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Full Length Research Paper

Bridging *sd1* molecular knowledge with recent breeding strategies for the improvement of traditional rice varieties - a *japonica* case-study

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The rice semidwarfing gene, *sd1*, also known as the "green revolution gene", has been studied intensively due to its contribution to the increase of crop production. Although *sd1* breeding was extensively applied since the 1960s, the recent advances in the molecular basis of this gene allowed designing a more precise breeding strategy - marker assisted backcrossing (MAB) - to track *sd1* introgression in two traditional rice varieties. For selection of *sd1* plants we first confirmed the efficiency of specific markers based on *Os*200 x 2 gene sequence. Background selection was also performed with the help of microsatellites markers (SSR) and a total of 7 breeding lines were recovered containing a higher percentage of recurrent parent genome (RPG). Analysis of Covariance (ANCOVA) using mean progenitor plant height as covariate was performed to compare several agronomic and quality-related parameters in two different environments. The results suggest that plant height differs significantly between the two environments $F(1, 220) = 155.336$; $p < 0.001$. From the total variability of plant height we could conclude that 73% is due to the genotype, while 10.4% depends on the environment. In addition, the percentage of RPG seems negatively correlated with plant height ($p < 0.005$). MAB and background selection thus revealed as useful tools to assist breeding for semidwarfism in traditional rice varieties.

Key words: Rice, semidwarfism, *sd1*, environmental influence.

INTRODUCTION

After the rapid expansion of the world population in the 1960s, problems related to food shortage were on the top of the priorities (Sasaki et al., 2002) . A major break-through arose with 'IR8', the first high-yielding modern rice cultivar, released by the International Rice Research Institute (IRRI) in 1966 (Peng et al., 2000) leading to the so-called "rice green revolution". This variety known as "miracle rice", was derived from a cross between a Taiwanese native semidwarf variety, 'Dee-geo-woo-gen' (DGWG), which harbours the *semidwarf* 1 (*sd1*) gene and an Indonesian variety 'Peta' (Hargrove and Cabanilla, 1979). 'IR8' possessed the semidwarf phenotype caused by the *sd1* gene with a high tillering rate, erect leaves, high harvest index and high nitrogen responsiveness.

Although several dwarfing genes were reported on rice, they were not used in breeding due to associated problems of floret sterility, incomplete panicle exsertion or abnormal plant and grain development (Aquino and Jennings, 1966). The chromosomal location of *sd1* was established on chromosome 1 (Tsai, 1991) and it was later confirmed with the molecular mapping based on the existing rice RFLP (Restriction Fragment Length Polymorphism) map (Cho et al., 1994). Cho and colleagues (1994) reinforced the idea of using tightly linked markers (two RFLPs and one isozyme) to predict the stature in a young age and

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therefore, as an efficient way of detecting the presence of the sd1 gene.

"Green revolution" lines are shorter due to an abnormal level of the production to the plant growth hormone gibberellin (GA). The *sd1* gene encodes GA20 oxidase-2 (GA20ox-2) which catalyzes late steps of GA biosynthesis (Ashikari et al., 2002; Monna et al., 2002; Sasaki et al., 2002; Spielmeyer et al., 2002), providing evidences that this key enzyme is not functioning effectively in the *sd1* mutants. The *sd1* allele present in 'IR8' is a null allele, containing a 383 bp deletion from exon 1 to exon 2 that originates a stop codon. In contrast, the *japonica* varieties 'Jikkoku', 'Reimei' and 'Calrose 76' contain a single nucleotide replacement in SD1 leading to a single amino acid substitution (Ashikari et al., 2002; Monna et al., 2002; Sasaki et al., 2002; Spielmeyer et al., 2002). Asano et al. (2007) investigated the alleles employed in the generation of semidwarf varieties, by sequencing analysis of 57 semidwarf varieties. The results obtained suggest the existence of seven different sd1 alleles in the semidwarf varieties, three of which are known as 'DGWG', 'Jikkoku' and 'Reimei' alleles (Ashikari et al., 2002, Sasaki et al., 2002, Spielmeyer et al., 2002), while the remaining were newly described (Asano et al., 2007).

Semidwarfism introduction increased plants response to nitrogen inputs, with a higher yield performance but without culm elongation and lodging problems (Ashikari et al., 2002). Rice breeding for semidwarfism has been largely exploited since the 1960s. However, the recent knowledge of the *sd1* sequence (Monna et al., 2002), allowed us to design a more specific breeding strategy to improve traditional varieties. In this work, we have performed field experiments in two different environments with several breeding lines derived from two Portuguese traditional varieties. Most of these varieties are very tall, have low productivity and are sensitive to diseases, especially to blast (Magnaporthe *oryzae* B. Couch). However; they have good grain quality and are well-adapted to Portuguese preferences (Jayamani et al., 2007). Our breeding lines were obtained by MAB and background selection. We found that plant height differed significantly between the two tested environments (Mondego and Tejo riverbeds) $F(1, 220) = 155.336$; $p < 0.001$. From the total variability of plant height observed, we could conclude that 73% is derived from the genotype, while 10.4% depends on the environment and that the amount of RPG is highly correlated with plant height ($p < 0.005$).

MATERIALS AND METHODS

Plant material

The crossing scheme used and genes introgressed in this process are shown in Figure 1. The genotypes 'IR36' and 'Allorio' (IRGC284) were obtained from the International Rice Genebank held at IRRI, Philippines. 'Reiho' was kindly provided by Y. Fukuta from Japan International Research Centre for Agricultural Sciences (JIRCAS), while 'Strella' was kindly provided by Estação Agronómica Nacional (Portugal). For agronomical evaluation of the plants having *sd1* introgressed, we used seven breeding lines from the BC_2F_3 further described. We also used a commercial variety ('Ariete') and all the parents as controls for agronomical parameters.

Growth conditions in the field and data collection

All the genotypes were grown in 2007 in two different locations with distinct cultivation methods: Bico da Barca (BB) in Mondego region (N 40°17', W 008°68') with higher availability of man labour and in "Centro Operacional e Tecnológico do Arroz" (COTArroz) - Salvaterra de Magos (CO) in Tejo/Sorraia region (N 39°26', W 008°78') where cultivation is more mechanized. In 2007, the average temperature in rice season was 19.8°C in COTArroz with a rainfall of 95 mm; while in Bico da Barca the average temperature during rice season was 19.4°C and the precipitation 113.4 mm. The application of total nitrogen was the same in both locations (96 kg/ha).

All the genotypes (breeding lines, parents and control) were transplanted with 3 - 5 leaves, in each trial. Data regarding plant height, number of productive tillers, panicle length, grain length and length: breadth ratio (L/B), were collected in stage nine according to the Standard Evaluation System for rice (SES). A total number of 12 plants in each trial were sampled for statistical analysis. Regarding biometric parameters for quality analysis, 10 whole grains (after dehulling) were measured with the assistance of a photographic enlarger and millimetric graphic paper. In order to study the behaviour of *sd1* gene, we have also performed plant height measurements in six individuals throughout the breeding process (data was collected in greenhouse, with Temperature (°C) = 28, RH (%) = 60 and 12 h photoperiod).

DNA extraction and PCR analysis of the *sd1* gene

Total DNA was extracted from leaves of 30 day-old seedlings according to CTAB method (Doyle and Doyle, 1987) with slight changes as described by Jayamani et al. (2007).

For amplification of the *sd1* gene (GenBank accession number AB077025), we used specific markers previously described (Ellis and Spielmeyer, 2002). The PCR reaction was performed with 40 ng of template DNA, using PCR components from invitrogen (Carlsbad, CA, USA) and 5% of dimethyl sulfoxide (DMSO). The PCR conditions were set at 95°C for 3 min, followed by 15 cycles of 95°C for 1 min, 52°C for 1 min, 72°C for 1 min and 20 cycles of 95°C for 1 min, 52°C for 1 min, 72°C for 1 min (with additional 5 s in each cycle) and a final extension of 7 min at 72°C.

Genotype analysis for background selection

We tested 165 microsatellite (SSR) markers regularly distributed along the rice genome to select plants with RPG higher percentage. A total of 45 polymorphic markers were used for background selection (data not shown). A graphical representation of the selected genotypes (with higher RPG) was obtained in BC_2F_2 generation using software program GGT (van Berloo, 1999).

Statistical data analysis

Comparison of plant height between seven breeding lines was done using Analysis of Covariance (ANCOVA) with mean progenitor plant height as covariate. A two-way analysis of variance was also used to test the significance of the differences between breeding lines and environmental conditions for the mean number of productive tillers, panicle length, grain length and L/B. Assumptions of the

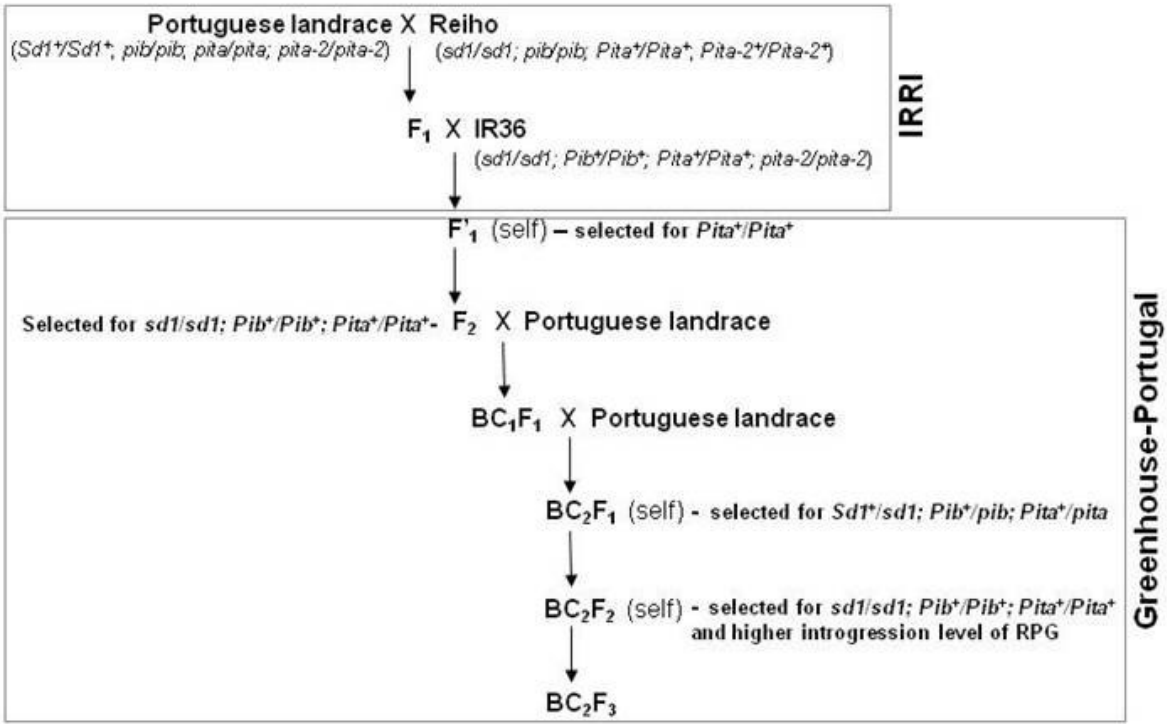

Figure 1. Breeding scheme used to improve the Portuguese landraces by marker-assisted backcross (MAB). The selection steps as well as the chosen genotypes are represented. In BC_2F_2 generation, we have selected genotypes according to their percentage of recurrent parent genome (RPG).

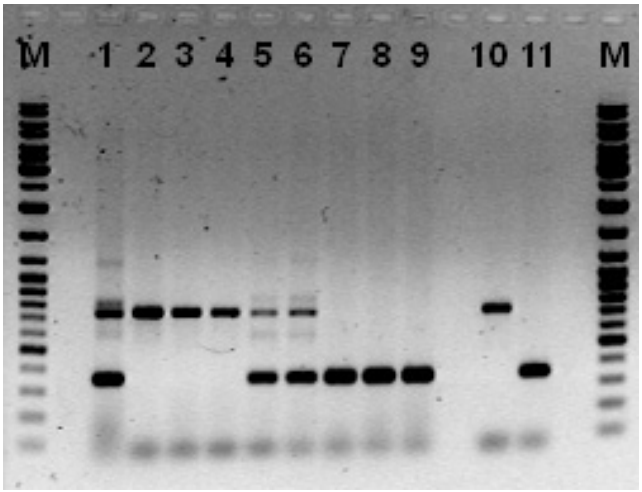

Figure 2. Analysis of *sd1* gene using specific primers in F_2 population. Lanes 1, 5 and 6- heterozygous plants; lanes 2, 3 and 4- homozygous wild type *Sd1* plants (731 bp); lanes 7, 8 and 9- homozygous mutated *sd1* plants (400 bp); lane 10- recurrent parent (wild type *Sd1*) and lane 11- donor parent (mutated *sd1*).

statistical tests, namely homocedasticity and homogeneity of slopes (for ANCOVA) were verified prior to the statistical analysis. All data analysis was performed with SPSS (v. 15, SPSS Inc, Chicago, IL). Statistically significant effects were assumed for $p < 0.05$.

Effect size of genotype and environment was calculated as the fraction of the total variability explained by the effect given by:

$$SizeEffect(\%) = \frac{Sum\ of\ Squares_{Effect}}{Sum\ of\ Squares_{Total}} \times 100\% \qquad (1)$$

where the sum of squares was given by the ANOVA analysis.

RESULTS

The donor varieties in our breeding program aimed for two main goals: increase of productivity associated with an improved architecture (this paper) and increase level of resistance to blast disease (*Magnaporthe oryzae*) (Negrão et al., in preparation). The genes of interest carried by each donor variety are indicated in Figure 1.

Selection of genotypes carrying *sd1* gene

In the breeding process, using specific primers, we have tracked the *sd1* allele with the expected Mendelian segregation (1: 2: 1) originated from 'IR36' ('DGWG' type) instead of the 'Reimei' source present in 'Reiho'. In this way, the allele could be tracked in a single PCR avoiding a further digestion step required to monitor the allele from 'Reimei'. An example of PCR analysis for selection purposes in F_2 generation is shown in Figure 2. In all steps

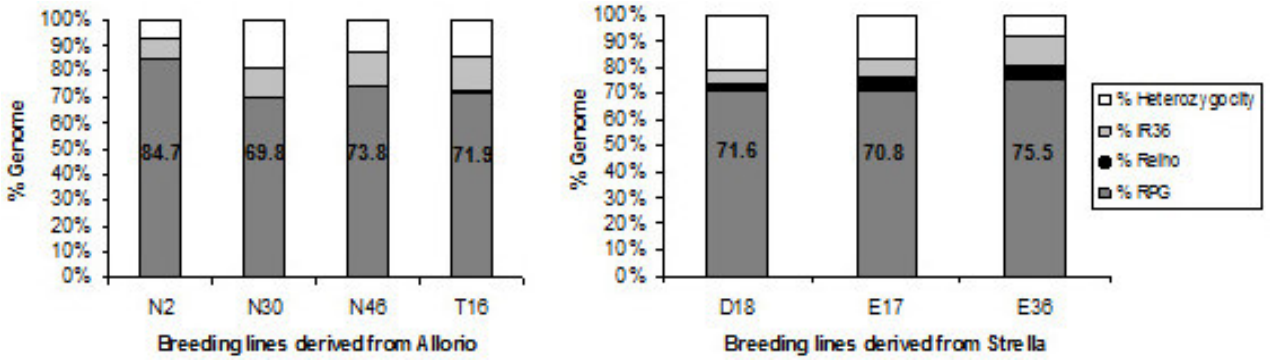

Figure 3. Graphical representation showing the percentage of recurrent parent genome (RPG) introgressed in the seven breeding lines derived from two Portuguese rice landraces used in this study.

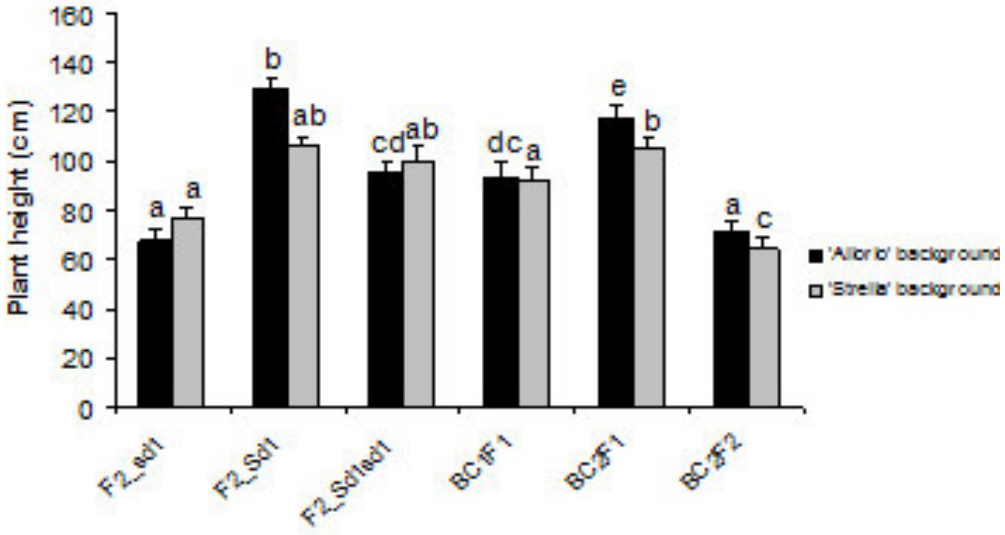

Figure 4. Evolution of plant height during the breeding process for semidwarfism. The plant height progress of two Portuguese rice landraces for progeny derived from 'Allorio' and 'Strella' are indicated in black and grey respectively. Six replicates were measured in all generations. The mean difference is significant at the 0.05 level. Means followed by different letter suffixes are significantly different as evaluated by Tukey-b's HSD tests. The plant height differed significantly between genotypes: $F(11, 59) = 17.149$; $p < 0.001$.

of *sd1* selection, we could identify the plants containing the mutated *sd1* allele in homozygous conditions.

Genotype analysis for background selection

Background selection was performed in the late stage of both crossing schemes ('Allorio'/'Reiho'//'IR36' and 'Strella'/'Reiho'//'IR36'). Based on the information obtained by background selection and GGT software, we could select a total of seven breeding lines with a percentage of RPG above 70%. We selected four breeding lines derived from 'Allorio' (N2, N30, N46 and T16) and three breeding lines from 'Strella' (D18, E17 and E36). In Figure 3, we can observe the percentage of the different genomes (RPG and donors parents).

Evolution of plant height throughout the breeding process

We analyzed plant height in several generations of the breeding process (different years in greenhouse conditions). We have collected data of F_2, BC_1F_1, BC_2F_1 and BC_2F_2 and performed ANCOVA. In the case of F_2 generation, we have distinguished the three possible allelic combinations of *sd1* to confirm the existing differences obtained by the presence of the mutated *sd1* gene. Figure 4 illustrates the evolution of plant height in F_2, BC_1F_1, BC_2F_1 and BC_2F_2 generations. Plant height differed

significantly between the breeding lines. When comparing the F_2 mutated *sd1* generation derived from 'Allorio' versus 'Strella', we can observe that although the plant height is lower in 'Allorio' background, it is not significantly different from 'Strella' F_2 genotypes. The same observation could be made for the remaining generations. For the 'Allorio' population, the mean height ranged from 68.1 cm (F_2 with mutated *sd1* in homozygocity) to 129.8 cm (F_2 wild type *Sd1*); while in 'Strella' population the mean ranged from 64.00 cm (BC_2F_2 generation) to 106.4 cm (F_2 wild type *Sd1*).

Comparison of the two different environments

We aimed to analyse how the environment could affect the plant performance in plants carrying *sd1* mutated gene and how the presence of this gene may affect agronomic parameters. For comparisons, both the recurrent parents and a control commercial variety 'Ariete' (widely cultivated in the two environments) were used as controls for the agronomical parameters. All the studied parameters can be observed in Figure 5.

The variation between the mean plant height of the different genotypes was highly significant: $F(9, 220) = 121.277$; $p < 0.001$. From the statistical analysis, we could observe that the effect of the genotype is dependent on the environment: $F(9, 220) = 2.758$; $p < 0.05$. In general and after considering the effect of the genotype, the plant height means differed significantly between the two environments $F(1, 220) = 155.336$; $p < 0.001$. In BB the plant height (M = 83.25; SD = 10.58) was higher than in CO (M = 75.475; SD = 12.258). We could also conclude that, as expected, on average, the breeding lines were shorter than the recurrent parents. From the total variability of plant height we found that 73% is due to the genotype, while 10.4% is the environmental contribution.

Regarding the number of productive tillers, we could conclude that the variation between genotypes was significant $F(9, 220) = 11.685$; $p < 0.001$ and also that a significant interaction exists between environment and genotypes: $F(9, 220) = 2.922$; $p < 0.05$. After deducting the effect of the genotype, we could also conclude that both environments caused significant differences in the number of productive tillers: $F(1, 220) = 205.985$; $p < 0.001$. In CO the mean of productive tillers was 12.55 (SD = 4.82), while in BB it was 20.83 (SD = 5.97). From the total variability of the number of productive tillers we could conclude that only 2.09% derives from the genotype, while 36.9% is due to the environmental effect.

We also measured the panicle length in both environments and the variation was highly significant between genotypes: $F(9, 220) = 36.935$; $p < 0.001$. In relation to environment, the differences observed between the two trial locations in terms of panicle length, were also significant: $F(1, 220) = 17.109$; $p < 0.001$. From the statistical analysis, we could observe that the effect of the genotype was highly dependent on the environment: $F(9, 220) = 5.576$; $p < 0.001$. The panicle length in CO was superior (M = 18.3233; SD = 2.39) to that in BB (M = 17.52; SD = 2.39) environment. The fraction of the variability observed in the panicle length was 2.75% due to the environment and 5.95% was due to the genotype. From this observation, we could conclude that in the case of panicle length most of the obtained variability results from the genotype x environment interaction and hence different genotypes respond differently to the environment.

We also tried to establish a relation between the two environments (CO and BB) and two of the most important quality parameters: grain length and L/B. Figure 5 illustrates the differences found in grain length (mm) between the two environment trials and the two progenies derived from the Portuguese rice landraces 'Allorio' and 'Strella'. When comparing the two environments, we observed that in CO the mean of grain length was 5.93 (SD = 0.51), while in BB this value was about 6.1 mm (SD = 0.55). From our results, we found a statistically significant interaction between genotype and environment: $F(9, 208) = 5.261$; $p < 0.001$, meaning that the differences between average grain length of the different genotypes depend on the environment where they were grown. There are also statistically significant differences between genotypes after considering the environment effect ($F(9, 208) = 111.693$; $p < 0.001$). The average grain length between the two environments was statistically significant after considering the genotype effect ($F(1, 208) = 33.637$; $p < 0.001$).

In L/B, the two environments also showed significant differences. In BB length to breadth ratio (M = 2.34; SD = 0.2569) was superior to CO (M = 2.28; SD = 0.2514). The variation between genotypes was significant $F(9, 208) = 81.088$; $p < 0.001$ and a significant interaction between environment and genotypes was observed: $F(9, 208) = 6.207$; $p < 0.001$. After deducing the effect of the genotype, we could also conclude that both environments caused significant differences in the L/B: $F(1, 208) = 10.563$; $p < 0.001$.

We have also analysed the weight of 100 grains and there was a significant interaction between genotype and environment for this parameter ($F(9, 208) = 5.486$; $p < 0.001$). There were statistically significant differences between the average weight of 100 grains for the different breeding lines ($F(1, 208) = 116.921$; $p < 0.001$). However, there were no significant differences in the average of weight of 100 grains between the two environments after taking into account the genotype and genotype x environment interaction effects ($F(1, 208) = 1.670$; $p = 0.198$).

In our study, we found two undesirable characteristics in two of the breeding lines, namely awning and seed shattering in N2 and E36, respectively. However, all the remaining five breeding lines showed promising characteristics.

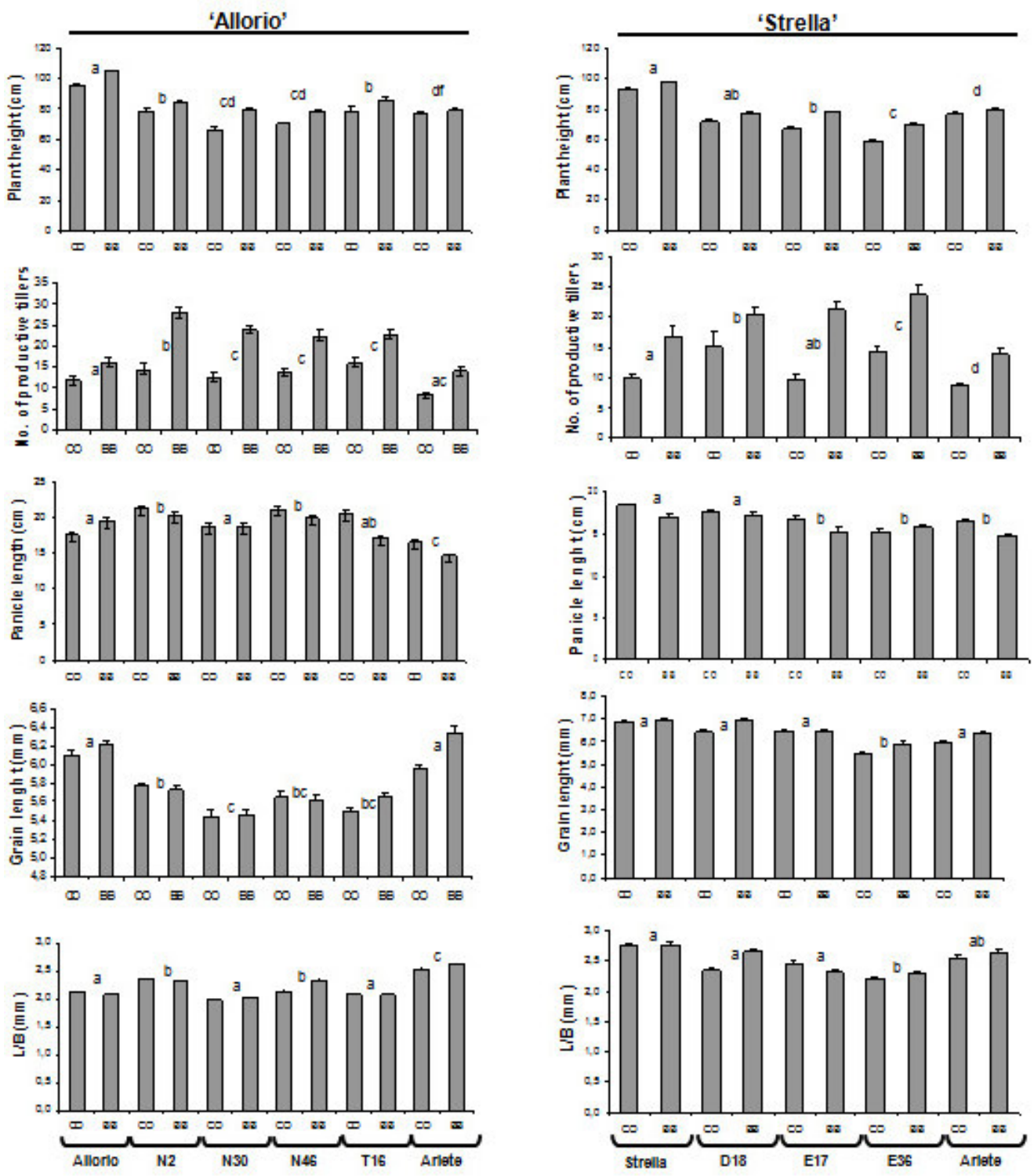

Figure 5. Comparison of the agronomic parameters registered in the two environments COTArroz (CO) and Bico da Barca (BB). Means followed by different letter suffixes are significantly different as evaluated by Tukey-b's HSD tests. The plant height differed significantly between genotypes: F (9, 220) = 121.277; $p < 0.001$. The genotype is dependent from the environment F (9,220) = 2.758; $p < 0.05$. The number of productive tillers differed significantly between genotypes F (9, 220) = 11.685; $p < 0.001$.; existing a significant interaction between genotype and environment F (9, 220) = 2.922; $p < 0.05$. Panicle length differed significantly between genotypes: F (9, 220) = 36.935; $p < 0.001$. The genotype is affected by the environment F (9, 220) = 5.576; $p < 0.001$. Grain length differed significantly between genotypes (F (9, 208) = 111.693; $p < 0.001$). The genotype is dependent from the environment F (9, 208) = 5.261; $p < 0.001$. Length to breadth ratio varies differentially between genotypes F (9, 208) = 81.088; $p<0.001$; genotypes are also affected by the environment F (9, 208) = 6.207; $p < 0.001$.

Table 1. Statistical correlation between the percentages of recurrent parent genome in each of the seven genotypes selected (see Figure 3) in relation to the various parameters analyzed in this work.

Parameters correlated with RPG	R*	*p*- value
Plant height	- 0.835	0.005
No. Productive tillers	0.041	0.915
Panicle length	- 0.423	0.256
Grain length	- 0.453	0.22
L/B	- 0.555	0.12

Correlation between the percentage of recurrent parent genome and the observed parameters

Based on the information supported by SSR markers and further analysis by GGT software we were able to perform statistical correlation between the amount of RPG present in BC_2F_2 (previous generation of this field trial) and the parameters examined in this study. In Table 1, we present the correlation between the percentages of RPG in the seven selected genotypes and the different parameters. From Table 1 we can conclude that there is a significant negative correlation between the amount of RPG and plant height, with this parameter presenting the strongest correlation coefficient. Also, it seems that the number of productive tillers is not correlated with the percentage of RPG (though it is marginally significant); in the remaining traits the correlation coefficient is also marginally significant.

DISCUSSION

Rice landraces offer vast genetic diversity for breeding purposes; however, most of the potential of this material remains unexplored (Neeraja et al., 2005; Tanksley and McCouch, 1997). Around the world, traditional varieties are much appreciated by local populations due to their excellent grain quality which is adapted to their organoleptic preferences. However, most of these landraces are not being fully explored because of their high stature, low productivity and susceptibility to diseases. In this context, we have conducted a study, in which two traditional Portuguese landraces were improved by MAB to introduce *sd1* gene. The breeding lines obtained were evaluated to monitor the influence of *sd1* gene in the plant agronomic behaviour.

Regarding lodging, the height-reducing effect of *sd1* clearly reduced this problem. Additionally, all breeding lines had a modern architecture, with more erect leaves and a stronger stem (data not shown). In a previous study (Murai et al., 2004), lodging resistance was reported as more related to breaking strength than to height reduction. Also, *sd1* is known to be more related to a height reduction of lower internodes (on a percentage basis) than with the upper internode (Murai and Yamamoto, 2001; Murai et al., 2004). Hence, the lodging reduction may be due to a thicker culm since panicle length was not significantly altered in our study. Further studies should be performed to confirm our hypothesis.

Since *sd1* is known to be related to the "green revolution", the effect of this gene in yield components is always a key question. Regarding panicle number per m^2, it was considered that *sd1* has a pleiotropic effect increasing this value in 'Taichung 65' genetic background, although in other varieties its effect is still not known (Murai et al., 2002). Murai and Yamamoto (2001) and Murai et al. (2002), pointed out that *sd1* gene reduces spikelet number per panicle, thus resulting in a smaller sink size. It is expected that quantitative trait loci (QTLs) related to the increase of spikelets' number per panicle are the main responsible for the high yield in semidwarf varieties (Murai et al., 2004). Nevertheless, the effective QTLs *spp1.1* and *spl1.1* related to the number of spikelets is located at the end of chromosome one (Yagi et al., 2001). Furthermore, *sd1* is also located in a region nearby the *spp1.1* QTL, meaning that both can interact and contribute to a yield increase. However, since we used a BC_2F_3 generation (which is still in recombination), we have only evaluated agronomical parameters not including the yield component, which will be further analysed.

In our study, we have evaluated several agronomical parameters in two distinct environments, CO in central region and BB in northern region, in Portugal. The main environmental difference between these two cultivation areas is in terms of the amount of precipitation and radiation (thus affecting evapotranspiration). However, we believe that the main reason for the different results we obtained was the type of cultivation and field management, rather than the slightly different environmental conditions.

From all the parameters, plant height is undoubtedly the factor more dependent on *sd1* influence. From our results, we could see a clear reduction in culm length of all the breeding lines when compared to their recurrent parents (tall landraces). This fact has been described earlier, since *sd1* reduces culm length mainly due to a decrease of internodes length (Xia et al., 1993). Also, all the obtained breeding lines had a plant height similar (on average) to commercial varieties (Figure 5). Hence, we

could verify the effectiveness of the introduction of *sd1* gene in the reduction of plant height, as well as in the improvement of the architecture of the selected plants which inherited shorter and erected leaves, approaching ideotype architecture.

Regarding the number of productive tillers, we observed differences in both environments, with a significant interaction between environmental effect and genotypes. Curiously, the total variability of the number of productive tillers was mostly derived from the environment (36.9%), which we believe may correspond to field management conditions. Furthermore, in BB region there was in average a higher number of productive tillers, which could be due to a better control of weed growth. Another interesting point was that in all breeding lines the number of productive tillers was higher when compared to their respective parent and even to the commercial variety. This aspect, when aiming for yield increase, could be explained by a possible pleiotropic effect of *sd1* gene at the apical meristem.

In this study, we could observe that the average panicle length was higher in all the breeding lines when compared to their recurrent genotype or even the commercial variety (except in E36 line). Foster and Rutger (1978) reported that *sd1* gene seems to have a small negative pleiotropic effect for panicle length. However in our study this was not the case, and in fact the introduction of *sd1* gene did not significantly alter panicle length which appeared to be dependent from the environment.

In rice, many genes control grain size. In general, the grain size of semidwarf varieties is smaller than that of normal varieties due to the smaller pleiotropic effects of *sd1* (Xia et al., 1994). Independent studies have tried to dissect the genetic basis of grain length, and six main QTLs and 12 pairs of epistatic QTLs have been described as related to this parameter (reviewed by Wan *et al.* 2006). In our study, the obtained breeding lines had smaller grain length when compared to their recurrent parent, especially in 'Allorio' background, a result that could be due to the inheritance of several QTLs. On the other hand, L/B was not very much affected by *sd1* introduction, which agrees with previous studies (Murai and Yamamoto, 2001, Murai et al., 2002).

While evaluating the breeding lines obtained from this MAB program, we could observe two undesirable traits in two lines, namely awning in N2 and seed shattering in E36. Although these traits are detrimental to rice cultivation, some studies have linked them to the presence of *sd1*. Regarding seed shattering, four loci have been mapped in rice, namely *sh1* on chromosome 11, *sh2* on chromosome 1, *Sh3* on chromosome 4, and *Sh4* on chromosome 3, and also QTLs for shattering have been reported on chromosomes 1, 3, 4, 7, 8, and 11 (Ji et al., 2006). Due to the fact that sh2 is located in chromosome 1 near sd1 gene (Oba et al., 1990), there was possibly a recombination event between these two gene regions in our line E36. In line N2, in which we observed awning, we believe that this may also be related to the introduction of *sd1* gene and its pleiotropic effect. In addition, all the breeding lines showed the weight of 100 grains lower than their recurrent parents which could also be related to a small pleiotropic effect of *sd1* gene. However further data is required to support these hypotheses. Another interesting aspect was the fact that, from the parameters related to the percentage of RPG, only plant height appeared to be negatively correlated and with a statistical significance.

With the help of MAB, we have successfully introduced semidwarfism into Portuguese traditional tall landraces. Besides this achievement, a major conclusion arising from our study was that *sd1* gene introduction is likely to have pleiotropic effects on other agronomical traits such as the number of productive tillers, panicle length or grain length. The results so far obtained are therefore highly promising and confirm the efficiency of this marker-assisted selection for *sd1* introgression, by significantly shortening the breeding process period from 3 months to 3 weeks of development. This aspect is very important in temperate growing conditions where only one life cycle per year is possible. Through marker-assisted selection, it is possible to identify plants to grow to maturity when they are only 2 weeks old, thus allowing a much better space management in greenhouse conditions.

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Abbreviations

MAB, Marker assisted backcrossing; **SSR,** simple sequence repeat or microsatellites markers; **RPG,** recurrent parent genome; **ANCOVA,** analysis of covariance; **IRRI,** international rice research institute; **DGWG,** dee-geo-woo-gen; ***sd1*,** semidwarf 1 gene; **RFLP,** restriction fragment length polymorphism; **RPG,** recurrent parent genome; **CO,** COTArroz; **BB,** bico da barca; **GA,** gibberellic acid; **L/B,** length to breadth ratio; **SES,** standard evaluation system; **PCR,** polymerase chain reaction; CTAB, cetyltrimethyl ammonium bromide; **DMSO,** dimethyl sulfoxide; **SD,** standard deviation; **M,** mean; **QTLs,** quantitative trait loci.

REFERENCES

Aquino RC, Jennings PR (1966). Inheritance and significance of dwarfism in an indica rice variety. Crop Sci. 6: 551-554.

Asano K, Takashi T, Miura K, Qian Q, Kitano H, Matsuoka M, Ashikari M (2007). Genetic and molecular analysis of utility of *sd1* alleles in rice breeding. Breed. Sci. 57 (1): 53-58.

Ashikari M, Sasaki A, Ueguchi-Tanaka M, Itoh H, Nishimura A, Datta S, Ishiyama K, Saito T, Kobayashi M, Khush GS, Kitano H, Matsuoka M (2002). Loss-of-function of a rice gibberellin biosynthetic gene, GA20 oxidase (GA20ox-2), led to the rice green revolution. Breed. Sci. 52 (2): 143-150.

Cho YG, Eun MY, McCouch SR, and Chae YA (1994). The semidwarf gene, *sd*-1, of rice (*Oryza sativa* L.) .II. Molecular mapping and marker-assisted selection. Theor. Appl. Gen. 89 (1): 54-59.

Doyle JJ, Doyle JL (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytoch. Bull. 19: 1-15.

Ellis MH, Spielmeyer W (2002). Perfect markers for the semidwarfing gene *sd1* in rice. Int. Rice Res. Notes 27(2): 13-14.

Hargrove TR, Cabanilla VL (1979). The impact of semi-dwarf varieties on Asian rice-breeding program. BioScience, 29: 731-735.

Jayamani P, Negrão S, Martins M, Maçãs B, Oliveira MM (2007). Genetic relatedness of Portuguese rice accessions from diverse origins as assessed by microsatellite markers. Crop Sci. 47(2): 879-886.

Ji HS, Chu SH, Jiang W, Cho YI, Hahn JH, Eun MY, McCouch SR, Koh HJ (2006). Characterization and mapping of a shattering mutant in rice that corresponds to a block of domestication genes. Genetics, 173(2): 995-1005.

Monna, L, Kitazawa N, Yoshino R, Suzuki J, Masuda H, Maehara Y, Tanji M, Sato M, Nasu S, and Minobe Y (2002). Positional cloning of rice semidwarfing gene, *sd-1*: Rice Green revolution gene encodes a mutant enzyme involved in gibberellin synthesis. DNA Res. 9(1): 11-17.

Murai M, Yamamoto H (2001). Allelic relationships and height effects of rice dwarfing genes from cv. Dee-geo-woo-gen, Calrose 76 and Reimei determined in a constant genetic background. SABRAO J. Breed. Gen. 33: 21-30.

Murai M, Komazaki T, Sato S (2004). Effects of *sd1* and *Ur1* (Undulate rachis-1) on lodging resistance and related traits in rice. Breed. Sci. 54(4): 333-340.

Murai M, Takamure I, Sato S, Tokutome T, Sato Y (2002). Effects of the dwarfing gene originating from Dee-geo-woo-gen on yield and its related traits in rice. Breed. Sci. 52(2): 95-100.

Neeraja CN, Hariprasad AS, Malathi S, Siddiq EA (2005). Characterization of tall landraces of rice (*Oryza sativa* L.) using gene-derived simple sequence repeats. Curr. Sci. 88(1): 149-152.

Oba S, Kikuchi F, Maruyama K (1990). Genetic analysis of semidwarfness and grain shattering of Chinese rice (*Oryza sativa*) variety Ai-Jio-Nan-Te. Jpn. J. Breed. 40: 13-20.

Peng S, Laza RC, Visperas RM, Sanico AL, Cassman KG, and Khush GS (2000) Grain yield of rice cultivars and lines developed in the Philippines since 1966. Crop Sci. 40(2): 307-314.

Sasaki A, Ashikari M, Ueguchi-Tanaka M, Itoh H, Nishimura A, Swapan D, Ishiyama K, Saito T, Kobayashi M, Khush GS, Kitano H, Matsuoka M (2002). Green revolution: a mutant gibberellin-synthesis gene in rice. Nature, 416(6882): 701-702.

Spielmeyer W, Ellis MH, Chandler PM (2002). Semidwarf (*sd-1*), green revolution rice, contains a defective gibberellin 20-oxidase gene. Proc. Nat. Acad. Sci. USA, 99(13): 9043-9048.

Tanksley SD, McCouch SR (1997). Seed banks and molecular maps: unlocking genetic potential from the wild. Science, 277(5329): 1063-1066.

Tsai KH (1991). Chromosomal location of gene *sd*-1 examined with isogenic translocation lines of Taichung 65. Rice Gen. Newsl. 8: 109-110.

Van Berloo R (1999). GGT: Software for the display of graphical genotypes. J. Hered. 90(2): 328-329.

Xia B, Oba S, Kikuchi F, Imai K (1993). Character expression of the semidwarfing gene *sd-1* in rice (*Oryza sativa* L.) III.Association between the semidwarfism and grain Numbers per panicle. J. Gen. Genomics, 20(6): 552-560.

Xia B, Oba S, Kikucrhi F, Takeda K (1994). Character expression of the semidwarfing gene *sd-1* in rice (*Oryza sativa* L.). J. Gen. Genomics, 21(1): 59-66.

Yagi T, Nagata K, Fukuta Y, Tamura K, Ashikawa I, Terao T (2001). QTL mapping of spikelet number in rice (*Oryza sativa* L.). Breed. Sci. 51(1): 53-56.