Alleles Affecting 30 Traits for Productivity in Two Japonica Rice Varieties, Koshihikari and Nipponbare (Oryza sativa L.)

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Abstract: We identified chromosome regions affecting traits (CRATs) for 30 productivity-related traits using 2 sets of chromosome segment substitution lines (CSSLs). One was established using Nipponbare as the donor and Koshihikari as the background variety (Kos-Nip); and the other using Koshihikari as the donor and Nipponbare as the background (Nip-Kos). We identified 249 and 181 CRATs for 30 traits in Kos-Nip and Nip-Kos CSSLs, respectively. Donor alleles in 75 (Nipponbare) and 82 (Koshihikari) CRATs had positive effects on productivity. Among them, some CRATs represented superior effects as compared with the alleles in indica varieties Kasalath and Nona Bokra. On chromosome 1, a CRAT for panicle number (PN1) increased yield by about 1.4 times compared with Koshihikari. PN1 increased leaf area, while maintaining the SPAD value, which is an indicator of photosynthetic ability. Therefore, PN1 might have a pleiotropic effect on sink size and source ability, and thus could consequently improve yield. In only 29 CRATs for 13 traits (12% of total), corresponding CRATs with contrasting effects were detected in the 2 sets of CSSLs. These results suggested that the effect of a gene, might be affected by the genetic background. By using a database of single-nucleotide polymorphisms in Koshihikari and Nipponbare, we could narrow the candidate genes for 28/29 CRATs; to minimum 6 genes for CRAT related to plant height at the early stage and diameter at stem. Thus japonica rice could be a useful genetic donor for improving the productivity of another japonica variety.

Key words: CSSLs, Panicle number, Productivity, QTL analysis, Rice, Yield.

A Japanese premium rice, Koshihikari has been registered in 1956, and it is a widely cultivated in Japan and numerous other countries (Yamamoto and Ogawa, 1992; Takeuchi et al., 2008). Its yield is lower than that of modern Japanese high yielding varieties such as Dontokoi and Akichikara (Koga et al., 1987; Uehara et al., 1995; Yamauchi, 2000); therefore, the improvement in productivity is one of the most important breeding targets for Koshihikari. Nipponbare belongs to the average yield varieties of Japan (Saitoh et al., 2000). Compared to Koshihikari, Nipponbare plants tend to be shorter, although their biomass is higher due to larger tiller numbers (Matsuo and Mochizuki, 2009). The photosynthetic rate of a flag leaf is similar in the 2 varieties (Sasai and Ishii, 1992).

Rice productivity is complex and is determined by various traits. Sink size and source ability are the main factors related to the yield (Li et al., 1998; Sheehy et al., 2001; Ujiie and Ishimaru, 2013). The sink size of plant is represented by panicle number, grain number per panicle, and grain weight (Nagata et al., 2002). The production of photosynthate in a flag leaf is the main source (Cock and Yoshida, 1972; Ishimaru et al., 2007; Takai et al., 2010) and is largely dependent on the leaf area and nitrogen content per area (Mae, 1997; Ohsumi et al., 2007; Ujiie et al., 2012). The chlorophyll meter (SPAD) value has been used as an indicator of nitrogen content (Peng et al., 1995; Ida, 2006). Biomass size and harvest index mean the amount of assimilation products and the proportion of biomass allocated to grain, respectively (Peng et al., 2000; Ujiie et al., 2012). Biomass is related to plant height, tiller (panicle) number, and stem diameter (Niklas and Enquist, 2001; Ishimaru et al., 2004).

Generally, in japonica varieties, productivity-related traits such as source ability and biomass are lower than in indica varieties (Maruyama and Tajima, 1988; Peng et al., 1998). The genetic variation in these traits is narrow in japonica
varieties compared with indica and japonica varieties (Zhang et al., 1992). A number of genetic materials such as backcross inbred lines and chromosome segment substitution lines (CSSLs) have been developed in rice. For the majority of them, indica varieties have been crossed with japonica varieties (Wan et al., 2004; Ebitani et al., 2005; Takai et al., 2007; Suralta et al., 2008). Using these materials, much genetic information on productivity has been stocked (e.g., Li et al., 1997; Ishimaru et al., 2001; Yue et al., 2006; Madoka et al., 2008; Takai et al., 2010; Ujiie et al., 2012; Ujiie and Ishimaru, 2013).

Using CSSLs with chromosome segments of Kasalath or Nona Bokra on a Koshihikari background (Koshihikari/Kasalath and Koshihikari/Nona Bokra), our group previously identified many chromosome regions affecting traits (CRATs) for productivity (Madoka et al., 2008; Ujiie et al., 2012; Ujiie and Ishimaru, 2013). In particular, we identified important CRATs influencing grain number per panicle, harvest index, and flag leaf area. In contrast, we were unable to identify CRATs for increasing biomass or panicle weight at maturity. A CRAT that increased panicle number by 23% relative to Koshihikari was detected in Koshihikari/Kasalath CSSLs.

Using Koshihikari and Nipponbare, two kinds of CSSLs have been developed (Hori et al., 2010). One was established using Nipponbare as the donor and Koshihikari as the background (Kos-Nip), and the other vice versa (Nip-Kos). By comparing these two sets of CSSLs, the existence and effects of CRATs can be confirmed. The complete genomes of Nipponbare and Koshihikari were sequenced (80.1% genome coverage) in 2005 and 2010, respectively (International Rice Genome Sequencing Project, 2005; Yamamoto et al., 2010). Between them, 67,051 single-nucleotide polymorphisms (SNPs) have been identified. The average SNP density was 1 SNP per 5.7 kb, but the density varied with the chromosome. For example, only 178 SNPs were detected on chromosome 9. These CSSLs are useful for prompt and easy narrowing of candidate genes based on the genomic information. Between Nipponbare and Koshihikari, quantitative trait loci (QTL) analysis for 50 agronomic traits has been conducted using backcross inbred lines (BILs) (Hori et al., 2012). However, most of these traits are strongly influenced by heading date, and thus the existence and performance of Nipponbare alleles on a Koshihikari background could not be detected using this BILs.

Many studies have shown that genetic donors can carry superior alleles despite poorer performance observed in these varieties (Xiao et al., 1998; Cai and Morishima, 2002; Madoka et al., 2008). Therefore, superior alleles for productivity in japonica rice might prove useful on other japonica backgrounds. In the studies with genetic materials from japonica varieties, grain quality has been the main target but there is poor genetic information about productivity (Kobayashi et al., 2007; Tabata et al., 2007; Kobayashi and Tomita, 2008; Takeuchi et al., 2008; Wada et al., 2008). In this study, we identified CRATs for 30 productivity-related traits using 2 sets of CSSLs between Koshihikari and Nipponbare. Additionally, the effects of Nipponbare alleles were compared with those of alleles in indica varieties such as Kasalath and Nona Bokra.

**Materials and Methods**

1. **Plant materials**

Two CSSLs were used in this study, one containing chromosomal segments of Nipponbare on a Koshihikari background (Kos-Nip), and a second containing chromosomal segments of Koshihikari on Nipponbare (Nip-Kos) (Hori et al., 2010). Forty-three and 49 seeds of Kos-Nip and Nip-Kos CSSLs, respectively, were sown in a greenhouse on 27 April 2009. Seedlings were transplanted into the paddy field in Tsukuba, Japan (latitude 37ºN), on 27 May, at one plant per hill with a spacing of 18 × 30 cm. Compound fertilizer (N : P₂O₅ : K₂O = 14 : 14 : 14%) and fused phosphate (P₂O₅ = 20%) were applied as a basal dressing at the rate of 40 and 80 g m⁻², respectively. In 2010, Koshihikari and a CSSL harboring a CRAT for panicle number located on chromosome 1 (tentatively named PN1) (Fig. 4) were grown for additional testing. Seedlings were transplanted into the paddy field in Tsukuba, Japan (latitude 37ºN), on 27 May, at one plant per hill with a spacing of 18 × 30 cm. Twenty-five plants were grown for each line. Compound fertilizer (N : P₂O₅ : K₂O = 14 : 14 : 14%) and fused phosphate (P₂O₅ = 20%) were applied as a basal dressing at the rate of 40 and 80 g m⁻², respectively. In 2010, Koshihikari and a CSSL harboring a CRAT for panicle number located on chromosome 1 (tentatively named PN1) (Fig. 4) were grown for additional testing. Seeding and transplanting were conducted on 26 April and 25 May, respectively. Fifteen plants were grown for each CSSL, and the other cultivation conditions were the same as in 2009. Daily mean air temperatures at Tsukuba during the growing season in 2009, 2010, and an average year are shown in Fig. 1; data were obtained from the Japan Meteorological Agency.

2. **Evaluation of traits**

The area and length of 3 flag leaves per plant were
measured for each line at heading. After drying at 80°C for 3 days, leaf weight was measured. Specific leaf area (SLA) was calculated from leaf area and weight (Ujiie and Ishimaru, 2013). SPAD values on flag leaves were determined at heading and 5 weeks after heading using a chlorophyll meter (SPAD-502; Minolta Camera Co., Ltd, Japan). Plant height was measured at heading and 37 days after transfer. At heading, the height of the auricle at the second leaf below the flag leaf (−2 leaf height) was measured for the highest tiller of each plant. The length and diameter of the harvested panicle were measured immediately. After harvesting, plants were cut at 40 cm above the ground, and the diameters at the stems were measured. Using the same plants, crown width was measured at 15 cm above the ground. Three tillers were harvested per plant just before heading, dried at 80°C for 3 days, and weighed. At maturity, when the secondary rachis branch located on the lower primary rachis branch changed to yellow, the tillers were harvested from plants not sampled before heading. The harvest index was calculated from the ratio of grain biomass to the total biomass. The yield components were evaluated for undamaged typical plants and the yield was calculated from these components. The size and whiteness of rice grains were measured using a grain quality inspector (RGQI 20A; Satake Co., Ltd, Japan). Each trait was represented by the average of 7 plants, with the exception of traits related to biomass, which were measured for 5 plants.

3. Statistical analysis for determining CRATs

CRATs were determined by comparing phenotypes with genotypes of simple sequence repeat (SSR) markers (Hori et al., 2010). A probability level (P) of 0.05 was used as the threshold for the detection of a CRAT using the method of Madoka et al. (2008). The effect of a CRAT was expressed as a percentage change of the value determined in host varieties (Koshihikari in Kos-Nip CSSLs or Nipponbare in Nip-Kos CSSLs).

Results

1. Phenotypic variation in the 2 sets of CSSLs

In all of the traits analyzed here, we observed phenotypic variation in both CSSLs (Fig. 2). In some traits, for example, plant height at heading, range of frequency distributions in Kos-Nip were quite different from those in Nip-Kos (plant height at heading: 112.0 – 135.5 cm in Kos-Nip and 95.3 – 107.6 cm in Nip-Kos CSSLs). For these traits, each CSSL had values that were similar to those observed for the background variety, but not the donor variety. For the majority of traits that had similar distributions, values in Koshihikari were similar to those of Nipponbare (e.g., grain length of Koshihikari and Nipponbare were 5.07 and 5.06 mm, respectively). In plant body weight, a significant difference was detected between Koshihikari and Nipponbare, but not between Kos-Nip and Nip-Kos.

2. Correlations between 30 traits

Among the 30 traits, 6 were negatively correlated in Kos-Nip while only 1 pair was negatively correlated in Nip-Kos. In contrast, 57 and 43 pairs of traits were positively correlated in Kos-Nip and Nip-Kos, respectively (threshold level was P < 0.001, Fig. 3). In both CSSLs, 4 traits related to size of the biomass (biomass at heading and maturity, plant body weight, and panicle weight) were correlated with each other. These 4 traits were also positively correlated with panicle number. Panicle number was correlated with 9 traits in Kos-Nip (yield, total grain number, loss in SPAD value, plant height at heading, diameter at panicle neck, and 4 traits related to biomass) and 8 traits in Nip-Kos (yield, total grain number, −2 leaf height, grain width). The largest number of correlations was observed for total grain number in Nip-Kos. Ten and 7 correlations involving yield were detected in Kos-Nip and Nip-Kos CSSLs, respectively. While filled grain ratio was negatively correlated with grain whiteness in Nip-Kos CSSLs, there were no positive correlations detected in either CSSL. SLA, crown width, and length under panicle neck were not correlated with other traits. In Kos-Nip CSSLs, the days to heading was positively correlated with the area, weight or length of flag leaf, and plant height at heading (data not shown; threshold was the same as in Fig. 3). Moreover, this trait was negatively correlated with harvest index, SPAD value at heading and grain whiteness. In Nip-Kos CSSLs, days to heading were positively correlated with filled grain ratio but negatively with grain whiteness.

3. Detection of CRATs

A total of 249 and 181 CRATs were detected for 30 traits in Kos-Nip and Nip-Kos CSSLs, respectively (Figs. 4, 5, Tables 1, 2). Among them, 75 and 92 CRATs in Kos-Nip and Nip-Kos, respectively, had positive effects on productivity by donor’s allele. In the Kos-Nip CSSLs, a CRAT for panicle number was identified on the short arm of chromosome 1 (tentatively named PN1), which overlapped a CRAT for grain yield and total grain number (Fig. 4). The CRAT improved both yield (35%) and total grain number (40%) via a 37% increase in panicle number (Table 1). In the Nip-Kos CSSLs, a chromosome region related to PN1 was associated with a 28% decrease in yield and a 19% decrease in total grain number, but was not associated with panicle number (Fig. 5, Table 2). Compared to Koshihikari, Nipponbare alleles in PN1 were also associated with increased biomass at heading by 56% and at maturity by 16%, increase in panicle weight by 27%, and increase in plant body weight by 7%. Corresponding CRATs with contrasting effects were not detected in the
Fig. 2. Frequency distributions of 30 productivity-related traits in Nipponbare/Koshihikari/Koshihikari and Nipponbare/Koshihikari/Nipponbare CSSLs. Blue and red bars indicate CSSLs containing Nipponbare chromosomal segments on Koshihikari background (Kos-Nip CSSLs) and CSSLs that the host variety switched places with the donor (Nip-Kos CSSLs), respectively.
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We compared chromosomal positions of CRATs for 22 traits with those of QTLs or CRATs in four previous reports (Table 1) (Madoka et al., 2008; Hori et al., 2012; Ujiie et al., 2012; Ujiie and Ishimaru, 2013). For the fifteen traits, total 37 overlapping among QTLs or CRATs were detected. Almost one third part of QTLs that was detected in BILs between Nipponbare and Koshihikari overlapped with CRATs in Kos-Nip CSSLs (Hori et al., 2012). Similarly, ratios of overlap with our CRATs were 10% or 20% in the CRATs by Kasalath and Nona Bokra alleles, respectively. In this study, we detected unique and superior CRATs by Nipponbare alleles. For example, CRATs on chromosome 2 and 11 increased biomass at heading by 24% and harvest index by 8%, respectively, and one on chromosome 7 decreased −2 leaf height by 15%.

4. Candidate genes

Twenty-nine CRATs had corresponding CRATs in the alternative CSSL with contrasting effects (Table 3). For example, the Nipponbare allele in a CRAT on chromosome 10 decreased plant body weight by 14%, whereas the Koshihikari allele increased body weight by 14%, compared with host varieties. Some CRATs also have
Fig. 4. Positions of CRATs for 30 traits on the genetic map of Nipponbare/Koshihikari//Koshihikari CSSLs. Bars indicate locations of CRATs. Putative CRATs data were analyzed using trait measurements and genotype data from SSR markers. A probability level of 0.05 was used as the threshold for the detection of CRATs.

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Fig. 4 (Continued). Positions of CRATs for 30 traits on the genetic map of Nipponbare/Koshihikari/Koshihikari CSSLs. Bars indicate locations of CRATs. Putative CRATs data were analyzed using trait measurements and genotype data from SSR markers. A probability level of 0.05 was used as the threshold for the detection of CRATs.
Fig. 5. Positions of CRATs for 30 traits on the genetic map of Nipponbare/Koshihikari//Nipponbare CSSLs. Bars indicate locations of CRATs. Putative CRATs data were analyzed using trait measurements and genotype data from SSR markers. A probability level of 0.05 was used as the threshold for the detection of CRATs. (Continued on next page).
Fig. 5 (Continued). Positions of CRATs for 30 traits on the genetic map of Nipponbare/Koshihikari//Nipponbare CSSLs. Bars indicate locations of CRATs. Putative CRATs data were analyzed using trait measurements and genotype data from SSR markers. A probability level of 0.05 was used as the threshold for the detection of CRATs.
Table 1. Effects of CRATs for the 30 productivity-related traits by Nipponbare alleles in the Koshihikari background.

| Traits                          | Number of CRATs detected | Chromosome number | Nearest markers of putative CRATs | CRAT-dependent change (%) | Agreed QTLs or CRATs |
|---------------------------------|--------------------------|-------------------|-----------------------------------|---------------------------|----------------------|
| Yield and yield components      |                          |                   |                                   |                           |                      |
| Yield                           | 14                       | 1                 | RM3252-RM8068                     | +35                       |                      |
|                                 |                          | 3                 | RM2505                            | -22                       |                      |
|                                 |                          | 4                 | RM2700                            | -27                       |                      |
|                                 |                          | 5                 | RM5709-RM2431                     | -21                       |                      |
|                                 |                          | 6                 | RM4674-RM19139                    | -19                       |                      |
|                                 |                          | 7                 | P530H65-35-P17G10-24              | -22                       |                      |
|                                 |                          | 8                 | RM1364-RM1306                     | -16                       |                      |
|                                 |                          | 9                 | RM22215                           | -19                       |                      |
|                                 |                          | 10                | RM4487-RM3496                     | -17                       |                      |
|                                 |                          | 11                | RM761-RM26341                     | -14                       |                      |
|                                 |                          | 12                | RM5521-RM5926                     | -17                       |                      |
| Total grain number              | 9                        | 1                 | RM3252-RM8068                     | +40 Kasalath allele      |                      |
|                                 |                          | 3                 | RM2505                            | -21                       |                      |
|                                 |                          | 4                 | RM7472-RM7181                     | -21                       |                      |
|                                 |                          | 5                 | RM5529-T362_02                    | -28 Kasalath allele      |                      |
|                                 |                          | 6                 | P530H65-35-P17G10-24              | -24                       |                      |
|                                 |                          | 7                 | RM1364-RM1306                     | -15                       |                      |
|                                 |                          | 8                 | RM2701-RM1815                     | -14                       |                      |
|                                 |                          | 9                 | RM1761-RM26341                    | -16                       |                      |
|                                 |                          | 10                | RM7221-RM5926                     | -24                       |                      |
| Panicle number                  | 5                        | 1                 | RM3252-RM8068                     | +37                       |                      |
|                                 |                          | 2                 | RM6938-RM2008                     | +19                       |                      |
|                                 |                          | 3                 | P530H65-35-P17G10-24              | -10                       |                      |
|                                 |                          | 4                 | RM4674-RM19139                    | -4                        |                      |
| Grains number per panicle       | 4                        | 1                 | RM3252-RM8068                     | +8                        |                      |
|                                 |                          | 2                 | RM6938-RM12985                    | +4                        |                      |
|                                 |                          | 3                 | RM2482                            | +4                        |                      |
|                                 |                          | 4                 | RM1761-RM26341                    | +15                       |                      |
| Filling grain ratio             | 6                        | 1                 | RM3252-RM8068                     | -8                        |                      |
|                                 |                          | 2                 | RM6938-RM12985                    | +4                        |                      |
|                                 |                          | 3                 | RM4674-RM19139                    | -4                        |                      |
|                                 |                          | 4                 | RM2482                            | +4                        |                      |
| 1,000-grain weight              | 7                        | 1                 | RM3252-RM8068                     | +4                        |                      |
|                                 |                          | 2                 | RM5521-RM1859                     | +4                        |                      |
|                                 |                          | 3                 | RM1761-RM26341                    | +15                       |                      |
|                                 |                          | 4                 | RM2221-RM5926                     | +4                        |                      |
| Biomass                         |                          | 1                 | RM3252-RM8068                     | +56                       |                      |
| Biomass at heading              | 5                        | 2                 | RM6938-RM12985                    | +17                       |                      |
|                                 |                          | 3                 | RM1940-RM6726                     | +2                        |                      |
|                                 |                          | 4                 | RM2482                            | +4                        |                      |
|                                 |                          | 5                 | RM4108-RM7365                     | +2                        |                      |
|                                 |                          | 6                 | P530H65-35-P17G10-24              | +5                        |                      |
|                                 |                          | 7                 | RM26830-RM1355                    | +5                        |                      |
|                                 |                          | 8                 | RM2756                            | +17                       |                      |
|                                 |                          | 9                 | RM7472-RM2008                     | +19                       |                      |
| Biomass at maturity             | 12                       | 1                 | RM3252-RM8068                     | +56                       |                      |
|                                 |                          | 2                 | RM5521-RM1859                     | +4                        |                      |
|                                 |                          | 3                 | RM1761-RM26341                    | +15                       |                      |
|                                 |                          | 4                 | RM2701-RM1815                     | -14                       |                      |
|                                 |                          | 5                 | RM1364-RM1306                     | -15                       |                      |
|                                 |                          | 6                 | RM2221-RM5926                     | -21                       |                      |
|                                 |                          | 7                 | RM4487-RM3496                     | -11                       |                      |
|                                 |                          | 8                 | RM7472-RM2008                     | -14                       |                      |
|                                 |                          | 9                 | RM6938-RM12985                    | -22 Kasalath allele      |                      |
| Panicle weight                  | 8                        | 1                 | RM3252-RM8068                     | +27                       |                      |
|                                 |                          | 2                 | RM1940-RM6726                     | +17                       |                      |
|                                 |                          | 3                 | RM2701-RM1815                     | +17                       |                      |
|                                 |                          | 4                 | RM1364-RM1306                     | +17                       |                      |
|                                 |                          | 5                 | RM2221-RM5926                     | -17                       |                      |
|                                 |                          | 6                 | RM4487-RM3496                     | -22 Kasalath allele      |                      |
|                                 |                          | 7                 | RM7472-RM2008                     | -22                       |                      |
|                                 |                          | 8                 | RM6938-RM12985                    | -15                       |                      |
|                                 |                          | 9                 | RM1940-RM6726                     | +2                        |                      |
|                                 |                          | 10                | RM7418-RM21224                    | +8                        |                      |
|                                 |                          | 11                | RM1364-RM1306                     | -15                       |                      |
|                                 |                          | 12                | RM3252-RM8068                     | +7                        |                      |
| Plant body weight               | 12                       | 1                 | RM3252-RM8068                     | +4                        |                      |
|                                 |                          | 2                 | RM1940-RM6726                     | +17                       |                      |
|                                 |                          | 3                 | RM2701-RM1815                     | +17                       |                      |
|                                 |                          | 4                 | RM1364-RM1306                     | +17                       |                      |
|                                 |                          | 5                 | RM2221-RM5926                     | -17                       |                      |
|                                 |                          | 6                 | RM4487-RM3496                     | -22 Kasalath allele      |                      |
|                                 |                          | 7                 | RM7472-RM2008                     | -22                       |                      |
|                                 |                          | 8                 | RM6938-RM12985                    | -15                       |                      |
|                                 |                          | 9                 | RM1940-RM6726                     | +2                        |                      |
|                                 |                          | 10                | RM1364-RM1306                     | -15                       |                      |
|                                 |                          | 11                | RM26830-RM1355                    | +5                        |                      |
|                                 |                          | 12                | RM3252-RM8068                     | +17                       |                      |

1) Putative CRATs data were analyzed using trait measurements and genotype data from previously reported SSR markers (Hori et al., 2010).
2) CRAT-dependent changes are expressed as the percentage change of the value determined in Koshihikari.
3) Reported by Madoka et al. (2009).
4) Reported by Hori et al. (2012).
5) Reported by Ujiie et al. (2012).
6) Reported by Ujiie and Ishimaru (2013). (Continued on next page).
Table 1 (Continued). Effects of CRATs for the 30 productivity-related traits by Nipponbare alleles in the Koshihikari background.

| Traits                        | Number of CRATs detected | Chromosome number | Nearest markers of putative CRATs | CRAT-dependent change (%) | Agreed QTLs or CRATs |
|-------------------------------|--------------------------|-------------------|-----------------------------------|---------------------------|----------------------|
| Harvest index                 |                          |                   |                                   |                           |                      |
| Leaf area                     |                          |                   |                                   |                           |                      |
| Leaf weight                   |                          |                   |                                   |                           |                      |
| SLA                           |                          |                   |                                   |                           |                      |
| Leaf length                   |                          |                   |                                   |                           |                      |
| SPAD value at heading         |                          |                   |                                   |                           |                      |
| SPAD value at maturity        |                          |                   |                                   |                           |                      |
| Loss in SPAD value            |                          |                   |                                   |                           |                      |
| Morphological traits          |                          |                   |                                   |                           |                      |

1) Putative CRATs data were analyzed using trait measurements and genotype data from previously reported SSR markers (Hori et al., 2010).
2) CRAT-dependent changes are expressed as the percentage change of the value determined in Koshihikari.
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Table 1 (Continued). Effects of CRATs for the 30 productivity-related traits by Nipponbare alleles in the Koshihikari background.

| Traits                        | Number of CRATs detected | Chromosome number | Nearest markers of putative CRATs | CRAT-dependent change (%) | Agreed QTLs or CRATs |
|-------------------------------|--------------------------|-------------------|----------------------------------|--------------------------|----------------------|
| Plant height at heading      | 13                       | 6                 | P525F142 –9                      | –                        | –                    |
|                              |                          | 7                 | RM381 –5                         | –                        | –                    |
|                              |                          | 7                 | RM5691–RM2752 –5                 | –                        | –                    |
|                              |                          | 10                | RM5539 –3                        | –                        | –                    |
|                              |                          | 11                | RM2221–RM5926 –5                 | –                        | –                    |
| -2 leaf height               | 5                        | 6                 | P525F142 –12 –12 Kasalath allele | –                        | –                    |
|                              |                          | 6                 | P530H05–P17G10–24                | –4 –12                  | –                    |
|                              |                          | 7                 | RM1364–RM1306 –15                | –                        | –                    |
|                              |                          | 9                 | RM2482 –14                       | –                        | –                    |
|                              |                          | 12                | RM5455–RM6905 –12                | –                        | –                    |
| Panicle length               | 7                        | 1                 | RM1349 –6                        | –                        | –                    |
|                              |                          | 3                 | RM1038–RM2187 –6                 | –                        | –                    |
|                              |                          | 6                 | RM1210–00070220 –7 Nipponbare allele (BILs) | –6 –15 | – |
|                              |                          | 7                 | RM25519 –5                       | –                        | –                    |
| Length under panicle neck    | 13                       | 1                 | RM2522–RM8068 –52 Kasalath allele | –                        | –                    |
|                              |                          | 2                 | RM6375–RM12983 –12               | –                        | –                    |
|                              |                          | 2                 | RM6933–RM6923 –21                | –                        | –                    |
|                              |                          | 3                 | RM1038–RM2187 –17                | –                        | –                    |
|                              |                          | 4                 | RM1205 –16                       | –                        | –                    |
|                              |                          | 6                 | P525F142 –20                     | –                        | –                    |
|                              |                          | 6                 | P530H05–P17G10–24                | –15                     | –                    |
|                              |                          | 7                 | RM1364–RM1306 –15                | –                        | –                    |
|                              |                          | 9                 | RM1817 –8                        | –                        | –                    |
|                              |                          | 10                | RM2571–RM1859 –5                 | –                        | –                    |
| Diameter at panicle neck     | 7                        | 1                 | RM2522–RM8068 –14                | –                        | –                    |
|                              |                          | 3                 | RM1038–RM2187 –18                | –                        | –                    |
|                              |                          | 4                 | RM2700 –11                       | –                        | –                    |
|                              |                          | 4                 | RM1205 –10 Kasalath allele       | –                        | –                    |
|                              |                          | 6                 | P530H05–P17G10–24                | –8 –15                  | –                    |
|                              |                          | 8                 | RM7057–RM2666 –7                 | –                        | –                    |
|                              |                          | 10                | RM5271–RM1859 –8                 | –                        | –                    |
| Diameter at stem             | 6                        | 4                 | RM1205 –10 Kasalath allele       | –                        | –                    |
|                              |                          | 5                 | RM4674–RM19139 –6 Kasalath allele | –6 –15 | – |
|                              |                          | 6                 | P525F142 –7                       | –                        | –                    |
|                              |                          | 7                 | RM1364–RM1306 –6                 | –                        | –                    |
|                              |                          | 8                 | RM4487–RM4966 –6                 | –                        | –                    |
|                              |                          | 9                 | P501E099–RM25766 –8                 | –                        | –                    |
|                              |                          | 11                | RM1761–RM26541 –23               | –                        | –                    |
| Crown width                  | 5                        | 4                 | RM1205 –27                       | –                        | –                    |
|                              |                          | 6                 | B1012E06–16 –25                  | –                        | –                    |
|                              |                          | 7                 | RM25788 –25                      | –                        | –                    |
|                              |                          | 9                 | RM2482 –29                       | –                        | –                    |
|                              |                          | 10                | RM55519 –28                      | –                        | –                    |
| Characteristics of grain     |                          | 7                 | RM1349 –2                        | –                        | –                    |
| Grain length                 |                          | 2                 | RM6375–RM12983 +2                | –                        | –                    |
|                              |                          | 2                 | RM6933–RM6923 –2                 | –                        | –                    |
|                              |                          | 3                 | RM1038–RM2187 –2                 | –                        | –                    |
|                              |                          | 4                 | RM2700 –11                       | –                        | –                    |
|                              |                          | 4                 | RM1205 –10 Kasalath allele       | –                        | –                    |
|                              |                          | 6                 | P525F142 –20                     | –                        | –                    |
|                              |                          | 7                 | RM1364–RM1306 –6                 | –                        | –                    |
|                              |                          | 8                 | RM4487–RM4966 –6                 | –                        | –                    |
|                              |                          | 9                 | P501E099–RM25766 –8                 | –                        | –                    |
|                              |                          | 11                | RM7221–RM1859 –1                 | –                        | –                    |
| Grain width                  | 10                       | 1                 | RM2522–RM8068 +2 Kasalath allele | –                        | –                    |
|                              |                          | 1                 | RM12204–P20E09–14 +2             | –                        | –                    |
|                              |                          | 2                 | RM6375–RM12983 +2                | –                        | –                    |
|                              |                          | 3                 | RM1038–RM2187 –2                 | –                        | –                    |
|                              |                          | 4                 | RM2700 –11                       | –                        | –                    |
|                              |                          | 5                 | RM3529–T1362_02 +4               | –                        | –                    |
|                              |                          | 6                 | P530H05–P17G10–24                | –6 –14                  | –                    |
|                              |                          | 9                 | RM1817 –8                        | –                        | –                    |
|                              |                          | 10                | RM3252–RM8068 –2                 | –                        | –                    |
| Grain thickness              | 7                        | 1                 | RM12204–P20E09–14 +4             | –                        | –                    |
|                              |                          | 3                 | RM2595 –2                        | –                        | –                    |
|                              |                          | 4                 | RM7472–RM7181 +4                 | –                        | –                    |
|                              |                          | 6                 | RM8120–00070220 +2               | –                        | –                    |
|                              |                          | 9                 | P501E099–RM25766 –4               | –                        | –                    |
|                              |                          | 11                | RM7221–RM1859 +5                 | –                        | –                    |
| Grain whiteness              | 9                        | 1                 | RM2522–RM8068 –4                 | –                        | –                    |
|                              |                          | 3                 | RM1038–RM2187 –18                | –                        | –                    |
|                              |                          | 5                 | RM3252–T1362_02 +16              | –                        | –                    |
|                              |                          | 6                 | P530H05–P17G10–24                | –6 –14                  | –                    |
|                              |                          | 7                 | RM2581 –5                        | –                        | –                    |
|                              |                          | 7                 | RM5691–RM2752 –5                 | –                        | –                    |
|                              |                          | 8                 | RM7202 –4                        | –                        | –                    |
|                              |                          | 9                 | P501E099–RM25766 –5               | –                        | –                    |
|                              |                          | 10                | RM5271–RM1859 –4                 | –                        | –                    |

1) Putative CRATs data were analyzed using trait measurements and genotype data from previously reported SSR markers (Hori et al., 2010).
2) CRAT-dependent changes are expressed as the percentage change of the value determined in Koshihikari.
3) Reported by Madoka et al. (2008).
4) Reported by Hori et al. (2012).
5) Reported by Ujiie et al. (2012).
6) Reported by Ujiie and Ishimaru (2013). (Continued on next page).
Table 2. Effects of CRATs for the 30 productivity-related traits by Koshihikari alleles in the Nipponbare background.

| Traits                      | Number of CRATs detected | Chromosome number | Nearest markers of putative CRATs | CRAT-dependent change (%) |
|-----------------------------|--------------------------|-------------------|----------------------------------|---------------------------|
| **Yield and yield components** |                          |                   |                                  |                           |
| Yield                       | 5                        | 1                 | RM3252-RM8068                    | -28                       |
|                             |                          | 1                 | RM11686                          | +28                       |
|                             |                          | 2                 | RM6938                           | -24                       |
|                             |                          | 5                 | RM3529-OJ111A10-16               | -30                       |
| Total grain number          | 2                        | 1                 | RM3252-RM8068                    | -19                       |
|                             |                          | 1                 | RM1005                           | -23                       |
| **Panicle number**          | 0                        |                   |                                  |                           |
| **Grain number per panicle**| 2                        | 1                 | RM12204                          | -12                       |
|                             |                          | 7                 | RM2732-RM1364                    | -23                       |
| **Filling grain ratio**     | 2                        | 2                 | RM12983-RM1379                   | -6                        |
|                             |                          | 3                 | RM15504-RM2595                   | -15                       |
| **1,000-grain weight**      | 12                       | 1                 | RM3252-RM8068                    | -1                        |
|                             |                          | 1                 | RM12204                          | +3                        |
|                             |                          | 2                 | RM12983-RM1379                   | -2                        |
|                             |                          | 2                 | RM6933-RM6923                    | +4                        |
|                             |                          | 4                 | RM1295                           | +5                        |
|                             |                          | 4                 | RM5799-RM2431                    | -2                        |
|                             |                          | 5                 | RM3529-OJ111A10-16               | -4                        |
|                             |                          | 6                 | RM8120-0007029                   | -2                        |
|                             |                          | 6                 | P17G10-24                       | -3                        |
|                             |                          | 7                 | RM2732-RM1364                    | +3                        |
|                             |                          | 9                 | P501E999-RM23766                 | -2                        |
|                             |                          | 11                | RM1355                           | -4                        |
| **Biomass**                 |                          |                   |                                  |                           |
| Biomass at heading          | 3                        | 2                 | RM12983-RM1379                   | +18                       |
|                             |                          | 7                 | RM2732-RM1364                    | +29                       |
|                             |                          | 12                | RM2197                           | +23                       |
| Biomass at maturity         | 2                        | 1                 | RM6740-T1368_01                  | -16                       |
|                             |                          | 10                | RM7217                           | +12                       |
| Panicle weight              | 2                        | 1                 | RM6740-T1368_01                  | -22                       |
|                             |                          | 9                 | RM24616-RM2482                   | -25                       |
| Plant body weight           | 3                        | 2                 | RM12983-RM1379                   | +14                       |
|                             |                          | 7                 | RM1306                           | +14                       |
| Harvest index               | 10                       | 1                 | RM11686                          | -10                       |
|                             |                          | 2                 | RM6375                           | -8                        |
|                             |                          | 4                 | RM7472-RM7181                    | -12                       |
|                             |                          | 4                 | RM5799-RM2431                    | -9                        |
|                             |                          | 5                 | T1362_02-RM4674                  | -12                       |
|                             |                          | 6                 | RM8120-0007029                   | -12                       |
|                             |                          | 6                 | P17G10-24                       | -14                       |
|                             |                          | 7                 | RM2732-RM1364                    | -12                       |
|                             |                          | 8                 | RM22215-RM3702                   | -5                        |
|                             |                          | 10                | RM333                            | -9                        |
| **Source ability in flag leaf** |                          |                   |                                  |                           |
| Leaf area                   | 5                        | 1                 | RM3252-RM8068                    | +19                       |
|                             |                          | 1                 | RM6740-T1368_01                  | +15                       |
|                             |                          | 1                 | P20E09-14                        | +12                       |
|                             |                          | 2                 | RM6375                           | +20                       |
| Leaf weight                 | 4                        | 1                 | P20E09-14                        | +10                       |
|                             |                          | 2                 | RM6375                           | +19                       |
|                             |                          | 10                | RM12204                          | +11                       |
| SLA                         | 4                        | 2                 | RM6375                           | +19                       |
|                             |                          | 10                | RM12204                          | +19                       |
| Leaf length                 | 13                       | 1                 | RM3252-RM8068                    | -7                        |
|                             |                          | 1                 | RM12204                          | -7                        |
|                             |                          | 2                 | RM6375                           | -9                        |
|                             |                          | 2                 | RM6375                           | -12                       |
|                             |                          | 3                 | RM1306-RM2595                    | -9                        |
|                             |                          | 4                 | RM1295                           | -9                        |
|                             |                          | 5                 | T1362_02-RM4674                  | -10                       |
|                             |                          | 6                 | P501E09-9-RM23766                | -7                        |
|                             |                          | 6                 | P17G10-24                       | -8                        |
|                             |                          | 7                 | RM2381                           | -12                       |
|                             |                          | 8                 | RM22215-RM3702                   | -8                        |
|                             |                          | 9                 | P501E999-RM23766                 | -5                        |
|                             |                          | 9                 | RM24616-RM2482                   | -6                        |
| SPAD value at heading       | 7                        | 1                 | RM11686                          | -5                        |
|                             |                          | 4                 | RM7472-RM7181                    | -5                        |
|                             |                          | 7                 | RM2381                           | -4                        |
|                             |                          | 7                 | RM2732-RM1364                    | -9                        |
|                             |                          | 9                 | P501E999-RM23766                 | -4                        |
|                             |                          | 10                | RM3251                           | -4                        |
|                             |                          | 10                | RM25539                          | -4                        |
| SPAD value at maturity      | 7                        | 1                 | RM3252-RM8068                    | +19                       |
|                             |                          | 3                 | RM4106-RM6923                    | -17                       |
|                             |                          | 3                 | RM1038-RM2595                    | -17                       |
|                             |                          | 6                 | RM8120-0007029                   | +15                       |
|                             |                          | 6                 | P17G10-24                       | +16                       |
|                             |                          | 7                 | RM4106-RM2595                    | +18                       |
| Loss in SPAD value          | 13                       | 1                 | RM3252-RM8068                    | -17                       |
|                             |                          | 1                 | RM11686                          | -16                       |
|                             |                          | 2                 | RM6375                           | -16                       |
|                             |                          | 5                 | T1362_02-RM4674                  | -17                       |
|                             |                          | 6                 | RM8120-0007029                   | -15                       |
|                             |                          | 6                 | P17G10-24                       | -15                       |
|                             |                          | 10                | RM2381                           | -20                       |

1) Putative CRAT data were analyzed using trait measurements and genotype data from previously reported SSR markers (Hori et al., 2010).
2) CRAT-dependent changes are expressed as the percentage change of the value determined in Nipponbare. (Continued on next page)
Table 2 (Continued).  Effects of CRATs for the 30 productivity-related traits by Koshihikari alleles in the Nipponbare background.

| Traits                               | Number of CRATs detected | Chromosome number | Nearest markers of putative CRATs | CRAT-dependent change (%)*1 |
|--------------------------------------|--------------------------|-------------------|----------------------------------|-----------------------------|
| Loss in SPAD value                   |                          |                   |                                  |                             |
|                                      | 13                       | 7                 | RM2752-RM1364                    | –19                         |
|                                      |                          | 9                 | P561E99-6-RM23766               | –15                         |
|                                      |                          | 10                | RM2571                          | –14                         |
|                                      |                          | 10                | RM25319                         | –15                         |
|                                      |                          | 11                | RM1355                          | –14                         |
|                                      |                          | 12                | RM2752-RM28070                   | –17                         |
|                                      |                          |                   |                                  |                             |
| Morphological traits                 |                          |                   |                                  |                             |
| Plant height at early stage          | 9                        | 1                 | RM11686                         | +5                          |
|                                      |                          | 3                 | RM1036-RM2187                   | +10                         |
|                                      |                          | 6                 | RM253142                       | +4                          |
|                                      |                          | 7                 | RM2752-RM1364                   | +11                         |
|                                      |                          | 8                 | RM7057                          | +7                          |
|                                      |                          | 8                 | RM3459-RM3549                   | +13                         |
|                                      |                          | 9                 | RM24039-RM1817                  | +9                          |
|                                      |                          | 10                | RM2571                          | +9                          |
|                                      |                          | 12                | RM2197                          | +6                          |
| Plant height at heading              | 5                        | 1                 | RM2552-RM8068                   | –4                          |
|                                      |                          | 1                 | RM11686                         | –6                          |
|                                      |                          | 4                 | RM7099-RM2451                   | –5                          |
|                                      |                          | 5                 | RM3529-OJ1111A10-16             | –7                          |
|                                      |                          | 7                 | RM2752-RM1364                   | +3                          |
| –2 leaf height                       | 4                        | 3                 | RM4108-RM3649                   | +17                         |
|                                      |                          | 7                 | RM2752-RM1364                   | +18                         |
|                                      |                          | 8                 | RM22215-RM3762                  | +21                         |
|                                      |                          | 10                | RM7217                          | +15                         |
| Panicle length                       | 7                        | 1                 | RM2552-RM8068                   | –15                         |
|                                      |                          | 1                 | RM11686                         | –10                         |
|                                      |                          | 4                 | RM1295                          | –12                         |
|                                      |                          | 5                 | T1382_02-RM4674                 | –14                         |
|                                      |                          | 7                 | RM2391                          | –14                         |
|                                      |                          | 7                 | RM2752-RM1364                   | –13                         |
|                                      |                          | 9                 | RM24039-RM1817                  | –11                         |
|                                      |                          | 10                | RM2571                          | –12                         |
|                                      |                          | 10                | RM333                           | –5                          |
| Length under panicle neck            | 10                       | 3                 | RM4108-RM3649                   | –25                         |
|                                      |                          | 3                 | RM1036-RM2187                   | –19                         |
|                                      |                          | 4                 | RM3509-RM2431                   | –20                         |
|                                      |                          | 5                 | RM2537-RM1939                   | –14                         |
|                                      |                          | 7                 | RM2391                          | –14                         |
|                                      |                          | 7                 | RM1306                          | –22                         |
|                                      |                          | 8                 | RM22215-RM3762                  | –20                         |
|                                      |                          | 10                | RM333                           | –25                         |
|                                      |                          | 11                | RM3625                          | –14                         |
|                                      |                          | 11                | RM1355                          | –17                         |
|                                      |                          | 10                | RM7217                          | +10                         |
|                                      |                          | 11                | RM1761-RM26123                  | +9                          |
|                                      |                          | 12                | RM2972                          | +18                         |
| Diameter at panicle neck             | 3                        | 3                 | RM4108-RM3649                   | +8                          |
|                                      |                          | 7                 | RM2752-RM1364                   | +10                         |
|                                      |                          | 8                 | RM22215-RM3762                  | +15                         |
|                                      |                          | 8                 | RM3459-RM3549                   | +6                          |
|                                      |                          | 10                | RM2571                          | +9                          |
|                                      |                          | 10                | RM2552-RM8068                   | +7                          |
|                                      |                          | 11                | RM1761-RM26123                  | +8                          |
| Diameter at stem                     | 7                        | 3                 | RM4108-RM3649                   | +8                          |
|                                      |                          | 7                 | RM2752-RM1364                   | +10                         |
|                                      |                          | 8                 | RM22215-RM3762                  | +15                         |
|                                      |                          | 8                 | RM3459-RM3549                   | +6                          |
|                                      |                          | 10                | RM2571                          | +9                          |
|                                      |                          | 10                | RM2552-RM8068                   | +7                          |
|                                      |                          | 11                | RM1761-RM26123                  | +8                          |
| Crown width                          | 0                        | 0                 |                                  |                             |

Characteristics of grain

| Grain length                          | 5                        | 1                 | RM352-RM8068                    | +1                          |
|                                      |                          | 1                 | RM11686                         | +2                          |
|                                      |                          | 4                 | RM1355                          | +2                          |
|                                      |                          | 9                 | RM24039-RM1817                  | +1                          |
|                                      |                          | 11                | RM333                           | –2                          |
| Grain width                           | 13                       | 1                 | RM352-RM8068                    | –1                          |
|                                      |                          | 1                 | RM11686                         | +2                          |
|                                      |                          | 2                 | RM3509-RM2431                   | –1                          |
|                                      |                          | 3                 | RM4108-RM3649                   | +2                          |
|                                      |                          | 5                 | RM1306-RM2431                   | –1                          |
|                                      |                          | 5                 | RM3529-OJ1111A10-16             | –1                          |
|                                      |                          | 6                 | RM3625-0007020                  | –2                          |
|                                      |                          | 6                 | T1382_02-RM4674                 | –1                          |
|                                      |                          | 7                 | RM2752-RM1364                   | +1                          |
|                                      |                          | 8                 | RM22215-RM3762                  | –1                          |
|                                      |                          | 9                 | P561E99-6-RM23766               | –1                          |
|                                      |                          | 10                | RM333                           | –1                          |
|                                      |                          | 11                | S0185_16-RM187, RM7221-RM5926   | +1                          |
| Grain thickness                       | 10                       | 1                 | RM352-RM8068                    | +2                          |
|                                      |                          | 1                 | RM11686                         | +1                          |
|                                      |                          | 2                 | RM3509-RM2431                   | –1                          |
|                                      |                          | 3                 | RM3625-0007020                  | –1                          |
|                                      |                          | 4                 | RM1355                          | +2                          |
|                                      |                          | 5                 | T1382_02-RM4674                 | +1                          |
|                                      |                          | 7                 | RM2752-RM1364                   | +1                          |
|                                      |                          | 7                 | RM24039-RM1817                  | +7                          |
|                                      |                          | 9                 | RM2571                          | +15                         |
|                                      |                          | 12                | RM2972                          | +11                         |

1) Putative CRAT data were analyzed using trait measurements and genotype data from previously reported SSR markers (Hori et al., 2010).
2) CRAT-dependent changes are expressed as the percentage change of the value determined in Nipponbare.
different strengths between corresponding alleles. For a CRAT related to harvest index on chromosome 7, effects differed depending on the genetic background (+6% or −12%). For CRATs detected in both Kos-Nip and Nip-Kos CSSLs, the number of SNPs resulting in amino acid substitutions is indicated in Table 3. The CRATs for yield and total grain number overlapping PN1 included 84 genes containing SNPs resulting in amino acid substitutions. There was no candidate genes identified in the CRAT for 1,000-grain weight on chromosome 9 based on this criterion.

**Discussion**

The productivity of Koshihikari could be improved by the introduction of CRATs originating from the japonica variety, Nipponbare. We identified 75 CRATs for which Nipponbare alleles had positive effects on 20 traits related to productivity. When Koshihikari was the host, Nipponbare

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**Table 3. Reversal CRATs between Koshihikari and Nipponbare.**

| Traits                        | Chr. | Nearest markers of putative CRATs | CRAT-dependent change (%) | Size of region (Mbp) | Number of genes containing SNP |
|-------------------------------|------|----------------------------------|--------------------------|----------------------|-------------------------------|
| **Yield and yield components** |      |                                  |                          |                      |                               |
| Yield                         | 1    | RM3252-RM8068                    | +35 (−28)                | 11.0                 | 84                            |
| Total grain number            | 1    | RM3252-RM8068                    | +40 (−19)                | 11.0                 | 84                            |
| 1,000-grain weight            | 1    | RM3252-RM8068                    | +4 (−1)                  | 11.0                 | 84                            |
|                               | 2    | RM6375-RM12983                   | +4 (−2)                  | 16.0                 | 10                            |
|                               | 6    | P530H05-35-P17G10-24             | +3 (−3)                  | 10.9                 | 10                            |
|                               | 9    | P501E099-RM23766                 | +2 (−2)                  | 10.6                 | 0                             |
|                               | 11   | RM26890-RM1355                   | +5 (−4)                  | 19.1                 | 44                            |
| **Biomass**                   |      |                                  |                          |                      |                               |
| Biomass at maturity           | 10   | RM5271-RM1859                    | −10 (+12)                | 17.3                 | 32                            |
| Plant body weight             | 7    | RM1364-RM1306                    | −21 (+14)                | 7.1                  | 57                            |
|                               | 10   | RM5271-RM1859                    | −14 (+14)                | 17.3                 | 32                            |
| Harvest index                 | 6    | RM8120-0007O29                   | +7 (−12)                 | 6.7                  | 10                            |
|                               | 7    | RM1364-RM1306                    | +6 (−12)                 | 7.1                  | 57                            |
| **Source ability in flag leaf** |   |                                  |                          |                      |                               |
| SPAD value at maturity        | 3    | RM1038-RM2187                    | −43 (+31)                | 6.3                  | 8                             |
|                               | 6    | P530H05-35-P17G10-24             | −18 (+16)                | 10.9                 | 10                            |
|                               | 10   | RM5271-RM1859                    | −22 (+26)                | 17.3                 | 32                            |
| Loss in SPAD value           | 5    | RM3529-T1362_02                  | +41 (−17)                | 20.3                 | 7                             |
|                               | 6    | P530H05-35-P17G10-24             | +18 (−15)                | 10.9                 | 10                            |
|                               | 10   | RM5271-RM1859                    | +29 (−21)                | 17.3                 | 32                            |
|                               | 11   | RM26890-RM1355                   | +16 (−14)                | 19.1                 | 44                            |
|                               | 12   | RM3453-RM6905                    | +15 (−17)                | 12.0                 | 35                            |
| **Morphological traits**      |      |                                  |                          |                      |                               |
| Plant height at early stage   | 1    | T1368_01-RM11686                 | −8 (+5)                  | 8.5                  | 18                            |
|                               | 8    | RM4487-RM5496                    | −7 (+13)                 | 7.8                  | 6                             |
| Plant height at heading       | 1    | RM3252-RM8068                    | +2 (−4)                  | 11.0                 | 84                            |
|                               | 5    | RM3529-T1362_02                  | +5 (−7)                  | 20.3                 | 7                             |
|                               | 7    | RM3691-RM2752                    | −5 (+3)                  | 20.8                 | 66                            |
| −2 leaf height               | 7    | RM1364-RM1306                    | −15 (+18)                | 7.1                  | 57                            |
| Diameter at panicle neck      | 10   | RM5271-RM1859                    | −8 (+10)                 | 17.3                 | 32                            |
| Diameter at stem              | 7    | RM1364-RM1306                    | −6 (+10)                 | 7.1                  | 57                            |
|                               | 8    | RM4487-RM3496                    | −6 (+6)                  | 7.8                  | 6                             |

1) Putative CRATs data were analyzed using trait measurements and genotype data from previously reported SSR markers (Hori et al., 2010).
2) CRAT-dependent changes are expressed as the percentage change of the value determined in Koshihikari and Nipponbare (in parentheses).
3) The number of genes with a CRAT that contain at least one SNP associated amino acid substitution.
had some unique CRATs compared to other indica donors, Kasalath and Nona Bokra (Madoka et al., 2008; Ujiie et al., 2012; Ujiie and Ishimaru, 2013). For example, 3 positive CRATs identified in this study for biomass at heading (Fig. 4) were not detected in other CSSLs between Koshihikari and indica varieties (Ujiie et al., 2012). The maximum effect of these 3 CRATs was increased by 56% compared with Koshihikari, and was stronger than those of Kasalath allele (+11%) (Table 1). For biomass at maturity, a positive CRAT was detected only from the Nipponbare allele (Fig. 4, Table 1). Additionally, 82 positive CRATs by Koshihikari alleles affecting 17 traits were identified on the Nipponbare background (Fig. 5, Table 2). These results support the idea that specific japonica varieties can serve as sources of superior and/or unique alleles that positively impact the productivity of other japonica varieties.

CRAT for panicle number on chromosome 1 (tentatively named PN1) significantly increased panicle number by 37%, total grain number by 40%, and also yield by 35% compared with Koshihikari (Fig. 4, Table 1). In modern japonica varieties, including Koshihikari, source ability is the main factor limiting increases in yield (Kusutani et al., 1993), and a partial expansion in sink size might not impact final grain yield (Sheehy et al., 2001; Ujiie and Ishimaru, 2013). Actually, a CRAT for sink size, 1,000-grain weight chromosome 2 significantly increased by 4% but did not affect on final yield (Fig. 4, Table 1). Similarly, GS3, a strong allele for grain size, has also been shown to not have effects on yield (Yu et al., 1997; Li et al., 2000; Hua et al., 2002; Xing et al., 2002). PN1 strongly impacted final yield, and this result suggested that higher sink size associated with PN1 could improve source capacity. An increase in tiller number (equal to panicle number) led to an increase in leaf area per plant via higher its number (Peng et al., 2008). Source ability after heading is mainly photosynthate production by flag leaves (Cock and Yoshida, 1972; Ishimaru et al., 2007; Takai et al., 2010), and this production is determined by leaf area and nitrogen content per area (Mae, 1997; Ohsumi et al., 2007; Ujiie et al., 2012). Nitrogen content per leaf area is closely related to photosynthetic rate, and SPAD value is used as an indicator (Peng et al., 1995; Ida, 2006). In the CSSL with PN1, the SPAD value was the same, but a flag leaf area was significantly larger by 21% than that in Koshihikari (Table 1). Therefore, PN1 might increase total source ability per plant by increasing the number and area of flag leaves by maintaining photosynthetic ability, too. On the other hand, the effect of PN1 was not stable for environmental change. In the complement experiment in 2010, the effect of PN1 was a third of that observed in 2009 (+12%, P = 0.06 NS; data not shown). Phenotype was determined by both genetic and environmental factors (Wang et al., 1999; Li et al., 2003). High temperature condition at the vegetative stage shortened the tillering period and then reduced the tiller number (similar to panicle number) (Oka, 1955; Matsushima et al., 1966; Kakizaki, 1976). Comparing to in 2009, the temperature at the vegetative stage was higher in 2010 (+1.6 to +2.7°C) (Fig. 1). Actually, days to heading of Koshihikari in 2010 was 4 days shorter than that in 2009. In Koshihikari and SL-PN1, heading date was almost the same in 2009. In 2010, panicle number in Koshihikari and SL-PN1 was significantly decreased by 12% and 27%, respectively, as compared with that in 2009. The high temperature in 2010 might reduce the effect of PN1 on panicle number.

Most of the positive CRATs related to source ability did not improve yield in Koshihikari in which source ability is the main limiting factor for yield (Kusutani et al., 1993). A CRAT on chromosome 2 increased flag leaf area by 20% maintaining SPAD value, but did not affect final yield (tentatively named LA2) (Fig. 4, Table 1). There are at least two possible explanations for these results. First, it is possible that CRATs include gene(s) with negative effects on sink or carbohydrate translocation. However, importantly, we did not observe negative effects of CRATs related to sink size in the region overlapping with LA2. The second is the possibility that source ability per plant may not increase to the level that could impact on yield by these CRATs. Actually, the increase in leaf area might contain negative effects on source ability per plant, such as an increase in respiration rate and deterioration in light-intercepting characteristics (Peng et al., 2008). On the other hand, PN1 increased the area and the number of flag leaves (+21 and +37%, respectively) and consequently improved yield by 35%. This difference between these two CRATs might be caused by the combination of expansion of area and the increase in the number of flag leaves by PN1.

A total of 181 CRATs for 30 traits were detected using Nip-Kos CSSLs, but for only 29 CRATs associated with 13 traits, their correspondent ones with opposite effect were identified in Kos-Nip CSSLs (Table 3). Hori et al. (2010) reported similar results for CRATs for pre-harvest sprouting resistance. They concluded that the cause of this phenomenon was due to partial lack of substituted chromosome. It was difficult to think that a partial lack might happen on substituted chromosome responding to 88% of the CRATs detected here. Some of QTLs were not detected in different host varieties. A QTL associated with heading has been identified on chromosome 10 on the Nipponbare background after crossed with Kasalath, but was not detected on the Koshihikari background (Yano et al., 2001; Ebitani et al., 2005). These results suggest that the effect of an allele, in many cases, might be determined not only by itself but also by the genetic background. On the other hand, correspondent alleles were detected in 12% of CRATs (alleles) and these alleles might be effective
without influencing the genetic background.

In BILs between Koshihikari and Nipponbare, the number of days to heading has been correlated with 33 traits, including yield, plant height, and flag leaf area (Hori et al., 2012). Similar results have been reported for a number of traits on various genetic materials (Mei et al., 2003; Kwon et al., 2008; Takeuchi et al., 2008). The main cause of these results was that heading date strongly affected other traits. According to Hori et al. (2012), most QTLs for agronomic traits are located near 2 QTLs associated with the number of days to heading. In this study, days to heading was correlated with 7 traits in Kos-Nip and 2 in Nip-Kos (data not shown; threshold level $P < 0.001$), and accounted for less than 13% and 5%, respectively, of all correlations detected in this study. Because most CSSLs did not contain 2 QTLs for days to heading, and we could evaluate their phenotype without the effect of heading date. For example, panicle length has been correlated with days to heading in 3 experiments using BILs (Hori et al., 2012), whereas we did not find a significant correlation in either set of CSSLs. In an experiment using BILs, the essential effects of QTLs could be influenced by linked QTLs for heading date. When an analysis for productivity is conducted, we should use CSSLs at least for traits strongly affected by heading date.

We compared chromosomal positions of CRATs in this study and QTLs or CRATs detected using other genetic materials with the same host, Koshihikari (Madoka et al., 2008; Hori et al., 2012; Ujiie et al., 2012; Ujiie and Ishimaru, 2013). Among 15 QTLs for 6 traits detected with BILs between Nipponbare and Koshihikari (Hori et al., 2012), 4 QTLs overlapped with CRATs detected using Kos-Nip CSSLs (Table 1). It was thought that these CRATs might be stable for environmental change. In the CSSLs using Kasalath and Nona Bokra as a donor, 13 and 9 overlapping among CRATs for 21 and 9 traits, respectively, were detected (Madoka et al., 2008; Ujiie et al., 2012; Ujiie and Ishimaru, 2013). These results suggested that common genes for determination of traits might be located in these chromosomal regions across genetic varieties as a donor. On the other hand, CRATs that did not overlap with others might be unique ones by Nipponbare alleles. Since this study was based on a single field experiment, the mapping results could have been affected by environmental conditions. The interaction of the CRATs with the environment remains to be revealed by further studies.

Using the database of SNPs identified between Koshihikari and Nipponbare (Yamamoto et al., 2010), primary candidate for causal genes could be estimated without narrowing in chromosome region. The size of a CRAT for plant height at heading on chromosome 5 was estimated to be 20 Mbp by analysis of SSR markers, and included 1,681 genes. In this region, there were only 7 genes containing SNPs with amino acid substitutions. These genes were primary candidates for causal genes, but the causal gene is not always included in this list. On the other hand, a CRAT for 1,000-grain weight detected on chromosome 9 has no SNP with amino acid substitution. In addition to the variation in coding region, numerous studies reported that the variations in non-coding region could determine phenotypic variations (e.g., Ishimaru et al., 2004). Therefore, it is necessary to consider mutations in non-coding regions as a second candidate for CRAT. By the combination between these data and positional cloning, we could be accelerated to narrow the number of candidate genes for a CRAT. Therefore, further development of this SNP database will likely valuable for the identification of important genes and direct functional analyses.

For most of the traits investigated here, the size of phenotypic variation in Kos-Nip CSSLs (Fig. 2) had a tendency to be smaller than that in Koshihikari/Kasalath and Koshihikari/Nona Bokra CSSLs (Madoka et al., 2008; Ujiie et al., 2012; Ujiie and Ishimaru, 2013). For example, the difference between maximum and minimum flag leaf area was 19.2 cm$^2$ in Koshihikari/Nona Bokra CSSLs, and it was 1.8 times that in Kos-Nip CSSLs (Fig. 2). For the SPAD value at heading, the maximum effects of the Nipponbare allele (+5%) were lower than those of Kasalath (+11%) and Nona Bokra allele (+21%) (Ujiie et al., 2012). Similar tendencies were observed for 8 other traits (e.g., flag leaf area, grain number per panicle, plant height, and grain width) (Madoka et al., 2008; Ujiie et al., 2012; Ujiie and Ishimaru, 2013). One explanation for these results could be that Nipponbare is a variety closely related to Koshihikari (Ohta et al., 2006). As compared with 2 CSSLs with indica donors, CRATs with strong effects were not identified for traits with smaller phenotypic variation in Kos-Nip CSSLs (Table 1). If a donor variety is closely related to the host, the number of traits to detect superior CRATs might be few. However, we noted strong and unique CRATs for several traits with Nipponbare alleles (e.g., CRATs for biomass at heading, harvest index and −2 leaf height). Additionally, we detected some CRATs that improved the traits related to productivity of Nipponbare by Koshihikari alleles, such as CRATs for SPAD value at maturity and diameter at the panicle neck. Therefore, we could detect useful alleles from various varieties including those closely related to the host.

We identified 249 and 181 CRATs for 30 productivity-related traits that were associated with Nipponbare and Koshihikari alleles, respectively, under each opposite genetic background (Figs. 4 and 5). For 88% of CRATs identified here, opposite effects were not observed within the same region of CSSLs generated from the alternate crosses (Table 3). The existence and effects of CRATs might be affected by the genetic background. There were superior and/or unique CRATs that were strongly affected.
by Nipponbare alleles than by alleles of Kasalath and Nona Bokra. Therefore, improvement in the productivity using a closely related variety might be possible through appropriate choice of a donor variety.

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