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# Identification and mapping QTLs of bolting time in purple cai-tai (*Brassica rapa* L. var. purpurea)

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**Bolting time is a crucial agronomic trait for yield and quality in purple cai-tai (*Brassica. rapa* L. var. purpurea), but the genetic mechanism controlling the procedure remains unknown. In the present study, a double haploid (DH) population derived from two inbred lines of purple cai-tai 4-1 and 040-3 was constructed to identify the quantitative trait loci (QTLs) of bolting time. Genetic linkage map was performed by JoinMap version 3.0 using SSR, SRAP and ESTP molecular markers. A total of one hundred and thirty-eight molecular markers were integrated into ten linkage groups (LGs), which were anchored to the corresponding chromosome of the *B. rapa* reference genome. The genetic linkage map covers 1253.1 cM, with an average distance of 9.08 cM between two adjacent markers. Five quantitative trait loci (QTLs) were identified to control bolting time and explaining variations from 17.7 to 44.2%. The genetic results of bolting time will be useful for future breeding of late bolting in purple cai-tai.**

**Key words:** Purple cai-tai (*Brassica rapa* L. var. *purpurea*), bolting time, quantitative trait loci (QTL).

## INTRODUCTION

*Brassica rapa* is consisted of various vegetables such as Chinese cabbage, non-heading Chinese cabbage, and turnip. Non-heading Chinese cabbage includes economically important vegetable taxa with a wide range of morphologies, such as pakchoi (*Brassica campestris* ssp. *chinensis* Makino var. communis Tsen et Lee), purple cai-tai (*B. rapa* L. var. *purpurea*, Canjie et al., 2019), rosette bok choy (*B. campestris* ssp. *chinensis* Makino var. rosularis Tsen et Lee), and taicai (*B. campestris* ssp. *chinensis* Makino var. tai-tsai Hort). Purple cai-tai is a natural early bolting mutant which bolting earlier without vernalization, and it is an important vegetable in the middle and lower reaches of Yangtze river.

In *B. rapa*, one of the most important agronomic traits is bolting because premature bolting significantly affects the quality and yield of the economic products (Kitamoto et al., 2014). Bolting times are regulated by multiple genes. In *Arabidopsis thaliana*, over 300 regulatory genes for bolting and flowering time have been isolated (Bouché et al., 2016). Many QTLs of bolting and flowing have been charactered in *B. rapa*. In the past two decades (Nishioka et al., 2005; Lou et al., 2007, 2011; Li et al., 2009; Kakizaki et al., 2011; Li et al., 2015).

During the elucidation of the genomes of crop species, it is crucial to assign molecular markers to the linkage

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groups (LGs) and construct genetic maps. A number of genetic linkage maps have been produced for *B. rapa* based on diverse marker types including Restriction Fragment Length Polymorphisms (RFLPs), Random Amplified Polymorphic DNA (RAPD), Simple sequence Repeats (SSR), Amplified Fragment Length Polymorphisms (AFLPs), and Sequence-related amplified polymorphism (SRAP) (Kim et al., 2006; Suwabe et al., 2006; Soengas et al., 2007; Yan et al., 2009; Honghao et al., 2014; Haidong et al., 2016).

SRAP has some advantages, such as simple, a apposite throughput rate, targeting open-reading frames (ORFs), and so on (Uzun et al., 2009). SSR markers are useful to construct high-density maps because of its high polymorphism levels, its co-dominant character, its abundance and wide distribution during the genome and the utility as convenient anchor points in the integration of intraspecific and interspecific consensus maps (Acher et al., 2004). Expressed sequence tag polymorphism (ESTP) markers are transferable between species and between genera (Brown et al., 2001).

Although many QTLs about bolting have been isolated and characterized with molecular markers, the report of QTL and the markers based on sequence-tagged Polymerase chain reaction (PCR) mapped in purple cai-tai is limited (Canjie et al., 2019), especially those which may provide anchors to the genome of *B. rapa* and are readily transferable to other populations. Thus, the objective of this research was to identify QTLs controlling bolting in two years. Our results should be useful to understand the genetic mechanism about the bolting in purple cai-tai, and contribute to breeders for designing effective strategies for better cultivar.

## MATERIALS AND METHODS

### Plant materials and DNA isolation

Double haploid (DH) population consists of 140 individual DH lines was employed for trait assay and genetic mapping. The population was developed from microspore culture of F1 buds of the cross between 040-3, a cultivar with early bolting which was derived from 040 and 4-1, a high inbred line with late bolting, which was obtained by seven generations of self-pollination of cultivar Daguzi. The plants of parents, F1 and 140 individual DH lines were cultivated in an open field at the Institute of Economic Crops of Hubei Academy of Agricultural Science, Wuhan, China (30.57°N, 114.3°E) from September of 2012 to April of 2013, and September of 2013 to April of 2014. The bolting time (that is, days after sowing to appearance of macroscopic floral bud) was judged by the observation recorded every third day (Wang et al., 2018) in 2013, 2014 spring.

### Detection of DNA polymorphism

DNA was isolated from fresh and young leaves of the parental and 140 DH lines according to the protocol published by Guillemaut and Laurence (1992). 106 SSR markers, and 4 ESTP markers and 652 SRAP markers were used to filtrate the polymorphism of the two parents and $F_1$. The experiment of SRAP was carried out following the procedure reported by Li and Quiros (2001), with minor modifications. SSR and ESTP markers were obtained as described by Choi et al. (2007) and HyeRan et al. (2009) (Supplementary Table 1). PCR was performed in a 10 µl reaction mixture containing 2 µl DNA template (40 ng), 1 µl 10 × PCR buffer ($MgCl_2$), 0.2 µl forward primer (10 µM), 0.2 µl reverse primer (10 µM), 0.8 µL dNTPs (10 mM), 0.2 µl TaqDNA polymerase (2.0 U/µl), and 5.6 µL $ddH_2O$ (Biomed Tec Co., Beijing, China). PCR conditions were as the follows:an initial denaturation step at 94°C for 4 min, followed by 35 cycles of DNA amplification(94°C for 30 s, 60°C for 30 s, and 72°C for 60 s), with a final 7 min extension at 72°C (Mastercycler nexus, Eppendorf, German). The PCR products were separated by electrophoresis on 9% polyacrylamide gels (acrylamide/ bisacrylamide = 29:1) and screened with silver staining (Choi et al. 2007).

### Linkage analysis, map construction and QTL analysis

A scoring system was applied for the reproducibly polymorphic makers among the parent lines in the DH population. Linkage assay and the construction of maps were carried out by JoinMap Version3.0 (Stam, 1993; Van Ooijen and Voorrips, 2001). SSR and ESTP markers previously mapped (Yan et al., 2011) were utilized for the identification of LGs in the LOD groups with a threshold range of 3.0–8.0. The annotation of LGs was identical with the second generation of referenced LGs in *B. rapa* (A1–A10). A composite interval mapping (CIM) reported by Zeng (1994) was employed for the analysis of QTLs for bolting time by a QTL Cartographer (version 2.5) (Basten et al., 2002). In order to estimate the appropriate significance threshold of a logarithm of odds (LOD), a test of 1,000-permutation was carried out via the QTL Cartographer.

## RESULTS

### Polymorphism screening of primers between parents

In order to construct the genetic linkage map, the two parents and $F_1$ were filtrated for polymorphism with 652 SRAP markers and 106 SSRmarkers, and 4 ESTP markers. In total, 183 (24.02%) out of 762 primers (or primer combinations, abbreviated as PCs), including128 SRAP PCs, 42 SSR PCs, and 3 ESTP, produced polymorphic loci. A total of 129 polymorphic loci were selected with the help of 128 polymorphic SRAP primer combinations. Meanwhile, 42 SSR and 3 ESTP polymorphic loci were obtained. All these obtained polymorphic markers were employed for the assay of DH population (Supplementary Table 2).

### Construction of genetic linkage map

A total of 140 DH individuals from $F_1$ progenies of two purple cai-tai “4-1” and “040-3” were used for genotyping and linkage analysis. There were 25.0% of 184 polymorphic markers not assigned. As shown in Figure 1, a total of 138 markers were anchored to 10 LGs which spanned 1253.1 cM of map distance with an average distance of 9.08 cM. The location of 10 LGs on their corresponding chromosomes (A1-A10) was confirmed via 32 SSR and 2 ESTP markers of which the map positions were already known on the reference maps of *B. rapa*.

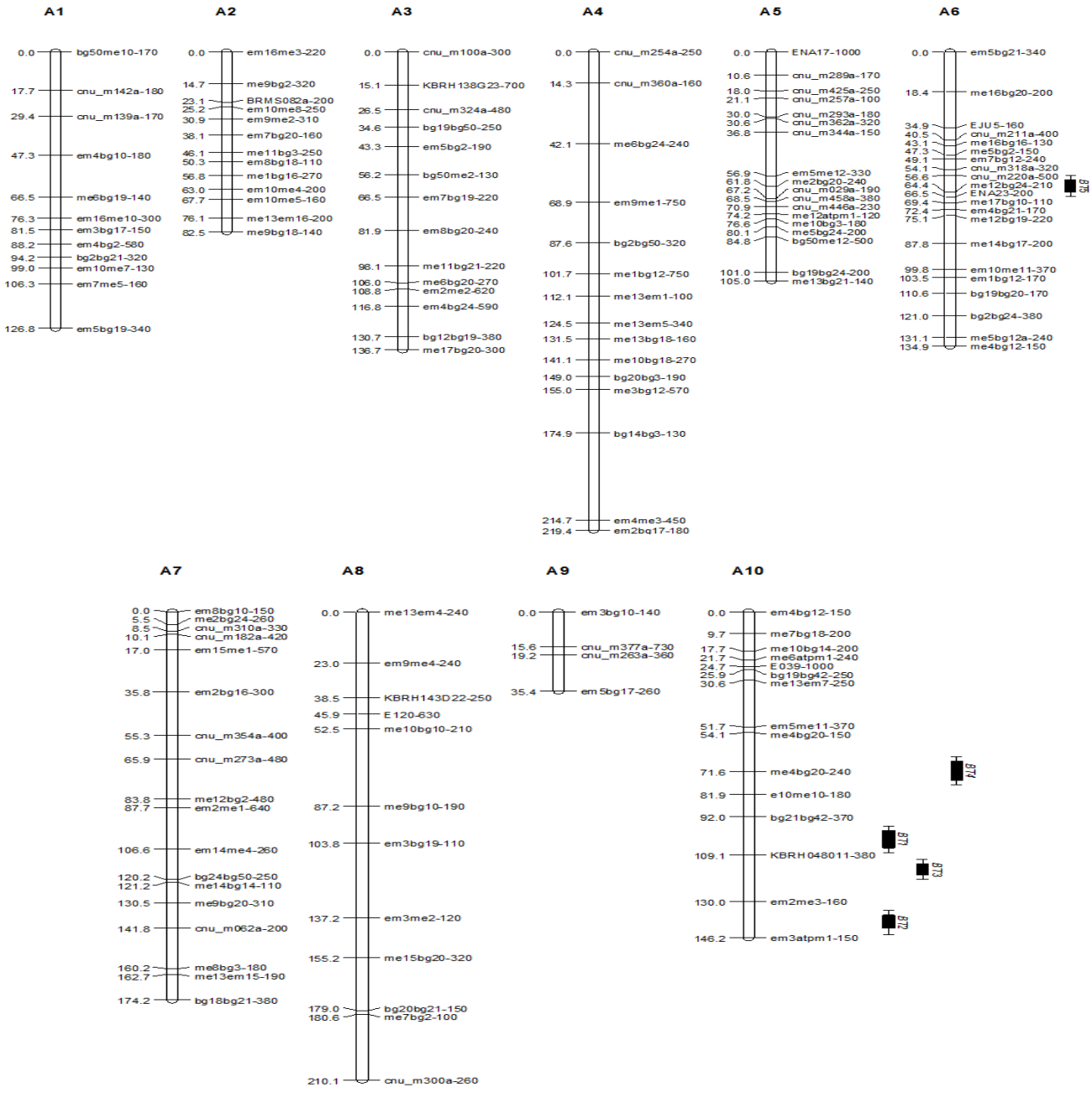


**Figure 1.** Genetic linkage map and localization of QTLs of bolting time traits on a population of 140 DH lines of purple cai-tai.

The length range of individual LGs varied from 35.4 cM (A9) to 219.4 cM (A4).

## QTL analysis for bolting time

As shown in Table 1, the parental lines, 040-3 and 4-1, revealed significant difference of the bolting time. All seven plants of 040-3 exhibited stably bolting time at 40 DAS in 2013 spring and 41 DAS in 2014 spring, respectively. For 4-1 of late bolting parent line, bolting time was detected between narrow ranges from 127 to 129 DAS in 2013 spring, and 128 to 132 DAS in 2014 spring, respectively. These results indicated that the genetic background of these two purple cai-tai lines is nearly homozygous with little environmental effect. Further, the average bolting time of the $F_1$ showed 96±2.2, 97±3.2 DAS which is slightly larger than that of the mid-parent (84±1 DAS, 85.5±1.4 DAS) in 2013 and 2014 spring, respectively. The bolting time of 137 out of the 140 $F_2$ DH progenies were checked that revealed a continuous distribution from 57 to 141 DAS in 2013

**Table 1.** Variation in bolting and flowering time of parents and F2 population.

| Environment | Generations | No. of plants | Bolting time (days) |
|---|---|---|---|
| Spring-2013 | 040-3 | 7 | 40 |
| | 4-1 | 10 | 128±1 |
| | F1 | 10 | 96±2.4 |
| | Mean of F2 population | 137 | 94.7±3.5 |
| | Range of F2 population | 137 | 57-141 |
| Spring-2014 | 040-3 | 10 | 41 |
| | 4-1 | 10 | 130±1.4 |
| | F1 | 10 | 97±3.2 |
| | Mean of F2 population | 137 | 93.1±2.8 |
| | Range of F2 population | 137 | 55-140 |

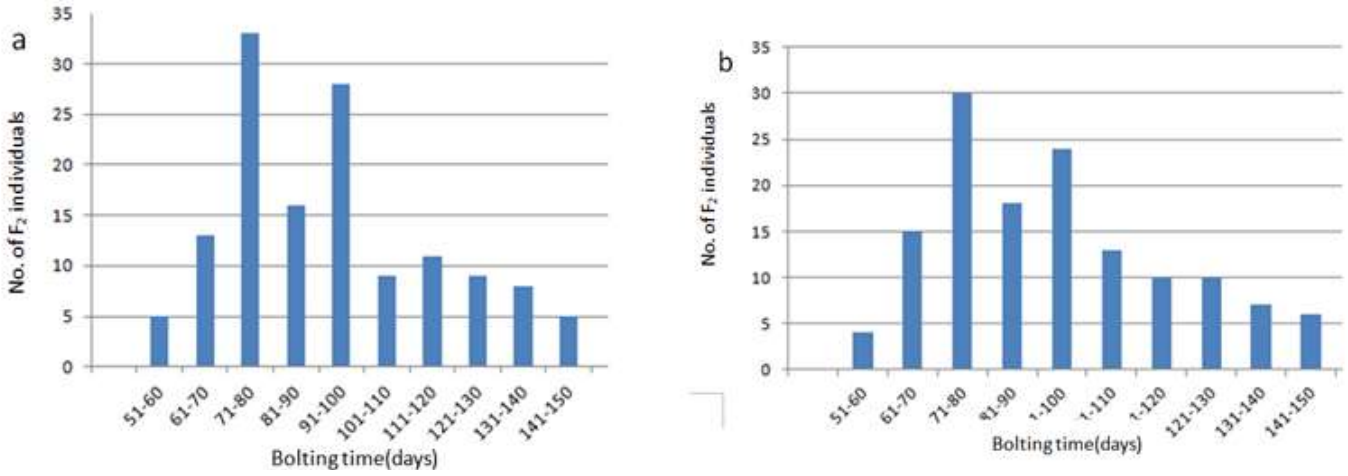


**Figure 2.** Frequency distribution of bolting time in the F2 population. (a) 2013 spring, (b) 2014 spring.

spring, and from 55 to 140 DAS in 2014 spring. The other 3 of the 140 $F_2$ DH progenies died before bolting. These results suggested that the bolting time in purple cai-tai probably be controlled by quantitative trait locus.

The frequency distributions of bolting time in the $F_2$ populations revealed continuous distribution, also showing that bolting time are quantitative traits controlled by polygenes (Figure 2). QTL analysis was performed individually for each of the 2013 and 2014 tests. Five QTLs for bolting were detected in A6 and A10 (four regions). The largest QTL effect (LOD of 11.73) on bolting time, named as BT1, was detected between the loci KBRH048O11-380 and bg21bg42-370 on A10, which explained approximately 44.2% phenotypic variation. Other four QTLs, named as BT2, BT3, BT4 and BT5, were mapped in A10 and A6 chromosome explaining 42.7, 41.6, 34.2, and 17.7% phenotypic variation, respectively. Remarkably, BT1, BT2, BT4 were detected twice in 2013 and 2014, but BT3 only in 2013 and BT5 only in 2014 (Table 2).

## DISCUSSION

A genetic linkage map was constructed via a segregating population of 140 purple cai-tai DH lines. This linkage map contains 104 SRAP, 32 SSR, and 2 ESTP markers which were grouped on 10 LGs, and each LG was anchored to the corresponding chromosome of the *B. rapa* reference map based on the common SSR and ESTP (Yan et al., 2011; HyeRan et al., 2009; Su Ryun Choi et al., 2007). It indicates that this map can be integreted into orther genetic linkage map of *B. rapa* and be useful for other researchers. Covered with a total genetic distance of 1253.1 cM, the linkage map in the present study is comparable to the published sequenced BAC anchored reference genetic map which is 1,123.3 cM (HyeRan et al., 2009) and the sequence-based genetic linkage map which is 1234.2 cM illustrated by Yan et al. (2011). The genetic map lengths differences among various reports are attributed to the scoring errors for the most parts. In addition, the differences have also

**Table 2.** QTL detected for the bolting time traits based on CIM (composite interval mapping) analysis.

| QTLs | Years | Marker interval | Group | QTL position | Peak LOD[a] | Addition effect |
|---|---|---|---|---|---|---|
| BT1 | 2013 | bg21bg42-370-- KBRH048011-380 | A10 | 102.049 | 11.73 | 15.42 |
| BT1 | 2014 | bg21bg42-370-- KBRH048011-380 | A10 | 102.049 | 11.24 | 14.53 |
| BT2 | 2013 | em2me3-160--em3atpm1-150 | A10 | 138.992 | 10.77 | 15.69 |
| BT2 | 2014 | em2me3-160--em3atpm1-150 | A10 | 138.992 | 10.54 | 15.23 |
| BT3 | 2013 | KBRH048011-380--em2me3-160 | A10 | 116.12 | 11.06 | 15.24 |
| BT4 | 2013 | me4bg20-150--e10me10-180 | A10 | 72.605 | 10.64 | 13.82 |
| BT4 | 2014 | me4bg20-150--e10me10-180 | A10 | 72.605 | 9.93 | 12.51 |
| BT5 | 2014 | cnu_m220a-500--ENA23-200 | A6 | 61.628 | 5.0 | 10.12 |

been reported to be caused by the type of markers, number of individuals, number of markers, recombination frequency, LOD values, and the software employed (Gosselin et al., 2002). The density of marks in the linkage map in the present study is more lower than the maps of Xiaowu et al. (2011) and Lei et al. (2018), so it needs to add marks to this linkage map for further research.

In total, five QTL affecting bolting time were identified in this study. The QTL BT5 near the marker cnu_m220a in A6 is similar with the qFT6.1 in *B. rapa* L. (Yating et al., 2016), it is a new QTL or the same QTL, need further verification. There is no similarity of the other four QTLs BT1, BT2, BT3 and BT4 with the previous studies (Jonathan et al., 1995; Hidetoshi et al., 2001; Yating et al., 2016) ,they may be new QTLs and subject to be further verify. The number of loci influencing bolting is different with previous genetic analyses (Jonathan H et al., 1995; Hidetoshi et al., 2001), that mostly attributable to different population, number of individuals, number of markers, and so on. In the future, a common linkage map will be employed to comparatively assay these QTLs for the elucidation of the genetics of bolting in *Brassica* crops. Moreover, it might make a contribution to the breeding of novel cultivars with controlled bolting.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ACKNOWLEDGMENTS

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**Supplementary Table 1.** Primers for SSR, ESTP and SRAP marker assays.

| Marker assay name | Assay type | Forward primer | Reverse primer |
|---|---|---|---|
| KBRH138G23 | SSR | TTTGACATCGTGCAATGCTA | TTGGGCTGGTCCTGAAGATA |
| KBRH139B23 | SSR | ATCTCATGGTTGGTTCACCG | ATTTCCAAAACACACACGCA |
| KBRH143D22 | SSR | GATGTGATACTTTGGCGACGG | TGAAGGATAATATGGTCTTGGCC |
| KBRH143F19 | SSR | GCATGCAAGCTTGGAACTGAT | CAGTCACGCTTTCTGACGAAAA |
| KBRH143H15 | SSR | TCTGCATCAAAATGCTAAAATGA | TGATCTTTTAGAAACAAAGATCGAG |
| KBRH143K20 | SSR | CAAATGTCTCAAGACACATAAACCA | CTAAAGCAGCAATTGGGTGTTC |
| KBRH048O11 | SSR | GCCTCTACCTGGCTTCAGCA | TCATTTGGCGCATACTTCCA |
| EJU3 | SSR | CCTCTTTTAATTCAAACAAGAAATCA | TTCGGACAATGGCAGTGATA |
| EJU5 | SSR | GGCACGTACATGGAGGATTC | TGTTGGTCGAGCTGTTTCAG |
| ENA17 | SSR | CAGTTATTTCGCCTCGTCT | TATTTGTGGTCTGTTATTGGA |
| ENA18 | SSR | TTAAAATGAAACCCACCCGA | TGTTGGGCAACATCCATTTA |
| ENA23 | SSR | GCTGTGCCAGTTCCTCTTTC | TCATTCCAAATGGCCTTACC |
| ENA28 | SSR | GGAGTCCGAGCGTTATGAAT | CTTCATCGACCCACCTTGTT |
| ENA4 | SSR | ACTTCTCTTTATTCACTTCCCA | GAGGGTGGTTGGTTCATT |
| ENA6 | SSR | CTCGTCTTCTTCACCTACAAC | CTGACATCTTTCTCACCCAC |
| cnu_m008a | SSR | GTTGCTGGGCTTGCAGTTAT | GAGCGTACCAGCAACCTCTC |
| cnu_m016a | SSR | GGTGAATGGAATCTTGTCTTGA | CCCAACAATCCCAGAAACAC |
| cnu_m020a | SSR | GGCTCTCCTCATCGTCAAAA | AATTCCGATTGCGACAAAAC |
| cnu_m029a | SSR | TACCCATTGGTGTCCTCCAG | TCGTTCTCGAATGTGAATTGTC |
| cnu_m030a | SSR | GAAACAAATTATTTAAAAATCAGACCA | TGGAACAATCCGTAAAACTATGC |
| cnu_m034a | SSR | TCACCGCCATAATTTGATCC | CCCTCTCAACAAGGTATGCAA |
| cnu_m037a | SSR | CCTAGTTCCTTGCACTCATGC | TTGTCTTTCAGATTGAAAACTTCG |
| cnu_m038a | SSR | GGCATGTGTCAATGAGTTGG | CTCCCACTCCTCCATTCAAC |
| cnu_m044a | SSR | TGTTTTGATCTTTACTGTTTTTGGA | AATGTTTTTATATCACTATTGCCAAAT |
| cnu_m046a | SSR | GCTAAAGGTTTAGTCCAAATAGGATTC | GCAAAATGATGCCCCATAAA |
| cnu_m050a | SSR | AGCCCAAGCTCGTATTCCTT | AAAATCGGGACAACCACCTA |
| cnu_m052a | SSR | GGAATCCTACGGAAGAGCAA | AAGGTAACGGTGGCAGTGAG |
| cnu_m062a | SSR | ATCGGCGCTGGTTATGTCA | CTAGGCTGCCCTTTCCGATT |
| cnu_m068a | SSR | CCATATGACTAACTTGACACTTTTGAA | TTCCCGGAAAGTCTTCTTGG |
| cnu_m073a | SSR | TGGCATTGACAGAGCTAGTA | TTTATTTAGTTTCATACCCT |
| cnu_m090a | SSR | GCAAAGATCGGCGAAGAAGA | TGCAGACACATTCGAACAAACA |
| cnu_m098a | SSR | TGCGACCCAAGTAGGTGAAAC | TGTCTCTCGCTCATTCATCCAA |
| cnu_m100a | SSR | AAAGTTCACACAAATGATTTTGATATT | TTTTCTAGGAATGGTCCAAACTT |
| cnu_m114a | SSR | AGTCGGAGGAAACGCGAAATTA | CGAAATAAAGACAGACAGAGACATCCA |
| cnu_m119a | SSR | ACACCTACTTGTTTCCATCCAAAT | CGGGTATTTGCGTTGTTTCC |
| cnu_m132a | SSR | CCATGGCCTCTCGTATTGCT | CCAACGGAGTGTTCCCAATC |
| cnu_m139a | SSR | TCAAGCGCAACAAACATTGG | TGGTGTTAGGGTTTAAGGTTGTGG |
| cnu_m142a | SSR | GACCTTCGGTTCAGGGTATGG | CTGAACGGTCAATTTGTTTGG |
| cnu_m146a | SSR | TCATACCAACGTGCTTTGAAGA | GTGTGGCCGGATCTGATCTA |
| cnu_m148a | SSR | CACAAGCATTCTACCATAGCAAAGTC | TGCACATATGGCATGTTGTTTG |
| cnu_m149a | SSR | GGAAGCCTCTGTGCGAAAAA | TGCCGACGATTTGATAGAGGA |
| cnu_m157a | SSR | CCGCAGTTGATCCATTAGCC | ACGCTGCATCCACATGAAAC |
| cnu_m172a | SSR | GGAATGGAACACCGGATTAGC | TCGGATCTGATTTGTCGGATTT |
| cnu_m173a | SSR | TGTATTCCATTATTTCCGACTAACCT | CCGCATTTTAAAAACGTGAGAAA |
| cnu_m179a | SSR | TGGTTACACCTAGTTCCTTGCACTC | GGCCTTTGCCCGTTTAGTTTTA |
| cnu_m182a | SSR | TTCATCACCGTCTTATGTTGTGC | GGCAGGTGGAATATGTGGAAAT |
| cnu_m207a | SSR | GGACCCGGAATACCTCAAAAGA | CATCAATAGCTCCGACACAATCC |
| cnu_m211a | SSR | TGTAAAGTTGTGCAAGGATTGTG | TGGGTTTGTGAAAATATGGTGAAA |
| cnu_m215a | SSR | CCAACCATTTCGTTAGTCAACC | TTACGCATGTACCTGCACTAAAAA |
| cnu_m220a | SSR | ATCAGAACCGAATCCGACCA | CAATGGTTGCAATGTTATTTGGA |

**Supplementary Table 1.** Contd.

| | | | |
|---|---|---|---|
| cnu_m225a | SSR | TTGCGTTTTCTCGTCGTCAA | CCCCGAGATAAATGGCACAC |
| cnu_m241a | SSR | AATGCTGTGTCCATGACCAA | CGGGCATCCACCTAATTTGT |
| cnu_m246a | SSR | AAAGCCATCCATCCATCAAGC | GATGCAACATTTGACTGTGTTAGAGC |
| cnu_m252a | SSR | TGAAAATCAACACGAACACACAGA | CTCGTGGGGGAATGAGTGAG |
| cnu_m254a | SSR | AAGCTTGAGCTTCCAGCCTTC | ATCAGTGCCGGCCTTGAATA |
| cnu_m256a | SSR | TTGAAATACATGATACCCCAACCA | CCGTTTTCAGGGCACAGTTT |
| cnu_m257a | SSR | TGCATGATGTTCATGTCTTGTAAA | TCCTTCTGTAAACCGGTTGTAATTT |
| cnu_m263a | SSR | GAGGAAGTACGGCAAGAAACCA | AGGACACATGTCCACATGAAAA |
| cnu_m268a | SSR | TCATTGGTGAAGAACCCACAAA | GCGACCATAAAAAGAGAGTGAGAA |
| cnu_m273a | SSR | ATAAGGGCATCGCCTCAACA | TGCACGCATCCACATAAACA |
| cnu_m277a | SSR | GCCATGAGCATTCGGTTAGG | TGAACTCTGGTTGGATTGACGA |
| cnu_m280a | SSR | TGTTACCACAGGAACCGTTCAA | CTTGGGCACACCATCATCTG |
| cnu_m284a | SSR | TCGGTTAAATCGAGTAACGATG | TTTCAGGACCTAGACGTTACCAA |
| cnu_m286a | SSR | AGTTGCCCCTATTCATGCAC | AATGCGTTCATGTGGGGATA |
| cnu_m288a | SSR | GCGTTTCGTCCTCTTCTCAC | TTACCCACCTTGGCTTCATC |
| cnu_m289a | SSR | CCCCTGGACTCCGTTTATCT | GATCTACGACGATCGGATGC |
| cnu_m293a | SSR | AAAAAGAAATGGATATTGTGTGAAA | CCTGGATCAAGACCACGAAG |
| cnu_m295a | SSR | GCTGCCTAATAGGGTGCTTG | AGAGCGCATTCAAGTCTGGT |
| cnu_m296a | SSR | TCTCGTCGCTCTGAATTGTG | TTGTGAAATCAAAGCAAAAAGG |
| cnu_m300a | SSR | AATTAGCGCGATAACATAAATAAAAA | AAATTGCTTAACTATTAAAACTGCAAA |
| cnu_m308a | SSR | GTTTGGGCCATCATGAAAAA | TGGTTGCAAAATGTCACAGAA |
| cnu_m310a | SSR | GGCAGGTGGAATATGTGGAA | GCACTATCATCATCAAACAGAACA |
| cnu_m316a | SSR | TCAAGCATGTCCTTAAAACTCTGA | GCGTTCACGTTTCCCATATC |
| cnu_m318a | SSR | TTATCAACATATTTTCAATCATTCCA | GCTTTGGACTATGCTTCTAAAGTACG |
| cnu_m320a | SSR | TTTTTCCTTTGGCTTAAACTGA | GCCAAAGCCACAAGATAACAT |
| cnu_m321a | SSR | TTGAATAATGACCCCAAATATCA | TCAATAGGTATTAACCAATTCTACCG |
| cnu_m324a | SSR | TTTTCAACTCCCACATGCAC | TGGGTATGTGCCAAATTGTTT |
| cnu_m327a | SSR | TTCTTGACCAAAAGAATCATGG | CTAACACGGGGAAAAGCAGA |
| cnu_m332a | SSR | TCGAACCGAAGTAAATAACGGACT | TTTCGCCCACTGACGCTATT |
| cnu_m338a | SSR | GCAACGATGAATCCCTAAACGA | AAATCCTCCCACTGTTTCCGAT |
| cnu_m344a | SSR | CCCAAATACGAAAACAAAGTTTGAC | AGGATCTCATCCGCTTTCCA |
| cnu_m354a | SSR | AAAGAAAACAAAGTGTCATTGTCTCA | TCTACCGGTTGAACCAGAGTTTTT |
| cnu_m356a | SSR | CGCATTTTCGCCGTCATTA | ACATCAGGCCGTCCCACTAA |
| cnu_m360a | SSR | ATCAGTGCCGGCCTTGAATA | AAGCTTGAGCTTCCAGCCTTC |
| cnu_m362a | SSR | CCTCTGCTGAAGGAGGCAAA | AGGTGGCTCTAGCGGAAGGT |
| cnu_m364a | SSR | ACCTGCCACCCTGTCAAAAC | GCACTAACCGTCCCTCTCCTC |
| cnu_m371a | SSR | TTTTTGGGTTTCTTCTCAAAATGC | ACTCCAGCGAATTTGGCTTT |
| cnu_m372a | SSR | CCAGTGGCCAATACGAAACC | TGATGGAGAGTGGGTTGTGC |
| cnu_m377a | SSR | TCAGTTGTCGGATCGTCTATG | CACTTATCTTTCCTTTGAAGTTGTTG |
| cnu_m379a | SSR | ACACCACTAAAACATTGCCATA | ACCGAAGGAGACTGCAAAGA |
| cnu_m384a | SSR | TGAAGGTGATGATGACGATGA | TCATGGTCTACAAAGACATACGG |
| cnu_m396a | SSR | TCATCATTAAAATGAGTTAAAATTCG | TTTTGGTGATCTTTTCTAAATTTTTC |
| cnu_m397a | SSR | TCTTCAAGTCAAAATACTCACATTCA | AAACGACAAATACATATGACAGTTTTA |
| cnu_m398a | SSR | TGACATTCGCATCAGATTTGT | TTGGGCTTCACGCATAAGAT |
| cnu_m400a | SSR | CGAGTTTTTGTGTGTACGTATAGTAAT | CCAAAGTGCGTAAAGGAAGG |
| cnu_m402a | SSR | GCCGACTCCTAGTGAGGAAA | TGTGTTTTGGGCTCAAAGGT |
| cnu_m409a | SSR | TTCCGGTCACTTCTAGCTTCA | TTTTGGTGGTTAGTATGTCGCTAT |
| cnu_m416a | SSR | TGGTGGGTCGTAACAGATGA | GCTCGCTTCCCAAATATGAA |
| cnu_m425a | SSR | TCGTTTGACCAACCGTACAA | CTTGCCAGCGTTGATACAGA |
| cnu_m439a | SSR | CCCTACGGACGGATGAGTAA | TCTGAGTGGCACCAGCATTA |
| cnu_m442a | SSR | CGATTTGGACAATGACTAGTGG | AACGCCATGGAAACAGAAAC |
| cnu_m446a | SSR | CACGTACGTCTTGGATGAATAAA | ATCTCACGTGGAGCACCATT |

**Supplementary Table 1.** Contd.

| | | | |
|---|---|---|---|
| cnu_m457a | SSR | CTGCTCCTTCACGTTTTCATCA | ACGGACAGCAACAACAACAAGA |
| cnu_m458a | SSR | GGGGTGAATCTTGGATGAGG | CTGACGGATTCCCAACGAAT |
| cnu_m459a | SSR | CAAAGCCGGATTTCTTTTAGCA | TTTAAAAGTATTCTAACAAATCCGTTG |
| E039 | ESTP | CTTGAGTGCTCAGGTCAAAGC | GAACCCTTACCCCCAAGACTAC |
| E120 | ESTP | ATCATAACCCTCAGGTTTGACATC | ACATCAAGCTCCTCTCTGGGTA |
| E129 | ESTP | AGATGGTAAAAGAGCACAAGCC | TTCAAGCTACCGATCCAACTG |
| E138 | ESTP | TGCTATCACAGTAGGGATTGCTT | CACTCCCACTCCTCCTAGTCC |
| atpm1 | SRAP | CTCTTGGTGATTTCAGCCAC | |
| bg10 | SRAP | CGTTTCTTCTCGCATTTCTC | |
| bg12 | SRAP | TCTAAGACCTCCACAGTAAG | |
| bg14 | SRAP | GCGTGGAAGCTGGAAGTCAAC | |
| bg16 | SRAP | TGATACCACTTGCGATACCA | |
| bg17 | SRAP | TGGTATCGCAAGTGGTATCA | |
| bg18 | SRAP | GCAAGTCTCTCAGGTTATTC | |
| bg19 | SRAP | GCTCTTCATCAGTTCTTGGT | |
| bg2 | SRAP | GACCAAATATAAAACACTAACTA | |
| bg20 | SRAP | TCCTCTCCACTTTTGTCTTC | |
| bg21 | SRAP | AACTCGCTTGCTTAGATATG | |
| bg24 | SRAP | CACCTTTTCCTACTCCTATC | |
| bg3 | SRAP | GGAACACTTAATGGTACGGT | |
| bg42 | SRAP | ACACATAATCTTCTACAAATAC | |
| bg50 | SRAP | AAGTCGTTGTAGTATAGTGG | |
| me1 | SRAP | TGAGTCCAAACCGGATA | |
| me2 | SRAP | TGAGTCCAAACCGGAGC | |
| me3 | SRAP | TGAGTCCAAACCGGAAT | |
| me4 | SRAP | TGAGTCCAAACCGGACC | |
| me5 | SRAP | TGAGTCCAAACCGGAAG | |
| me6 | SRAP | TGAGTCCAAACCGGACA | |
| me7 | SRAP | TGAGTCCTTTCCGGTAA | |
| me8 | SRAP | TGAGTCCAAACCGGACG | |
| me9 | SRAP | TGAGTCCTTTCCGGTCC | |
| me10 | SRAP | TGAGTCCAAACCGGACT | |
| me11 | SRAP | TGAGTCCTTTCCGGTGC | |
| me12 | SRAP | TGAGTCCAAACCGGTAG | |
| me13 | SRAP | TGAGTCCAAACCGGTCA | |
| me14 | SRAP | TGAGTCCAAACCGGTAA | |
| me15 | SRAP | TGAGTCCAAACCGGTGC | |
| me16 | SRAP | TGAGTCCAAACCGGAAC | |
| me17 | SRAP | TGAGTCCAAACCGGCAT | |
| em1 | SRAP | GACTGCGTACGAATTAAT | |
| em2 | SRAP | GACTGCGTACGAATTTGC | |
| em3 | SRAP | GACTGCGTACGAATTGAC | |
| em4 | SRAP | GACTGCGTACGAATTTGA | |
| em5 | SRAP | GACTGCGTACGAATTAAC | |
| em6 | SRAP | GACTGCGTACGAATTGCA | |
| em7 | SRAP | GACTGCGTACGAATTCAA | |
| em8 | SRAP | GACTGCGTACGAATTCAC | |
| em9 | SRAP | GACTGCGTACGAATTACG | |
| em10 | SRAP | GACTGCGTACGAATTGAT | |
| em11 | SRAP | GACTGCGTACGAATTATG | |
| em12 | SRAP | GACTGCGTACGAATTCGA | |
| em13 | SRAP | GACTGCGTACGAATTTAG | |

**Supplementary Table 1.** Contd.

| | | |
|---|---|---|
| em14 | SRAP | GACTGCGTACGAATTTCG |
| em15 | SRAP | GACTGCGTACGAATTGTC |
| em16 | SRAP | GACTGCGTACGAATTGGT |

**Supplementary Table 2.** Detail of the primers for detection of DNA polymorphism and assigned linkage group.

| Marker assay name | Assay type | Polymorphism | Assigned linkage group |
|---|---|---|---|
| KBRH138G23-700 | SSR | O | A3 |
| KBRH139B23 | SSR | × | × |
| KBRH143D22 | SSR | O | × |
| KBRH143F19 | SSR | × | × |
| KBRH143H15 | SSR | O | × |
| KBRH143K20-320 | SSR | O | A7 |
| KBRH048O11 | SSR | O | × |
| EJU3 | SSR | × | × |
| EJU5-130 | SSR | O | A6 |
| ENA17 | SSR | O | × |
| ENA18 | SSR | × | × |
| ENA23-200 | SSR | O | A6 |
| ENA28 | SSR | × | × |
| ENA4 | SSR | O | × |
| ENA6 | SSR | × | × |
| cnu_m008a | SSR | × | × |
| cnu_m016a | SSR | × | × |
| cnu_m020a | SSR | O | × |
| cnu_m029a-190 | SSR | O | A5 |
| cnu_m030a | SSR | × | × |
| cnu_m034a | SSR | × | × |
| cnu_m037a | SSR | × | × |
| cnu_m038a | SSR | × | × |
| cnu_m044a | SSR | × | × |
| cnu_m046a | SSR | × | × |
| cnu_m050a | SSR | × | × |
| cnu_m052a | SSR | × | × |
| cnu_m062a-200 | SSR | O | A7 |
| cnu_m068a | SSR | × | × |
| cnu_m073a | SSR | O | × |
| cnu_m090a | SSR | O | × |
| cnu_m098a | SSR | × | × |
| cnu_m100a-300 | SSR | O | A3 |
| cnu_m114a | SSR | × | × |
| cnu_m119a | SSR | × | × |
| cnu_m132a | SSR | × | × |
| cnu_m139a | SSR | O | × |
| cnu_m142a-180 | SSR | O | A1 |
| cnu_m146a | SSR | O | × |
| cnu_m148a | SSR | × | × |
| cnu_m149a | SSR | × | × |
| cnu_m157a | SSR | × | × |
| cnu_m172a | SSR | × | × |

**Supplementary Table 2.** Contd**.**

| | | | |
|---|---|---|---|
| cnu_m173a | SSR | × | × |
| cnu_m179a | SSR | × | × |
| cnu_m182a-420 | SSR | O | A7 |
| cnu_m207a | SSR | × | × |
| cnu_m211a | SSR | O | × |
| cnu_m215a | SSR | × | × |
| cnu_m220a-500 | SSR | O | A6 |
| cnu_m225a | SSR | × | × |
| cnu_m241a | SSR | × | × |
| cnu_m246a | SSR | × | × |
| cnu_m252a | SSR | × | × |
| cnu_m254a | SSR | O | × |
| cnu_m256a | SSR | × | × |
| cnu_m257a-100 | SSR | O | A5 |
| cnu_m263a-360 | SSR | O | A9 |
| cnu_m268a | SSR | × | × |
| cnu_m273a | SSR | O | × |
| cnu_m277a | SSR | × | × |
| cnu_m280a | SSR | × | × |
| cnu_m284a | SSR | × | × |
| cnu_m286a | SSR | × | × |
| cnu_m288a | SSR | × | × |
| cnu_m289a-170 | SSR | O | A5 |
| cnu_m293a-180 | SSR | O | A5 |
| cnu_m295a | SSR | × | × |
| cnu_m296a | SSR | × | × |
| cnu_m300a-260 | SSR | O | A8 |
| cnu_m308a | SSR | × | × |
| cnu_m310a | SSR | O | × |
| cnu_m316a | SSR | × | × |
| cnu_m318a-320 | SSR | O | A6 |
| cnu_m320a | SSR | × | × |
| cnu_m321a | SSR | × | × |
| cnu_m324a | SSR | O | × |
| cnu_m327a | SSR | O | × |
| cnu_m332a | SSR | × | × |
| cnu_m338a | SSR | × | × |
| cnu_m344a-150 | SSR | O | A5 |
| cnu_m354a-400 | SSR | O | A7 |
| cnu_m356a | SSR | × | × |
| cnu_m360a | SSR | O | × |
| cnu_m362a-320 | SSR | O | A5 |
| cnu_m364a | SSR | × | × |
| cnu_m371a | SSR | × | × |
| cnu_m372a | SSR | × | × |
| cnu_m377a-730 | SSR | O | A9 |
| cnu_m379a | SSR | × | × |
| cnu_m384a | SSR | O | × |
| cnu_m396a | SSR | × | × |
| cnu_m397a | SSR | × | × |
| cnu_m398a | SSR | × | × |
| cnu_m400a | SSR | × | × |

**Supplementary Table 2.** Contd**.**

| | | | |
|---|---|---|---|
| cnu_m402a | SSR | × | × |
| cnu_m409a | SSR | × | × |
| cnu_m416a | SSR | O | × |
| cnu_m425a-250 | SSR | O | A5 |
| cnu_m439a | SSR | × | × |
| cnu_m442a | SSR | × | × |
| cnu_m446a-230 | SSR | O | A5 |
| cnu_m457a | SSR | × | × |
| cnu_m458a-380 | SSR | O | A5 |
| cnu_m459a | SSR | × | × |
| hri_mBRMS082a-200 | SSR | O | A2 |
| E039-1000 | ESTP | O | A10 |
| E120-630 | ESTP | O | A8 |
| E129 | ESTP | × | × |
| E138 | ESTP | O | × |
| bg50me10-170 | SRAP | O | A1 |
| em4bg10-180 | SRAP | O | A1 |
| me6bg19-140 | SRAP | O | A1 |
| em16me10-300 | SRAP | O | A1 |
| em3bg17-150 | SRAP | O | A1 |
| em4bg2-580 | SRAP | O | A1 |
| bg2bg21-320 | SRAP | O | A1 |
| em10me7-130 | SRAP | O | A1 |
| em7me5-160 | SRAP | O | A1 |
| em5bg19-340 | SRAP | O | A1 |
| em16me3-220 | SRAP | O | A2 |
| me9bg2-320 | SRAP | O | A2 |
| em10me8-250 | SRAP | O | A2 |
| em9me2-310 | SRAP | O | A2 |
| em7bg20-160 | SRAP | O | A2 |
| me11bg3-250 | SRAP | O | A2 |
| em8bg18-110 | SRAP | O | A2 |
| me1bg16-270 | SRAP | O | A2 |
| em10me4-200 | SRAP | O | A2 |
| em10me5-160 | SRAP | O | A2 |
| me13em16-200 | SRAP | O | A2 |
| me9bg18-140 | SRAP | O | A2 |
| bg19bg50-250 | SRAP | O | A3 |
| em5bg2-190 | SRAP | O | A3 |
| bg50me2-130 | SRAP | O | A3 |
| em7bg19-220 | SRAP | O | A3 |
| em8bg20-240 | SRAP | O | A3 |
| me11bg21-220 | SRAP | O | A3 |
| me6bg20-270 | SRAP | O | A3 |
| em2me2-620 | SRAP | O | A3 |
| em4bg24-590 | SRAP | O | A3 |
| bg12bg19-380 | SRAP | O | A3 |
| me17bg20-300 | SRAP | O | A3 |
| me6bg24-240 | SRAP | O | A4 |
| em9me1-750 | SRAP | O | A4 |
| bg2bg50-320 | SRAP | O | A4 |
| me1bg12-750 | SRAP | O | A4 |

**Supplementary Table 2.** Contd**.**

| | | | |
|---|---|---|---|
| me13em1-100 | SRAP | O | A4 |
| me13em5-340 | SRAP | O | A4 |
| me13bg18-160 | SRAP | O | A4 |
| me10bg18-270 | SRAP | O | A4 |
| bg20bg3-190 | SRAP | O | A4 |
| me3bg12-570 | SRAP | O | A4 |
| bg14bg3-130 | SRAP | O | A4 |
| em4me3-450 | SRAP | O | A4 |
| em2bg17-180 | SRAP | O | A4 |
| em5me12-330 | SRAP | O | A5 |
| me2bg20-240 | SRAP | O | A5 |
| me12atpm1-120 | SRAP | O | A5 |
| me10bg3-180 | SRAP | O | A5 |
| me5bg24-200 | SRAP | O | A5 |
| bg50me12-500 | SRAP | O | A5 |
| bg19bg24-200 | SRAP | O | A5 |
| me13bg21-140 | SRAP | O | A5 |
| em5bg21-340 | SRAP | O | A6 |
| me16bg20-200 | SRAP | O | A6 |
| me16bg16-130 | SRAP | O | A6 |
| me5bg2-150 | SRAP | O | A6 |
| em7bg12-240 | SRAP | O | A6 |
| me12bg24-210 | SRAP | O | A6 |
| me17bg10-110 | SRAP | O | A6 |
| em4bg21-170 | SRAP | O | A6 |
| me12bg19-220 | SRAP | O | A6 |
| me14bg17-200 | SRAP | O | A6 |
| em10me11-370 | SRAP | O | A6 |
| em1bg12-170 | SRAP | O | A6 |
| bg19bg20-170 | SRAP | O | A6 |
| bg2bg24-380 | SRAP | O | A6 |
| me5bg12-240 | SRAP | O | A6 |
| me4bg12-150 | SRAP | O | A6 |
| em8bg10-150 | SRAP | O | A7 |
| me2bg24-260 | SRAP | O | A7 |
| em15me1-570 | SRAP | O | A7 |
| em2bg16-300 | SRAP | O | A7 |
| me12bg2-480 | SRAP | O | A7 |
| em2me1-640 | SRAP | O | A7 |
| em14me4-260 | SRAP | O | A7 |
| bg24bg50-250 | SRAP | O | A7 |
| me14bg14-110 | SRAP | O | A7 |
| me9bg20-310 | SRAP | O | A7 |
| me8bg3-180 | SRAP | O | A7 |
| me13em15-190 | SRAP | O | A7 |
| bg18bg21-380 | SRAP | O | A7 |
| me13em4-240 | SRAP | O | A8 |
| em9me4-240 | SRAP | O | A8 |
| me10bg10-210 | SRAP | O | A8 |
| me9bg10-190 | SRAP | O | A8 |
| em3bg19-110 | SRAP | O | A8 |
| em3me2-120 | SRAP | O | A8 |

**Supplementary Table 2.** Contd**.**

| | | | |
|---|---|---|---|
| me15bg20-320 | SRAP | O | A8 |
| bg20bg21-150 | SRAP | O | A8 |
| me7bg2-100 | SRAP | O | A8 |
| em3bg10-140 | SRAP | O | A9 |
| em5bg17-260 | SRAP | O | A9 |
| em4bg12-150 | SRAP | O | A10 |
| me7bg18-200 | SRAP | O | A10 |
| me10bg14-200 | SRAP | O | A10 |
| me6atpm1-240 | SRAP | O | A10 |
| bg19bg42-250 | SRAP | O | A10 |
| me13em7-250 | SRAP | O | A10 |
| em5me11-370 | SRAP | O | A10 |
| me4bg20-150 | SRAP | O | A10 |
| me4bg20-240 | SRAP | O | A10 |
| e10me10-180 | SRAP | O | A10 |
| bg21bg42-370 | SRAP | O | A10 |
| em2me3-160 | SRAP | O | A10 |
| em3atpm1-150 | SRAP | O | A10 |
| em8bg19-130 | SRAP | O | × |
| me12bg10-240 | SRAP | O | × |
| em1bg10-260 | SRAP | O | × |
| bg12bg50-370 | SRAP | O | × |
| me14bg10-240 | SRAP | O | × |
| me17bg24-330 | SRAP | O | × |
| me5bg12-270 | SRAP | O | × |
| bg18bg20-120 | SRAP | O | × |
| me3bg18-140 | SRAP | O | × |
| me14bg21-190 | SRAP | O | × |
| bg2bg42-270 | SRAP | O | × |
| me1atpm1-160 | SRAP | O | × |
| me11bg18-330 | SRAP | O | × |