Marker-assisted selection strategy to pyramid two or more QTLs for quantitative trait-grain yield under drought

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Abstract

Background: Marker-assisted breeding will move forward from introgressing single/multiple genes governing a single trait to multiple genes governing multiple traits to combat emerging biotic and abiotic stresses related to climate change and to enhance rice productivity. MAS will need to address concerns about the population size needed to introgress together more than two genes/QTLs. In the present study, grain yield and genotypic data from different generations (F3 to F8) for five marker-assisted breeding programs were analyzed to understand the effectiveness of synergistic effect of phenotyping and genotyping in early generations on selection of better progenies.

Results: Based on class analysis of the QTL combinations, the identified superior QTL classes in F3/BC1F3/BC2F3 generations with positive QTL x QTL and QTL x background interactions that were captured through phenotyping maintained its superiority in yield under non-stress (NS) and reproductive-stage drought stress (RS) across advanced generations in all five studies. The marker-assisted selection breeding strategy combining both genotyping and phenotyping in early generation significantly reduced the number of genotypes to be carried forward. The strategy presented in this study providing genotyping and phenotyping cost savings of 25–68% compared with the traditional marker-assisted selection approach. The QTL classes, Sub1 + qDTY1.1 + qDTY2.1 + qDTY3.1 and Sub1 + qDTY2.1 + qDTY3.1 in Swarna-Sub1, Sub1 + qDTY1.1 + qDTY1.2 + qDTY2.2 and Sub1 + qDTY2.2 + qDTY3.1 in IR64-Sub1, qDTY2.2 + qDTY4.1 in Samba Mahsuri, Sub1 + qDTY3.1 + qDTY6.1 + qDTY6.2 and Sub1 + qDTY6.1 + qDTY6.2 in TDK1-Sub1 and qDTY12.1 + qDTY3.1 and qDTY2.2 + qDTY3.1 in MR219 had shown better and consistent performance under NS and RS across generations over other QTL classes.

Conclusion: "Deployment of this procedure will save time and resources and will allow breeders to focus and advance only germplasm with high probability of improved performance. The identification of superior QTL classes and capture of positive QTL x QTL and QTL x background interactions in early generation and their consistent performance in subsequent generations across five backgrounds supports the efficacy of a combined MAS breeding strategy".

Keywords: Drought, Drought yield QTLs, Marker-assisted selection breeding strategy, Pyramiding, Rice
major interactions, i) interaction between genes for the same trait, ii) genes for different traits, and iii) interactions of genes with environments and genetic background restricting the use of QTLs in introgression programs (Kumar et al. 2014; Wang et al. 2012; Xue et al. 2009; Almeida et al. 2013; Elangovan et al. 2008; Cuthbert et al. 2008; Heidari et al. 2011; Bennett et al. 2012). Selection of an appropriate donor/recipient to create desirable variability (Mondal et al. 2016; Dixit et al. 2014) and precise selection under variable conditions, environments, and stress intensity levels is must. A large population size is generally required for selecting appropriate plants possessing the needed gene combinations, desired plant type, and higher yield. An integration of modern, novel, and affordable breeding strategies with knowledge of associated mechanisms, interactions, and associations among related or unrelated traits/factors is necessary in rice breeding improvement programs.

The conventional breeding approach involving a series of phenotyping and genotyping screening of a large population to obtain desired variability and a high frequency of favorable genes in combination was earlier followed by several drought breeding program (Kumar et al. 2014). A conventional breeding approach involving sequential selection of large segregating populations for biotic (bacterial late blight, blast) and abiotic stresses (drought, submergence) across generations helped breeders to develop breeding lines combining tolerance of both stresses. Superior lines in terms of acceptable plant type, grain yield, and quality traits and stable performance under different environments are promoted for release (Kumar et al. 2014; Sandhu and Kumar 2017).

Modern molecular breeding strategies have been implemented to practice a more precise, quick and cost-effective breeding strategy compared to traditional conventional rice breeding improvement programs. Previously, many QTLs for grain yield under drought using different strategies such as selective/whole-genome genotyping, bulk segregant analysis (Vikram et al. 2011; Yadaw et al. 2013; Mishra et al. 2013; Sandhu et al. 2014; Ghimire et al. 2012) have been identified. The successful introgression and pyramiding of the identified genetic regions in different genetic backgrounds using marker-assisted backcrossing (Yadaw et al. 2013; Mishra et al. 2013; Sandhu et al. 2014; Venuprasad et al. 2009; Sandhu et al. 2013; Sandhu et al. 2015) has been reported. Accurate repetitive phenotyping in multi-locations and multi-environments under variable growing conditions is required to evaluate the performance and adaptability of the developed MAB products. There have been several examples of introgression of single genes for both biotic and abiotic stresses (gall midge – Das and Rao 2015; blast – Miah et al. 2016; brown plant hopper – Jairin et al. 2009; submergence – Septiningsih et al. 2009) in the background of popular high-yielding varieties as well as introgression of more than one gene for biotic stresses (\(xa5 + xa13 + Xa21\) - Singh et al. 2001, Kottapalli et al. 2010; \(Xa21 + xa13\) - Singh et al. 2011) for oligogenic traits controlled by major genes.

Several major large-effect QTLs such as \(qDTY_{1.1}\) (Vikram et al. 2011; Ghimire et al. 2012), \(qDTY_{2.1}\) (Venuprasad et al. 2009), \(qDTY_{2.2}\) (Venuprasad et al. 2007; Swamy et al. 2013), \(qDTY_{3.1}\) (Venuprasad et al. 2009), \(qDTY_{4.1}\) (Swamy et al. 2013), \(qDTY_{6.1}\) (Venuprasad et al. 2012), \(qDTY_{10.1}\) (Swamy et al. 2013), and \(qDTY_{12.1}\) (Bernier et al. 2007) for grain yield under reproductive-stage (RS) drought stress have been identified. A total of 28 significant marker trait associations were detected for yield-related trait in genome wide association study of japonica rice under drought and non-stress conditions (Volante et al. 2017). Moreover, each of these identified QTLs has shown a yield advantage of 300–500 kg ha\(^{-1}\) under RS drought stress depending upon the severity and timing of the drought occurrence. However, in order to provide farmers with an economic yield advantage under drought, it is necessary that two or more such QTLs be combined to obtain a targeted yield advantage of 1.0 t ha\(^{-1}\) under severe RS drought stress (Sandhu and Kumar 2017; Kumar et al. 2014).

Polygenic traits governed by more than one gene within the identified QTLs do not follow the simple rule of single gene introgression. The positive/negative interactions of alleles within QTLs and with the genetic background (Dixit et al. 2012a, b), pleiotropic effect of genes and linkage drag (Xu and Crouch 2008; Vikram et al. 2015; Vikram et al. 2016; Bernier et al. 2007; Venuprasad et al. 2009; Vikram et al. 2011; Venuprasad et al. 2012) played an important role in determining the effect of introgressed loci. The reported linkage drag of the \(qDTY\) QTLs has been successfully broken and individual QTLs have been introgressed into improved genetic backgrounds (Vikram et al. 2015). To identify an appropriate number of plants with positive interactions and high phenotypic expression, MAB requires genotyping and phenotyping of large numbers of plants/progenies in each generation from F\(_2\) onwards. In this case, MAB for more than two genes/QTLs is not a cost-effective approach. The population size to be genotyped and phenotyped for complex traits such as drought increases significantly as two or more QTLs are considered for introgression. To enhance breeding capacity to develop climate-resilient rice cultivars, there is a strong need to develop a novel, cost/labor-effective, and high-throughput breeding strategy. The effective integration of molecular knowledge into breeding programs and making MAB cost-effective enough to be fully adapted by small- or moderate-sized breeding programs are still a challenge.
In the present study, we closely followed the marker-assisted introgression of two or more QTLs for RS drought stress in the background of rice varieties; Swarna-Sub1, IR64-Sub1, Samba Mahsuri, TDK1-Sub1, and MR219 from F3 to F6/F7/F8 generations. Class analysis for different combinations of QTLs for yield under RS drought stress as well as under irrigated control conditions was performed with the aim to understand the effectiveness of synergistic effect of phenotyping and genotyping in early generations on selection of better progenies. We hypothesized that a QTL class that has performed well in an early generation may maintain its performance across generations/years and seasons.

Results

Performance of lines introgressed with QTLs for grain yield under drought

The pyramided lines with either a single gene or in combination of genetic loci associated with grain yield under drought produced a grain yield advantage over the recipient parent across backgrounds and generations (Fig. 1a to j). The pyramided lines with two or more QTLs had shown a high grain yield advantage in Swarna-Sub1 (Table 1), IR64-Sub1 (Table 2), Samba Mahsuri (Table 3), TDK1-Sub1 (Table 4), and MR219 (Table 5) backgrounds. In a Swarna-Sub1 background, a grain yield advantage of 76.2–2478.5 kg ha⁻¹ and 395.7–2376.3 kg ha⁻¹ under non-stress (NS) in Sub1 + qDTY1.1 + qDTY2.1 + qDTY3.1 and Sub1 + qDTY2.1 + qDTY3.1 pyramided lines, respectively, was observed. Under RS drought stress, a grain yield advantage of 292.4–1117.8 and 284.2–2085.5 kg ha⁻¹ in Sub1 + qDTY1.1 + qDTY2.1 + qDTY3.1 and Sub1 + qDTY2.1 + qDTY3.1 pyramided lines, respectively, was observed (Table 1). In an IR64-Sub1 background, the pyramided lines (Sub1 + qDTY1.1 + qDTY2.2) showed a grain yield advantage ranging from 213.5 to 1571.4 kg ha⁻¹ and 170.4 to 864.7 kg ha⁻¹ under NS and RS drought stress, respectively. Under RS drought stress, the pyramided lines (Sub1 + qDTY3.1 + qDTY2.3 + qDTY3.2) showed a grain yield advantage of 217.1 to 719.1 kg ha⁻¹ in an IR64-Sub1 background (Table 2). The grain yield advantage ranged from 48.0 to 2216.9 kg ha⁻¹ and 95.5 to 1296.4 kg ha⁻¹ under NS and RS drought stress conditions, respectively, in Samba Mahsuri introgressed with qDTY2.2 + qDTY4.1 (Table 3). In TDK1-Sub1 pyramided lines (Sub1 + qDTY3.1 + qDTY6.1 + qDTY6.2), the grain yield advantage ranged from 65.2 to 792.0 kg ha⁻¹ and 155.9 to 2429.5 kg ha⁻¹ under NS and RS drought stress conditions, respectively (Table 4). The pyramided lines with qDTY2.1 + qDTY3.1 and qDTY2.2 + qDTY3.1 showed a grain yield advantage of 735.1–1012.8 kg ha⁻¹ and 324.0–1240.9 kg ha⁻¹, respectively, under NS and 672.3–1059.5 kg ha⁻¹ and 571.4–1099.3 kg ha⁻¹, respectively, under RS drought stress conditions in an MR219 background (Table 5).

Performance of pyramided lines in the F₃ generation

Mean performances of QTL classes from F₃ to F₈ of Swarna-Sub1, IR64-Sub1, Samba Mahsuri, TDK1-Sub1, and MR219 pyramided lines are shown in Tables 1, 2, 3, 4, and 5, respectively.

In a Swarna background, two classes (Sub1 + qDTY1.1 + qDTY2.1 + qDTY3.1, Sub1 + qDTY1.1 + qDTY2.2 + Sub1 + qDTY2.2 + qDTY3.1) showed higher performance in F₃ under both NS and RS drought stress (Table 1). In an IR64-Sub1 background, three classes (Sub1 + qDTY1.1 + qDTY1.2, Sub1 + qDTY1.1 + qDTY2.2, Sub1 + qDTY2.2 + qDTY1.2) showed higher performance under NS and RS drought stress both, whereas Sub1 + qDTY3.2 + qDTY2.3 + qDTY1.2 performed better under RS drought stress only in F₃ (Table 2). In Samba Mahsuri background, the QTL class qDTY2.2 + qDTY4.1 showed a higher performance than a single QTL under both NS and RS drought stress in F₃ (Table 3). In a TDK1-Sub1 background, the classes consisted of pyramided lines with Sub1 + qDTY3.1 + qDTY6.1 + qDTY6.2 and Sub1 + qDTY6.1 + qDTY6.2 showed a stable and high effect across variable growing conditions in F₃ (Table 4). In the MR219 background, pyramided lines having qDTY12.1 + qDTY3.1 and qDTY2.2 + qDTY3.1 showed significant yield advantage under both NS and RS drought stress (Table 5).

Validation of MAB-selected class performance in subsequent generations

The performance of pyramided line classes identified as superior in the F₃ generation was found to be consistent and higher than other QTL classes throughout F₄, F₅, F₆, F₇, and F₈ generations (except where the number of lines per class was less) across all five studied backgrounds in the present study. The high mean grain yield QTL classes in the F₃ generation, Sub1 + qDTY1.1 + qDTY2.1 + qDTY3.1 and Sub1 + qDTY2.1 + qDTY3.1 in a Swarna background (Table 1), qDTY2.2 + qDTY4.1 in a Samba Mahsuri background (Table 3), and Sub1 + qDTY3.1 + qDTY6.1 + qDTY6.2 and Sub1 + qDTY6.1 + qDTY6.2 in a TDK1-Sub1 background (Table 4) had maintained their high mean grain yield performance from the F₄ to F₈ generations over other QTL classes. The low mean yield performers in the F₃ generation, Sub1 + qDTY1.1, Sub1 + qDTY1.1 + qDTY3.1, or Sub1 + qDTY1.1 + qDTY2.2 in a Swarna-Sub1 background (Table 1), qDTY2.2 in a Samba Mahsuri background (Table 3), and qDTY6.1 + qDTY6.1 and Sub1 + qDTY6.2 + qDTY3.1 in a TDK1-Sub1 background (Table 4), were observed to be lower yielders in each of the generations from F₄ to F₈. The significant high grain yield advantage of Sub1 + qDTY1.1 + qDTY1.2, Sub1 + qDTY1.1 + qDTY2.2, Sub1 + qDTY2.2 + qDTY12.1, and Sub1 + qDTY3.2 +
Fig. 1 (See legend on next page.)
qDTY2.3 in an IR64-Sub1 background (Table 2) and of qDTY12.1 + qDTY4.1 and qDTY2.2 + qDTY3.1 in an MR219 background (Table 5) was consistent from the F4 to F7 generation. QTL classes Sub1 + qDTY12.1 + qDTY2.2 + qDTY3.1, Sub1 + qDTY24.1 + qDTY4.1, and qDTY12.1 + qDTY2.3 + qDTY12.1 + Sub1 in an IR64-Sub1 background showed lower yield from F3 to subsequent generations (Table 2). The low grain yield performance of qDTY12.1 + qDTY2.2 and qDTY2.2 + qDTY3.1 + qDTY12.1 under RS drought stress in MR219 was maintained from the F4 to F7 generation (Table 5). None of the inferior QTL classes identified in F3 outperformed the identified superior QTL combination class or combination classes in any advanced generation under NS as well as under variable intensities of RS drought stress in different seasons/years across generations from F4 to F7/F8.

Cost effectiveness of the early generation selection

The genotyping cost for the whole population considering all QTL classes from F3 to F7/F8 ranged from USD 9225 to USD 21760 whereas the genotyping cost accounting for further advancement and screening (F4 to F7/F8) of only superior classes in F3 varied from USD 5730 to USD 8978 (Table 6). A genotyping cost savings of USD 12443, 3720, 14,780, 2273, and 6225 was observed in Swarna-Sub1, IR64-Sub1, Samba Mahsuri, TDK1-Sub1, and MR219 backgrounds, respectively, with a range of savings of USD 2273 to USD 14780 in all five backgrounds.

The phenotyping cost for the whole population ranged from USD 29197 to USD 157455 whereas it was USD 20225 to USD 50507 in the case of selected classes (Table 7). A phenotyping cost savings of USD 60023, 8973, 10,963, 106,948, and 30,029 was observed in Swarna-Sub1, IR64-Sub1, Samba Mahsuri, TDK1-Sub1, and MR219 backgrounds, respectively, with phenotyping cost savings of USD 14780, 2273, and 6225 in IR64-Sub1, Samba Mahsuri, TDK1-Sub1, and MR219 backgrounds, respectively.

Interaction among QTLs and with background

In our study, qDTY1.1 showed positive interactions with qDTY2.2, qDTY2.3, and qDTY3.1 whereas qDTY1.2 showed positive interactions with qDTY2.2, qDTY2.3, and qDTY3.1. qDTY3.1 showed positive interactions with qDTY1.1, qDTY2.2, qDTY5.2, qDTY5.3, and qDTY6.2 at least in the genetic backgrounds that we studied in the present experiment. Such information will be helpful to breeders in selecting QTL combinations in their MAB programs.

Discussion

Phenotypic evaluation of QTLs pyramided lines

The yield reduction in RS drought stress experiments was 45, 77, 79, and 97% in F3, F5, F7, and F7 generations, respectively, in Swarna-Sub1 introgression lines as compared to the mean yield of the NS experiments. In IR64-Sub1, the yield reduction was 22, 96, 82, and 97% in F3, F5, F7, and F7 generations, respectively. In the Samba Mahsuri background, the mean yield reduction was 66, 98, and 98% in F3, F5, F7, and F7 generations, respectively, in the RS drought stress experiment compared with NS experiments. A grain yield reduction of 68, 93, 98, and 96% was observed in F4, F6, F7, and F8 generations, respectively. In the Samba Mahsuri background, the mean yield reduction was 66, 98, and 98% in F3, F5, F7, and F7 generations, respectively, in the RS drought stress experiment compared with NS experiments. A grain yield reduction of 68, 93, 98, and 96% was observed in F4, F5, F6, and F7 generations, respectively. Accurate standardized phenotyping under RS drought stress assists breeders in rejecting inferior QTL classes in F3 itself and is the basis of success of the combined MAS breeding approach. It is evident from the yield reduction as well as the water table depths (Fig. 2a-e) that the stress level in RS drought stress experiments ranged from moderate to severe drought stress intensity at the reproductive stage in most of the cases. DTF of majority of pyramided lines was less than that of recipient lines under RS but not under NS. Some of the selected progenies showed early DTF than recipient under NS and this may have resulted from linkages of the drought resistant QTLs with earliness (Vikram et al. 2016). Most of the progenies showed similar PHT as that of recipient cultivars under NS but higher PHT under RS because of their increased ability to produce biomass under RS (data not presented).
Selection of superior QTLs class in early generation

In a marker-assisted QTL introgression/pyramiding program, it would be very valuable to explore QTL combinations with high performance in early generations. The F₂ generation is highly heterogeneous; therefore, screening of a large population size is essential to maximize the exploitation of genetic variation (Kahani and Hittalmani 2015). Sometimes, based on the availability of resources, fields for phenotyping, as well as capacity of breeding programs, breeders have to reduce the population size, which may lead to a loss of existing positive genetic variability in the population (Govindaraj et al. 2015).

In the present study, the screening of a large-sized F₃ population was carried out under control (NS) and RS drought stress conditions. The classification of the population in different classes based on QTL combinations in Table 1.}

### Table 1 Mean comparison of QTL classes of grain yield (kg ha⁻¹) across F₃ to F₈ generations under reproductive-stage drought stress and irrigated non-stress control conditions in Swarna-Sub1 background at IRRI, Philippines

| QTL class | 2012DS | 2012DS | 2012DS | 2012DS | 2012DS | 2012WS | 2013DS | 2013DS | 2014DS | 2014DS | 2015WS | 2015WS | 2016DS |
|-----------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| NS_Med    | F₃     | F₃     | F₃     | F₃     | F₃     | F₃     | F₃     | F₃     | F₃     | F₃     | F₃     | F₃     | F₃     |
| RS_Med    |        |        |        |        |        |        |        |        |        |        |        |        |        |
| RS_Late   |        |        |        |        |        |        |        |        |        |        |        |        |        |
| NS_Late   |        |        |        |        |        |        |        |        |        |        |        |        |        |
| NS        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| RS        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| NS        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| RS        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| NS        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| RS        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| MS        |        |        |        |        |        |        |        |        |        |        |        |        |        |

Population size

|          | 663    | 366    | 304    | 91     | 84     | 754    | 432    | 432    | 432    | 52     | 52     | 52     |

A: qDTY₁.₁  
B: Sub1+ qDTY₁.₁  
C: DTY₁.₁  
D: Sub1+ qDTY₂.₁  
E: qDTY₂.₁  
F: Sub1+ qDTY₂.₁  
G: Sub1  
H: qDTY₁.₁+ qDTY₂.₁  
I: Sub1+ qDTY₁.₁+ qDTY₂.₁  
J: qDTY₁.₁+ qDTY₃.₁  
K: Sub1+ qDTY₁.₁+ qDTY₃.₁  
L: qDTY₂.₁+ qDTY₃.₁  
M: qDTY₂.₁+ qDTY₂.₁+ Sub1  
N: qDTY₂.₁+ qDTY₂.₁+ qDTY₃.₁  
O: Sub1+ qDTY₂.₁+ qDTY₂.₁+ qDTY₃.₁  
X: Parent  

The letter display are QTL class labels ordered by mean grain yield of QTL class. Means followed by the same letter (within a column) are not significantly different, DS dry season, WS wet season, NS non-stress, RS reproductive-stage drought stress, Med medium duration, Late late duration, X recipient parent (no QTL).
### Table 2

Mean comparison of QTL classes of grain yield (kg ha\(^{-1}\)) across F\(_3\) to F\(_7\) generations under reproductive-stage drought stress and irrigated non-stress control conditions in IR64-Sub1 background at IRRI, Philippines

| QTL class | QTL          | 2013WS | 2013WS | 2014DS | 2014DS | 2014WS | 2015DS | 2015DS | 2015WS | 2015WS | 2015WS | 2015WS |
|-----------|--------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
|           |              | F\(_3\) | F\(_3\) | F\(_4\) | F\(_4\) | F\(_5\) | F\(_6\) | F\(_7\) | F\(_7\) | F\(_7\) | F\(_7\) | F\(_7\) |
| A         | Sub1 + qDTY\(_{1,1}\) + qDTY\(_{1,2}\) + qDTY\(_{12,1}\) | 4137 ac | 3621 cde | 7553 bdf | 584 g | – – – – – – |
| B         | Sub1 + qDTY\(_{1,1}\) + qDTY\(_{1,2}\) + qDTY\(_{2,2}\) + qDTY\(_{12,1}\) | 3640 ac | 2605 a | 1996 b | 196 ab | – – – – – – |
| C         | Sub1 + qDTY\(_{1,1}\) + qDTY\(_{1,2}\) + qDTY\(_{2,2}\) | 4986 c | 2734 ab | 5996 abc | 377 def | – – – – – – |
| D         | Sub1 + qDTY\(_{1,1}\) + qDTY\(_{1,2}\) | 4418 cd | 3054 abc | 7709 cef | 232 abc | 3585 ab | 5192 a | 477 bcd | – – – – – – |
| E         | Sub1 + qDTY\(_{1,1}\) + qDTY\(_{1,2}\) + qDTY\(_{2,2}\) | 3589 ac | 2634 abc | – – – – – – |
| F         | Sub1 + qDTY\(_{1,1}\) + qDTY\(_{2,2}\) | 4953 ac | 3169 ab | 7637 bdf | 367 def | 3347 ab | 5292 b | 4105 a | 188 a |
| G         | Sub1 + qDTY\(_{1,1}\) | 4413 ac | 1057 bc | 7892 cd | 232 ab |
| H         | Sub1 + qDTY\(_{1,1}\) + qDTY\(_{2,2}\) | 4001 ac | 2760 abc | 6660 abe | 245 b |
| I         | Sub1 + qDTY\(_{1,1}\) + qDTY\(_{2,2}\) + qDTY\(_{12,1}\) | 5370 cb | 3352 abc | 8790 b | 259 b |
| J         | Sub1 + qDTY\(_{1,1}\) | 4380 cd | 2690 abd | 6117 ab | 214 b |
| K         | Sub1 + qDTY\(_{2,2}\) + qDTY\(_{12,1}\) | 4395 cd | 3130 bc | 6512 ab | 308 ae |
| L         | Sub1 + qDTY\(_{2,2}\) | 4252 cd | 3767 e | 7893 cf | 223 abc |
| M         | Sub1 + qDTY\(_{2,2}\) + qDTY\(_{12,1}\) | 3168 ac | 3084 abe | 8532 cef | 194 b |
| N         | Sub1 + qDTY\(_{2,2}\) | 3145 ab | 2602 a | 7080 bde | 244 abcd |
| O         | Sub1 + qDTY\(_{2,2}\) | 3670 ac | 2746 abd | 7145 abf | 416 c |
| P         | Sub1 + qDTY\(_{2,2}\) + qDTY\(_{12,1}\) | 3109 ac | 2728 abd | 7798 bdf | 197 b |
| Q         | Sub1 + qDTY\(_{2,2}\) + qDTY\(_{12,1}\) | 3055abcd | 2526 a | 6441 ab | 220 b |
| R         | Sub1 + qDTY\(_{2,2}\) + qDTY\(_{12,1}\) | 2845 ac | 2931 abc | 6496 abc | 168 b |
| S         | Sub1 + qDTY\(_{2,2}\) + qDTY\(_{12,1}\) | 1688 ac | 2891 abe | 5319 a | 304 ae |
| T         | Sub1 + qDTY\(_{2,2}\) | 3444 ac | 3427 be | 6230 ad | 124 b |
| X         | Parent | 3620 ac | 2305 a | 6066 abf | 87 abc |

The letter display are QTL class labels ordered by mean grain yield of QTL class. Means followed by the same letter (within a column) are not significantly different, DS dry season, WS wet season, NS non-stress, RS reproductive-stage drought stress, X recipient parent (no QTL).

### Table 3

Mean comparison of QTL classes of grain yield (kg ha\(^{-1}\)) across BC1F\(_3\) to BC1F\(_8\) generations under reproductive-stage drought stress and irrigated non-stress control conditions in Samba Mahsuri background at IRRI, Philippines

| QTL class | QTL          | 2013DS | 2013DS | 2014WS | 2014WS | 2015WS | 2015WS | 2015WS | 2016DS | 2016DS |
|-----------|--------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
|           |              | F\(_3\) | F\(_3\) | F\(_6\) | F\(_6\) | F\(_7\) | F\(_7\) | F\(_7\) | F\(_7\) | F\(_7\) |
| A         | qDTY\(_{2,2}\) | 2020 a | 1069 bc | 3405 b | 3327 b | 44 a | – – – – – – |
| B         | qDTY\(_{4,1}\) | 1900 a | 894 b | 3340 b\(^1\) | 4727 d\(^1\) | 184 b\(^1\) | 5643 b\(^1\) | 33 a |
| C         | qDTY\(_{2,2}\) + qDTY\(_{4,1}\) | 2916 b | 1296 c | 3270 b | 4161 c | 110 ba | 4999 a | 216 b |
| X         | Parent | 2742 b | 0 a | 2137 a | 1945 a | 15 a | 4051 a | 39 a |

Trial Mean

|               | F\(_7\) | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
|---------------|--------|----|----|----|----|----|----|----|----|----|
| F- value      | 1.59   | 2.88 | 3.22 | 2.68 | 4.32 | 1.54 | 1.53 |    |    |    |
| p-value       | 0.2996 | 0.006 | 0.0006 | 0.0011 | 0.0363 | 0.0404 | 0.0004 | 0.5566 | 0.5585 | 18 a |

The letter display are QTL class labels ordered by mean grain yield of QTL class. Means followed by the same letter (within a column) are not significantly different, DS dry season, WS wet season, NS non-stress, RS reproductive-stage drought stress, X recipient parent (no QTL).
Table 4  Mean comparison of QTL classes of grain yield (kg ha\(^{-1}\)) across BC\(_{2F3}\) to BC\(_{2F8}\) generations under reproductive-stage drought stress and irrigated non-stress control conditions in TDK-Sub1 background at IRRI, Philippines

| QTL class | QTL | 2013WS | 2014DS | 2014WS | 2015DS | 2015WS | 2016DS |
|-----------|-----|--------|--------|--------|--------|--------|--------|
|           | RS  | NS     | RS     | NS     | NS     | NS     | RS     | NS     |
| BC\(_{2F3}\) | BC\(_{2F4}\) | BC\(_{2F4}\) | BC\(_{2F5}\) | BC\(_{2F6}\) | BC\(_{2F6}\) | BC\(_{2F7}\) | BC\(_{2F8}\) | BC\(_{2F8}\) |

| Population size | 843 | 231 | 231 | 48 | 48 | 48 | 60 | 60 | 60 |

A  
Sub1 + qDTY\(_{6.1}\) + qDTY\(_{6.2}\) + qDTY\(_{3.1}\)
B  
qDTY\(_{6.1}\) + qDTY\(_{6.2}\) + qDTY\(_{3.1}\)
C  
Sub1 + qDTY\(_{6.1}\) + qDTY\(_{6.2}\)
D  
Sub1 + qDTY\(_{6.1}\) + qDTY\(_{3.1}\)
E  
Sub1 + qDTY\(_{6.2}\) + qDTY\(_{3.1}\)
F  
qDTY\(_{6.1}\) + qDTY\(_{6.2}\)
G  
qDTY\(_{6.1}\) + qDTY\(_{3.1}\)
H  
Sub1 + qDTY\(_{6.2}\) + qDTY\(_{3.1}\)
I  
Sub1 + qDTY\(_{6.2}\)
J  
Sub1 + qDTY\(_{6.1}\)
K  
Sub1 + qDTY\(_{2.2}\)
L  
Sub1 + qDTY\(_{2.2}\)
M  
Sub1 + qDTY\(_{3.1}\)
N  
qDTY\(_{2.2}\)
X  
Parent

The letter display are QTL class labels ordered by mean grain yield of QTL class. Means followed by the same letter (within a column) are not significantly different, RS dry season, WS wet season, NS non-stress, RS reproductive-stage drought stress, X recipient parent (no QTL)

Table 5  Mean comparison of QTL classes of grain yield (kg ha\(^{-1}\)) across BC\(_{1F3}\) to BC\(_{1F7}\) generations under reproductive-stage drought stress and irrigated non-stress control conditions in MR219 background at IRRI, Philippines

| QTL class | QTL | 2013DS | 2014DS | 2015DS |
|-----------|-----|--------|--------|--------|
|           | NS  | RS     | NS     | RS     |
| BC\(_{1F3}\) | BC\(_{1F4}\) | BC\(_{1F5}\) | BC\(_{1F7}\) | BC\(_{1F7}\) |

| Population size | 214 | 214 | 620 | 620 | 70 | 70 |

A  
qDTY\(_{12.1}\)
B  
qDTY\(_{12.1}\) + qDTY\(_{2.2}\)
C  
qDTY\(_{12.1}\) + qDTY\(_{2.2}\) + qDTY\(_{3.1}\)
D  
qDTY\(_{2.2}\)
E  
qDTY\(_{2.2}\) + qDTY\(_{3.1}\)
F  
qDTY\(_{2.2}\) + qDTY\(_{3.1}\) + qDTY\(_{12.1}\)
G  
qDTY\(_{3.1}\)
H  
qDTY\(_{12.1}\) + qDTY\(_{3.1}\)
I  
qDTY\(_{3.1}\)
J  
qDTY\(_{2.2}\)
K  
qDTY\(_{3.1}\)
X  
Parent

The letter display are QTL class labels ordered by mean grain yield of QTL class. Means followed by the same letter (within a column) are not significantly different, DS dry season, WS wet season, NS non-stress, RS reproductive-stage drought stress, X recipient parent (no QTL)
each generation (F3 to F7/F8) followed by class analysis to see the performance of each QTL class across generation advancement proved to be an effective approach in identifying best-bet QTL combination classes across five high-yielding genetic backgrounds. The performance of the genotypes in a particular QTL class was consistent from F3 to F7/F8 generations in all five studied background in the present study. The advancement of the classes with high mean grain yield performance in the F3 generation in addition to the MAB approach involving stepwise phenotyping and genotyping screening suggested this as being a cost/labor- and resource-effective breeding strategy. The lesser number of genotypes in advanced generations can be screened more precisely in a large plot size with more replications. The current cost-effective high-throughput phenotyping platform (Comar et al. 2012; Andrade-Sanchez et al. 2014; Sharma and Ritchie 2015; Bai et al. 2016) can be used for precise breeding and physiological studies considering the small population size. Even at the F3 level, some heterozygosity will be observed when more genes are involved in the introgression program. However, in our study, we did not observe any change in performance of QTL classes found superior in F3, indicating the F3 generation to be suitable to conduct class analysis and reject inferior classes.

Table 6  Comparison of genotyping cost (USD) considering advancement of all QTL classes versus advancement of only higher performing F3 generation QTL classes

| Background     | Generation | Number of QTL classes | Population size | Cost (USD) | Total genotyping cost (USD) | Savings (USD) |
|----------------|------------|-----------------------|-----------------|------------|----------------------------|---------------|
|                |            |                       | Based on all classes | Based on selected classes | Based on all classes | Based on selected classes | Based on all classes | Based on selected classes |
| Swarna-Sub1    | F3         | 15                    | 754             | 5655       | 5655                        | 21,420        | 8978            | 12,443           |
|                | F4         | 15                    | 754             | 5655       | 795                         |               |                 |                  |
|                | F5         | 10                    | 432             | 3240       | 795                         |               |                 |                  |
|                | F6         | 10                    | 432             | 3240       | 795                         |               |                 |                  |
|                | F7         | 6                     | 432             | 3240       | 810                         |               |                 |                  |
|                | F8         | 5                     | 52              | 300        | 127.50                      |               |                 |                  |
| IR64-Sub1      | F3         | 20                    | 467             | 7005       | 7005                        | 12,105        | 8385            | 3720             |
|                | F4         | 19                    | 194             | 2910       | 690                         |               |                 |                  |
|                | F5         | 13                    | 64              | 960        | 270                         |               |                 |                  |
|                | F6         | 12                    | 64              | 960        | 270                         |               |                 |                  |
|                | F7         | 7                     | 18              | 270        | 150                         |               |                 |                  |
| Samba Mahsuri  | BC1F3      | 3                     | 42              | 210        | 210                         |               |                 | 14,780           |
|                | BC1F4      | 3                     | 3000            | 15,000     | 3200                        |               |                 |                  |
|                | BC1F5      | 3                     | 1200            | 6000       | 3200                        |               |                 |                  |
|                | BC1F6      | 3                     | 70              | 350        | 220                         |               |                 |                  |
|                | BC1F7      | 2                     | 20              | 100        | 75                          |               |                 |                  |
|                | BC1F8      | 2                     | 20              | 100        | 75                          |               |                 |                  |
| TDK1-Sub1      | BC2F3      | 14                    | 843             | 6323       | 6323                        | 9225          | 6954            | 2272             |
|                | BC2F4      | 7                     | 231             | 1733       | 323                         |               |                 |                  |
|                | BC2F5      | 7                     | 48              | 360        | 105                         |               |                 |                  |
|                | BC2F6      | 7                     | 48              | 360        | 105                         |               |                 |                  |
|                | BC2F7      | 5                     | 60              | 450        | 98                          |               |                 |                  |
|                | BC2F8      | 5                     | 60              | 450        | 98                          |               |                 |                  |
| MR219          | BC1F3      | 7                     | 214             | 1605       | 1605                        | 11,955        | 5730            | 6225             |
|                | BC1F4      | 7                     | 620             | 4650       | 1800                        |               |                 |                  |
|                | BC1F5      | 7                     | 620             | 4650       | 1800                        |               |                 |                  |
|                | BC1F6      | 7                     | 70              | 525        | 262.50                      |               |                 |                  |
|                | BC1F7      | 7                     | 70              | 525        | 262.50                      |               |                 |                  |

The genotyping cost was calculated considering five markers per QTL (one peak/near the peak, two right-hand-side flanking markers, and two left-hand-side flanking markers) and USD 0.50 per data point.
In addition to the modern next-generation genotyping strategies (Barba et al. 2014; Rius et al. 2015; Dhanapal and Govindaraj 2015) and agricultural system models (Antle et al. 2016), several breeding strategies involving correlated traits as selection criteria in early generations (Senapati et al. 2009), grain yield (Kumar et al. 2014), secondary traits (Mhike et al. 2012), genetic variance, heritability (Almeida et al. 2013), path coefficient analysis, selection tolerance index (Dao et al. 2017), and yield index (Raman et al. 2012) have been suggested for use in breeding programs. The consistent performance of pyramided lines with specific QTL combinations across generations (F₃ to F₇/F₈) in five backgrounds in the present study validates the potential of the suggested combined MAS breeding approach presented in the current study. The integration of accurate phenotyping and the selection of the best class representing the genetic variability of the whole population in early generations are critical steps for the practical implementation of this ultimate novel breeding strategy. Keeping a large F₃ population size depending upon the number of genes/QTLs being introgressed and precise phenotyping to exploit the hidden potential of each genotype in each QTL class could maximize the potential output of each class in early generations. The most logical QTL-class performance-derived novel breeding strategy could be

### Table 7

Comparison of phenotyping cost (USD) considering advancement of all QTL classes versus advancement of only higher performing F₃ generation QTL classes

| Background          | Generation | Population size | Phenotyping cost (USD) | Total phenotyping cost (USD) | Savings (USD) |
|---------------------|------------|----------------|------------------------|------------------------------|--------------|
|                     |            | Based on all classes | Based on selected classes | Based on all classes | Based on selected classes |               |
| Swarna-Sub1         | F₃         | 754            | 27,280                 | 103,330                     | 60,023       |
|                     | F₄         | 754            | 27,280                 | 3835                         |              |
|                     | F₅         | 432            | 15,630                 | 3835                         |              |
|                     | F₆         | 432            | 15,630                 | 3835                         |              |
|                     | F₇         | 432            | 15,630                 | 3907                         |              |
|                     | F₈         | 52             | 1881                   | 1561                         |              |
| IR64-Sub1           | F₃         | 467            | 16,896                 | 29,197                       | 8973         |
|                     | F₄         | 194            | 7019                   | 1664                         |              |
|                     | F₅         | 64             | 2316                   | 651                          |              |
|                     | F₆         | 64             | 2316                   | 651                          |              |
|                     | F₇         | 18             | 651                    | 362                          |              |
| Samba Mahsuri       | BC₁F₃      | 42             | 1520                   | 157,455                      | 106,948      |
|                     | BC₁F₄      | 3000           | 108,540                | 50,507                       | 53,033       |
|                     | BC₁F₅      | 1200           | 43,165                 | 23,155                       |              |
|                     | BC₁F₆      | 70             | 2533                   | 1592                         |              |
|                     | BC₁F₇      | 20             | 724                    | 543                          |              |
|                     | BC₁F₈      | 20             | 724                    | 543                          |              |
| TDK1-Sub1           | BC₂F₃      | 843            | 30,500                 | 44,501                       | 10,963       |
|                     | BC₂F₄      | 231            | 8358                   | 1556                         |              |
|                     | BC₂F₅      | 48             | 1737                   | 507                          |              |
|                     | BC₂F₆      | 48             | 1737                   | 507                          |              |
|                     | BC₂F₇      | 60             | 2171                   | 470                          |              |
|                     | BC₂F₈      | 20             | 2171                   | 470                          |              |
| MR219               | BC₁F₃      | 214            | 7743                   | 57,671                       | 30,029       |
|                     | BC₁F₄      | 620            | 22,432                 | 8683                         |              |
|                     | BC₁F₅      | 620            | 22,432                 | 8638                         |              |
|                     | BC₁F₆      | 70             | 2533                   | 1266                         |              |
|                     | BC₁F₇      | 70             | 2533                   | 1266                         |              |

The phenotyping cost of USD 36.18 per entry was calculated considering two replications and screening under NS and RS drought stress with plot size of 1.54 m² (IRRI Standard drought screening costing).

**Population size and validation of combined breeding strategy**

In addition to the modern next-generation genotyping strategies (Barba et al. 2014; Rius et al. 2015; Dhanapal and Govindaraj 2015) and agricultural system models (Antle et al. 2016), several breeding strategies involving correlated traits as selection criteria in early generations (Senapati et al. 2009), grain yield (Kumar et al. 2014), secondary traits (Mhike et al. 2012), genetic variance, heritability (Almeida et al. 2013), path coefficient analysis, selection tolerance index (Dao et al. 2017), and yield index (Raman et al. 2012) have been suggested for use in breeding programs. The consistent performance of pyramided lines with specific QTL combinations across generations (F₃ to F₇/F₈) in five backgrounds in the present study validates the potential of the suggested combined MAS breeding approach presented in the current study. The integration of accurate phenotyping and the selection of the best class representing the genetic variability of the whole population in early generations are critical steps for the practical implementation of this ultimate novel breeding strategy. Keeping a large F₃ population size depending upon the number of genes/QTLs being introgressed and precise phenotyping to exploit the hidden potential of each genotype in each QTL class could maximize the potential output of each class in early generations. The most logical QTL-class performance-derived novel breeding strategy could be
adopted to optimize the breeding efficiency of small-to moderate-sized breeding programs in rice breeding improvement programs. Further, the strategy could be equally useful to other crops in which major genes/ QTLs determine the expression of traits and QTL x QTL or QTL x genetic background interactions have been identified.

We were able to understand the effectiveness of early generation selection in the marker-assisted introgression program for drought because the breeding program maintained systematic data for both genotyping and phenotyping conducted over the past six or more years. It was only after we successfully identified the best lines coming from each introgression program after successful

Fig. 2 Soil water potential measured by parching water table level in experiments (a) Swarna-Sub1 pyramided lines with qDTY1.3, qDTY2.1, and qDTY3.2 in different generations; b IR64-Sub1 pyramided lines with qDTY1.3, qDTY2.3, qDTY3.2, and qDTY3.2 in different generations; c Samba Mahsuri pyramided lines with qDTY2.2 and qDTY4.1 in different generations; d TDK1-Sub1 pyramided lines with qDTY3.1, qDTY6.1, and qDTY6.2 in different generations; and (e) MR219 pyramided lines with qDTY2.2, qDTY3.1, and qDTY12.1 in different generations using polyvinyl chloride (PVC) pipe
multi-location evaluation that we realized that, as the breeding program will need to bring in more and more genes for multiple traits to address each of the new emerging climate-related challenges, modifications that allow plant breeders to make large-scale rejections in the early generation will become necessary. The effectiveness of the combined MAS strategy is evident from the result that, in none of the five cases were the superior QTL class combinations identified in F3 outperformed by inferior classes identified in F4 in any advanced generation under both NS and variable intensities of RS drought stress in different seasons/years across generations from F4 to F7/F8/F9.

Cost-effectiveness of combined breeding strategy
Breeding practices are challenged by being laborious, time consuming, and non-economical, requiring large land space and a large population size (Sandhu and Kumar 2017), being imprecise, and having unreliable phenotyping screening (Bhat et al. 2016); hence, an economical, fast, accurate, and efficient breeding selection system is required to increase yield potential and productivity (Khan et al. 2015). The cost-benefit balance (Bhat et al. 2016) must be considered in increasing genomic, molecular marker breeding approach (Table 6). Although the cost-benefit of the combined MAS breeding strategy will always be inversely proportional to the number of superior QTL class combinations identified for advancement in F3 and subsequent generations, the cost savings will increase as the number of genes included in the introgression program increases because of the rejection of a larger proportion of the total population early in the F3 generation. This procedure will save time, labor, resources, and space and will allow breeders to focus only on germplasm with higher value. This will reduce the population size for phenotypic and genotypic selection in advanced generations compared with the traditional molecular marker breeding approach (Table 6). Although the cost-benefit of the combined MAS breeding strategy will always be inversely proportional to the number of superior QTL class combinations identified for advancement in F3 and subsequent generations, the cost savings will increase as the number of genes included in the introgression program increases because of the rejection of a larger proportion of the total population early in the F3 generation. This procedure will save time, labor, resources, and space and will allow breeders to focus only on germplasm with higher value. This will reduce the population size for phenotypic and genotypic selection in advanced generations compared with the traditional molecular marker assisted breeding strategies (Price 2006; McNally et al. 2009; Yadaw et al. 2013; Sandhu et al. 2014; Brachi et al. 2012; Begum et al. 2015). It will be practical and realistic only if the phenotyping, genotyping, and class analysis in early generations are accurate.

Interactions among QTLs and with background
The QTLs for grain yield under drought have shown QTL x QTL (Sandhu et al. 2018) as well as QTL x genetic background interactions (Dixit et al. 2012a, b; Sandhu et al. 2018). Many such interactions that may occur between QTL x QTL and QTL x genetic background are unknown. Such positive/negative interactions affecting grain yield under normal or RS situation can be captured through approach that combines selection based on phenotyping and genotyping in the early generations. The current study clearly demonstrated the success of selection based on combining phenotyping and genotyping in identifying better progenies in early generation thereby reducing the number of progenies to be advanced. Number of plants to be generated and evaluated in the early generations will depend upon the number of QTLs/genes to be introgressed together, size of introgressed QTLs region as well as availability of closely linked markers for each of the QTLs. The QTLs for grain yield under drought have shown undesirable linkages with low yield potential, very early maturity duration, tall plant height (Vikram et al. 2015). At IRRI, studies were undertaken to break the undesirable linkages of QTLs with tall plant height, very early maturity duration and low yield potential (Vikram et al. 2015). Such improved lines were used in the MAS introgression program. The drought tolerant donors N22, Dular, Apo, Way Rarem, Kali Aus, Aday Sel that are source of identified QTLs do not possess good grain quality. Even though, we did not study the linkage of qDTYs with grain quality, the introgressed lines released as varieties in IR64, Swarna backgrounds in India and Nepal did not reveal any adverse effect on grain quality. The yield superiority of lines with two or more QTLs under both NS and RS drought stress over the five high-yielding backgrounds clearly indicated that qDTY QTLs identified at IRRI are free from undesirable linkage drag and can be successfully used in MAB programs targeting yield improvement under RS drought stress. Further, in Swarna-Sub1, IR64-Sub1, and TDK-Sub1, the highest yielding classes identified were the classes possessing both Sub1 and combinations of the drought QTLs. The yield superiority of such classes across these three backgrounds over all the generations clearly indicated that tolerance of submergence and drought can be effectively combined even though they are governed by different physiological mechanisms. In the QTL study undertaken at IRRI, qDTY1.1 showed a significant mean yield advantage in MTU1010 and IR64 (Sandhu et al. 2015); qDTY2.2 in Pusa Basmati 1460, MTU1010, and IR64 (Venuprasad et al. 2007; Swamy et al. 2013; Sandhu et al. 2013; Sandhu et al. 2014); qDTY3.2 in Vandana and IR64 (Dixit et al. 2012b; Sandhu et al. 2014); qDTY4.2 in Sabitri (Yadaw et al. 2013); qDTY5.1 in IR72 (Venuprasad et al. 2009); and qDTY12.1 in Vandana (Bernier et al. 2007), Sabitri (Mishra et al. 2013), Kalinga, and Anjali backgrounds. Similar interaction of qDTY2.3 and qDTY4.2 with qDTY12.1 in a Vandana background (Dixit et al. 2012b); qDTY2.2 and qDTY3.1 with qDTY12.1 in an MRQ74 background (Shamsudin et al. 2016); and
qDTY2.2 + qDTY4.1 in an IR64 background (Swamy et al. 2013) was observed. The interaction of identified QTLs with other QTLs in more than two backgrounds supports the usefulness of such QTL classes in MAS. In all five of these cases, through genotyping and phenotyping we were able to identify QTL class combinations with positive interactions and higher yield. As more data are generated across different backgrounds and interactions are established, breeders will have the ability to identify and forward only selected classes without phenotyping from F3 onward.

Pyramiding of multiple QTLs associated with multiple traits

With the identification of gene-based/closely linked markers for different biotic stresses (bacterial blight, blast, brown planthopper, gall midge) and abiotic stresses (submergence, drought, phosphorus deficiency, cold, anaerobic germination, high temperature), the MAB program is moving forward to introgress more genes/QTLs to develop climate-resilient and better rice varieties. For effective tolerance to develop a variety combining tolerance of biotic and abiotic stresses – bacterial leaf blight (three genes – xa5, xa13, Xa21), blast (two – pi2, pi9), brown planthopper (two – BPH3, BPH17), gall midge (two – Gm4, Gm8), drought (three – qDTY1.1, qDTY2.1, qDTY3.1), and submergence (Sub1) – researchers will need introgression and the combination of 13–15 genes/QTLs in gene combinations mentioned here or in other combinations depending upon the prevalence of a pathotype/biotype in different regions. The number of genes to be introgressed is likely to increase as exposure of rice to high temperature at the reproductive stage will probably increase in most rice-growing regions. The introgression of 10–15 genes will not only require a larger initial population in F2 and F3 but will also lead to increased positive/negative interactions between genes/QTLs. With capacity development, as more and more breeding programs adopt marker-assisted introgression of more genes, the combined MAS strategy will be of great help to plant breeders in reducing the number of plants that they should handle in each generation and make their breeding program cost-effective.

Conclusions

The selection of QTL classes with a high mean yield performance and positive interactions among loci and with background in the early generation and consistent performance of QTL classes in subsequent generations across five backgrounds supports the effectiveness of a combined MAS breeding strategy. The challenge ahead is the appropriate estimation of the precise population size to be used for QTL class analysis in the early F3 generation to maintain genetic variability as the number of genes/QTLs increases further. Integration of a cost-effective, efficient, designed, statistics-led early generation superior QTL class selection-based breeding strategy with new-era genomics such as genotyping by sequencing and genomic selection could be an important breakthrough to build up a scientific next-generation breeding program.

Methods

The study was conducted at the International Rice Research Institute (IRRI), Philippines, to introgress QTLs for grain yield under RS drought stress in the background of improved high-yielding widely grown but drought-susceptible varieties from India (Swarna, IR64, Samba Mahsuri), Lao PDR (TDK1), and Malaysia (MR219).

Five sets of introgressed populations were used:

1. Swarna-Sub1 pyramided lines with qDTY1.1, qDTY2.1, qDTY3.1, qDTY4.1, and qDTY5.1
2. IR64-Sub1 pyramided lines with qDTY1.1, qDTY1.2, qDTY2.2, qDTY2.3, qDTY3.2, and qDTY4.1
3. Samba Mahsuri pyramided lines with qDTY2.2 and qDTY4.1
4. TDK1-Sub1 pyramided lines with qDTY3.1, qDTY6.1, and qDTY6.2
5. MR219 pyramided lines with qDTY2.2, qDTY3.1, and qDTY12.1

Three steps were employed for the development of a cost-effective, reliable, and resource-efficient combined MAS breeding strategy: (1) grain yield and genotypic data across F3, F4, F5, F6, F7, and F8/fixed lines for all five sets were compiled; (2) class analysis was carried out to develop a combined MAS breeding strategy; and (3) the performance of the superior classes was monitored across advanced generations to validate the combined MAS breeding strategy.

The screening of all five population sets was carried out under NS control and RS drought stress conditions. For the NS experiments, 5-cm water depth level was maintained throughout the rice growing season until physiological maturity. For the screening under RS drought stress, irrigation was stopped at 30 days after transplanting (DAT). The last irrigation was provided at 24 DAT and there was no standing water in the field when drought was initiated at 30 DAT. The stress cycle was continued until severe stress symptoms were observed. Monitoring of soil water potential was carried out by placing perforated PVC pipes at 100-cm soil depth in the field in a zig-zag manner. After the initiation of stress, the water table level was recorded daily. When approximately 70% of the lines exhibited severe leaf rolling or
witling, one life-saving irrigation with a sprinkler system was provided. Then, a second cycle of the stress was initiated. The water table level was measured from all the pipes until the rice crop reached 50% maturity.

Molecular marker work was carried out following the procedure as described in Sandhu et al. (2014). For genotyping, a total of 754, 754, 432, 432, 432, and 52 plants were phenotyped and genotyped in F3 (NS, RS), F4 (NS), F5 (NS, RS), F6 (NS, RS), F7 (NS), and F8 (NS, RS) generations, respectively. In the IR64-Sub1 background, 467, 194, 64, 64, and 18 plants were phenotyped and genotyped in F3 (NS, RS), F4 (NS, RS), F5 (NS), F6 (NS, RS), and F7 (NS, RS) generations, respectively. In the Samba Mahsuri background, a total of 42, 3000, 1200, 70, 20 and 20 plants were phenotyped and genotyped in BC1F3 (NS, RS), BC1F5 (NS, RS), BC1F7 (NS, RS), and BC1F8 (NS, RS) generations respectively. In the TDK-1Sub1 background, 843, 231, 48, 48, 60 and 60 plants were phenotyped and genotyped in BC2F3 (RS), BC2F5 (NS, RS), BC2F7 (NS, RS), and BC2F8 (NS, RS) generations, respectively. A total of 214, 620, 620, 70, and 70 plants were phenotyped and genotyped in BC1F3 (NS, RS), BC1F4 (NS, RS), BC1F6 (NS, RS), and BC1F8 (NS, RS) generations, respectively, in the MR219 background. Data on plant height, days to 50% flowering, and grain yield were recorded following the procedure of Venuprasad et al. (2009). The detailed description on QTLs and markers used in the present study in each background is presented in Additional file 1: Table S1. The general schematic scheme followed for QTL introgression and pyramiding program, phenotyping and genotyping screening is shown in Additional file 1: Figure S1.

Analytical approach to reveal a combined MAS breeding strategy

The grain yield data from F3, F4, F5, F6, F7, and F8 generations across seasons and NS (control) and RS drought stress conditions for all five sets of pyramided populations were compiled and categorized into classes based on the genotypic QTL information. Class analysis using SAS v9.2 was attempted to see the mean grain yield performance of QTL classes across generation advancement.

Genotyping and phenotyping cost calculation

The genotyping cost of USD 36.18 per entry (two replications, screening under NS and RS drought stress with plot size of 1.54 m2) (IRRI Standard drought screening costing) including the cost of land preparation, land rental, irrigation, electricity, field layout, seeding, transplanting, maintenance cost, resource input (fertilizer), pesticides, herbicides, field supplies, harvesting, threshing, drying, data collection, and labor was used to calculate the cost savings for phenotyping. The genotyping cost was calculated for the whole population across successive generations (F3 to F7/F8) and compared with the genotyping cost (F3 to F7/F8) considering only the QTL classes that performed better in F5. The genotyping cost was calculated considering five markers per QTL (one peak/near the peak, two right-hand-side flanking markers, and two left-hand-side flanking markers) using USD 0.50 per data point (Xu et al. 2002; Xu 2010).

Statistical analysis

**Mean comparison of QTL genotype classes**

Hypothesis about no differences among genotype means of QTL genotype classes for each background under NS and RS drought stress in each season was performed in SAS v9.2 (SAS Institute Inc. 2009) using the following linear model.

\[ y_{ijkl} = \mu + r_k + b(r)_{kl} + q_i + g(q)_{ij} + e_{ijkl} \]

where \(\mu\) represents the population mean, \(r_k\) represents the effect of the \(k^{th}\) replicate, \(b(r)_{kl}\) is the effect of the \(k^{th}\) block within the \(k^{th}\) replicate, \(q_i\) corresponds to the effect of the \(i^{th}\) QTL, \(g(q)_{ij}\) symbolizes the effect of the \(j^{th}\) genotype nested within the \(i^{th}\) QTL, and \(e_{ijkl}\) corresponds to the error (Knapp 2002). The effects of QTL class and the genotypes within QTL were considered fixed and the replicates and blocks within replicates were set to random.

Additional file

**Additional file 1: Table S1.** QTLs and markers information’s in marker assisted introgression program in different backgrounds. **Figure S1.** General schematic scheme for QTL introgression and pyramiding program, phenotyping and genotyping screening. In case of Swarna-Sub1 and IR64-Sub1 no backcross was attempted. In case of Samba Mahsuri and MR219, one backcross was attempted. In case of TDK1-Sub1 two backcross was attempted. (DOCX 269 kb)

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Availability of data and materials

The relevant supplementary data has been provided with the manuscript.

Authors’ contributions

AK conceived the idea of the study and was involved in critical revision and final approval of the version to be published; NS was involved in conducting the experiments, analysis, interpretation of the data, and drafting the manuscript; SD, SY, BPMs, and NAAS were involved in developing populations and conducting the experiments. All authors read and approved the final manuscript.
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