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UPDATE FOR ELITE JAPONICA VARIETY

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ABSTRACT

A new concept called Variety Update for rice molecular breeding was introduced. We updated four traits of *japonica* elite variety Koshihikari and bred four new varieties of Koshihikari in this work. The idea of Variety Update was from computer software and digital informatics. To improve on less desirable traits of elite variety only through replacing a small chromosomal fragment, was our concept.

Key words: Variety Update, SNP marker, Rice breeding, QTL, Trait

INTRODUCTION

The origin of agriculture led to the domestication of many plant species and to the exploitation of natural resources. It took almost 10,000 years for food grain production to reach 1 billion tons, in 1960, and only 40 years to reach 2 billion tons, in 2000 (Khush, 2001). This unprecedented increase, which has been named the "Green Revolution," resulted from improved crop varieties, combined with the application of agronomic practices. It was known that the semi-dwarf gene *sd1* played the main role in the green revolution. Gene *sd1* produces strong rice culm, with the result that a rice plant has lodging tolerance and is a high-yield type. Since the Green Revolution, many rice varieties with *sd1* have been bred and used in rice production in various Asian countries to increase food supply. China, to counter this dearth, built up its hybrid rice-breeding system with the *sd1* gene to develop higher-yield varieties.

However, rice varieties in Japan have shown no change, even in the Green Revolution. Bred 50 years ago, Koshihikari is still the most popular rice variety even now. It has taken over about 38% of rice paddy areas in Japan even at present; while other paddies have been generally inadaptable for the cultivation of Koshihikari. This indicates it has been the optimum rice variety for the last five decades in Japan. Koshihikari is considered to be of very good eating quality, but has lower yields. This fact indicates that the breeding technology of the Green Revolution only played a role in increasing the yield of rice variety, but could not improve the eating quality with *sd1* gene only.

The goal of key technology in crop breeding is to make certain progress in new varieties. Looking at the huge numbers of rice varieties bred every year, however, it appears that farmers (users) were not shown what traits were improved in the new varieties. Rice farmers in Japan would certainly never use the old-variety Koshihikari if an improved Koshihikari existed - just as a computer user would never depend on a ten-year-old laptop.

Since the two agriculturally important subspecies of rice, called *indica* and *japonica,* were published (IRGSP, 2005, Han and Zhang, 2008), DNA markers used in genetic analysis, QTL finding, and map-based cloning are easily made. In rice breeding, DNA marker selection methods were established and used in the actual breeding. In this paper, we show a new concept that is different from DNA marker selection methods or MAS (Marker-Assisted Selection), called "Variety Update."

MATERIALS AND METHODS

The idea of Variety Update is from computer software, the internet, iPhone and others that improve on old versions. This concept involved two steps: one, to find out any bugs, undesirable traits, defects and so on; and the other, to solve such problems, and to add new functions, thereby achieving progress. Koshihikari is the preferred rice variety in Japan but it has some defects such as weak lodging tolerance, lower yield, low regeneration ability, strong photosensitivity and others. Here, we attempted a procedure we call "Variety Update" to improve the less desirable traits of Koshihikari.

Updating the Lodging Tolerance of Koshihikari

We identified the position of culm length QTL in the semi-dwarf gene *sd1* region in chromosome 1. The QTL showed that the chromosome fragment of *indica* variety HABATAKI in the *sd1* region has the effect of reducing Koshihikari culm length by some 15 cm. We decided to use the chromosome fragment to update the lodging tolerance of Koshihikari. Semi-dwarf gene *sd1* was isolated (Sasaki et al., 2002), and we bred the introgression line ILA with Koshihikari background, which has the chromosomal fragment of HABATAKI's *sd1* region. We crossed ILA and Koshihikari to make the F1 plant and made the F2 segregation population ILAF2 from the seeds of the F1 plant. We selected a plant ILAF2-1, which is heterozygous in the *sd1* locus and homozygous Koshihikari type in the vicinity of *sd1* locus. Then the seeds of plant ILAF2-1 were used to make the second segregation population ILAF3. A plant ILAF3-1 was selected from the segregation population of ILAF3. ILAF3-1 is heterozygous in the *sd1* locus but homozygous Koshihikari type in both sides of *sd1*.

Finally, an F4 plant ILAF4-1 was selected from a segregation population ILAF4 derived from the seed of ILAF3-1. ILAF4-1 is homozygous HABATAKI type in the *sd1* locus and homozygous Koshihikari type in others. ILAF4-1 is the new Koshihikari variety, called Koshihikari Eichi 4, which is the result of this Variety Update for the lodging tolerance of Koshihikari.

Updating the Grain Yield of Koshihikari

We had the idea to increase grain yield of Koshihikari by making its panicle size larger. We found a QTL *Yq1* in chromosome 1. The allele of HABATAKI in the *Yq1* region has the effect of increasing the grain number of the main panicle. After that, we isolated the QTL as *Gn1a* gene (Ashikari et al., 2005). We bred the introgression line ILB with Koshihikari background, which has the chromosomal fragment of HABATAKI's *Gn1a* region. We crossed ILB and Koshihikari to obtain the F1 plant and made the F2 segregation population ILBF2 by growing the seeds of the F1. We selected a plant ILBF2-1, which is heterozygous in the *Gn1a* locus and homozygous Koshihikari type in the vicinity of *Gn1a* locus. Then the

seeds of plant ILBF2-1 were used to make the second segregation population ILBF3. A plant ILBF3-1 was selected from the segregation population of ILBF3. ILAF3-1 is heterozygous in the *Gn1a* locus, but homozygous Koshihikari type in both sides of *Gn1a*.

Lastly, an F4 plant ILBF4-1 was selected from a segregation population ILBF4, which was derived from the seeds of ILBF3-1. ILBF4-1 is homozygous HABATAKI type in the *Gn1a* locus and is homozygous Koshihikari type in others. ILBF4-1 is the new Koshihikari variety called Koshihikari Eichi 2, being the result of Variety Update in the grain yield of Koshihikari.

Updating the Callus Regeneration Ability of Koshihikari

Koshihikari is one of the most important *japonica* varieties used for food production, such as the *indica* variety IR64. However, Koshihikari has only very low regeneration ability of callus. For future gene function and genome function research, we decided to update this trait. We found a QTL *PSR1* in chromosome 1. The allele of *indica* variety Kasalath has the effect of increasing the ability of callus regeneration of Koshihikari. We isolated the QTL as *PSR1* gene after that (Nishimura et al., 2005). We bred the introgression line ILC with Koshihikari background, which has the chromosomal fragment of Kasalath's *PSR1* region; crossed ILC and Koshihikari to obtain the F1 plant; and made the F2 segregation population ILCF2. We selected a plant ILCF2-1 which is heterozygous in the *PSR1* locus and homozygous Koshihikari type in the vicinity of *PSR1* locus. Subsequently, the seeds of plant ILCF2-1 were used to make second-segregation population ILCF3. A plant ILCF3-1 was selected from the segregation population of ILCF3. ILCF3-1 is heterozygous in the *PSR1* locus, but homozygous Koshihikari type in both sides of *PSR1*.

Finally, an F4 plant ILCF4-1 was selected from a segregation population ILCF4 derived from the seed of ILCF3-1. ILCF4-1 is homozygous Kasalath type in the *PSR1* locus and homozygous Koshihikari type in others. ILCF4-1 is the new Koshihikari variety called Koshihikari Eichi 1, which is the result of this Variety Update for the callus regeneration ability of Koshihikari.

Changing the Photoperiod Sensitivity of Koshihikari

As mentioned, Koshihikari is the preferred variety in Japan, shown by about 38% of rice paddies being used for its rice production. However, Koshihikari could not be adapted to all areas in the country. The reason is that maturity will be too late if the variety is grown in northern Yamagata Prefecture (northern latitude of 38 degrees). And, its maturity will be too early if grown in southern Aichi Prefecture (southern latitude of 35 degrees). Therefore, we decided to change the photoperiod sensitivity of Koshihikari. A QTL gene *Hd1* is a known gene for photoperiod sensitivity in *japonica* varieties and had been isolated (Yano et al., 2000). We found a QTL *QTS1* in *Hd1* region of chromosome 6. The allele of indica variety HABATAKI in this region has the effect of changing the photoperiod sensitivity of Koshihikari. We bred the introgression line ILD with Koshihikari background, which has the chromosomal fragment of HABATAKI's *Hd1* region; and crossed ILD and Koshihikari to have the F1 plant and make the F2 segregation population ILDF2. We selected a plant ILDF2-1 from the segregation population ILDF2, which is heterozygous in the *Hd1* locus and homozygous Koshihikari type in the vicinity of *Hd1* locus. Next, the seeds of plant ILDF2-1 were used to make second-segregation population ILDF3. A plant ILDF3-1 was selected from the segregation population of ILDF3. ILDF3-1 is heterozygous in the *Hd1* locus, but homozygous Koshihikari type in both sides of *Hd1*.

Finally, an F4 plant ILDF4-1 was selected from a segregation population ILDF4, which was derived from the seeds of ILDF3-1. ILDF4-1 is homozygous HABATAKI type in the *Hd1* locus and is homozygous Koshihikari type in others. ILDF4-1 is the new Koshihikari variety called Koshihikari Eichi 3, which is the result of this Variety Update.

RESULTS

Results of Updating the Lodging Tolerance of Koshihikari

ILAF4-1 is homozygous HABATAKI type in the semi-dwarf gene *sd1* locus and homozygous Koshihikari type in others. The F5 seed from the ILAF4-1 was used to test the phenotype in the rice fields. The generation after that, derived from seeds of the ILAF4-1

plant, is called the new-variety Koshihikari Eichi 4. The culm length (stem length excluding panicle) of Koshihikari Eichi 4 was 82.9 cm whereas that of Koshihikari grown in Chiba Prefecture, Japan was 96.9 cm. The difference of plant height between Koshihikari Eichi 4 and Koshihikari was 14 cm, which is apparent in the rice field (Fig.1-A,B,C). Koshihikari Eichi 4 has a shorter culm length and better lodging tolerance than that of Koshihikari (Fig.1 D). However, there are no differences between Koshihikari and Koshihikari Eichi 4 excluding the above traits (Fig.1 F). We also measured the length of chromosomal fragment transferred from HABATAKI included *sd1* locus. The length of this fragment is from 90-840 kb.

Results of Updating the Grain Yield of Koshihikari

ILBF4-1 is homozygous HABATAKI type in the *Gn1a* locus and is homozygous Koshihikari type in others. The F5 seeds from the ILBF4-1 were used to test the phenotype in the rice fields. The generation after that from selfing plant ILBF4-1 is called Koshihikari Eichi 2 as a new variety. The grain number of main panicles of Koshihikari Eichi 2

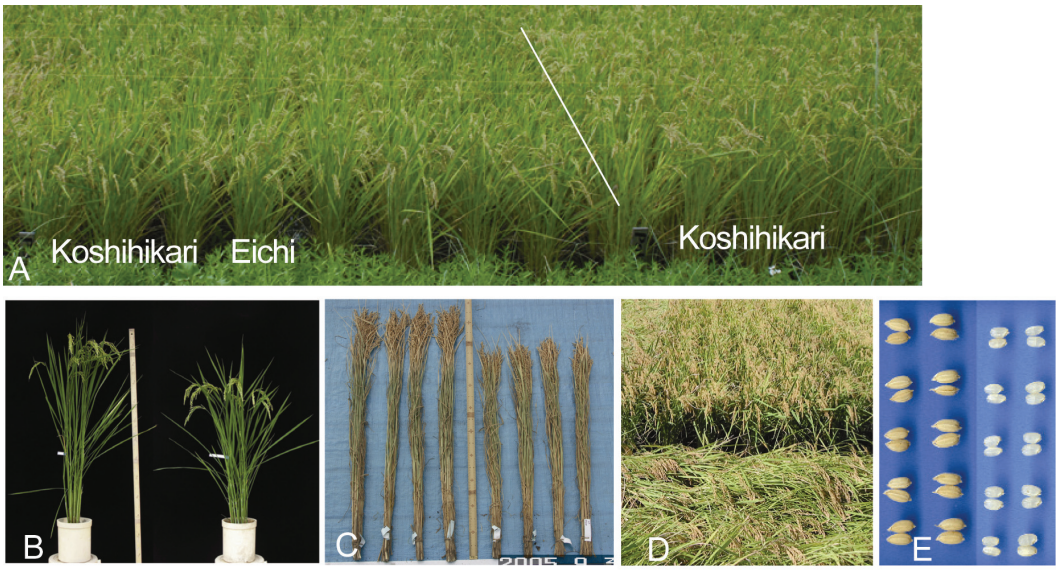


Fig.1. The differences between Koshihikari and Koshihikari Eichi 4. A: The plant height in rice field. B: Koshihikari Eichi 4 (right) and Koshihikari (left). C: The harvested plants, Koshihikari Eichi 4 (right) and Koshihikari (left). D: The lodging tolerance, Koshihikari Eichi 4 (upper), Koshihikari (lower). E: The seeds (left) and brown rice (right) of Koshihikari Eichi 4 (right) and Koshihikari (left).

averaged 196 when grown in Nagoya, Japan in 2006, whereas Koshihikari was 171 grains. There are also differences in panicle grain density between Koshihikari Eichi 2 (10.0 grains/cm) and Koshihikari (8.89 grains/cm) (Fig.2 C). However, there are no significant differences between Koshihikari and Koshihikari Eichi 2 excluding the above traits (Fig.2 A, B, D). We also measured the length of chromosomal fragment transferred from HABATAKI in the Koshihikari Eichi 2 included *Gn1a* locus. The length of this fragment is from 37kb-245 kb .

Results of Updating the Callus Regeneration Ability of Koshihikari

ILCF4-1 is homozygous Kasalath type in the *PSR1* locus and is homozygous Koshihikari type in others. The F5 seeds from the ILCF4-1 were used to test the phenotype in the rice fields. The generation after that from plant ILBF4-1 is the new variety called Koshihikari Eichi 1. The regeneration ability of Koshihikari Eichi 1 is higher than that of Koshihikari and similar to that of gene donor Kasalath (Fig. 3 C). However, there are no significant differences of the agricultural traits for Koshihikari and Koshihikari Eichi 1 excluding the above traits (Fig. 3 A, B).



Fig.2. The differences between Koshihikari and Koshihikari Eichi 2. A: The plant in rice field. B: The plant type, Koshihikari Eichi 2 (right) and Koshihikari (left). C: The panicles Koshihikari Eichi 2 (lower) and Koshihikari (upper). D: The seeds (left) and brown rice (right) of Koshihikari Eichi 2 (right) and Koshihikari (left).

Results of Changing the Photoperiod Sensitivity of Koshihikari

ILDF4-1 is homozygous HABATAKI type in the *Hd1* locus and homozygous Koshihikari type in others. The F5 seed from the ILDF4-1 was used to test the phenotype in the rice fields. The generation after that from plant ILDF4-1 is called Koshihikari Eichi 3 as the new variety. The heading date of Koshihikari Eichi 3 was July 27, 2006 when grown in Nagoya, Japan, whereas that of Koshihikari was August 7 of that year. The time from sowing to heading of Koshihikari Eichi 3 averaged 77 days whereas for Koshihikari, it was 88 (Fig.4 A, B). There are also different culm lengths for Koshihikari and Koshihikari Eichi 3, because of the different heading dates (Fig. 4 B). However, there are no significant differences between Koshihikari and Koshihikari Eichi 3 excluding the above traits.

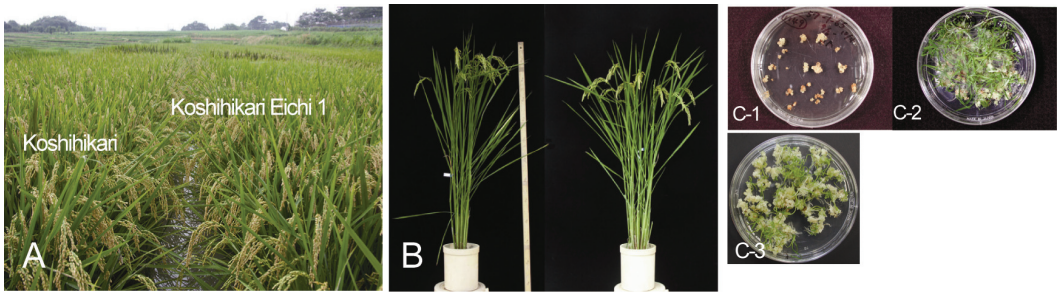


Fig.3. The differences between Koshihikari and Koshihikari Eichi 1. A: The plant in rice field. B: The plant type, Koshihikari Eichi 1 (right) and Koshihikari (left). C: The regeneration ability, Koshihikari (C-1), Kasalath (C-2), Koshihikari Eichi 1 (C-3).

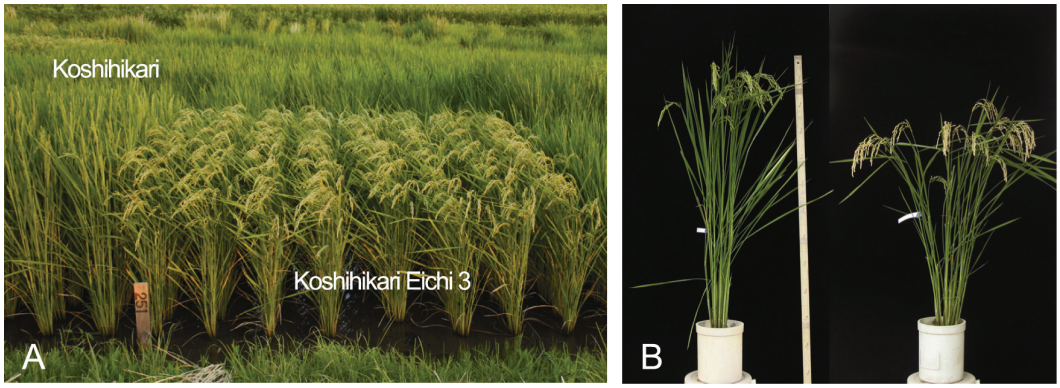


Fig.4. The differences between Koshihikari and Koshihikari Eichi 3. A: The plant in rice field, Koshihikari Eichi 3 headed earlier than Koshihikari. B: The plant type, Koshihikari Eichi 3 (right) and Koshihikari (left).

DISCUSSION

The aim of the work reported herein was to establish a new marker selection method call Variety Update, and to make certain progress in new varieties. The idea is from computer software or digital informatics (e.g. we attempt to update the *japonica* elite variety Koshihikari). Our goal was to improve Koshihikari through replacing one of its small chromosomal fragments with that of a donor's having a better gene (allele). It was most important in this concept to only modify target traits of Koshihikari. That meant we avoided as much as possible, changing other traits. Therefore we tried to keep the chromosomes of Koshihikari in the new-bred variety by replacing a very small chromosomal fragment of Koshihikari.

Deciding Which Trait(s) Should be Updated

The first step for our Variety Update was to find out any "bugs" in the elite variety. In other words, to decide which trait or traits should be modified. And next was to find the donor genome by QTL analysis. It was tempting to consider that many traits of Koshihikari should be modified, because it is a well-known and appreciated variety long used in rice production in Japan. We decided that the target traits for modification are: lodging tolerance, panicle size, callus regeneration ability, and photoperiod sensitivity. The traits of disease resistance and/or insect tolerance were also attractive targets for updating, also to improve the variety. However, it is necessary to discover less desirable traits of elite variety for updating by one's own knowledge and experiences in such methodology.

Selecting a Donor Genome

The important step in our method was to find a donor genome including a gene that has the desired effect of updating the targeted trait. Therefore we carried out the QTL analysis with the segregation population derived from Koshihikari and the donors selected. The results from the QTL analysis will show the effect of the donor genome in each detected QTL.

Therefore, the biggest QTL with the biggest effect should be selected for the update. However, one should retry the QTL analysis if the effect of the donor allele was ineffective.

Using Introgression Lines

Introgression lines were bred by backcross and DNA marker selection methods: cross the Koshihikari and the donor lines to obtain the F1 plant and then backcross with Koshihikari. The introgression line was selected that has only the donor chromosomal fragment in the target region. Other chromosomes and chromosomal fragments except the target region should be homozygous-type of Koshihikari.

We used 140 SNP markers to search all chromosomal regions and showed the introgression lines ILA, ILB, ILC and ILD do not have the donor chromosomal fragment except for the target regions. However, this is not to disavow the possibility that some donor chromosomal fragments exist still between the SNP markers. It is better to remove all such small donor chromosomal fragments in the IL genome by using more DNA markers and selecting markers distant equally.

Obtaining Gene Information of the Detected QTL Locations

To use only a very small chromosomal fragment to update less desirable traits of Koshihikari, one must know the given position of the gene in the chromosome. While the orthodox way is to identify it, information from published research papers will also help one identify gene position. Much information was available on this research; and the major genes *sd1, Gn1a, PSR1, Hd1* for these traits had been isolated (Sasaki et al., 2002, Nishimura et al., 2005, Ashikari et al., 2005, Yano et al., 2000). The locations in each region for these genes were the best references to identify each fragment of donor chromosome. In the final analysis one should identify a given position for each gene, with this methodology. It is also an effective way to discover a new gene, should your own results differ from those of others.

The Donor Chromosomal Fragment Size in the Updated Varieties

Our policy in this Variety Update method was to replace a minute DNA fragment of Koshihikari, to avoid changing other Koshihikari traits. Therefore, one needs genotype a multitude of plants from the segregation population. However, it would be expensive to genotype a large population. In our experiment we genotyped from 5,000 to 20,000 plants for each target trait. One point of genotype will cost USD1 so one target will cost $10,000 to $40,000 for genotyping if one genotypes two points for one plant.

It was not clear how big the donor DNA fragment should be for the replacement. Size in this method would depend on whether there are undesirable genes in the target region. It is no problem should the fragment be bigger if there is no undesirable gene there, and the cost will be less. Therefore, the size also depends on the relation between the donor genome and the elite variety Koshihikari. Even if that was unclear, we were trying to keep the size of the donor DNA fragment to less than 500kb for our Koshihikari Eichi series.

We updated four traits for Koshihikari and bred 4 new varieties called Koshihikari Eichi 1, Koshihikari Eichi 2, Koshihikari Eichi 3 and Koshihikari Eichi 4. These four varieties were applied for and allowed as the new Koshihikari varieties used in food production by the Japanese government. However, as said above, some small DNA fragment of donor may still exist between markers. Thus, these varieties also have to be under observation for a time.

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