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EATING QUALITIES OF HIGH-YIELDING *japonica*-RICE LINES CARRYING *Ur1* GENE

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SUMMARY

An incompletely dominant gene *Ur1* (Undulate rachis-1) on chromosome 6 increases spikelet number per panicle, which enlarges sink size and can increase yield in rice. Murai 79 (denoted by "79") and other four prospective *Ur1*-carrying *japonica* inbred lines possess not only remarkably high yielding-abilities but also various heading times from extremely late 79 to early "J3". Their eating qualities were examined by comparing them with two *japonica* check varieties, viz. a representative variety of southern Japan 'Hinohikari', and early-maturing 'Koshihikari'. According to sensory eating-quality test, 79 was less sticky and higher in hardness than Hinohikari, which may be due to its high amylose content in milled rice. Its overall evaluation and taste were not significantly different from those of Hinohikari. "Mido" was measured by the specific apparatus, is considered as the most reliable indicator for overall evaluation. The 79 had higher values of mido, which may cause its high eating-quality despite its high amylose content. Additionally, 79 has an advantage to lessen the high-temperature damage for brown-rice appearance, because its maturing temperatures were lower than those of middle heading Hinohikari in the experimental years. Late-heading and middle-heading lines, "7E" and "47" were not significantly different from Hinohikari regarding overall evaluation, taste, stickiness, hardness and flavor in 2010. J3 was not significantly different from either Hinohikari or Koshihikari in overall evaluation and taste, and was lower in hardness than Koshihikari. Regarding amylose content, J3, Hinohikari and Koshihikari were similar to one another, while 47 was lower than Hinohikari. On the other hand, "53" was lower than Hinohikari in overall evaluation and flavor. Consequently, 79, 7E, 47 and J3 could be candidates for extremely late-heading, late-heading, middle-heading and early-heading commercial varieties, respectively, and also be mid-mother lines for developing high-yielding varieties with high eating quality.

Keywords: Eating quality, amylose content, *Ur1* gene, commercial variety, rice breeding, high yield, heading time, *Oryza sativa*.

Key findings: The eating qualities of the five high-yielding *Ur1*-carrying lines with various heading times were compared with those of 'Hinohikari' and/or 'Koshihikari' by sensory eating-quality test. Extremely late-heading 79 was higher in hardness and less sticky than Hinohikari, due to its high amylose content in milled rice, but not significantly different from Hinohikari in overall evaluation, taste and flavor. Middle-heading 47, late-heading 7E, and early-heading J3 were not significantly different from Hinohikari in overall evaluation and the other five test items; regarding amylose content, J3 and 47 were similar to or lower than Hinohikari.

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INTRODUCTION

An incompletely dominant gene *Ur1* (Undulate rachis-1) on chromosome 6 of rice increases spikelet number per panicle, due to the increases of number of secondary branches per primary branch, number of spikelets per single secondary branch, and number of primary branches per panicle (Nagao and Takahashi, 1963; Sato and Shinjyo, 1991; Murai and Iizawa, 1994; Murai, 1999; Murai *et al*., 2014). *Ur1* enlarges sink size and is able to increase yield in both the *Ur1*/*Ur1* and *Ur1*/+ genotypes (Murai *et al*., 1997, 2002, 2003, 2004 and 2005 b; Murai 1999).

The high-yielding *Ur1*-carrying line Murai 79 (Murai *et al*., 2005 a), denoted by "79", and three other *Ur1*-carrying *japonica* inbred lines were developed from the same F_1 of 'Nisihikari' × the isogenic line of Taichung 65 carrying both *Ur1* and *sd1-d* (dee-geo-woo-gen dwarf gene), which possess various heading times from extremely late to middle in southern Japan (Kamimukai *et al*., 2020). In addition, an early-heading *Ur1*-carrying line "J3" was developed from the F_1 hybrid of 'Koshihikari' × 79 in pedigree method. According to yield tests for the five *Ur1*-carrying lines (Kamimukai *et al*., 2020; Table 14), they were higher-yielding by 23 to 70% than 'Hinohikari', a representative variety for the normal season cropping in southern Japan.

Koshihikari occupied the highest percentage (33.9%) of the total rice-cultivated area of Japan in 2019, followed by 'Hitomebore', Hinohikari, 'Akitakomachi', and 'Nanatsuboshi' (Rice Stable Supply Support Organization, 2020). All of the latter four varieties involve Koshihikari in their ancestry, and inherit low amylose content and stickiness from Koshihikari (Saito *et al.***,** 1989; Yamamoto and Ogawa, 1992; Sasaki *et al.*, 1993; Yoshimura *et al.*, 2002; The Miyazaki breeding group of rice, 2005), reflecting palatability for rice in recent Japanese people.

Sensory eating-quality test for cooked rice was performed for the five *Ur1*-carrying lines and two check varieties Hinohikari and Koshihikari, which involved the six ordinary test items, viz. taste, stickiness, hardness, flavor, external appearance and overall evaluation (for example, see Table 2).

According to Kurasawa *et al.* (1972), Inatsu (1988), Azuma *et al.* (1994) Okadome *et al.* (1999), and Cui *et al.* (2000), amylose content was positively correlated with hardness, but was negatively correlated with stickiness and overall evaluation in sensory eating-quality tests, in which various Japanese varieties were used; protein content was positively correlated with hardness, but was negatively correlated with stickiness and overall evaluation. However, the correlations, mentioned above, seem to have not been high. Azuma *et al.* (1994) reported low and non-significant correlations between amylose content and overall evaluation in the varieties possessing various eating qualities. Hence, factors other than amylose content and protein content may affect eating quality. We measured the contents of these substances in milled rice of the five *Ur1*-carrying lines, and Hinohikari and three other varieties.

TOYO RICE CORPORATION devised and manufactures an apparatus (containing some versions) which indicates a degree of rice taste named "Mido" by measuring the thickness of the water retention membrane on the surface of partially cooked rice (personal communication from the above corporation). Kawamura *et al*. (1996) suggested that this apparatus was more reliable to estimate eating qualities of rice samples, compared with the three other types of apparatus examined. According to Azuma *et al.* (1994) and Sato *et al*. (2003), mido values were positively correlated with overall evaluation of sensory eating-quality test among Japanese varieties. The apparatus was employed to measure mido values of some of the *Ur1*-carrying lines and varieties.

From the results obtained from sensory eating-quality test, analyses of amylose and protein contents, and measurement of mido, the eating qualities of the five *Ur1*-carrying lines are examined, and possibilities of high-yielding and high eating-quality varieties with various heading times are discussed.

MATERIALS AND METHODS

High yielding prospective lines carrying *Ur1* gene

The developing processes and maturities of the five *Ur1*-carrying lines (Kamimukai *et al*, 2020) are summarized as follows.

"79" (Murai 79, Murai *et. al.* 2005 a) is one of the recombinant inbred lines (RILs) developed from the F_1 between 'Nishihikari' and an isogenic line of Taichung 65 carrying both *Ur1* and *sd1-d* (dee-geo-woo-gen dwarf). 79 can be regarded as extremely late in heading time, which was later in 80%-heading by 16.5 days in average than a check variety Hinohikari in the six experimental years (Table 12, and data in 2004, 2005 and 2011). In this study, the generations of 79 were F_{14} and later.

"53" (Malangen *et al*., 2013) and "47" are two other well-ripened RILs carrying *Ur1*, which were intermediate in 80%-heading between Koshihikari and Hinohikari (Tables 12 and 14), and can be regarded as the middle and rather early, and the just middle, respectively, in heading in Kochi Prefecture. The generation of 47 was F_{13} in 2010 and 2011. Generations of 53 in the three experimental years were F_{13} or later.

"7E" is a sister line of 79 originating from another F_3 plant from the same F_2 plant as that of 79. From the F_3 generation, selections for earliness, low amylose content, higher panicle weight per plant (yield) and better appearance of brown rice were performed on the basis of plant and/or progeny line. The 7E was nine days later in 80% heading than Hinohikari, and 5 days earlier than 79 in 2010 (Tables 12 and 14). Accordingly, it can be regarded as the late heading in Kochi Prefecture as well as southern Japan. The generation of 7E was F_{11} in 2010.

The "J3" is an early-heading *Ur1*-carrying line developed from the F_1 hybrid of Koshihikari × 79 by pedigree method. J3 was almost the same as Koshihikari in 80%-heading date (Tables 12 and 14). Its generations used were F_9 and F_{10}.

Japonica varieties used in the experiments

'Hinohikari' (abbreviated as "Hi") is a representative variety for normal season cropping in southern Japan, possessing high eating-quality, low amylose content, and a rather long culm (Yagi *et al*., 1990; The Miyazaki Breeding Group, 2005). Its maturity is classified as the middle in Kyushu District, and as the middle and rather late in Kochi Prefecture (Agricultural Production Bureau, Ministry of Agriculture, Forestry and Fisheries, Japan, 1997; Table 12).

'Koshihikari' ("Kos") is a representative high eating-quality variety in Japan, but is long-culmed and readily lodged (Agricultural Production Bureau, Ministry of Agriculture, Forestry and Fisheries, Japan, 1997). It is cultivated almost over Japan except the northern part of Northeast Japan, Hokkaido Prefecture and Okinawa Prefecture, and occupied 33.9% of the total rice area of Japan in 2019 (Rice Stable Supply Support Organization, 2020). Its maturity is classified as the early and rather late in Kochi Prefecture (Agricultural Production Bureau, Ministry of Agriculture, Forestry and Fisheries, Japan, 1997; Table 12)

'Nishihikari' ("Ni") is a short-culmed variety possessing the highest lodging resistance in Kyushu District, (Nishiyama, 1982; Agricultural Production Bureau, Ministry of Agriculture, Forestry and Fisheries, Japan; 1997). Its maturity is classified as the middle and rather late in Kyushu District, and as late in Kochi Prefecture (ditto; Table 12). Its eating quality is the middle and rather high (Nishiyama, 1982).

'Nipponbare' ("Nip") had been the first leading variety from 1970 to 1978 in Japan (The Committee for Researching Rice Distribution, 1991). At present, its cultivated area is restrictive, due to its eating quality lower than that of Kos (Yamamoto and Ogawa, 1992). Its maturity is classified as the middle and rather early in Tokushima Prefecture, a neighboring prefecture of Kochi Prefecture (Agricultural Production

Bureau, Ministry of Agriculture, Forestry and Fisheries, Japan; 1997; Table 12).

Cultivations in the experimental field

The experiments were conducted in an experimental paddy field of the Faculty of Agriculture (present name: the Faculty of Agriculture and Marine Science), Kochi University (Nankoku 33°35′ N, 7 m above sea level) in 2004, 2005, 2009, 2010, 2011 and 2018. In each experimental year, seedlings were transplanted at a spacing of 30 cm × 15 cm (22.2 hills/m^2) with two seedlings per hill, from 3rd to 12th of May. Total amounts of N, applied together with K_2O and P_2O_5 elements, varied from 8.00 g/m^2 in 2018 to 18.00 g/m^2 in both 2009 and 2010 (Table 1). The ways of cultivation in 2005 and 2010 are described in detail in Kamimukai *et al.* (2020).

Sensory eating-quality test

For the rice samples in 2004 and 2005, cooking was conducted by the process as follows. Milled rice of 450 g was taken into well water in a bowl and washed with flowing water for about 5 minutes and then was settled for about 35 minutes. The water was added until the total weight of the milled rice (450 g) + water reached 1050 g. Electric rice cookers (RC-109VSS-1.0L, TOSHIBA CORPOLATION, Tokyo) were used to cook the milled rice + water. The eating quality tests described below were conducted at the Kochi Agricultural Research Station.

For the rice samples grown in 2009, 2010, and 2011, milled rice of 540 ml was taken into well water in a bowl for rice-cleaning and was stirred with a rice ladle for about 10 minutes under flowing water; and the soaked rice in the bowl was settled for about 30 minutes. The soaked rice was taken into a kitchen cup with scale, and the water was added until the volume of the milled rice + water reached 965 ml. Electric rice cookers (NCJ-10UF-1.01L, Sanyo Electric Co., Ltd., Osaka) were used to cook the milled rice + water. The eating quality tests described below were conducted at the Faculty of Agriculture, Kochi University.

In a sensory eating-quality test, each panelist evaluated the cooked rice according to six items, viz. taste, stickiness, hardness, flavor, external appearance and overall evaluation. Grading (−3 to +3) in each test item was performed, regarding the value of the standard variety Hi (or Kos only in 2005) as 0. In terms of overall evaluation, taste, flavor and external appearance, the way of scoring was as follows: +3 much better, +2 better, +1 slightly better, 0 same as standard, −1 slightly worse, −2 worse, −3 much worse. For stickiness, +3 much more sticky, +2 more sticky, +1 slightly more sticky, 0 same as standard, −1 slightly less sticky, −2 less sticky, and −3 much less sticky. For hardness, +3 much higher, +2 higher, +1 slightly higher, 0 same as standard, −1 slightly lower, −2 lower, −3 much lower. For the rice samples grown in 2004 and 2005, numbers of panelists were 16 and 13, respectively, ranging in age from twenties to fifties, and men were more than women. For the rice samples grown in 2009, 2010 and 2011, numbers of panelists were 11 or 13, 11 and 9, respectively, and most of men and women were twenties in age.

Statistical analyses applied to the results of the sensory eating-quality tests are explained using Table 2 as an example. For each test item such as overall evaluation in each line such as 79, its difference from Hi (=0) was tested: 1) the 95% confidence interval for the mean value of 79 in overall evaluation was calculated; 2) when the confidence interval included 0, the difference between 79 and Hi in overall evaluation was not statistically significant, and *vice versa*; 3) in fact, the difference of −0.4 was not statistically significant at the 5% level of probability. Moreover, to compare 79 and 53 in each test item, analysis of variance was performed, using the interaction between the effect of the panelists (n = 16, df = 15) and the effect of the lines (n = 2, df = 1) as error (df = 15), and the $LSD_{(5\%)}$ was calculated from the error variance and the t-value of df 15 at the probability of 5%.

Measurements of amylose and protein contents

The rice samples grown in 2009, 2010 and 2018 were used for measuring amylose and protein contents (%) to the total dry weight of milled rice. Amylose contents were measured by Auto Analyzer II (in 2009 and 2010) and Auto Analyzer Synca (in 2018), both manufactured by BLTEC Co., Ltd., Osaka. Protein contents were measured by the near infrared spectrometers, NIR 6500 (in 2009and 2010) and Infratec NOVA (in 2018), both manufactured by Foss Japan Co., Ltd., Tokyo. For each of amylose and protein contents, the measurement for each rice sample of line/variety was repeated two times and three times, respectively, in 2018, and both 2009 and 2010.

Measurement of Mido

Mido was measured by TOYO RICE TASTE DEGREE METER MODEL MA-90B for the rice samples grown in 2004 and 2005, and by RICE TASTE DEGREE METER MODEL MA-90R2 for the rice samples grown in 2018, which have been manufactured by TOYO RICE CORPORATION (previous name: Toyo Rice Cleaning Machine Co., Ltd) (Table 10). The process of measuring mido by the former meter (personal communication from the above corporation) is summarized as follows. The 33 g of milled rice (90% milling) is placed in boiled water for 10 minutes; after allowing it to cool for 3 minutes, the thickness of water retention membrane on the surface of the partially cooked rice is measured electromagnetically; and it is expressed as a percentage by regarding the thickness of 'Nipponbare' grown in Shiga Prefecture from 1986 to 1988 as 70. A modified process of measuring mido from that in the former meter is applied to the latter meter. Measurement of mido was repeated three times for each rice sample of each line/variety in the three experimental years.

Grading of brown rice

Appearances of brown-rice samples of 79, J3, Kos, Nip, Ni, and Hi grown in 2009, those of 79, 7E, 47, 53, Kos, Ni and Hi grown in 2010, and those of 79 and Hi grown in 2018 were graded by two or three agriculture-products inspectors possessing the national certification (Table 11). The appearances were classified into nine grades: the first to fourth grades, the fifth and sixth ones, the seventh and eighth ones, and the ninth one, which corresponded to the first-class rice, the second-class rice and the third-class rice and the rice below the preceding three classes, respectively. The latter way of classification is usually applied in the trade of rice in Japan.

RESULTS

Sensory eating-quality test

Results of sensory eating-quality test for 79, and it and other *Ur1*-carrying lines were shown in Tables 2, 3, 4, and 7. Regarding overall evaluation and taste, 79 was not significantly different from Hi, in all of the eating-quality tests in 2004, 2005, 2009 and 2010. In terms of stickiness, 79 was less than Hi in all of the four experimental years, being statistically significant in the three experimental years except 2004. On the other hand, 79 was higher in hardness than Hi in the four experimental years, being statistically significant in 2009 and 2010. Similarly, 79 was significantly higher in external appearance than Hi in 2009 and 2010. Significant differences in flavor were not noticed between 79 and Hi in the four experimental years.

As shown in Tables 2 and 3, 53 was lower than Hi regarding both overall evaluation and taste in the two experimental years, although the difference in taste was not statistically significant in 2004. As for stickiness, 53 was significantly lower than Hi in 2005. In hardness, 53 was not significantly different from Hi in the two experimental years. In external appearance, 53 was significantly lower than Hi in 2005. It is noteworthy that 53 was significantly lower in flavor than both Hi and 79 in both years.

As shown in Tables 5 and 6, J3 was not significantly different from either Hi or Kos in each of overall evaluation and taste in the two experimental years. In terms of stickiness, J3 was not significantly different from Kos in the two experimental years, and was significantly lower than Hi in 2009. As for hardness, J3 and Hi were significantly lower than Kos in both years. J3 and Kos were higher in external appearance than Hi in both years. Significant differences in flavor were not noticed among the three line-varieties in both years.

As shown in Table 6, 47 was not significantly different from either Hi or Kos in overall evaluation and all of other five test items in 2010. Furthermore, 47 was not significantly different from Hi in the five test items except for its lower hardness than that of Hi in 2011 (Table 8). 7E was not significantly different from Hi in all of the five test items except for its significantly higher external appearance than Hi in 2010 (Table 7).

Amylose and protein contents

Table 9 shows amylose and protein contents in milled rice of the five *Ur1*-carrying lines, Hi and the three other ordinary varieties grown in 2009, 2010 and 2018. 79 was higher in amylose content by 2.3% to 6.2% than Hi in the three experimental years. In each of 79 and Hi, the amylose content in 2010 was higher than those in 2009 and 2018, although the order between 79 and Hi was constant in the three experimental years. Six and eight of all of the five *Ur1*-carrying lines and four ordinary varieties were in the order 79 ≥ Ni ≥ Nip > Hi ≥ Kos > J3 (79 > Nip) in 2009, and were in the order 79 > 7E ≥ 53 ≥ Ni > J3 > Kos ≥ Hi > 47 (7E > Ni) in 2010, respectively, where "≥" indicates that the former is higher than the latter although being not significant statistically. Ni was higher than the three other ordinary varieties in 2009 and the two others in 2010, respectively. Kos and Hi were the lowest among the ordinary varieties examined in each of the two years, but were not significantly different from each other. 79 was higher than not only Ni but also the other four *Ur1*-carrying lines in 2010. 7E and 53 were intermediate between 79 and Hi in 2010. J3 was significantly lower than Kos in 2009, but was significantly higher than Kos in 2010. 47 was 1.1% lower than Hi, and the lowest in 2010.

Regarding protein content (Table 9), six and eight of all of the nine lines-varieties were in the order J3 > Kos ≥ Nip = Ni ≥ 79 = Hi in 2009 and Ni > J3 ≥ 79 = 47 = Kos > 7E ≥ 53 ≥ Hi (7E > Hi) in 2010, respectively. Correlation coefficient between values in 2009 and those in 2010 among 79, J3, Kos, Nip, Ni, and Hi, which were commonly grown in the two years, was not significant statistically (r = 0.157), suggesting that order among the lines-varieties was not consistent between the 2 years. Regarding coefficient of variation in 2010, value in this trait (6%) was lower that in amylose content (13%). Therefore, varietal variation was lower in protein content than in amylose content.

Mido values

Table 10 shows mido values of all or some of 53, 79, Kos, Ni and Hi in 2004, 2005 and 2018. Of the five lines-varieties, and five were in the order Kos > 79 ≥ Hi > 53 > Ni in 2004; and four were in the order Hi > Ni > 79 > 53 in 2005; and Hi ≥ 79 in 2018. 79 was not significantly different from Hi in 2004 and 2018, but was significantly lower than Hi in 2005. 53 was significantly lower than Hi in both 2004 and 2005. Difference between Kos and Hi (2.0) was little but significant. Ni was significantly lower than Hi in 2004 and 2005.

Grading of brown-rice appearance

Table 11 shows grades of brown-rice appearance in almost all or some of 79, 7E, 47, 53, J3, Kos, Nip, Ni and Hi in 2009, 2010 and 2018. 79 was significantly higher-graded in this trait than Hi and J3, but it was not significantly different from Ni, Nip and Kos in 2009. 79 was higher-graded than Hi in 2018. In 2010, however, 79, 7E, 47, Ni and Hi were classified at the lowest grade, and 53 was at the second lowest grade. Its cause was the increased frequencies of milky-white, white-core and white-belly grains in 7E, 53, 47 and Hi, and the increase of green-immature grains additionally in Hi, while the increased frequency of white-core grains and that of white-base grains were the major causes in 79 and Ni, respectively, on the basis of the visual observations by the agriculture-products inspectors (data in detail is not shown). Accordingly, various kinds of damaged grains caused the serious deteriorations of grade in the six lines-varieties. Table 12 shows daily maximum and daily minimum temperatures in the second 10-days and the third 10-days of July, the first 10-days to the third 10-days of both August and September in 2009, 2010 and 2018, and the averages of those for the 17 years from 2003 to 2019; and 80%-heading dates of the five

Ur1-carring lines and the four ordinary varieties. Both the daily maximum and minimum temperatures from the second 10-days of August to the second 10-days of September in 2010 were higher by 0.4 °C to 2.4 °C than the respective averages of the 17 years, with the exception of the daily minimum temperature at the second 10-days of September. Additionally, the daily maximum and minimum temperatures were high at the first 10-days of August in 2010, because early or middle August is the highest in temperature ordinarily in each year in Japan. These 1 + 1/3 months almost included the maturing periods of 79, 7E, 47, Ni and Hi. Hence, this high-temperature period may have caused the deteriorations of grade in these five lines-varieties, by affecting grain filling. J3 was significantly lower-graded than Kos in each of the two years. In 2009, J3 was classified at the lowest grade, due to the increased frequencies of milky-white, white-core and white-base grains. However, both the daily maximum and daily minimum temperatures from the second 10-days of July to the second 10-days of August, which almost overlapped its maturing period, were lower than or similar to the respective averages of the 17 years, with the exception of the daily minimum temperature at the second 10-days of July. Hence, this deterioration in J3 could not be regarded as high-temperature damage during grain filling, and its cause is not able to be specified at present.

DISCUSSION

Kurasawa *et al.*, (1972) measured amylose content (%) against the total starch in various rice varieties of Japan, in which the highest variety 'San-in 52' (22.1%) differed from the second lowest one (Kos) by 3.8%. The amylose content of Hi was similar to that of Kos (Table 9). Differences in amylose content between 79 and Hi were 2.3 to 6.2% (3.9% in average). Therefore, it is inferred that 79 possesses the highest level of amylose content among Japanese *japonica* varieties. This is consistent with the results that 79 was lower in stickiness but higher in hardness than Hi (Tables 2, 3, 4, and 7). Regarding overall evaluation as well as taste, 79 was not significantly different from Hi in each experimental year. In mido, 79 was not significantly different from Hi in two of the three experimental years (Table 10), suggesting that the high values of mido contributed to the high eating quality in 79. In 53, on the other hand, the lower values of mido may be related with the lower values of both overall evaluation and taste. Hence, the thickness of the water retention membrane may be related with eating quality, independently from amylose content.

In southern Japan, grains of brown rice damaged by high temperature such as white-back, white-base and milky-white grains appeared when maturing temperature was too high in summer (Iwashita *et al*., 1973). According to Tashiro and Wardlaw (1991), milky-white, white-back and opaque grains were induced by high temperatures, and the sensitive duration for inducing the damaged grains was from heading to 24 days after heading. In the case of Hi, the ratio of first-class rice to the total amount of prefectural rice production in Kochi Prefecture as well as Fukuoka Prefecture decreased to about 10% in 2010, in which the summer was unusually warm

(Miyazaki, 2014; Sakata, 2014). In fact, the daily maximum temperature and daily minimum temperature were sufficiently high to inflict damage to brown-rice appearance at each of the first 10-days of August to the first 10-days of September in 2010 at the experimental paddy field (Table 12). This period almost overlapped the maturing durations of Hi in Kochi Prefecture. Table 13 shows averages of daily maximum or daily minimum temperatures for 30 days after 80%-heading in 79 and Hi in 2009, 2010, and 2018. The average daily maximum and minimum temperatures after 80%-heading in the three years were 0.1 °C to 1.8 °C and 1.2 °C to 1.7 °C lower, respectively, in 79 than in Hi. Consequently, 79 has higher possibility of avoiding high temperature damage to brown-rice appearance than the middle-heading variety, from the meteorological point of view. In 2010, the maturing temperatures for both 7E and 47 were too high to evaluate their brown-rice appearances without high-temperature damage. Hence, grading of brown-rice appearance for them should be performed in years when their maturing temperatures are not high or rather low.

53 was lower in both overall evaluation and taste than Hi, which is consistent with its lower values of mido (Table 11), and it had a disagreeable smell like that from scorched rice. Hence, 53 could not be a commercial variety.

J3 was lower in hardness than Kos (Tables 5 and 6), although they were almost identical in amylose content (Table 9). Nevertheless, they were not significantly different from each other in the other five test items. From the view point of consumers, for example, aged people have difficulty in chewing and swallowing more or less in general. J3’s high eating quality with lower hardness might be favorable for such people. Kos has the primary disadvantage of long culm, e.g., 88.0 cm in the experimental field in 2010 (Kamimukai *et al*. 2020; Table 14), being readily lodged: difficulty of harvest, viviparity and/or grade-down of brown-rice appearance are frequently accompanied by lodging. J3 was 17.5 cm shorter in culm length than Kos, suggesting its higher lodging resistance. However, J3 was lower-graded than Kos in brown-rice appearance. In Kochi Prefecture, seedlings of Kos are transplanted to paddy fields by farmers in mid-March at the earliest, and reaches heading in late June. The averages of daily maximum and minimum temperatures from the third 10-days of June to the second 10-days of July for the 17 years from 2003 to 2019, and those from the second 10-days of July to the second 10-days of August (Table 12) are calculated. The average values of maximum and minimum temperatures for the 17 years are lower in the former duration (max. = 27.9 °C, min. = 22.5 °C) than in the latter duration (max. = 30.5 °C, min. = 24.1 °C). Accordingly, J3 should be tried to grow in this earliest cropping season, and its brown-rice appearance should be graded in addition to a yield test for it.

47 was not significantly different from either Hi or Kos regarding all of the six test items in 2010 (Table 6). 7E was not significantly different from Hi in every test item except its higher external appearance (Table 7). Hence, these two lines seem to possess high eating qualities, even though further eating-quality tests are necessary to ascertain the above results, not only in the experimental field but also in

other warm regions of Japan. Grading for brown-rice samples from other cultivation tests should be performed, because normal evaluations for the lines were not able to be conducted due to the unusually higher temperature during their grain filling.

According to Takeuchi *et al*. (2008), QTL(s) for all/some of overall evaluation, taste, stickiness, hardness, and glossiness was detected at the distal end of the short arm of chromosome 3, which seems to be identical with the QTL for stickiness reported by Kobayashi and Tomita (2008). Other QTLs for stickiness and/or amylose content were detected at the sites on the long arm of chromosome 3 and chromosome 6 (Takeuchi *et al*., 2008) and at the sites on chromosome 1 and chromosome 2 (Kobayashi and Tomita, 2008). At all of the above QTLs, except that on the long arm of chromosome 3, alleles originating from Kos were positive for better eating-quality. 47 had high eating-quality and the amylose content lower than Kos (Tables 6, 8 and 9). The amylose contents of the parents of 47 were 20.3% in Ni and 13.9% in the isogenic line of Taichung 65 carrying both *Ur1* and *sd1-d,* in which the data were taken from rice samples harvested in the same experimental paddy field in 2000 (Murai unpublished). Hence, the low amylose content of 47 seems to be inherited mainly from Taichung 65. Both Taichung 65 (‘Kameji’ × ‘Shinriki’) and Nishihikari (‘Asominori’ × Saikai 128) are genealogically distant from Kos (Norin 22 × Norin 1) (Nishiyama, 1982; Yamamoto and Ogawa, 1992; Agricultural Production Bureau, Ministry of Agriculture, Forestry and Fisheries, Japan, 1997). Why was high-eating-quality 47 with the low amylose content obtained, without involving Kos and its relatives in the parentage? To answer this query, genetic analyses by applying the QTLs mentioned above may be effective.

Culm length was in the order Kos > Hi > 79 ≥ 7E ≥ J3 > 53 > 47 > Ni (Kamimukai *et al*. 2020; Table 14). 79 (72.3 cm) was shorter by 8.7 cm than Hi. Regarding lodging resistance, however, the former was not significantly different from the latter, because the effect of the lower height on enhancing the resistance was cancelled by heavier panicles in 79 (Murai unpublished). In Hi, its evaluation in lodging resistance varies from the highly resistant to the middle and rather weak among several prefectures in southern Japan, such as the highly resistant in Kochi Prefecture (Agricultural Production Bureau, Ministry of Agriculture, Forestry and Fisheries, Japan, 1997). In this study, serious lodging preventing process of harvest and inducing viviparity has not been observed not only in 79 but also in 7E and J3, implying that they possess actually sufficient lodging resistances. 7E and J3 were not significantly different from 79 in culm length, suggesting that they are not so different from 79 in lodging resistance. Moreover, 47 (63.5 cm) was similar in culm length to Ni which possesses the highest-ranked lodging-resistance (Nishiyama, 1982). No lodging has been observed in 47, like in Ni. Thus, 47 may possess high lodging-resistance.

Tests for field resistance to leaf blast have been conducted for the four *Ur1*-carying lines except 53 (Murai unpublished). 7E and J3 were classified as susceptible, but 47 as resistant. 79 was judged as intermediate between susceptible and middle tolerant. Nevertheless, 79 was classified as intermediate between susceptible and resistant in panicle-blast resistance, although tests in this trait have not been conducted for the other lines.

Consequently, 7E, 47, and J3 could be candidates for late-heading, middle-heading and early-heading commercial varieties, respectively, and also be mid-mother lines for developing high-yielding varieties with high eating quality. In particular, 47 possesses high resistances to both lodging and field resistance to leaf blast.

For 79, sensory eating-quality test was repeated several times (Tables 2, 3, 4 and 7; Kamimukai *et al*. 2017), confirming its high eating quality. Its high yielding ability was ascertained by growing it under five cultivated conditions involving various fertilizer levels (Kamimukai *et al*. 2020). Furthermore, 79 was used in trial cultivations by farmers. Such cultivation tests under various environmental conditions and eating-quality tests should also be performed for 7E, 47 and J3, in order to make sure of their practical utilities.

Characteristics of the five *Ur1*-carrying lines regarding yield, eating quality and other agronomic traits are summarized in Table 14, according to the results of the present study and Kamimukai *et al.* (2020). In addition, the field resistances to leaf blast in all the seven lines-varieties, and the field resistances to brown spot in the six lines-varieties except 53 (Murai unpublished) are shown in the table.

In Japan, people aged 70 and above accounted for 21.5% of the total population in 2019, which is the highest percentage in the world (Statistics Bureau of Japanese Government, 2019). In general, the people in this age bracket has difficulty in chewing and swallowing more or less. According to the levels of this difficulty, gruel, "nanhan" (semisoft rice) and ordinary cooked rice are served for aged people in Japan. Nanhan is the name of a cooked rice used in Japan, having softness intermediate between gruel and ordinary cooked rice, which is boiled with an intermediate amount of water between those of the two kinds of cooked rice. The nanhan made from 79 was better for serving such people than that made from Hi, due to its higher hardness and lower stickiness (Kamimukai *et al.* 2017). Results about the *nanhan* made from 79 will be reported in detail in the next paper.

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Table 1. Total amounts of N, P_2O_5 and K_2O (basal + top-dressing) applied by chemica fertilizers in the six experimental years.

Year	N (g/m^2)	P_2O_5 (g/m^2)	K_2O (g/m^2)
2004	16.00	16.00	16.00
2005	12.00	12.00	12.00
2009	18.00	18.00	18.00
2010	18.00	18.00	18.00
2011	17.00	17.00	17.00
2018	8.00	5.13	6.14

Table 2. Sensory eating-quality test for 53 and 79 in 2004, in which evaluation (+ or −) in each test item was performed, regarding the value of the standard variety Hi as 0.

Test item		79	53	LSD (5%)
Overall evaluation	Average	−0.4 a	−0.5 a	0.6
	Significance of difference from Hi	ns	*	
Taste	Average	−0.4 a	−0.4 a	0.5
	Significance of difference from Hi	ns	ns	
Stickiness	Average	−0.4 a	−0.4 a	0.6
	Significance of difference from Hi	ns	ns	
Hardness	Average	0.3 a	0.4 a	0.6
	Significance of difference from Hi	ns	ns	
Flavor	Average	0.1 a	−0.4 b	0.5
	Significance of difference from Hi	ns	*	
External appearance	Average	0.1 a	−0.2 a	0.5
	Significance of difference from Hi	ns	ns	

Values followed by the same letter within test item trait are not significantly different at the 5% level, determined by the LSDs in the table.
*, ** Significantly different from Hi at the 5 and 1% levels, respectively.
ns: Not significantly different from Hi.

Table 3. Sensory eating−quality test for 53, 79 and Hi in 2005, in which evaluation (+ or −) in each test item was performed, regarding the value of the standard variety Kos as 0.

Test item		79	53	Hi	LSD (5%)
Overall evaluation	Average	0.2 a	−0.8 b	−0.1 a	0.7
	Significance of difference from Kos	ns	*	ns	
Taste	Average	0.3 a	−0.9 b	0.1 a	0.7
	Significance of difference from Kos	ns	**	ns	
Stickiness	Average	−0.1 b	−0.3 b	0.5 a	0.6
	Significance of difference from Kos	ns	Ns	*	
Hardness	Average	−0.1 a	−0.5 a	−0.4 a	0.6
	Significance of difference from Kos	ns	**	**	
Flavor	Average	−0.1 a	−0.7 b	−0.1 a	0.5
	Significance of difference from Kos	ns	**	ns	
External appearance	Average	0.1 a	−1.4 b	−0.1 a	0.6
	Significance of difference from Kos	ns	**	ns	

Note: Rice sample of Kos grown by early-April transplanting in Kochi Agricultural Research Station, in which chemical fertilizer was applied at the late of 5 g/m^2 for each of N, P_2O_5, and K_2O in total, was used.
*, ** Significantly different from Kos at the 0.05 and 0.01 levels, respectively.
ns: Not significantly different from Kos.
Values followed by the same letter within each test item are not significantly different at the 5% level, determined by the LSDs in the table.

Table 4. Sensory eating−quality test for 79 in 2009, in which evaluation (+ or −) in each test item was performed, regarding the value of the standard variety Hi as 0.

Test item	79	
Overall evaluation	0.6	ns
Taste	0.4	ns
Stickiness	−1.3	*
Hardness	1.5	**
Flavor	0.6	ns
External appearance	1.4	*

*, ** Significantly different from Hi at the 5 and 1% levels, respectively.
ns: Not significantly different from Hi.

Table 5. Sensory eating−quality test for J3 and Kos in 2009, in which evaluation (+ or −) in each test item was performed, regarding the value of the standard variety Hi as 0.

Test item		J3	Kos	LSD (5%)
Overall evaluation	Average	0.0 a	0.7 a	0.8
	Significance of difference from Hi	ns	ns	
Taste	Average	0.0 a	0.4 a	0.8
	Significance of difference from Hi	ns	ns	
Stickiness	Average	−0.8 a	−0.4 a	0.6
	Significance of difference from Hi	*	ns	
Hardness	Average	0.1 b	1.6 a	0.9
	Significance of difference from Hi	ns	**	
Flavor	Average	0.4 a	0.2 a	0.6
	Significance of difference from Hi	ns	ns	
External appearance	Average	0.6 a	1.2 a	0.8
	Significance of difference from Hi	*	**	

*, ** Significantly different from Hi at the 5 and 1% levels, respectively.
ns: Not significantly different from Hi.
Values followed by the same letter within each test item trait are not significantly different at the 5% level, determined by the LSDs in the table.

Table 6. Sensory eating-quality test for 47, J3 and Kos in 2010, in which evaluation (+ or −) in each test item was performed, regarding the value of the standard variety Hi as 0.

Test item		47	J3	Kos	LSD (5%)
Overall evaluation	Average	−0.1 a	0.0 a	0.5 a	0.7
	Significance of difference from Hi	ns	ns	*	
Taste	Average	−0.4 a	−0.2 a	0.2 a	0.9
	Significance of difference from Hi	ns	ns	ns	
Stickiness	Average	−0.3 a	−0.7 a	−0.3 a	0.8
	Significance of difference from Hi	ns	ns	ns	
Hardness	Average	0.3 ab	−0.2 b	1.0 a	0.9
	Significance of difference from Hi	ns	ns	**	
Flavor	Average	−0.3 a	0.4 a	0.3 a	0.9
	Significance of difference from Hi	ns	ns	ns	
External appearance	Average	0.6 a	1.0 a	1.3 a	0.7
	Significance of difference from Hi	ns	**	**	

*, ** Significantly different from Hi at the 5, and 1% levels, respectively.
ns: Not significantly different from Hi.
Values followed by the same letter within each test item are not significantly different at the 5% level, determined by the LSDs in the table.

Table 7. Sensory eating−quality test for 79, and 7E in 2010, in which evaluation (+ or −) in each test item was performed, regarding the value of the standard variety Hi as 0.

Test item		79	7E	LSD (5%)
Overall evaluation	Average	0.0 a	0.5 a	0.8
	Significance of difference from Hi	ns	ns	
Taste	Average	0.1 a	0.2 a	0.8
	Significance of difference from Hi	ns	ns	
Stickiness	Average	−0.7 a	0.0 a	0.8
	Significance of difference from Hi	*	ns	
Hardness	Average	1.5 a	0.2 b	0.5
	Significance of difference from Hi	**	ns	
Flavor	Average	0.0 a	0.3 a	1.0
	Significance of difference from Hi	ns	ns	
External appearance	Average	1.6 a	0.8 b	0.8
	Significance of difference from Hi	**	*	

*, ** Significantly different from Hi at the 5 and 1% levels, respectively.
ns: Not significantly different from Hi.
Values followed by the same letter within each test item are not significantly different at the 5% level, determined by the LSDs in the table.

Table 8. Sensory eating-quality test for 47 in 2011, in which evaluation (+ or -) in each test item was performed, regarding the value of the standard variety Hi as 0.

Test item	47	
Overall evaluation	-0.1	ns
Taste	0.2	ns
Stickiness	0.1	ns
Hardness	-1.2	**
Flavor	-0.3	ns
External appearance	0.0	ns

*, ** Significantly different from Hi at the 5 and 1% levels, respectively.
ns: Not significantly different from Hi.

Table 9. Amylose, and protein contents in milled rice of 79, 7E, 47, 53, J3, and Hi and three other ordinary varieties in 2009, 2010 and 2018.

Traits	Years	79	7E	47	53	J3	Kos	Nip	Ni	Hi	LSD (5%)
Amylose content [1] (%)	2009	14.6 a	—	—	—	11.4 e	12.0 d	13.8 b	14.2 ab	12.3 d	0.4
	2010	22.2 a	18.8 b	14.8 f	18.6 bc	17.4 d	16.2 e	—	18.3 c	15.9 e	0.3
	2018	17.8 a	—	—	—	—	—	—	—	14.6 b	0.4
Protein content [1] (%)	2009	6.4 b	—	—	—	7.3 a	6.8 b	6.5 b	6.5 b	6.4 b	0.5
	2010	5.1 b	5.0 bc	5.1 bc	4.7 cd	5.2 b	5.1 b	—	5.6 a	4.6 d	0.5
	2018	7.3 a	—	—	—	—	—	—	—	6.6 b	3.5

[1] Content of amylose or protein to whole weight of milled rice on the basis of dry matter weight.
Values followed by the same letter within each row are not significantly different at the 5% level, determined by the LSDs in the table.

Table 10. Mido values of 53, 79, and three ordinary varieties in 2004, 2005 and 2018.

Years	53	79	Kos	Ni	Hi	LSD (5%)
2004	63.4 c	69.6 b	71.5 a	59.5 d	69.5 b	1.5
2005	58.6 d	64.3 c	—	67.9 b	71.9 a	2.5
2018	—	69.8 a	—	—	70.4 a	3.9

Values followed by the same letter within each year are not significantly different at the 5% level, determined by the LSDs in the table.
—: Mido was not measured.

Table 11. Grading of brown–rice appearances of 79, 7E, 47, 57, J3, Kos, Nip, Ni and Hi in 2009, 2010 and 2018.

Year	79	7E	47	53	J3	Kos	Nip	Ni	Hi	LSD (5%)
2009	6.0 ab	—	—	—	9.0 c	7.0 b	5.5 a	5.0 a	8.5 c	1.1
2010	9.0 d	9.0 d	9.0 d	8.3 c	7.7 b	6.0 a	—	9.0 d	9.0 d	1.1
2018	7.0 a	—	—	—	—	—	—	—	8.3 b	0.9

Note: 1–4 (the first−class rice), 5–6 (the second−class rice) , 7–8 (the third−class rice) and 9 (below the third class): see the materials and methods.
—: Not grown in 2009, 2010 or 2018.
Values followed by the same letter within each year are not significantly different at the 5% level, determined by the LSDs in the table. Analysis of variance was performed, using the interaction between the effect of the agriculture-products inspectors (n = 2 in 2009 and 2018, and n = 3 in 2010), and the effect of the lines-varieties (n = 6, 8 and 2 in 2009, 2010 and 2018, respectively) as error (df = 5, 14 and 1 in 2009, 2010 and 2018, respectively); for example, the LSD(5%) was calculated from the error variance and the t-value of df 5 at the probability of 5% in 2009.

Table 12. Daily maximum and minimum temperatures in each of the second 10-days of July to the third 10-days of September in 2009, 2010 and 2018, and the respective averages of those from 2003 to 2019; and 80%-heading dates of the five *Ur1*-carring lines and the four check varieties.

Month	10-days Duration of month	2009			2010			2018			Max. temp. (°C)	Average from 2003 to 2019
		Max. temp. (°C)	Min. temp. (°C)	Line/variety: 80%-heading date	Max. temp. (°C)	Min. temp. (°C)	Line/variety: 80%-heading date	Max. temp. (°C)	Min. temp. (°C)	Line/variety 80%-headir date		
July	Second	29.0	24.2	Kos: 16th. J3: 17th.	28.5	23.6		31.4	24.7		29.3	23.6
	Third	29.1	23.5	Nip: 27th.	29.9	23.6	J3 & Kos: 23th. 53: 28th.	31.4	25.1		30.6	24.0
Aug.	First	30.2	24.2	Hi: 2nd. Ni: 4th.	31.0	24.2	47: 3th. Hi: 6th.	32.2	25.1	Hi: 1st	31.1	24.3
	Second	31.3	23.4	79: 16th.	31.4	25.5	Ni: 13th. 7E: 15th.	30.5	23.7		31.0	24.4
	Third	30.4	22.1		32.2	24.7	79: 20th.	31.0	25.0	79: 27th	30.4	23.6
Sep.	First	30.6	22.0		32.0	24.5		28.3	22.1		29.6	22.5
	Second	29.1	18.5		30.5	21.3		27.7	22.0		28.8	21.4
	Third	28.0	20.0		28.1	20.1		27.2	19.6		27.6	19.7

Source: Japan Meteorological Agency (http://www.jma.go.jp/jma/menu/report.html)., Site of observation: Nankoku-Nissho meteorological-observation point which is the nearest to the experimental paddy field.

Table 13. Averages of daily maximum and minimum temperatures for 30 days after 80% heading in 79 and Hi in 2009, 2010 and 2018.

Years	Daily Max. temp. (°C)		Daily Min. temp. (°C)	
	79	Hi	79	Hi
2009	30.3	30.7	21.5	23.1
2010	31.6	31.7	23.5	24.8
2018	29.3	31.1	23.1	24.5

Source: Japan Meteorological Agency (http://www.jma.go.jp/jma/menu/report.html).

Site of observation: Nankoku-Nissho meteorological-observation point which is the nearest to the experimental field.

Table 14. Characteristics of the five *Ur1*-carrying lines (summarized table).

Traits	79	7E	47	53	J3	Kos	Hi
Heading time in Kochi prefecture[1, 2]	Extremely late	Late	Middle	Middle & rather early	Early & rather late	Early & rather late	Middle & rather late
Yield (percentage to Hi in 2010)[1]	(135)	(136)	(135)	(130)	(135)	(98)	(100)
Yield [percentages to Hi in various fertilizer levels in 2003 and 2005][1]	[130 to 170]	—	—	[123 to 148]	—	—	[100]
Overall evaluation and other test items in eating-quality test[3]	High; hard & less sticky	High	High; Sticky	Low; low in flavor	High; less hard than Kos	High; Sticky	
Amylose content[4]	High	Intermediate	Low	Intermediate	Low	Low	Low
Mido[5]	High or rather high	—	—	Low	—	High	High
Culm length in 2010[1] (cm)	72.3	71.2	63.5	67.5	70.5	88.0	81.0
Field resistance to leaf blast[6]	Intermediate between susceptible and middle resistant[7]	Susceptible	Resistant	Susceptible	Susceptible	Susceptible	Susceptible
Field resistance to brown spot[6]	Middle resistant	Middle resistant	Resistant	—	Susceptible	Susceptible	Susceptible

—: Not grown or not measured.
[1] Kamimukai et al. (2020).
[2] Table 12.
[3] Table 2 to Table 8.
[4] Table 9.
[5] Table 10.
[6] Murai unpublished.
[7] Additionally, middle resistant to panicle blast (Murai unpublished).

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