and Improvement, InTechOpen, Rijeka, Croatia 2014, pp. 1–5.
[20] M. Ashikari, H. Sakakibara, S.Y. Lin, T. Yamamoto, T. Takashi, A. Nishimura, E.R. Angeles, Q. Qian, H. Kitano, M. Matsuoka, Cytokinin oxidase regulates rice grain production, Science 309 (2005) 741–745.
[21] T. Ookawa, H.T. Yano, M. Murata, K. Ando, T. Miura, H. Asano, K. Ochiai, Y. Ikeda, M. Nishitani, R. Ebitani, T. Ozaki, H. Angeles, E.R. Hirasawa, T.M. Matsuoka, New approach for rice improvement using a pleiotropic QTL gene for lodging resistance and yield, Nat. Commun. 1 (2010) 132.
[22] J.F.A. Griffiths, J.H. Miller, D.T. Suzuki, R.C. Lewontin, W.M. Gelbart, à Introduction, L'analyse Génétique, 4th edition de Boeck, Bruxelle, Belgium, 2006 (in French).
[23] Bioversity International, IRRI and WARDA, Descriptors for Wild and Cultivated Rice (Oryza Spp.), Bioversity International, Rome, Italy, 2007 (International Rice Research Institute, Los Baños, Philippines; WARDA, Africa Rice Center, Cotonou, Benin).
[24] Addinsoft, XLStat, Version 2015.6, New York, USA, https://www.xlstat.com/en/news/version-2015-6 2015.
[25] Integrated Breeding Platform (IBP), Breeding Management System (BMS), Version, 3.0.9, 2015, Mexico, D.F., Mexico, https://www.integratedbreeding.net/.
[26] D.L. Zhao, G.N. Atlin, L. Bastiaans, H.J. Spiertz, Cultivar weed-competitiveness in aerobic rice: heritability, correlated traits, and the potential for indirect selection in weed-free environments, Crop Sci. 1 (2006) 372–380.
[27] H.F. Robinson, R.E. Comstock, P.H. Harvey, Estimates heritability and the degree of dominance in corn, Agron. J. 41 (1949) 353–359.
[28] K. Mather, Biometrical Genetics, the Study of Continuous Variation, 2nd ed. Methuen, London, UK, 1949.
[29] M.A. Saghai-Maroof, K.M. Soliman, R.A. Jorgensen, R.W. Allard, Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics (ribosomal DNA spacer-length variation/restriction fragment-length polymorphisms/*Rrnl*/*Rrn2*), Proc. Natl. Acad. Sci. U. S. A. 81 (1984) 8014–8018.
[30] X. Perrier, J.P. Jacquemoud-Collet, DARwin Software, Version 6, http://darwin.cirad.fr/ 2006.

[31] D.S. Brar, G.S. Khush, Alien introgression in rice, Plant Mol. Biol. 35 (1997) 35–47.
[32] D. Fujita, K.R. Trijatmikoa, A.G. Taglea, M.V. Sapasapa, Y. Koidea, K. Sasakia, N. Tsakirpalogloua, R.B. Gannabana, T. Nishimurad, S. Yanagiharab, Y. Fukutab, T. Koshibad, I.H. Slamet-Loedina, T. Ishimarua, N. Kobayashia, *NAL1* allele from a rice landrace greatly increases yield in modern *indica* cultivars, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 20431–20436.
[33] B. Venkateswarlu, R.M. Visperas, Source-Sink Relationships in Crop Plants, IRRI, Los Baños, Philippines, 1987.
[34] B.P. Caton, A.E. Cope, M. Mortimer, Growth traits of diverse rice cultivars under competition: implications for screening for competitiveness, Field Crop Res. 80 (2003) 157–172.
[35] C. Bertrand, Y. Collard, J.D. Mackill, Marker-assisted selection: an approach for precision plant breeding in the twenty-first century, Philos. Trans. R. Soc. B-Biol. Sci. (2008) 557–572.
[36] P. Taylor, R. Lewontin, The Genotype/Phenotype Distinction, in: E.N. Zalta (Ed.), The Stanford Encyclopedia of Philosophy, 2017.