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## Genotypic differences in shoot silicon content and the impact on grain arsenic accumulation in rice

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Manuscripts

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3 1 Title Page:  
4 2 Genotypic differences in shoot silicon content and the impact on grain arsenic accumulation  
5 3 in rice  
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7 38 Partha Talukdar<sup>1</sup>, Sue E. Hartley<sup>2</sup>, Anthony J Travis<sup>1</sup>, Adam H. Price<sup>1</sup>, Gareth J. Norton<sup>1\*</sup>  
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25 46 **Abstract:**  
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28 47 Silicon in rice has been demonstrated to be involved in resistance to lodging, tolerance to  
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30 48 both drought and salinity, and also enhances resistance to pests and diseases. The aim of this  
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32 49 study was to determine the range of silicon content in a set of rice (*Oryza sativa* L.)  
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34 50 accessions, and to determine if the natural variation of shoot silicon is linked to the  
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36 51 previously identified silicon transporters (*Lsi* genes). Silicon content was determined in 50  
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38 52 field-grown accessions, representing all sub-populations of rice, with all accessions being  
39  
40 53 genotyped with 700K SNPs. SNPs within 10 kb of the *Lsi* genes were examined to determine  
41  
42 54 if any were significantly linked with the phenotypic variation. An XRF method of silicon  
43  
44 55 determination compared favourably with digestion and colorimetric analysis. There were  
45  
46 56 significant genotypic differences in shoot silicon ranging from 16.5 – 42.4 mg g<sup>-1</sup> of plant dry  
47  
48 57 weight, there was no significant difference between the rice sub-populations. Plants with  
49  
50 58 different alleles for SNPs representing *Lsi2* and *Lsi3* were significantly different for shoot  
51  
52 59 silicon content. Shoot silicon correlated negatively with grain arsenic in the *tropical* and  
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54 60 *temperate japonica* sub-population, suggesting that accessions with high shoot silicon have  
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3 61 reduced grain arsenic. This study indicates that alleles for *Lsi* genes are excellent candidate  
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5 62 genes for further study to explain the natural variation of shoot silicon in rice.  
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10 64 **Key words:** arsenic, natural variation, rice, silicon, XRF  
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## 66 **1 Introduction**

67 Global rice (*Oryza sativa* L.) production needs to increase continuously to ensure the world's  
68 food security (Hibberd et al., 2008). As a beneficial element, silicon alleviates biotic and  
69 abiotic stresses in rice which helps to maintain yield (Ma and Takahashi, 2002; Detmann et  
70 al., 2012; Meharg and Meharg, 2015). Silicon is mainly available as monosilicic acid ranging  
71 from 0.1 to 0.6 mM in the soil solution (Epstein, 1994; Ma and Takahashi, 2002). Previous  
72 studies have demonstrated that monosilicic acid is taken up by rice roots as an undissociated  
73 molecule and translocated into the shoots through the transpiration stream (Takahashi and  
74 Hino, 1978; Mitani-Ueno et al., 2005). It then polymerises on the surface of cells in the shoot  
75 in the form of a silica-cellulose double layer and silica-cuticle double layer. This silica-base  
76 layer improves resistance to lodging, salinity tolerance, drought tolerance, and enhances  
77 resistance to pests and diseases (Takahashi and Hino, 1978; Mitani-Ueno et al., 2005, Chen  
78 et al., 2011; Han et al., 2015).

79 Genetically rice can be classified into two major sub-species, *Japonica* and *Indica* (Chang,  
80 2003) and these have been further classified into 5 sub-populations; *indica*, *aus*, (both *Indica*  
81 sub-species) *tropical japonica*, *temperate japonica*, and *aromatic* (all three *Japonica* sub-  
82 species) (Garris et al., 2005; Zhao et al., 2011). Several previous studies indicate that there  
83 are differences in shoot silicon content between the *Indica* and *Japonica* sub-species of rice.  
84 Deren et al., (1992) showed that *Japonica* sub-species usually have a higher silicon content  
85 than *Indica* rice varieties, based on screening 10 accessions in the greenhouse and 18 under  
86 field conditions. A study conducted by Winslow (1992) revealed that African upland  
87 *Japonica* rice accessions had 50 to 100% higher silicon content in mature flag leaves than  
88 Asian upland *Indica* accessions. In addition to the differences at the subspecies level several  
89 studies have looked at genotypic differences in silicon content, showing ranges of 41 to 60  
90 mg g<sup>-1</sup> (Deren, 2001) and 28 to 61 mg g<sup>-1</sup> (Norton et al., 2010a). Ma et al., (2007a) also

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3 91 observed that silicon uptake by the root and the concentration silicon present in the shoot are  
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5 92 both higher in *Japonica* than *Indica* rice accessions, which they attributed to differences in  
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7 93 the expression of silicon transporter genes.

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9 94 Two types of silicon transporters have been identified in rice to date. A gene  
10  
11 95 (LOC\_Os02g51110) identified for silicic acid influx in rice is classified as an aquaporin  
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13 96 (Low silicon 1 or *Lsi1*) which is a member of the nodulin 26-like intrinsic protein (OsNIP2;  
14  
15 97 1) group of aquaporins (*Ma et al.*, 2006; *Ma et al.*, 2008). A homologue of *Lsi1*, known as  
16  
17 98 *Lsi6* (LOC\_Os06g12310; OsNIP2; 2), responsible for shoot and husk silicon distribution in  
18  
19 99 rice is also classified as an aquaporin (*Yamaji et al.*, 2008). The efflux of silicic acid through  
20  
21 100 the plasma membrane protein known as low silicon 2 (*Lsi2*; LOC\_Os03g01700) is an energy  
22  
23 101 dependent process in rice (*Ma et al.*, 2007b). A homologue of *Lsi2*, known as *Lsi3*  
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25 102 (LOC\_Os10g39980), is also an energy dependent active transporter involved in regulating  
26  
27 103 shoot silicon accumulation in rice (*Yamaji et al.*, 2015).

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31 104 It has been shown that arsenic, classified as a class one carcinogen, can be transported  
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33 105 through silicon transporters in rice (*Ma et al.*, 2008; *Zhao et al.*, 2010; *Mitani-Ueno et al.*,  
34  
35 106 2011). There are two different forms of arsenic present in rice: organic arsenic and inorganic  
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37 107 arsenic (*Williams et al.*, 2005). Organic arsenic is found in rice in two main types of  
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39 108 molecular species dimethylarsinic acid (DMA), and monomethylarsonic acid (MMA) as well  
40  
41 109 as tetramethylarsonium (*Williams et al.*, 2005; *Hansen et al.*, 2011). Inorganic arsenic is  
42  
43 110 found in rice as two molecular species; arsenate and arsenite (*Abedin et al.*, 2002; *Williams et*  
44  
45 111 *al.*, 2005). Arsenate is an analogue of phosphate and is taken up via phosphate transporters  
46  
47 112 while arsenite is taken up by silicic acid transporters in rice (*Abedin et al.*, 2002; *Ma et al.*,  
48  
49 113 2008). It has been shown that the silicon transporters *Lsi1*, *Lsi2* and *Lsi6* are also arsenic  
50  
51 114 transporters, using a combination of mutants and transgenic lines (*Ma et al.*, 2008; *Zhao et*  
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53 115 *al.*, 2010; *Mitani-Ueno et al.*, 2011). Several studies indicate that anaerobic rice cultivation  
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3 116 leads to increased mobilisation of soil arsenic in the form of arsenite, which causes  
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5 117 anaerobically-grown rice to accumulate more arsenic through silicon transporters (*Ma et al.*,  
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7 118 2008; *Xu et al.*, 2008; *Carey et al.*, 2010). Silicon fertilisation has also been shown to  
8  
9 119 decrease shoot and grain arsenic indicating that silicon could play an important role in  
10  
11 120 decreasing total arsenic uptake in rice (*Li et al.*, 2009; *Seyfferth and Ferdorf*, 2012).

13  
14 121 This study was designed to address four questions all related to the process of silicon and  
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16 122 arsenic accumulation in rice: How does the cultivation method affect silicon distribution in  
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18 123 different organs of rice plants? Are there significant genotypic differences in shoot silicon  
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20 124 concentration across a diverse panel of rice related to the 5 different sub-populations of rice?  
21  
22 125 Is there a relationship between natural variation in shoot silicon and arsenic content in rice?  
23  
24 126 Can natural variation in shoot silicon be linked to known silicon transporters in rice? The  
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26 127 results provide a deeper understanding of the natural variation in silicon content across rice  
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28 128 accessions and its relationship to arsenic accumulation in rice grains.  
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## 129 **2 Materials and Methods**

### 130 **2.1 Silicon content in different organs of rice (*Oryza sativa* L.) grown in flooded and** 131 **non-flooded conditions**

132 An experiment was conducted in a greenhouse at the University of Aberdeen, UK under both  
133 flooded and non-flooded conditions with four replicates for each treatment. One litre plastic  
134 pots were filled with soil (~530 g soil described in *Norton et al.*, 2013). For the flooded  
135 condition, a plastic liner was used to line the pots and hold the water within the pot whereas  
136 the non-flooded pots were kept without a liner to allow drainage of water through the pot.  
137 Five Italice Carolina (*temperate japonica*) seeds were sown in each pot, then thinned to one  
138 plant in each pot after two weeks. To maintain the flooded condition, tap water from the  
139 greenhouse was used to flood the pots to 2 cm above the soil surface when plants were 3  
140 weeks old. Every two weeks during the first four weeks of growth 100 mL of half strength  
141 Yoshida's nutrient solution was added (*Yoshida et al.*, 1976). The dose of Yoshida's nutrient  
142 solution was increased up to 100 mL of full strength solution every week after four weeks  
143 and continued until the filled grains had matured when samples were harvested.

144 At harvest, samples were collected from different parts of the mature plants: root, 3<sup>rd</sup> sheath,  
145 3<sup>rd</sup> node, 3<sup>rd</sup> leaf, 2<sup>nd</sup> sheath, 2<sup>nd</sup> node, 2<sup>nd</sup> leaf, flag sheath, 1<sup>st</sup> node, flag leaf, husk and  
146 unpolished grains. The sheath, node and leaves were taken from the main tiller, with the most  
147 recent leaf prior to the flag leaf designated 2<sup>nd</sup> leaf. Root samples were washed thoroughly  
148 with tap water followed by deionised water and confirmed to be free of soil particles by  
149 examining the roots under a microscope (Leica MZ8, 10445932, 16×/14B, PLAN 1.0X).  
150 Samples were dried at 80°C for 5 days until a constant weight was achieved. All samples  
151 were mixed and subsampled prior to being ball-milled. The silicon content was determined  
152 by Flow Injection Analyser (FIA) after alkali digestion.

## 153 **2.2 Genotypic differences in shoot silicon content of rice**

154 Seeds were obtained from the Rice Diversity Panel 1 (RDP1) (<http://ricediversity.org/>) (Ali et  
155 al., 2011; Eizenga et al., 2014). The classification of Zhao et al., (2011) was used for the sub-  
156 population classification of rice accessions. In 2009 a total of 312 accessions were cultivated  
157 at the experimental site in Bangladesh. Seedlings were transplanted by hand in a single 2m  
158 row of 10 hills, each hill (one seedling) 20 cm apart and each row 20 cm apart in a  
159 randomised complete block design with four replicates of each accession. The experimental  
160 site was kept flooded until the grains were filled. Plant material from the central six plants  
161 was harvested and used for chemical analysis. Detailed information about the experimental  
162 site and experimental methods are described in Norton et al., (2012). For shoot silicon  
163 analysis, fifty accessions (10 accessions from each rice sub-population) were randomly  
164 selected based on the initial sub-population assignment using single sequence repeat (SSR)  
165 markers (Ali et al., 2011) (Supplementary Table 1). Subsequently, after selection and silicon  
166 analysis, these accessions have been assigned to sub-populations based on the 700K SNP data  
167 (McCouch et al., 2016), these sub-population assignments are used for classification of the  
168 accessions in this study.

## 169 **2.3 Analysis of rice shoot silicon by FIA**

170 Plant material and certified reference material (CRM) were prepared for silicon analysis as  
171 described by Carneiro et al., (2007). A total of 1.5 g shoot material from each sample was  
172 sub-sampled at random and powderised using a ball mill (Retsch, MM200, Germany). From  
173 the powderised plant material, a sub-sample of 20 mg was weighed into a 50 mL  
174 polyethylene centrifuge tube (CORNING<sup>®</sup>, NY). To digest the sample, 0.6 mL of hydrogen  
175 peroxide (H<sub>2</sub>O<sub>2</sub>, > 30% W/V, Fisher Scientific) and 1.5 mL of sodium hydroxide (NaOH,  
176 solutions 50%, Fluka) were added and the samples were then vortexed (mixed using a vortex  
177 mixer). The samples were heated for 1 hour at 90°C in a water bath, then vortexed again and

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3 178 left overnight. The tubes were vortexed again after overnight extraction, then heated at 123°C  
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5 179 under a pressure of 0.15 MPa for 1 h. Samples were kept at room temperature for 2 h then  
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7 180 vortexed, followed by addition of 18.5 mL of ddH<sub>2</sub>O. Prior to analysis, samples were diluted  
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9 181 1:5 with Milli-Q water. Silicon content was measured using an FIA spectrophotometer  
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11 182 (Tecator FIAstar 5010) a wavelength of 410 nm (*Carneiro et al., 2007; Norton et al., 2010a;*  
12  
13 183 *Norton et al., 2010b*).

#### 16 184 **2.4 Analysis of rice shoot silicon by P-XRF**

18 185 A total of 1.5 g of dried shoot material for each rice accession was sub-sampled at random  
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20  
21 186 and powdered using a ball mill (Retsch, MM200, Germany). To perform the analysis of shoot  
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23 187 silicon by P-XRF, 19 accessions were selected at random from the 50 accessions for which  
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25 188 shoot silicon had been determined by FIA. For P-XRF analysis, 0.7 g of homogeneous  
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27 189 powder sample was compressed into 13 mm diameter pellets using a manual hydraulic press  
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29 190 with a 13mm die at a pressure of 10 tons (Specac, Orpington, United Kingdom). Shoot silicon  
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31 191 content (% of silicon dry shoot weight) was measured using a commercial P-XRF instrument  
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33 192 (Niton XL3t900 GOLDD analyzer: Thermo Scientific Winchester, UK), calibrated using Si-  
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35 193 spiked synthetic methyl cellulose and validated using Certified Reference Materials of NCS  
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37 194 DC73349 ‘Bush branches and leaves’ obtained from the China National Analysis Center for  
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39 195 Iron and Steel, as described in *Reidinger et al., (2012)*. The mean value of samples for each  
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41 196 accession was used for correlation analysis between P-XRF and FIA measurements.  
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#### 45 197 **2.5 Relationship between silicon and arsenic content in rice**

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47 198 The plant material used in this study was previously examined for grain arsenic content  
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49 199 (*Norton et al., 2012*) which provided an opportunity to examine the relationship between  
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51 200 shoot silicon and grain arsenic in rice. The relationship between shoot silicon (log  
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3 201 transformed) and grain arsenic (log transformed) was investigated for the 50 rice accessions  
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5 202 based on accession means.  
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## 7 203 **2.6 Single Marker Analysis**

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10 204 The accessions used in this study have been genotyped using a high-density SNP chip  
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12 205 (*McCouch et al., 2016*). SNPs for the accessions were extracted using PLINK (*Purcell et al.,*  
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14 206 *2007*). SNPs were extracted from 10 kb upstream of the start codon to 10 kb downstream of  
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16 207 the stop codon of the *Lsi1*, *Lsi2*, *Lsi3*, and *Lsi6* loci. SNPs were excluded from the analysis if  
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18 208 they were invariant or if minor alleles were present in less than 5% of the accessions. The  
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20 209 RDP1 population has a high degree of stratification by rice sub-population (*Zhao et al., 2011;*  
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22 210 *McCouch et al., 2016*). To overcome this stratification, sub-population assignment was used  
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24 211 (based on the 700 K SNP data; *McCouch et al., 2016*) as a factor in a two-way ANOVA, with  
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26 212 SNP base call as the other factor. The two-way ANOVA was used to determine if the  
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28 213 phenotype for the accession was significantly different for each SNP tested.  
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## 31 214 **2.7 Sequence alignments**

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35 215 Based on the result achieved from the single-marker analysis the sequence diversity of *Lsi2*  
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37 216 and *Lsi3* were investigated for 5 cultivars using BAM files produced after aligning sequence  
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39 217 reads against Nipponbare version 7 reference genome. The genome sequences of the cultivars  
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41 218 used in this study have been previously published (*Kawahara et al., 2013; Cardoso et al.,*  
42  
43 219 *2014; Schatz et al., 2014*). The accessions were from the following sub-populations; 2 *indica*  
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45 220 accessions (IR64 and Bala), 1 *aus* accession (DJ123) and 2 *tropical japonica* accessions  
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47 221 (Azucena and Nipponbare).The genomic DNA sequence was visualised using the IGV  
48  
49 222 (<https://www.broadinstitute.org/igv/>) to identify the difference of genomic DNA sequence  
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51 223 within *Lsi2* and *Lsi3* in 5 cultivars (*Thorvaldsdóttir et al., 2013; Robinson et al., 2011*). Using  
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3 224 Clustal Omega the DNA sequences of 5 cultivars were aligned for *Lsi2* and *Lsi3* separately  
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5 225 and showed in supplementary figure 3 and 4 respectively (*Sievers et al.*, 2011).  
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## 8 226 **2.8 Statistical analysis**

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10 227 Statistical significance was set at  $P < 0.05$  for all analyses, which were performed using  
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12 228 Minitab 16. The normality of distribution and homogeneity of variance of the data were  
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14 229 tested prior to one or two-way analysis of variance (ANOVA), as appropriate. Pearson  
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16 230 correlation analysis was used to investigate the relationship between measurements of shoot  
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19 231 silicon and grain arsenic.  
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## 232 **3 Results**

### 233 **3.1 Shoot silicon content in different organs of rice plants**

234 Flooding increased plant silicon content in the flag sheath, 1<sup>st</sup> node, flag leaf and husk  
235 compared to plants grown under non-flooded conditions (Fig. 1). The content of silicon in  
236 grain and root tissues were significantly lower than any in other organs of plants grown under  
237 either condition. There was a significant difference ( $P < 0.001$ ,  $F = 27.40$ ,  $R^2 = 78.20\%$ ) of  
238 silicon content between different organs of the plant in non-flooded conditions: The highest  
239 mean content was in the husks ( $46.8 \text{ mg g}^{-1}$ ), while the lowest was in the grain ( $3.5 \text{ mg g}^{-1}$ ).  
240 For plants grown under flooded conditions: The highest silicon content was observed in the  
241 flag leaf ( $67.3 \text{ mg g}^{-1}$ ) and the lowest was in the grains ( $4.4 \text{ mg g}^{-1}$ ).

### 242 **3.2 Genotypic difference in shoot silicon content of rice**

243 Fifty diverse rice accessions were examined by FIA to determine the difference in shoot  
244 silicon content of rice. There was a significant genotypic difference in shoot silicon content  
245 among the 50 accessions, where genotype explained 55% of the variation ( $P < 0.001$ ;  $F =$   
246  $5.80$ ;  $R^2 = 55.30\%$ ;  $df = 49$ ). The mean shoot silicon content of the 50 accessions was  $28.1$   
247  $\text{mg g}^{-1}$ , and the lowest mean shoot silicon was observed in Dhala Shita ( $16.5 \text{ mg g}^{-1}$ ) The  
248 highest mean shoot silicon was observed in Bala ( $42.4 \text{ mg g}^{-1}$ ) (Fig. 2). There was no  
249 significant difference for shoot silicon content of the 5-major rice sub-populations (Fig. 3).

250 Nineteen rice accessions were selected at random from the 50 accessions analysed by FIA,  
251 for measurement of shoot silicon content by P-XRF. The silicon content of four individual  
252 field grown replicates of each accession were measured separately by P-XRF and FIA and the  
253 mean value of each accession was used for correlation analysis. Using both methods,  
254 genotypic differences were observed between the accessions ( $P < 0.001$ ;  $F = 9.90$ ;  $df = 18$  for  
255 P-XRF;  $P < 0.001$ ;  $F = 7.30$ ;  $df = 18$  for FIA). Correlation analysis indicated that there was a

256 significant and large positive correlation between the two methods ( $r = 0.95$ ;  $P < 0.001$ ;  $df =$   
257 18) (Fig 4).

### 258 **3.3 Correlation between shoot silicon and grain arsenic in rice**

259 No significant correlation was observed between mean shoot silicon and mean shoot arsenic  
260 for all of the 50 accessions (supplementary Figure 1), or for within each of the 5 sub-  
261 populations. There was a weak negative correlation ( $r = -0.31$ ;  $P = 0.028$ ;  $df = 49$ )  
262 (supplementary Figure 2) between shoot silicon and grain arsenic content for all 50  
263 accessions. When correlation analysis was conducted separately for shoot silicon and grain  
264 arsenic on each of the sub-populations, significant negative correlations were found for the  
265 *temperate japonica* ( $r = -0.78$ ;  $P = 0.007$ ;  $df = 9$ ) and *tropical japonica* ( $r = -0.84$ ;  $P = 0.002$ ;  
266  $df = 9$ ) accessions (Fig. 5). No significant correlations were observed for the other 3 major  
267 rice sub-populations (*indica*, *aus* and *aromatic*).

### 268 **3.4 Testing accessions with different alleles of SNPs around and within *Lsi* genes for** 269 **variation in shoot silicon concentration**

270 A total of 10 SNPs from the SNP database are within 10 kb upstream and downstream of the  
271 *Lsi2* gene (selected SNPs for the rice accessions are presented in supplementary Table 2).  
272 Shoot silicon concentration for accessions with the different alleles for two of these SNPs  
273 was significantly different. SNP-3.434426 is located 2551 bp before the start codon, and  
274 revealed a significant difference between the C and T polymorphism ( $P = 0.006$ ), where the  
275 mean silicon content of accessions with the C allele was  $29.3 \text{ mg g}^{-1}$  while the mean silicon  
276 content of the accessions with the T allele was  $23.1 \text{ mg g}^{-1}$ . SNP-3.438416 is located 6541 bp  
277 before the start codon and revealed a significant difference between the A and C  
278 polymorphism ( $P = 0.008$ ), where the mean silicon content of the accessions with the A allele  
279 was  $29.6 \text{ mg g}^{-1}$  while the silicon content of the accessions with the G allele had a mean of

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3 280 23.1 mg g<sup>-1</sup>. Both SNPs group the accessions in a similar way, the only difference was more  
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5 281 missing SNP information for SNP-3.438416 (Fig. 6).  
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8 282 A total of 20 SNPs from the SNP database are within 10 kb upstream and downstream of the  
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10 283 *Lsi3* gene (selected SNPs for the rice accessions are presented in supplementary Table 3).  
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12 284 Shoot silicon concentration for accessions with the different alleles for one of these SNPs was  
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14 285 significantly different. SNP- 10.21340470 is located 5299 bp prior to the start codon, and  
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16 286 revealed a significant difference between the G and A polymorphism ( $P = 0.016$ ), where the  
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18 287 mean silicon content of accessions with the G allele was 28.4 mg g<sup>-1</sup> while the mean silicon  
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20 288 content of the accessions with the A allele was 35.6 mg g<sup>-1</sup> (Fig. 6).  
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23 289 There were 20 SNPs and 19 SNPs observed within 10 kb of *Lsi1* and *Lsi6* respectively.  
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25 290 However, at each of these SNPs the different alleles were not significantly different for shoot  
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27 291 silicon concentration.  
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30 292 To explore further, the sequence alignments of *Lsi2* and *Lsi3* were performed using available  
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32 293 high-quality genome sequences. The accessions used were Nipponbare, Azucena, IR64, Bala,  
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34 294 and DJ123 which are from the *tropical japonica*, *tropical japonica*, *indica*, *indica* and *aus*  
35  
36 295 rice subgroups respectively. From the sequence analyses of *Lsi2* and *Lsi3* a number of  
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38 296 polymorphisms within the genes were identified. For *Lsi2*, there was a synonymous SNP  
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40 297 substitution within the first exon, where DJ123 has “C” allele while the other 4 accessions  
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42 298 have “T” allele (Supplementary Figure 3). For *Lsi3*, 4 SNPs were detected in exons and 6  
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44 299 SNPs in introns (Supplementary Figure 4). There was only one non-synonymous SNP  
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46 300 observed in the first exon of *Lsi3* where DJ123 and Bala have “T” allele and other accessions  
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48 301 have “A” allele. The available 3000 rice genome sequence data indicates that this  
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50 302 polymorphism between “A” and “T” in *Lsi3* is associated with the *aus* sub-population in rice  
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52 303 where 15 accessions have “A” allele and 184 accessions have “T” allele (Alexandrov et al.,  
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3 304 2015). This non-synonymous polymorphism between “A” and “T” in *Lsi3* with the “T” allele  
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5 305 is very rarely observed in *indica* and *japonica* subpopulations of rice in 3000 rice genome  
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7 306 sequence data (*Alexandrov et al.*, 2015).  
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## 308 4 Discussion

309 In this study, genotypic differences in shoot silicon content were identified from field grown  
310 rice cultivars. However, no differences in shoot silicon were observed across the 5 different  
311 sub-populations of rice. Additionally, SNPs detected in the accessions were significantly  
312 linked to known silicon transporter genes in rice, which indicates that these genes are  
313 potentially involved in natural variation of silicon accumulation in rice.

314 Flooded conditions increased silicon content in the upper part of the plant (flag sheath, 1<sup>st</sup>  
315 node, flag leaf and husk) compared to the non-flooded conditions, which suggests that the  
316 uptake or translocation of shoot silicon into these plant organs might be controlled by  
317 different processes (compared to those determining silicon uptake in other tissues) which  
318 differ between aerobic and anaerobic conditions. It has been shown that silicon dissolution  
319 and bio-availability plays a significant role in the variation of silicon content in grasses  
320 (Quigley et al., 2017). Therefore, the difference in dissolved silicon in flooded and non-  
321 flooded conditions might affect the accumulation of silicon in the rice plants used in this  
322 study. It was also notable that there was no significant difference in silicon content in  
323 different tissues between the internodes (e.g. flag leaf, 2<sup>nd</sup> leaf) in non-flooded conditions but  
324 there was a significant difference between the silicon content of internodes under flooded  
325 conditions (Fig. 1). Previous studies have shown that transpiration is one of the most  
326 important factors responsible for higher *silicification* in plants and that transpirational flow is  
327 higher in anaerobic conditions than in aerobic ones (Mitani-Ueno et al., 2005; Kato and  
328 Okami, 2011; Kumar et al., 2017; McLarnon et al., 2017). Therefore, one potential  
329 explanation for increased silicon accumulation in the upper organs or developing organs of  
330 rice plants (e.g. flag sheath, 1<sup>st</sup> node, flag leaf and husk) grown in flooded soils is a higher  
331 transpirational flow in these plants. Importantly, the data presented in figure 1 shows that  
332 tissue silicon content is reasonably evenly distributed across tissues with only that from

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3 333 flooded plants in tissue associated with flowering and seed production significantly higher  
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5 334 than the rest. Since this reproductive tissue was removed from the field samples used in this  
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7 335 study we can be reassured that a mean value obtained from straw will be a good estimate of  
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9 336 the tissue concentration of the majority of rice plant.

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12 337 Fifty accessions from 5 different sub-populations (10 accessions from each sub-population)  
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14 338 were selected at random to examine the difference of shoot silicon content in rice and this  
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16 339 revealed highly significant differences of shoot silicon content. A genotypic difference in  
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18 340 shoot silicon content across a wide group of accessions has been observed previously (*Deren,*  
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20 341 2001; *Norton et al., 2010a*). The 2.6-fold difference of shoot silicon content in this study is  
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22 342 similar to the previous 2.2 fold range detected for genotypic differences of shoot silicon  
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24 343 content in rice (*Norton et al., 2010a*). However, the maximum value observed in our study is  
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26 344 slightly lower than that detected previously (42.4 mg g<sup>-1</sup> in this study, 60 mg g<sup>-1</sup> (*Deren,*  
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28 345 2001), 61 mg g<sup>-1</sup> (*Norton et al., 2010a*).

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31 346 The plant material used for determination of shoot silicon content in the 50 rice accessions  
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33 347 was grown in flooded, irrigated conditions (*Norton et al., 2012*). Previous studies estimated  
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35 348 that 27% - 44% of the silicon taken up by rice plants is supplied by irrigation, while the  
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37 349 remaining percentage must be supplied by soil constituents (*Desplanques et al., 2006;*  
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39 350 *Klotzbücher et al., 2015*). All the accessions tested in this study had a silicon content below  
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41 351 50 mg g<sup>-1</sup> which, according to *Dobermann and Fairhurst (2000)*, is below the critical level of  
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43 352 mineral deficiency for rice production. The low shoot silicon content (16.5 mg g<sup>-1</sup> to 42.4 mg  
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45 353 g<sup>-1</sup>) observed in this study may be due to removal of rice straw from the paddy field, which is  
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47 354 common practice in Bangladesh, and has been shown to contribute to lower shoot silicon in  
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49 355 the subsequent rice crop (*Seyfferth et al., 2013*). Future work should focus on linking the  
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51 356 flooded and non-flooded pot based experiment and the removal of straw at the field scale to  
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3 357 establish the importance of water management and field management on silicon accumulation  
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5 358 in field grown rice.

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7 359 Several studies have demonstrated that the *Japonica* sub-species of rice have higher shoot  
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9 360 silicon than *Indicas* (Winslow, 1992; Winslow et al., 1997; Ma et al., 2007a). These studies  
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11 361 may have been limited by the number of accessions that were screened. For example, Ma et  
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13 362 al., (2007b) only screened two rice accessions to examine the genotypic difference in silicon  
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15 363 uptake of rice. To improve the current understanding of silicon biology in rice, we  
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17 364 investigated field grown shoot samples of 50 rice accessions across 5 sub-populations. Within  
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19 365 the material tested in this study the data suggests that the natural variation observed in shoot  
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21 366 silicon is not governed by genetic differences between rice sub-populations, but rather is  
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23 367 largely due to the genetic differences within individual sub-groups.

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26 368 Data on more than 50 accessions would have opened the opportunity to conduct genome-  
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28 369 wide association (GWA) mapping where 200 accessions is considered a lower limit.  
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30 370 However, the FIA colorimetric method for the determination of silicon in rice shoots proved  
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32 371 not to be high throughput. However, in addition to the FIA method, a sub-set of samples  
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34 372 were also analysed by P-XRF. The two different methods were strongly correlated, but not  
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36 373 perfectly, and indicated that values for silicon content in samples measured by FIA were  
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38 374 slightly higher than those measured by P-XRF. The observation that both methods provide  
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40 375 comparable results highlights the conclusion that P-XRF can be used for silicon analysis to  
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42 376 detect and measure genotypic differences across populations, instead of the more laborious  
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44 377 and time-consuming alkali digestion method. Furthermore, a second advantage of P-XRF is  
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46 378 that it is a non-destructive method. This would make it much more suitable for future GWA  
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48 379 mapping studies.

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51 380 The plant material used in this study was previously used to examine the variation of shoot  
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53 381 and grain arsenic (Norton et al., 2012). The comparison of shoot silicon and grain arsenic in  
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3 382 this study is in agreement with previous studies where, in general, plants that had high shoot  
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5 383 silicon also had lower grain arsenic (*Seyfferth and Ferdorf, 2012; Norton et al., 2012; Norton*  
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7 384 *et al., 2013*). However, this study also adds more insight by taking into consideration the sub-  
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9 385 population structure of rice cultivars. The correlation between shoot silicon and grain arsenic  
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11 386 was sub-population specific. A strong relationship in between shoot silicon and grain arsenic  
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13 387 was observed in *temperate japonica* and a weaker one in *tropical japonica*, but was not  
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15 388 observed in *indica*, *aus* or *aromatic*. This important observation suggests that the genetic  
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17 389 regulation of arsenic content in rice grain is different in *temperate* and *tropical japonicas*  
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19 390 compared to the other rice sub-populations, implying that the silicon-transport-linked  
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21 391 pathway implicated for arsenic accumulation (*Ma et al., 2007b; Norton et al., 2012*) may be  
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23 392 less relevant in the *other* sub-populations.  
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27 393 The accessions used in the study have been genotyped using a 700K SNP chip (*McCouch et*  
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29 394 *al., 2016*). Single-marker analysis was used to test the candidacy of the known transporters of  
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31 395 silica in rice. The study indicated that two SNPs within 10 kb of *Lsi2* and one within 10 kb  
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33 396 of *Lsi3* were involved in contributing to the natural variation of shoot silicon accumulation in  
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35 397 rice (Fig. 6). The *Lsi2* gene has been shown to be pivotal for transport of silicon and  
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37 398 inorganic arsenic in studies conducted with mutants and transgenic plants (*Ma et al., 2006;*  
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39 399 *Ma et al., 2007b; Yamaji et al., 2008; Mitani-Ueno et al., 2011; Yamaji et al., 2015*). The  
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41 400 identification of differences in shoot silicon and the link with three SNPs close to the genes  
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43 401 further suggest that *Lsi2* and *Lsi3* are excellent candidate genes to explain the natural  
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45 402 variation observed in shoot silicon content of rice. When looking at the sequencing variation  
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47 403 of a number of diverse cultivars (which have been sequenced to a high depth) it is evident  
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49 404 that there is only a small number of polymorphisms within the genes (Supplementary figures  
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51 405 3 and 4). The highly conserved sequence for *Lsi2* may be due to its importance function for  
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53 406 silicon accumulation in rice. However, the accessions screened in this study are likely to have  
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3 407 greater sequence variation than the cultivars for which high quality sequence is available, and  
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5 408 therefore there may be greater sequence variation for *Lsi2* (and the other *Lsi* genes) than that  
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7 409 is represented in the 5 accessions reported here. A focus for future study will be to expand  
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9 410 sequence information to more accessions to more fully explore sequence variation associated  
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11 411 with the polymorphic SNPs presented in figure 6.  
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## 17 413 **5 Conclusion**

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20 414 This study has demonstrated strong genotypic differences in shoot silicon in a diverse  
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22 415 collection of rice cultivars, showing that there is potential to breed rice with increased silicon  
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24 416 content that could improve resistance to both biotic and abiotic stresses in rice, which would  
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26 417 help to maintain crop yields. The identification of significant SNPs linked with the shoot  
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28 418 silicon phenotype within 10 kb of known silicon transporters warrants further study to  
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30 419 investigate the impact of different alleles of these genes on silicon and arsenic accumulation  
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32 420 in rice. Furthermore, the XRF method of silicon determination could be applied to GWA  
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34 421 mapping studies that might reveal further candidate genes for silicon content in rice.  
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3 647 **Figure 1:** Silicon in different organs of rice (bars are the mean of four replicates and error  
4 648 bars represent standard error of the mean). Letters above the columns (upper-case = anaerobic  
5 649 and lower case = aerobic) indicate statistically significant differences in silicon content of  
6 650 different plant organs using Tukey's test in two conditions. \*denotes a significant difference  
7 651 between the two treatments for that plant organ.  
8 652

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10 653 **Figure 2:** Mean shoot silicon ( $\text{mg g}^{-1}$ ) of 50 rice accessions determined by FIA. Different  
11 654 symbols refer to the accessions belonging to the different sub-populations; circle = *aus*,  
12 655 square = *indica*, cross = *aromatic*, triangle = *tropical japonica*, upside down triangle =  
13 656 *temperate japonica*. Error bars indicate the standard error of the mean ( $n = 4$ ).

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15 657 **Figure 3:** Shoot silicon ( $\text{mg g}^{-1}$ ) content of 50 accessions in 5 different sub-populations of  
16 658 rice. ARO = *aromatic*, AUS = *aus*, IND = *indica*, TEJ = *temperate japonica* and TRJ =  
17 659 *tropical japonica*. The edges of each box show the upper and lower quantile and the bold line  
18 660 in the box shows the median value and the dotted line the mean value. The whiskers are the  
19 661 10<sup>th</sup> and 90<sup>th</sup> percentiles.

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21 662 **Figure 4:** Correlation of mean shoot silicon in 19 rice accessions determined by FIA and P-  
22 663 XRF. Error bars indicate the standard error of the mean ( $n = 4$ ). Dotted line is the 1:1 line.

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24 664 **Figure 5:** Correlation between shoot silicon ( $\text{mg g}^{-1}$ ) and grain arsenic ( $\mu\text{g kg}^{-1}$ ) in ARO=  
25 665 *aromatic*, AUS = *aus*, IND = *indica*, TEJ = *temperate japonica* and TRJ = *tropical japonica*  
26 666 subpopulations.

27 667 **Figure 6:** Variation in shoot silicon ( $\text{mg g}^{-1}$ ) between different SNPs within 10 kb of *Lsi2* and  
28 668 *Lsi3*

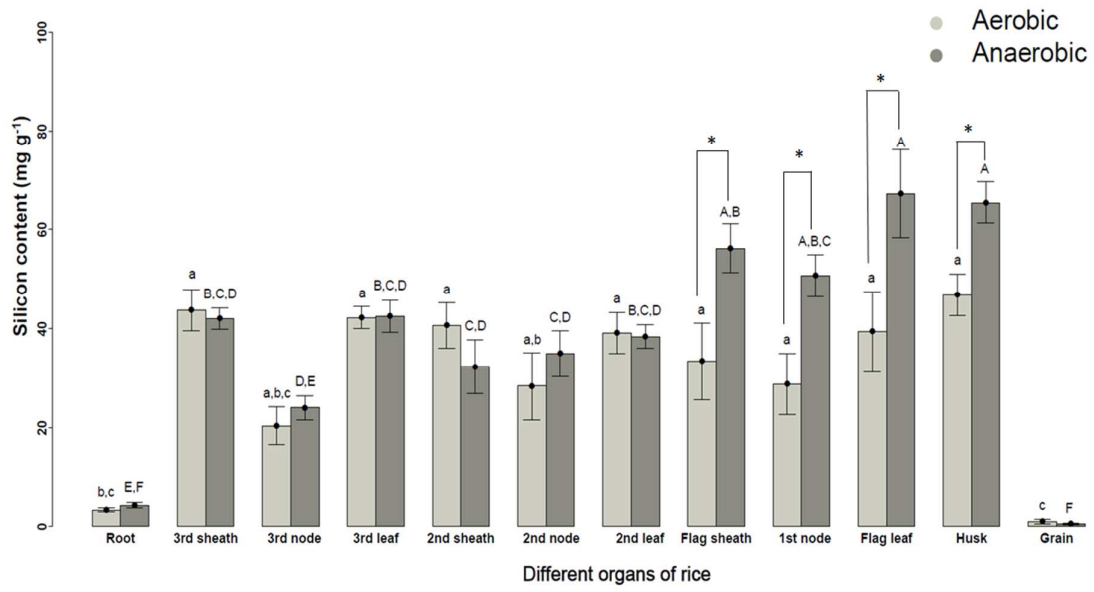
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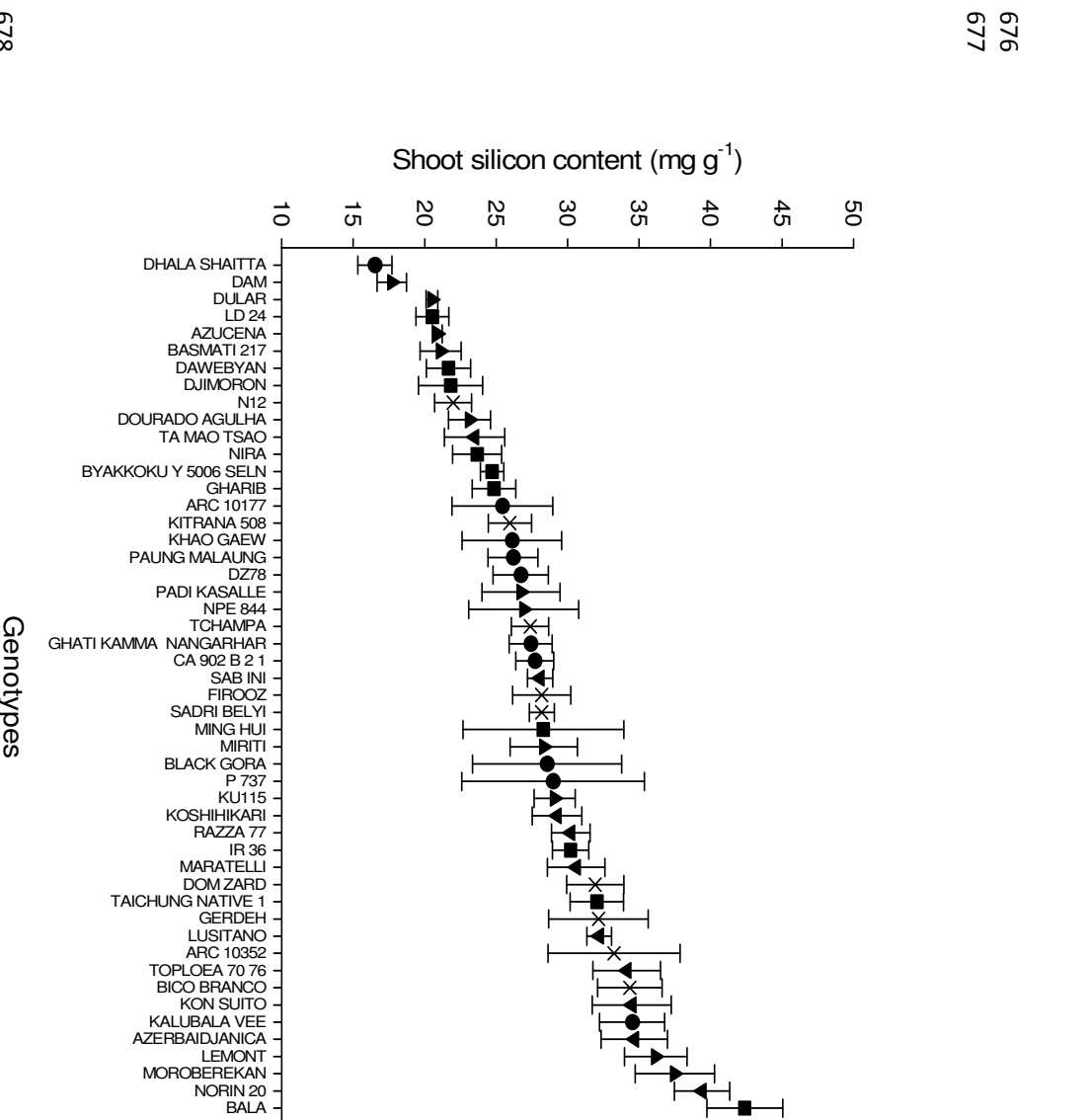
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675 **Figure 1**

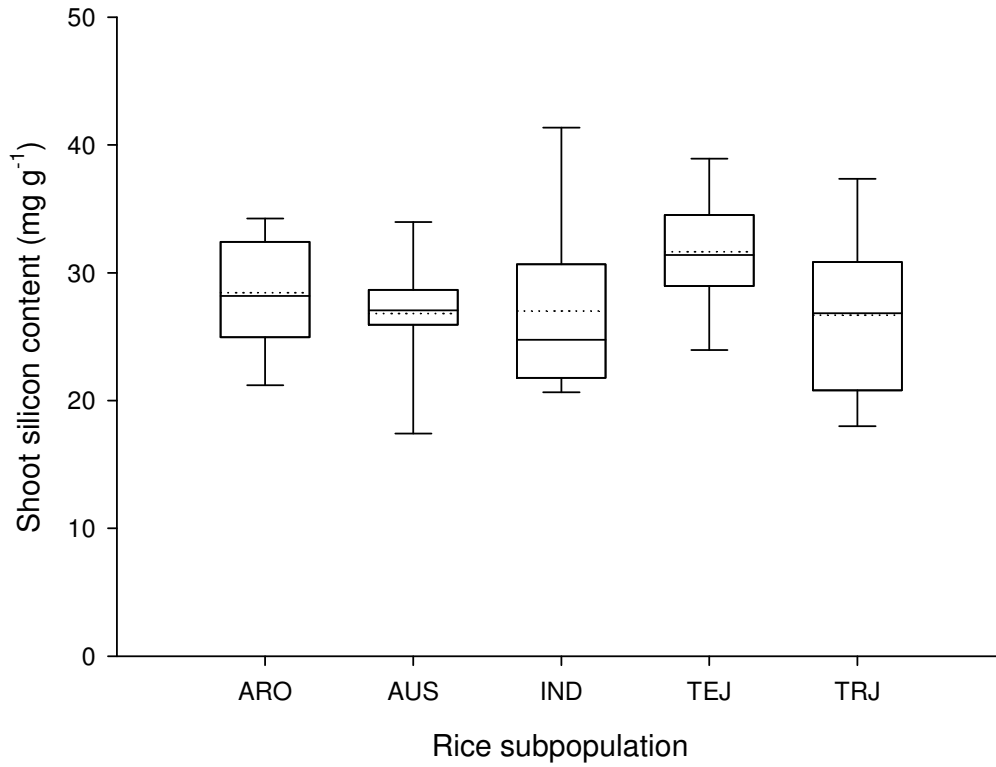


679 **Figure 2**

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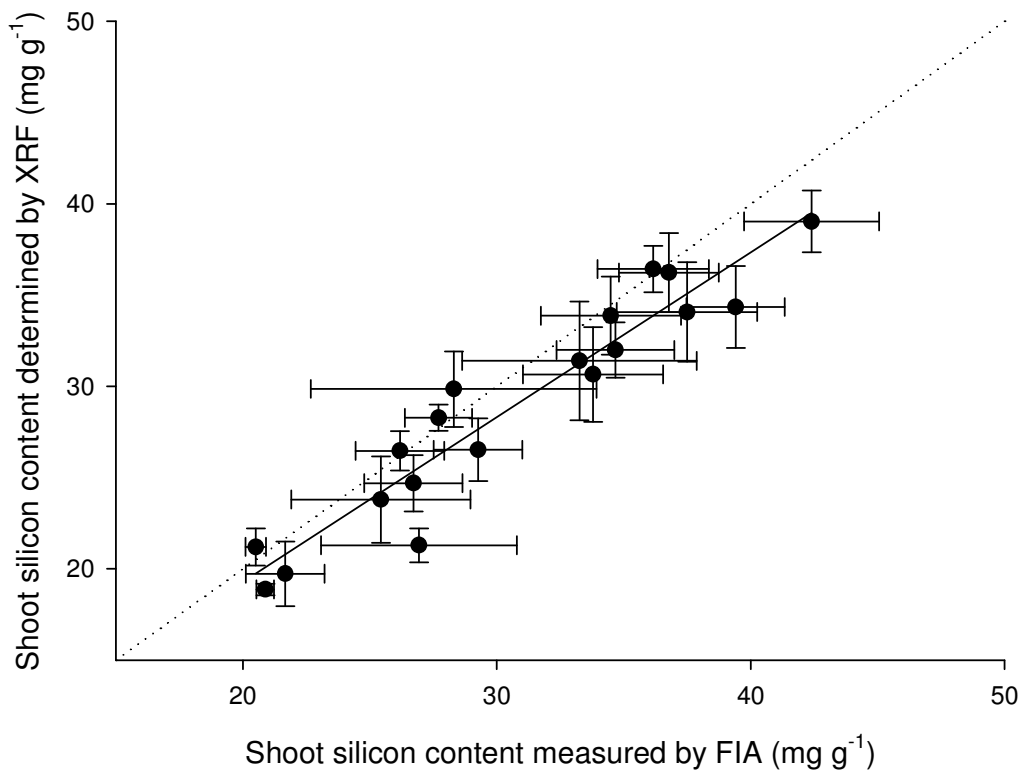


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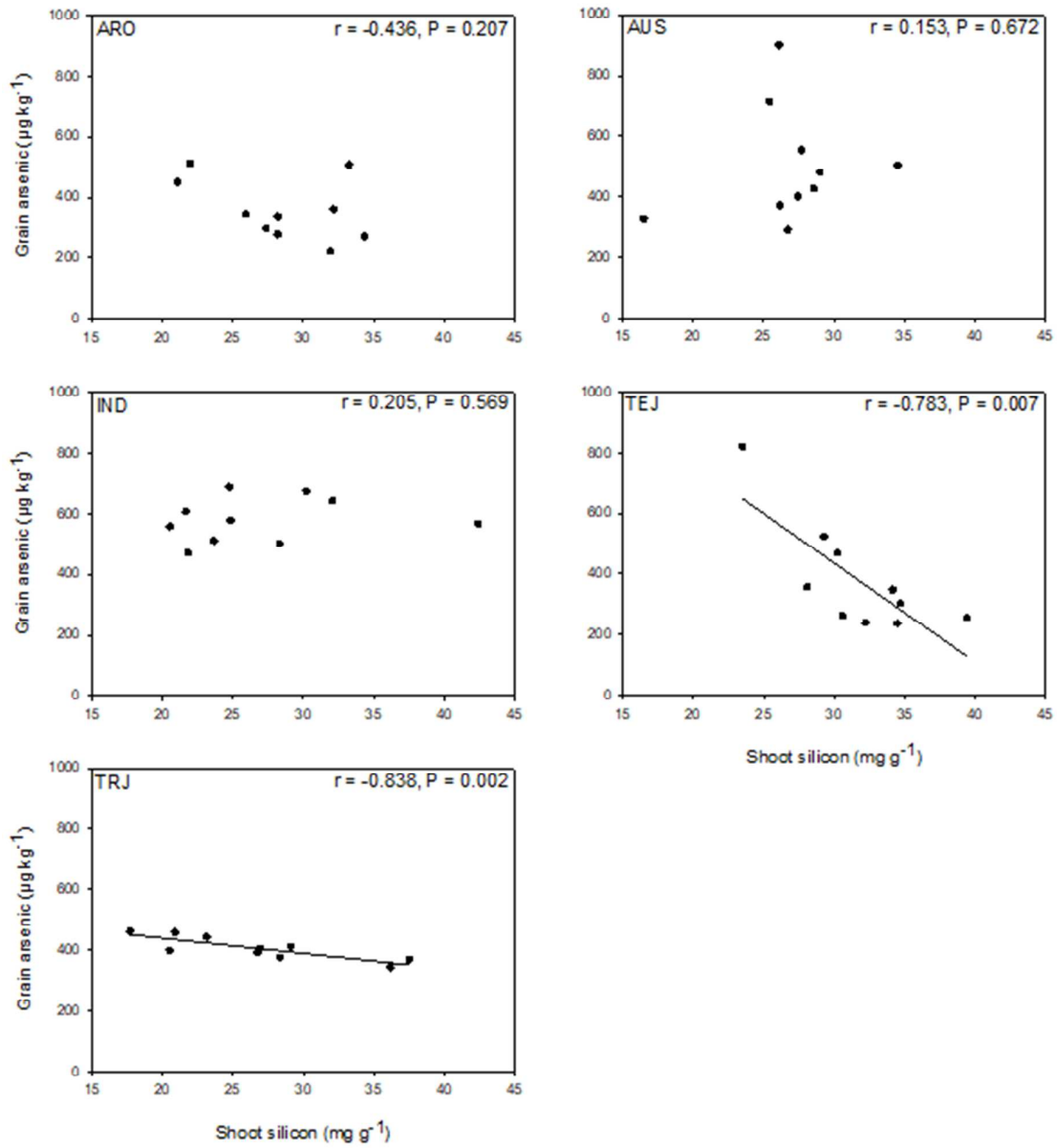
682 **Figure 3**

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685 **Figure 4**

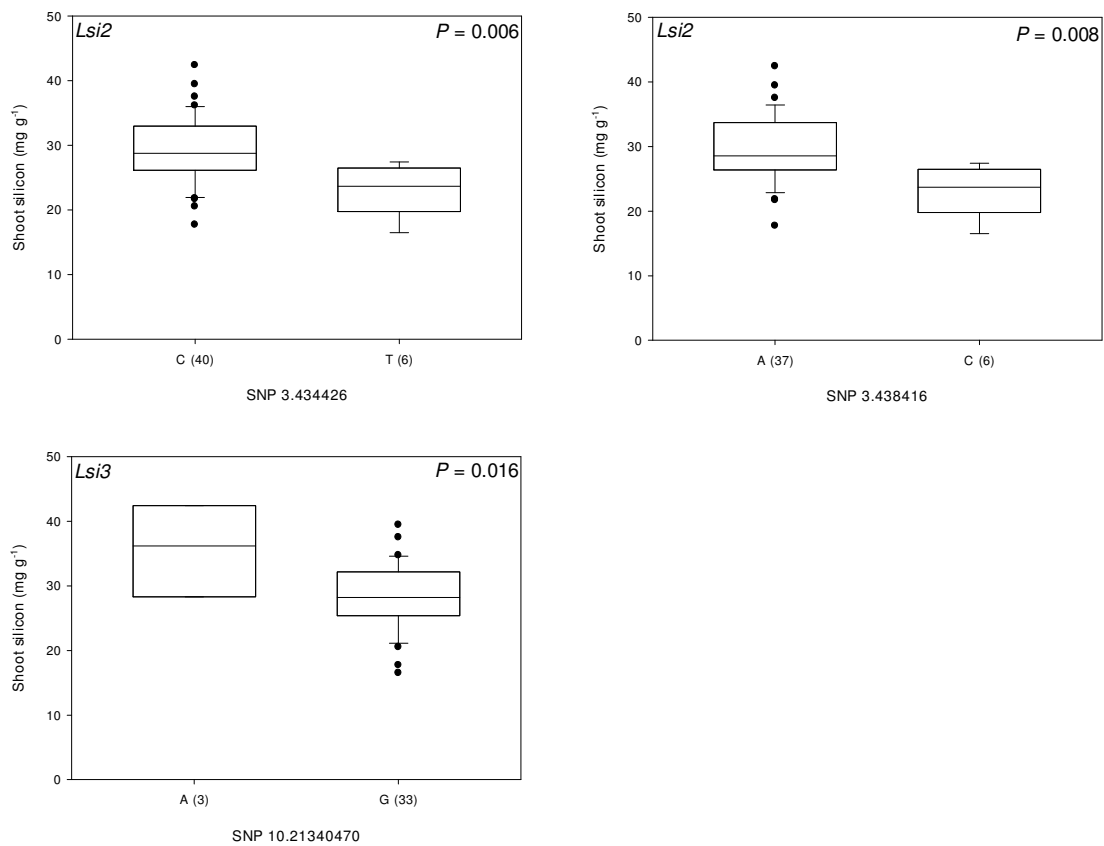


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687 **Figure 5**

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692 **Figure 6**

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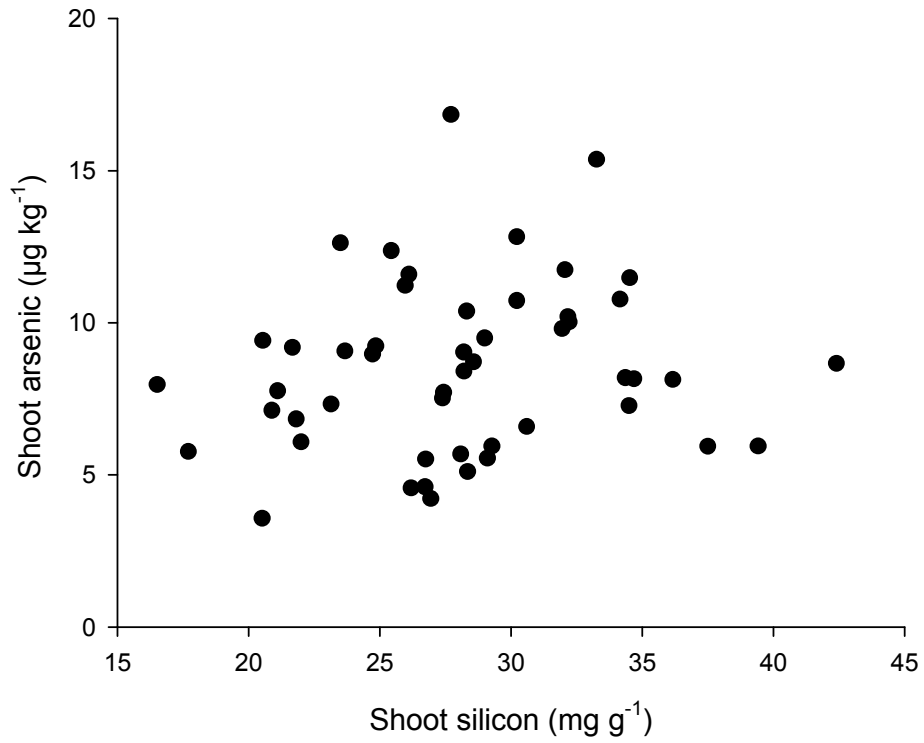
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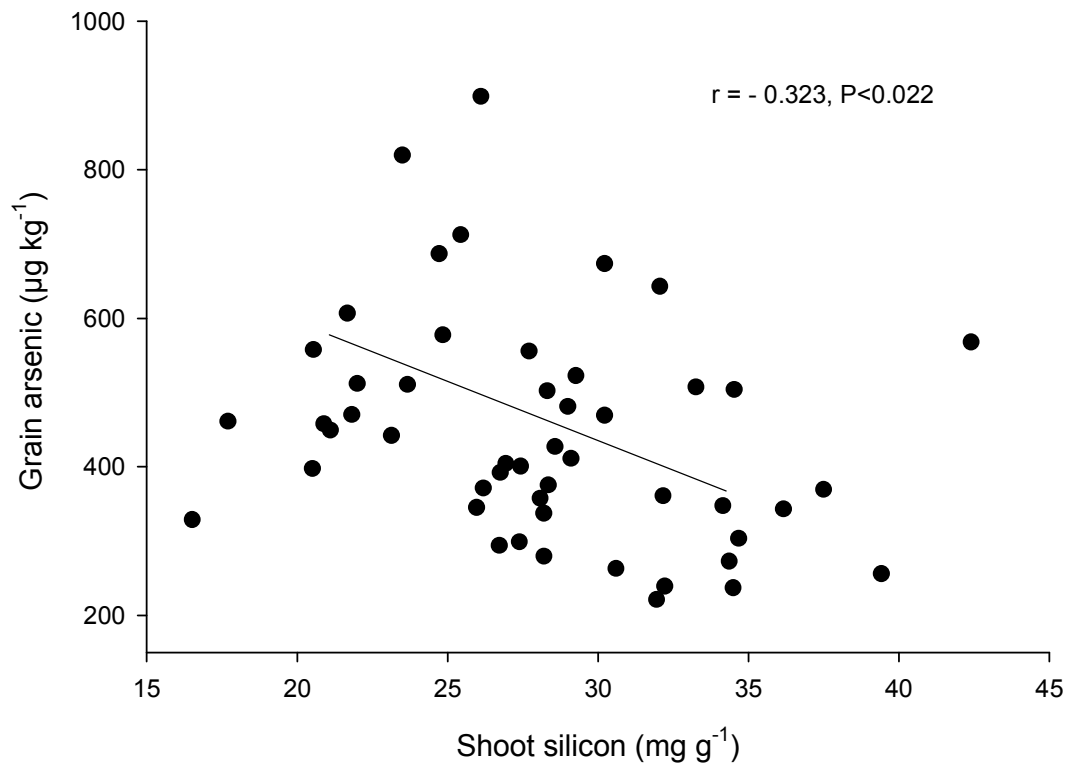
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3 706 **Supplementary Table 1:** Selected genotype from RDP1 for shoot silicon analysis

4 707 **Supplementary Table 2:** SNPs with 10 kb (upstream and downstream) of *Lsi2*. SNPs data  
5 taken from the High-density rice array (HDRA, 700k SNPs data) available at  
6 708 <http://ricediversity.org/data/index.cfm>. After each SNP name (in brackets) is the location of  
7 709 that SNP aligned to the Rice Genome Annotation Project – release 7 sequence available at  
8 710 <http://rice.plantbiology.msu.edu/>  
9 711

10 712 **Supplementary Table 3:** SNPs with 10 kb (upstream and downstream) of *Lsi3*. SNPs data  
11 taken from the High-density rice array (HDRA, 700k SNPs data) available at  
12 713 <http://ricediversity.org/data/index.cfm>. After each SNP name (in brackets) is the location of  
13 714 that SNP aligned to the Rice Genome Annotation Project – release 7 sequence available at  
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**Supplementary figure 1:** Correlation between shoot silicon (mg g<sup>-1</sup>) and shoot arsenic (µg kg<sup>-1</sup>) within 50 accessions of RDP1.



31 **Supplementary figure 2:** Correlation between shoot silicon ( $\text{mg g}^{-1}$ ) and grain arsenic ( $\mu\text{g kg}^{-1}$ ) within 50 accessions of RDP1.

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## Lsi2 (LOC\_Os03g01700)



10	Bala	AGCATCAGCAGCGCATGCACTAGCAGCTTAGCTACTGCGCGGTACCAGAGAGAGATCAT
11	IR64	AGCATCAGCAGCGCATGCACTAGCAGCTTAGCTACTGCGCGGTACCAGAGAGAGATCAT
12	DJ123	AGCATCAGCAGCGCATGCACTAGCAGCTTAGCTACTGCGCGGTACCAGAGAGAGATCAT
13	Nipponbare	AGCATCAGCAGCGCATGCACTAGCAGCTTAGCTACTGCGCGGTACCAGAGAGAGATCAT
14	Azucena	AGCATCAGCAGCGCATGCACTAGCAGCTTAGCTACTGCGCGGTACCAGAGAGAGATCAT
15		*****
16	Bala	CAGCTCAAGTAGCTAAGCTAGCTATAATCTGCTAGCTAGCTCGATCAGACACTTAATTAC
17	IR64	CAGCTCAAGTAGCTAAGCTAGCTATAATCTGCTAGCTAGCTCGATCAGACACTTAATTAC
18	DJ123	CAGCTCAAGTAGCTAAGCTAGCTATAATCTGCTAGCTAGCTCGATCAGACACTTAATTAC
19	Nipponbare	CAGCTCAAGTAGCTAAGCTAGCTATAATCTGCTAGCTAGCTCGATCAGACACTTAATTAC
20	Azucena	CAGCTCAAGTAGCTAAGCTAGCTATAATCTGCTAGCTAGCTCGATCAGACACTTAATTAC
21		*****
22	Bala	CTGCTAGGTGGTGGTCGATCGAAGAAGAAG <b>AAGATGAGTGAGCTTGCGTCGGCGCCAAG</b>
23	IR64	CTGCTAGGTGGTGGTCGATCGAAGAAGAAG <b>AAGATGAGTGAGCTTGCGTCGGCGCCAAG</b>
24	DJ123	CTGCTAGGTGGTGGTCGATCGAAGAAGAAG <b>AAGATGAGTGAGCTTGCGTCGGCGCCAAG</b>
25	Nipponbare	CTGCTAGGTGGTGGTCGATCGAAGAAGAAG <b>AAGATGAGTGAGCTTGCGTCGGCGCCAAG</b>
26	Azucena	CTGCTAGGTGGTGGTCGATCGAAGAAGAAG <b>AAGATGAGTGAGCTTGCGTCGGCGCCAAG</b>
27		*****
28	Bala	<b>GTGGCGCTTGATCGATCGCGTTCGCGGTGTTCTGGATGATGGCGGTGTTCCCGTCGGTG</b>
29	IR64	<b>GTGGCGCTTGATCGATCGCGTTCGCGGTGTTCTGGATGATGGCGGTGTTCCCGTCGGTG</b>
30	DJ123	<b>GTGGCGCTTGATCGATCGCGTTCGCGGTGTTCTGGATGATGGCGGTGTTCCCGTCGGTG</b>
31	Nipponbare	<b>GTGGCGCTTGATCGATCGCGTTCGCGGTGTTCTGGATGATGGCGGTGTTCCCGTCGGTG</b>
32	Azucena	<b>GTGGCGCTTGATCGATCGCGTTCGCGGTGTTCTGGATGATGGCGGTGTTCCCGTCGGTG</b>
33		*****
34	Bala	<b>CCGTTCTGCCGATCGGGCGGACGGCGGGGTCGCTGCTGAGCGCGGTGCTGATGGTGATA</b>
35	IR64	<b>CCGTTCTGCCGATCGGGCGGACGGCGGGGTCGCTGCTGAGCGCGGTGCTGATGGTGATA</b>
36	DJ123	<b>CCGTTCTGCCGATCGGGCGGACGGCGGGGTCGCTGCTGAGCGCGGTGCTGATGGTGATA</b>
37	Nipponbare	<b>CCGTTCTGCCGATCGGGCGGACGGCGGGGTCGCTGCTGAGCGCGGTGCTGATGGTGATA</b>
38	Azucena	<b>CCGTTCTGCCGATCGGGCGGACGGCGGGGTCGCTGCTGAGCGCGGTGCTGATGGTGATA</b>
39		*****
40	Bala	<b>TTCACGTGATCAGCCCCGACGACGCGTACGCCTCCATCGACCTCCCAATCCTGGGCCTC</b>
41	IR64	<b>TTCACGTGATCAGCCCCGACGACGCGTACGCCTCCATCGACCTCCCAATCCTGGGCCTC</b>
42	DJ123	<b>TTCACGTGATCAGCCCCGACGACGCGTACGCCTCCATCGACCTCCCAATCCTGGGCCTC</b>
43	Nipponbare	<b>TTCACGTGATCAGCCCCGACGACGCGTACGCCTCCATCGACCTCCCAATCCTGGGCCTC</b>
44	Azucena	<b>TTCACGTGATCAGCCCCGACGACGCGTACGCCTCCATCGACCTCCCAATCCTGGGCCTC</b>
45		*****
46	Bala	<b>CTCTTCGCCACCATGGTGGTGGGCAGCTACCTCCGGAACGCCGGGATGTTCAAGCACCTG</b>
47	IR64	<b>CTCTTCGCCACCATGGTGGTGGGCAGCTACCTCCGGAACGCCGGGATGTTCAAGCACCTG</b>
48	DJ123	<b>CTCTTCGCCACCATGGTGGTGGGCAGCTACCTCCGGAACGCCGGGATGTTCAAGCACCTG</b>
49	Nipponbare	<b>CTCTTCGCCACCATGGTGGTGGGCAGCTACCTCCGGAACGCCGGGATGTTCAAGCACCTG</b>
50	Azucena	<b>CTCTTCGCCACCATGGTGGTGGGCAGCTACCTCCGGAACGCCGGGATGTTCAAGCACCTG</b>
51		*****
52	Bala	<b>GGGCGTCTGCTGGCGTGGAAGAGCCAGGGCGGGCGGACCTCATGTGCCCGCTCTGCGTC</b>
53	IR64	<b>GGGCGTCTGCTGGCGTGGAAGAGCCAGGGCGGGCGGACCTCATGTGCCCGCTCTGCGTC</b>
54	DJ123	<b>GGGCGTCTGCTGGCGTGGAAGAGCCAGGGCGGGCGGACCTCATGTGCCCGCTCTGCGTC</b>
55	Nipponbare	<b>GGGCGTCTGCTGGCGTGGAAGAGCCAGGGCGGGCGGACCTCATGTGCCCGCTCTGCGTC</b>
56	Azucena	<b>GGGCGTCTGCTGGCGTGGAAGAGCCAGGGCGGGCGGACCTCATGTGCCCGCTCTGCGTC</b>
57		*****

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4 Bala **GTCACCGCCCTCGCCAGCGCCCTCTTACCAACGACACCTGCTGCGTCTCCTCACCGAG**  
5 IR64 **GTCACCGCCCTCGCCAGCGCCCTCTTACCAACGACACCTGCTGCGTCTCCTCACCGAG**  
6 DJ123 **GTCACCGCCCTCGCCAGCGCCCTCTTACCAACGACACCTGCTGCGTCTCCTCACCGAG**  
7 Nipponbare **GTCACCGCCCTCGCCAGCGCCCTCTTACCAACGACACCTGCTGCGTCTCCTCACCGAG**  
8 Azucena **GTCACCGCCCTCGCCAGCGCCCTCTTACCAACGACACCTGCTGCGTCTCCTCACCGAG**  
9 \*\*\*\*\*  
10 Bala **TTCGTCTCGAGCTCGCCGCGGAGCGCAACCTCCCCGCAAGCCCTTCTCTCGCCCTC**  
11 IR64 **TTCGTCTCGAGCTCGCCGCGGAGCGCAACCTCCCCGCAAGCCCTTCTCTCGCCCTC**  
12 DJ123 **TTCGTCTCGAGCTCGCCGCGGAGCGCAACCTCCCCGCAAGCCCTTCTCTCGCCCTC**  
13 Nipponbare **TTCGTCTCGAGCTCGCCGCGGAGCGCAACCTCCCCGCAAGCCCTTCTCTCGCCCTC**  
14 Azucena **TTCGTCTCGAGCTCGCCGCGGAGCGCAACCTCCCCGCAAGCCCTTCTCTCGCCCTC**  
15 \*\*\*\*\*  
16 Bala **GCCTCCAGCGCCAACATCGGCTCCGCCGCCACCCCATCGGCAACCCCCAGAACCTGGTC**  
17 IR64 **GCCTCCAGCGCCAACATCGGCTCCGCCGCCACCCCATCGGCAACCCCCAGAACCTGGTC**  
18 DJ123 **GCCTCCAGCGCCAACATCGGCTCCGCCGCCACCCCATCGGCAACCCCCAGAACCTGGTC**  
19 Nipponbare **GCCTCCAGCGCCAACATCGGCTCCGCCGCCACCCCATCGGCAACCCCCAGAACCTGGTC**  
20 Azucena **GCCTCCAGCGCCAACATCGGCTCCGCCGCCACCCCATCGGCAACCCCCAGAACCTGGTC**  
21 \*\*\*\*\*  
22 Bala **ATCGCCTTCAATAGCAAGATCACCTTCCCAAGTTCTCATGGGAATCTCCCGGCCATG**  
23 IR64 **ATCGCCTTCAATAGCAAGATCACCTTCCCAAGTTCTCATGGGAATCTCCCGGCCATG**  
24 DJ123 **ATCGCCTTCAATAGCAAGATCACCTTCCCAAGTTCTCATGGGAATCTCCCGGCCATG**  
25 Nipponbare **ATCGCCTTCAATAGCAAGATCACCTTCCCAAGTTCTCATGGGAATCTCCCGGCCATG**  
26 Azucena **ATCGCCTTCAATAGCAAGATCACCTTCCCAAGTTCTCATGGGAATCTCCCGGCCATG**  
27 \*\*\*\*\*  
28 Bala **CTCGTCGGGATGGCCGTC AACATGGTTCATGCTGCTCTGCATGTACTGGAGGGAGCTGGGC**  
29 IR64 **CTCGTCGGGATGGCCGTC AACATGGTTCATGCTGCTCTGCATGTACTGGAGGGAGCTGGGC**  
30 DJ123 **CTCGTCGGGATGGCCGTC AACATGGTTCATGCTGCTCTGCATGTACTGGAGGGAGCTGGGC**  
31 Nipponbare **CTCGTCGGGATGGCCGTC AACATGGTTCATGCTGCTCTGCATGTACTGGAGGGAGCTGGGC**  
32 Azucena **CTCGTCGGGATGGCCGTC AACATGGTTCATGCTGCTCTGCATGTACTGGAGGGAGCTGGGC**  
33 \*\*\*\*\*  
34 Bala **GGAGGGGCCGAGCTCAGCGTCGACGGCAAGCAGATGGAGGCGGTGGAGGAAGGCAGGTTCG**  
35 IR64 **GGAGGGGCCGAGCTCAGCGTCGACGGCAAGCAGATGGAGGCGGTGGAGGAAGGCAGGTTCG**  
36 DJ123 **GGAGGGGCCGAGCTCAGCGTCGACGGCAAGCAGATGGAGGCGGTGGAGGAAGGCAGGTTCG**  
37 Nipponbare **GGAGGGGCCGAGCTCAGCGTCGACGGCAAGCAGATGGAGGCGGTGGAGGAAGGCAGGTTCG**  
38 Azucena **GGAGGGGCCGAGCTCAGCGTCGACGGCAAGCAGATGGAGGCGGTGGAGGAAGGCAGGTTCG**  
39 \*\*\*\*\*  
40 Bala **CCGGCGTCGGCCAAGAGCACGCCGAGCTGAACGGCAACGGCAACACGATGATGTCGCTG**  
41 IR64 **CCGGCGTCGGCCAAGAGCACGCCGAGCTGAACGGCAACGGCAACACGATGATGTCGCTG**  
42 DJ123 **CCGGCGTCGGCCAAGAGCACGCCGAGCTGAACGGCAACGGCAACACGATGATGTCGCTG**  
43 Nipponbare **CCGGCGTCGGCCAAGAGCACGCCGAGCTGAACGGCAACGGCAACACGATGATGTCGCTG**  
44 Azucena **CCGGCGTCGGCCAAGAGCACGCCGAGCTGAACGGCAACGGCAACACGATGATGTCGCTG**  
45 \*\*\*\*\*  
46 Bala **GAGATGTCGGAGAACATAACGACCAAGCACCCATGGTTCATGCAGTGCACGGAGGCGCGG**  
47 IR64 **GAGATGTCGGAGAACATAACGACCAAGCACCCATGGTTCATGCAGTGCACGGAGGCGCGG**  
48 DJ123 **GAGATGTCGGAGAACATAACGACCAAGCACCCATGGTTCATGCAGTGCACGGAGGCGCGG**  
49 Nipponbare **GAGATGTCGGAGAACATAACGACCAAGCACCCATGGTTCATGCAGTGCACGGAGGCGCGG**  
50 Azucena **GAGATGTCGGAGAACATAACGACCAAGCACCCATGGTTCATGCAGTGCACGGAGGCGCGG**  
51 \*\*\*\*\*  
52 Bala **CGGAAGCTGTTCTCAAGAGCTTCGCGTACGTGGTGACGGTGGGGATGGTGGTGGCCTAC**  
53 IR64 **CGGAAGCTGTTCTCAAGAGCTTCGCGTACGTGGTGACGGTGGGGATGGTGGTGGCCTAC**  
54 DJ123 **CGGAAGCTGTTCTCAAGAGCTTCGCGTACGTGGTGACGGTGGGGATGGTGGTGGCCTAC**  
55 Nipponbare **CGGAAGCTGTTCTCAAGAGCTTCGCGTACGTGGTGACGGTGGGGATGGTGGTGGCCTAC**  
56 Azucena **CGGAAGCTGTTCTCAAGAGCTTCGCGTACGTGGTGACGGTGGGGATGGTGGTGGCCTAC**  
57 \*\*\*\*\*

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3 Bala ATGGTGGGGCTCAACATGTCGTGGACGGCCATCACCACGGCGCTGGCGCTGGTGGTGGTC  
4 IR64 ATGGTGGGGCTCAACATGTCGTGGACGGCCATCACCACGGCGCTGGCGCTGGTGGTGGTC  
5 DJ123 ATGGTGGGGCTCAACATGTCGTGGACGGCCATCACCACGGCGCTGGCGCTGGTGGTGGTC  
6 Nipponbare ATGGTGGGGCTCAACATGTCGTGGACGGCCATCACCACGGCGCTGGCGCTGGTGGTGGTC  
7 Azucena ATGGTGGGGCTCAACATGTCGTGGACGGCCATCACCACGGCGCTGGCGCTGGTGGTGGTC  
8 \*\*\*\*\*  
9 Bala GACTTCCGCGACGCCGAGCCGTGCCTGGACACCGTGTCTACTCGCTGCTCGTCTTCTTC  
10 IR64 GACTTCCGCGACGCCGAGCCGTGCCTGGACACCGTGTCTACTCGCTGCTCGTCTTCTTC  
11 DJ123 GACTTCCGCGACGCCGAGCCGTGCCTGGACACCGTGTCTACTCGCTGCTCGTCTTCTTC  
12 Nipponbare GACTTCCGCGACGCCGAGCCGTGCCTGGACACCGTGTCTACTCGCTGCTCGTCTTCTTC  
13 Azucena GACTTCCGCGACGCCGAGCCGTGCCTGGACACCGTGTCTACTCGCTGCTCGTCTTCTTC  
14 \*\*\*\*\*  
15 Bala TCCGGGATGTTTCATCACCCTCAGCGGCTTCAACAAGACGGGCTCCCGGGAGCCATCTGG  
16 IR64 TCCGGGATGTTTCATCACCCTCAGCGGCTTCAACAAGACGGGCTCCCGGGAGCCATCTGG  
17 DJ123 TCCGGGATGTTTCATCACCCTCAGCGGCTTCAACAAGACGGGCTCCCGGGAGCCATCTGG  
18 Nipponbare TCCGGGATGTTTCATCACCCTCAGCGGCTTCAACAAGACGGGCTCCCGGGAGCCATCTGG  
19 Azucena TCCGGGATGTTTCATCACCCTCAGCGGCTTCAACAAGACGGGCTCCCGGGAGCCATCTGG  
20 \*\*\*\*\*  
21 Bala GACTTCATGGCCCCCTACTCCAAGGTCAACAGCGTCGGCGGCATCTCCGCTCTCCGTC  
22 IR64 GACTTCATGGCCCCCTACTCCAAGGTCAACAGCGTCGGCGGCATCTCCGCTCTCCGTC  
23 DJ123 GACTTCATGGCCCCCTACTCCAAGGTCAACAGCGTCGGCGGCATCTCCGCTCTCCGTC  
24 Nipponbare GACTTCATGGCCCCCTACTCCAAGGTCAACAGCGTCGGCGGCATCTCCGCTCTCCGTC  
25 Azucena GACTTCATGGCCCCCTACTCCAAGGTCAACAGCGTCGGCGGCATCTCCGCTCTCCGTC  
26 \*\*\*\*\*  
27 Bala ATCATCTCTCCTCTCCAACCTCGCATCAAACGTACCAACGGTAATTATACATTCTAT  
28 IR64 ATCATCTCTCCTCTCCAACCTCGCATCAAACGTACCAACGGTAATTATACATTCTAT  
29 DJ123 ATCATCTCTCCTCTCCAACCTCGCATCAAACGTACCAACGGTAATTATACATTCTAT  
30 Nipponbare ATCATCTCTCCTCTCCAACCTCGCATCAAACGTACCAACGGTAATTATACATTCTAT  
31 Azucena ATCATCTCTCCTCTCCAACCTCGCATCAAACGTACCAACGGTAATTATACATTCTAT  
32 \*\*\*\*\*  
33 Bala TAATCCAGAATTAATGTATACTACTCCATATAAGTATAACTAATTAACATACAAAAATAT  
34 IR64 TAATCCAGAATTAATGTATACTACTCCATATAAGTATAACTAATTAACATACAAAAATAT  
35 DJ123 TAATCCAGAATTAATGTATACTACTCCATATAAGTATAACTAATTAACATACAAAAATAT  
36 Nipponbare TAATCCAGAATTAATGTATACTACTCCATATAAGTATAACTAATTAACATACAAAAATAT  
37 Azucena TAATCCAGAATTAATGTATACTACTCCATATAAGTATAACTAATTAACATACAAAAATAT  
38 \*\*\*\*\*  
39 Bala ATATGCGCATGCATGCAGTGCTTCTTATGGGTGATGAGGTGGCGAAGGCGGCGGCTGA  
40 IR64 ATATGCGCATGCATGCAGTGCTTCTTATGGGTGATGAGGTGGCGAAGGCGGCGGCTGA  
41 DJ123 ATATGCGCATGCATGCAGTGCTTCTTATGGGTGATGAGGTGGCGAAGGCGGCGGCTGA  
42 Nipponbare ATATGCGCATGCATGCAGTGCTTCTTATGGGTGATGAGGTGGCGAAGGCGGCGGCTGA  
43 Azucena ATATGCGCATGCATGCAGTGCTTCTTATGGGTGATGAGGTGGCGAAGGCGGCGGCTGA  
44 \*\*\*\*\*  
45 Bala TATCGCCGGCGGCGGTGACGACGTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGG  
46 IR64 TATCGCCGGCGGCGGTGACGACGTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGG  
47 DJ123 TATCGCCGGCGGCGGTGACGACGTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGG  
48 Nipponbare TATCGCCGGCGGCGGTGACGACGTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGG  
49 Azucena TATCGCCGGCGGCGGTGACGACGTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGG  
50 \*\*\*\*\*  
51 Bala GGAACCTGTCGCTGCTGGGGTCCGGCGGGAACCTGATAGTGTGCGAGCAGGCGAGGAGGG  
52 IR64 GGAACCTGTCGCTGCTGGGGTCCGGCGGGAACCTGATAGTGTGCGAGCAGGCGAGGAGGG  
53 DJ123 GGAACCTGTCGCTGCTGGGGTCCGGCGGGAACCTGATAGTGTGCGAGCAGGCGAGGAGGG  
54 Nipponbare GGAACCTGTCGCTGCTGGGGTCCGGCGGGAACCTGATAGTGTGCGAGCAGGCGAGGAGGG  
55 Azucena GGAACCTGTCGCTGCTGGGGTCCGGCGGGAACCTGATAGTGTGCGAGCAGGCGAGGAGGG  
56 \*\*\*\*\*

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3 Bala CGCCAGGAACGCCTACGACCTCACCTTCTGGCAGCACATCGTCTTCGGCGTCCCATCCA  
4 IR64 CGCCAGGAACGCCTACGACCTCACCTTCTGGCAGCACATCGTCTTCGGCGTCCCATCCA  
5 DJ123 CGCCAGGAACGCCTACGACCTCACCTTCTGGCAGCACATCGTCTTCGGCGTCCCATCCA  
6 Nipponbare CGCCAGGAACGCCTACGACCTCACCTTCTGGCAGCACATCGTCTTCGGCGTCCCATCCA  
7 Azucena CGCCAGGAACGCCTACGACCTCACCTTCTGGCAGCACATCGTCTTCGGCGTCCCATCCA  
8 \*\*\*\*\*  
9 Bala CCCTCATCGTCACCGCGTCGGCATACCCCTCATCGGCAAGATCTGATCTCATCTCATCG  
10 IR64 CCCTCATCGTCACCGCGTCGGCATACCCCTCATCGGCAAGATCTGATCTCATCTCATCG  
11 DJ123 CCCTCATCGTCACCGCGTCGGCATACCCCTCATCGGCAAGATCTGATCTCATCTCATCG  
12 Nipponbare CCCTCATCGTCACCGCGTCGGCATACCCCTCATCGGCAAGATCTGATCTCATCTCATCG  
13 Azucena CCCTCATCGTCACCGCGTCGGCATACCCCTCATCGGCAAGATCTGATCTCATCTCATCG  
14 \*\*\*\*\*  
15 Bala ACCCATCCAAATTAATTAATTATGAGATCGACAAACATCCAAGCTTGCTAGGCTCGTCGT  
16 IR64 ACCCATCCAAATTAATTAATTATGAGATCGACAAACATCCAAGCTTGCTAGGCTCGTCGT  
17 DJ123 ACCCATCCAAATTAATTAATTATGAGATCGACAAACATCCAAGCTTGCTAGGCTCGTCGT  
18 Nipponbare ACCCATCCAAATTAATTAATTATGAGATCGACAAACATCCAAGCTTGCTAGGCTCGTCGT  
19 Azucena ACCCATCCAAATTAATTAATTATGAGATCGACAAACATCCAAGCTTGCTAGGCTCGTCGT  
20 \*\*\*\*\*  
21 Bala CGTCGTCGTCGACCACCGTACCATATATATGCATGCATGCCACGCACGTATATATATGCT  
22 IR64 CGTCGTCGTCGACCACCGTACCATATATATGCATGCATGCCACGCACGTATATATATGCT  
23 DJ123 CGTCGTCGTCGACCACCGTACCATATATATGCATGCATGCCACGCACGTATATATATGCT  
24 Nipponbare CGTCGTCGTCGACCACCGTACCATATATATGCATGCATGCCACGCACGTATATATATGCT  
25 Azucena CGTCGTCGTCGACCACCGTACCATATATATGCATGCATGCCACGCACGTATATATATGCT  
26 \*\*\*\*\*  
27 Bala CCTCAACCTCAAGTCAATTAATTAAGAGAATGGATGAATGAATCAATGTTGTGTATGATT  
28 IR64 CCTCAACCTCAAGTCAATTAATTAAGAGAATGGATGAATGAATCAATGTTGTGTATGATT  
29 DJ123 CCTCAACCTCAAGTCAATTAATTAAGAGAATGGATGAATGAATCAATGTTGTGTATGATT  
30 Nipponbare CCTCAACCTCAAGTCAATTAATTAAGAGAATGGATGAATGAATCAATGTTGTGTATGATT  
31 Azucena CCTCAACCTCAAGTCAATTAATTAAGAGAATGGATGAATGAATCAATGTTGTGTATGATT  
32 \*\*\*\*\*  
33 Bala TCCTTTTTGTTTTGTTTTACCACCGTATATGTGCTGTGTGGTGTATGGCCGGCGAGCT  
34 IR64 TCCTTTTTGTTTTGTTTTACCACCGTATATGTGCTGTGTGGTGTATGGCCGGCGAGCT  
35 DJ123 TCCTTTTTGTTTTGTTTTACCACCGTATATGTGCTGTGTGGTGTATGGCCGGCGAGCT  
36 Nipponbare TCCTTTTTGTTTTGTTTTACCACCGTATATGTGCTGTGTGGTGTATGGCCGGCGAGCT  
37 Azucena TCCTTTTTGTTTTGTTTTACCACCGTATATGTGCTGTGTGGTGTATGGCCGGCGAGCT  
38 \*\*\*\*\*  
39 Bala TGA AATTGAATTGGCCTTGCTTGCATGCATGCATGGTGATCAGATCAGCTCAGCTAGCTA  
40 IR64 TGA AATTGAATTGGCCTTGCTTGCATGCATGCATGGTGATCAGATCAGCTCAGCTAGCTA  
41 DJ123 TGA AATTGAATTGGCCTTGCTTGCATGCATGCATGGTGATCAGATCAGCTCAGCTAGCTA  
42 Nipponbare TGA AATTGAATTGGCCTTGCTTGCATGCATGCATGGTGATCAGATCAGCTCAGCTAGCTA  
43 Azucena TGA AATTGAATTGGCCTTGCTTGCATGCATGCATGGTGATCAGATCAGCTCAGCTAGCTA  
44 \*\*\*\*\*  
45 Bala GCTAAATCTTGCTTAATTTTATCAAGTGTCAATCATGATGAGAAGAGAGGGAAC TAGATG  
46 IR64 GCTAAATCTTGCTTAATTTTATCAAGTGTCAATCATGATGAGAAGAGAGGGAAC TAGATG  
47 DJ123 GCTAAATCTTGCTTAATTTTATCAAGTGTCAATCATGATGAGAAGAGAGGGAAC TAGATG  
48 Nipponbare GCTAAATCTTGCTTAATTTTATCAAGTGTCAATCATGATGAGAAGAGAGGGAAC TAGATG  
49 Azucena GCTAAATCTTGCTTAATTTTATCAAGTGTCAATCATGATGAGAAGAGAGGGAAC TAGATG  
50 \*\*\*\*\*  
51 Bala GAGCTAGCTAGCTAGCAACACATGCAGTGTGTGCTAAGCAGTGCACCTGTCATGCTTAAA  
52 IR64 GAGCTAGCTAGCTAGCAACACATGCAGTGTGTGCTAAGCAGTGCACCTGTCATGCTTAAA  
53 DJ123 GAGCTAGCTAGCTAGCAACACATGCAGTGTGTGCTAAGCAGTGCACCTGTCATGCTTAAA  
54 Nipponbare GAGCTAGCTAGCTAGCAACACATGCAGTGTGTGCTAAGCAGTGCACCTGTCATGCTTAAA  
55 Azucena GAGCTAGCTAGCTAGCAACACATGCAGTGTGTGCTAAGCAGTGCACCTGTCATGCTTAAA  
56 \*\*\*\*\*  
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3 Bala TTTGCTTGCTTAATTCAACATCGTATTACATGTATGTTGATATGTGCATGTTCCCTCAAAA  
4 IR64 TTTGCTTGCTTAATTCAACATCGTATTACATGTATGTTGATATGTGCATGTTCCCTCAAAA  
5 DJ123 TTTGCTTGCTTAATTCAACATCGTATTACATGTATGTTGATATGTGCATGTTCCCTCAAAA  
6 Nipponbare TTTGCTTGCTTAATTCAACATCGTATTACATGTATGTTGATATGTGCATGTTCCCTCAAAA  
7 Azucena TTTGCTTGCTTAATTCAACATCGTATTACATGTATGTTGATATGTGCATGTTCCCTCAAAA  
8 \*\*\*\*\*  
9 Bala TACATGGAAAAAAATGTTTCTACACATCTCAATCGTATAGAGTATGTAGCATGTCTTGT  
10 IR64 TACATGGAAAAAAATGTTTCTACACATCTCAATCGTATAGAGTATGTAGCATGTCTTGT  
11 DJ123 TACATGGAAAAAAATGTTTCTACACATCTCAATCGTATAGAGTATGTAGCATGTCTTGT  
12 Nipponbare TACATGGAAAAAAATGTTTCTACACATCTCAATCGTATAGAGTATGTAGCATGTCTTGT  
13 Azucena TACATGGAAAAAAATGTTTCTACACATCTCAATCGTATAGAGTATGTAGCATGTCTTGT  
14 \*\*\*\*\*  
15 Bala TCAAACAAATATTTGTAGCCCTCTCTCTCTCTCTCTTTAGGTTTATTAATTAATTAGT  
16 IR64 TCAAACAAATATTTGTAGCCCTCTCTCTCTCTCTCTTTAGGTTTATTAATTAATTAGT  
17 DJ123 TCAAACAAATATTTGTAGCCCTCTCTCTCTCTCTCTTTAGGTTTATTAATTAATTAGT  
18 Nipponbare TCAAACAAATATTTGTAGCCCTCTCTCTCTCTCTCTTTAGGTTTATTAATTAATTAGT  
19 Azucena TCAAACAAATATTTGTAGCCCTCTCTCTCTCTCTCTTTAGGTTTATTAATTAATTAGT  
20 \*\*\*\*\*  
21 Bala CTGTTCAATGTCACCTCAGTTCATAGAGATGCTATACTTAATTAATTGCTCCATGTATGC  
22 IR64 CTGTTCAATGTCACCTCAGTTCATAGAGATGCTATACTTAATTAATTGCTCCATGTATGC  
23 DJ123 CTGTTCAATGTCACCTCAGTTCATAGAGATGCTATACTTAATTAATTGCTCCATGTATGC  
24 Nipponbare CTGTTCAATGTCACCTCAGTTCATAGAGATGCTATACTTAATTAATTGCTCCATGTATGC  
25 Azucena CTGTTCAATGTCACCTCAGTTCATAGAGATGCTATACTTAATTAATTGCTCCATGTATGC  
26 \*\*\*\*\*  
27  
28 Bala AGAGTTGTACTACCTTAAAATATCAGTATGCATGCTCTTGTTGCCATTCCAAAAGAACAA  
29 IR64 AGAGTTGTACTACCTTAAAATATCAGTATGCATGCTCTTGTTGCCATTCCAAAAGAACAA  
30 DJ123 AGAGTTGTACTACCTTAAAATATCAGTATGCATGCTCTTGTTGCCATTCCAAAAGAACAA  
31 Nipponbare AGAGTTGTACTACCTTAAAATATCAGTATGCATGCTCTTGTTGCCATTCCAAAAGAACAA  
32 Azucena AGAGTTGTACTACCTTAAAATATCAGTATGCATGCTCTTGTTGCCATTCCAAAAGAACAA  
33 \*\*\*\*\*  
34 Bala A  
35 IR64 A  
36 DJ123 A  
37 Nipponbare A  
38 Azucena A  
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**Supplementary figure 3.** Genomic sequence alignment of *Lsi2*. Regions highlighted in bold are predicted exon regions based on the Rice Genome Annotation Project – release 7 sequence available at <http://rice.plantbiology.msu.edu/>.

*Lsi3* (LOC\_Os10g39980)

Bala ACTCTCATCGCCACCACCACCTCCCACTCCAAGACGCTAGCTGCATTGTAGTGAGAGAGA  
 IR64 ACTCTCATCGCCACCACCACCTCCCACTCCAAGACGCTAGCTGCATTGTAGTGAGAGAGA  
 DJ123 ACTCTCATCGCCACCACCACCTCCCACTCCAAGACGCTAGCTGCATTGTAGTGAGAGAGA  
 Nipponbare ACTCTCATCGCCACCACCACCTCCCACTCCAAGACGCTAGCTGCATTGTAGTGAGAGAGA  
 Azucena ACTCTCATCGCCACCACCACCTCCCACTCCAAGACGCTAGCTGCATTGTAGTGAGAGAGA  
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Bala GAGGCGGCCATGGCGCTGGCGTCGCTGCCGAAGGTGGTGGTGGGGTCGGTGGCGTTCGGT  
 IR64 GAGGCGGCCATGGCGCTGGCGTCGCTGCCGAAGGTGGTGGTGGGGTCGGTGGCGTTCGGT  
 DJ123 GAGGCGGCCATGGCGCTGGCGTCGCTGCCGAAGGTGGTGGTGGGGTCGGTGGCGTTCGGT  
 Nipponbare GAGGCGGCCATGGCGCTGGCGTCGCTGCCGAAGGTGGTGGTGGGGTCGGTGGCGTTCGGT  
 Azucena GAGGCGGCCATGGCGCTGGCGTCGCTGCCGAAGGTGGTGGTGGGGTCGGTGGCGTTCGGT  
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Bala GTGTTCTGGATGCTGGCGGTGTTCCCGTCGGTGCCGTTCTGCGGATCGGGCGGACGGCG  
 IR64 GTGTTCTGGATGCTGGCGGTGTTCCCGTCGGTGCCGTTCTGCGGATCGGGCGGACGGCG  
 DJ123 GTGTTCTGGATGCTGGCGGTGTTCCCGTCGGTGCCGTTCTGCGGATCGGGCGGACGGCG  
 Nipponbare GTGTTCTGGATGCTGGCGGTGTTCCCGTCGGTGCCGTTCTGCGGATCGGGCGGACGGCG  
 Azucena GTGTTCTGGATGCTGGCGGTGTTCCCGTCGGTGCCGTTCTGCGGATCGGGCGGACGGCG  
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Bala GGGGCGTGCTGGGCGGGTGTGATGATCGTGTTCACGTGATCAGCGCCGACGACGCC  
 IR64 GGGGCGTGCTGGGCGGGTGTGATGATCGTGTTCACGTGATCAGCGCCGACGACGCC  
 DJ123 GGGGCGTGCTGGGCGGGTGTGATGATCGTGTTCACGTGATCAGCGCCGACGACGCC  
 Nipponbare GGGGCGTGCTGGGCGGGTGTGATGATCGTGTTCACGTGATCAGCGCCGACGACGCC  
 Azucena GGGGCGTGCTGGGCGGGTGTGATGATCGTGTTCACGTGATCAGCGCCGACGACGCC  
 \*\*\*\*\*

Bala TACGCCTCCATCGACCTCCCCATCCTGGGCTCCTCTTCGCCACCATGGTCGTGGCGGC  
 IR64 TACGCCTCCATCGACCTCCCCATCCTGGGCTCCTCTTCGCCACCATGGTCGTGGCGGC  
 DJ123 TACGCCTCCATCGACCTCCCCATCCTGGGCTCCTCTTCGCCACCATGGTCGTGGCGGC  
 Nipponbare TACGCCTCCATCGACCTCCCCATCCTGGGCTCCTCTTCGCCACCATGGTCGTGGCGGC  
 Azucena TACGCCTCCATCGACCTCCCCATCCTGGGCTCCTCTTCGCCACCATGGTCGTGGCGGC  
 \*\*\*\*\*

Bala TACCTCAAGAACGCCGGCATGTTACGGCACCTGGGCGGGTGTGGCGTGGCGGAGCCAG  
 IR64 TACCTCAAGAACGCCGGCATGTTACGGCACCTGGGCGGGTGTGGCGTGGCGGAGCCAG  
 DJ123 TACCTCAAGAACGCCGGCATGTTACGGCACCTGGGCGGGTGTGGCGTGGCGGAGCCAG  
 Nipponbare TACCTCAAGAACGCCGGCATGTTACGGCACCTGGGCGGGTGTGGCGTGGCGGAGCCAG  
 Azucena TACCTCAAGAACGCCGGCATGTTACGGCACCTGGGCGGGTGTGGCGTGGCGGAGCCAG  
 \*\*\*\*\*

Bala GGGGGGCGGACCTCATGTGCCGCTCTGCGTCGTACCGCGCTCGCCAGCGCGCTCTTC  
 IR64 GGGGGGCGGACCTCATGTGCCGCTCTGCGTCGTACCGCGCTCGCCAGCGCGCTCTTC  
 DJ123 GGGGGGCGGACCTCATGTGCCGCTCTGCGTCGTACCGCGCTCGCCAGCGCGCTCTTC  
 Nipponbare GGGGGGCGGACCTCATGTGCCGCTCTGCGTCGTACCGCGCTCGCCAGCGCGCTCTTC  
 Azucena GGGGGGCGGACCTCATGTGCCGCTCTGCGTCGTACCGCGCTCGCCAGCGCGCTCTTC  
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Bala ACCAACGACACCTGCTGCGTCGTCCTCACCGAGTTCGTGCTCGAGCTCGCCGCGGAGCGC  
 IR64 ACCAACGACACCTGCTGCGTCGTCCTCACCGAGTTCGTGCTCGAGCTCGCCGCGGAGCGC  
 DJ123 ACCAACGACACCTGCTGCGTCGTCCTCACCGAGTTCGTGCTCGAGCTCGCCGCGGAGCGC  
 Nipponbare ACCAACGACACCTGCTGCGTCGTCCTCACCGAGTTCGTGCTCGAGCTCGCCGCGGAGCGC  
 Azucena ACCAACGACACCTGCTGCGTCGTCCTCACCGAGTTCGTGCTCGAGCTCGCCGCGGAGCGC  
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1  
2  
3 Bala AACCTCCCGCGAAGCCGTTCCCTGCTCGCGCTCGCCACCAGCGCCAACATCGGCTCCAGC  
4 IR64 AACCTCCCGCGAAGCCGTTCCCTGCTCGCGCTCGCCACCAGCGCCAACATCGGCTCCAGC  
5 DJ123 AACCTCCCGCGAAGCCGTTCCCTGCTCGCGCTCGCCACCAGCGCCAACATCGGCTCCAGC  
6 Nipponbare AACCTCCCGCGAAGCCGTTCCCTGCTCGCGCTCGCCACCAGCGCCAACATCGGCTCCAGC  
7 Azucena AACCTCCCGCGAAGCCGTTCCCTGCTCGCGCTCGCCACCAGCGCCAACATCGGCTCCAGC  
8 \*\*\*\*\*  
9 Bala GCGACGCCCATCGGCAACCCGCGAGAACC TGGTCATCGCCTTCAACAGCAAGATCTCCTTT  
10 IR64 GCGACGCCCATCGGCAACCCGCGAGAACC TGGTCATCGCCTTCAACAGCAAGATCTCCTTT  
11 DJ123 GCGACGCCCATCGGCAACCCGCGAGAACC TGGTCATCGCCTTCAACAGCAAGATCTCCTTT  
12 Nipponbare GCGACGCCCATCGGCAACCCGCGAGAACC TGGTCATCGCCTTCAACAGCAAGATCTCCTTT  
13 Azucena GCGACGCCCATCGGCAACCCGCGAGAACC TGGTCATCGCCTTCAACAGCAAGATCTCCTTT  
14 \*\*\*\*\*  
15 Bala ATTTCCCTTCCCTCGGCATCCTCCCCGCCATGCTCGCCGGCATGGGGATCAACATGCTC  
16 IR64 ATTTCCCTTCCCTCGGCATCCTCCCCGCCATGCTCGCCGGCATGGGGATCAACATGCTC  
17 DJ123 ATTTCCCTTCCCTCGGCATCCTCCCCGCCATGCTCGCCGGCATGGGGATCAACATGCTC  
18 Nipponbare ATTTCCCTTCCCTCGGCATCCTCCCCGCCATGCTCGCCGGCATGGGGATCAACATGCTC  
19 Azucena ATTTCCCTTCCCTCGGCATCCTCCCCGCCATGCTCGCCGGCATGGGGATCAACATGCTC  
20 \*\*\*\*\*  
21 Bala ATGCTGCTCTGCATGTACTGGAAGGAGCTCGACGGCGGCGCCTGCAGCCCCGACGAGGTC  
22 IR64 ATGCTGCTCTGCATGTACTGGAAGGAGCTCGACGGCGGCGCCTGCAGCCCCGACGAGGTC  
23 DJ123 ATGCTGCTCTGCATGTACTGGAAGGAGCTCGACGGCGGCGCCTGCAGCCCCGACGAGGTC  
24 Nipponbare ATGCTGCTCTGCATGTACTGGAAGGAGCTCGACGGCGGCGCCTGCAGCCCCGACGAGGTC  
25 Azucena ATGCTGCTCTGCATGTACTGGAAGGAGCTCGACGGCGGCGCCTGCAGCCCCGACGAGGTC  
26 \*\*\*\*\*  
27 Bala GCCGCCGGCAAGCAGATGGAGGCCATCGAGGAGGGCCGCCGACGGCGCTCAACAACAAC  
28 IR64 GCCGCCGGCAAGCAGATGGAGGCCATCGAGGAGGGCCGCCGACGGCGCTCAACAACAAC  
29 DJ123 GCCGCCGGCAAGCAGATGGAGGCCATCGAGGAGGGCCGCCGACGGCGCTCAACAACAAC  
30 Nipponbare GCCGCCGGCAAGCAGATGGAGGCCATCGAGGAGGGCCGCCGACGGCGCTCAACAACAAC  
31 Azucena GCCGCCGGCAAGCAGATGGAGGCCATCGAGGAGGGCCGCCGACGGCGCTCAACAACAAC  
32 \*\*\*\*\*  
33 Bala AAGAAAGACGACGGCGACGCCGCCACCCCGCATCGCCGGAGGACGACGACGGCGGCGAC  
34 IR64 AAGAAAGACGACGGCGACGCCGCCACCCCGCATCGCCGGAGGACGACGACGGCGGCGAC  
35 DJ123 AAGAAAGACGACGGCGACGCCGCCACCCCGCATCGCCGGAGGACGACGACGGCGGCGAC  
36 Nipponbare AAGAAAGACGACGGCGACGCCGCCACCCCGCATCGCCGGAGGACGACGACGGCGGCGAC  
37 Azucena AAGAAAGACGACGGCGACGCCGCCACCCCGCATCGCCGGAGGACGACGACGGCGGCGAC  
38 \*\*\*\*\*  
39 Bala GCCGAGTCGATGATGTCGGAGAACATCTCGACGAAGCACCGGTGGTTTCATGCAGTGCTCG  
40 IR64 GCCGAGTCGATGATGTCGGAGAACATCTCGACGAAGCACCGGTGGTTTCATGCAGTGCTCG  
41 DJ123 GCCGAGTCGATGATGTCGGAGAACATCTCGACGAAGCACCGGTGGTTTCATGCAGTGCTCG  
42 Nipponbare GCCGAGTCGATGATGTCGGAGAACATCTCGACGAAGCACCGGTGGTTTCATGCAGTGCTCG  
43 Azucena GCCGAGTCGATGATGTCGGAGAACATCTCGACGAAGCACCGGTGGTTTCATGCAGTGCTCG  
44 \*\*\*\*\*  
45 Bala GAGCACCGGCGGAAGCTATTCCCTCAAGTCTTCGCGTACGTGGTCACCGTCGGCATGCTC  
46 IR64 GAGCACCGGCGGAAGCTATTCCCTCAAGTCTTCGCGTACGTGGTCACCGTCGGCATGCTC  
47 DJ123 GAGCACCGGCGGAAGCTATTCCCTCAAGTCTTCGCGTACGTGGTCACCGTCGGCATGCTC  
48 Nipponbare GAGCACCGGCGGAAGCTATTCCCTCAAGTCTTCGCGTACGTGGTCACCGTCGGCATGCTC  
49 Azucena GAGCACCGGCGGAAGCTATTCCCTCAAGTCTTCGCGTACGTGGTCACCGTCGGCATGCTC  
50 \*\*\*\*\*  
51 Bala GTCGCCTACATGCTCGGCCTCAACATGTCGTGGACCGCCATCACACC GCCCATCGCCCTC  
52 IR64 GTCGCCTACATGCTCGGCCTCAACATGTCGTGGACCGCCATCACACC GCCCATCGCCCTC  
53 DJ123 GTCGCCTACATGCTCGGCCTCAACATGTCGTGGACCGCCATCACACC GCCCATCGCCCTC  
54 Nipponbare GTCGCCTACATGCTCGGCCTCAACATGTCGTGGACCGCCATCACACC GCCCATCGCCCTC  
55 Azucena GTCGCCTACATGCTCGGCCTCAACATGTCGTGGACCGCCATCACACC GCCCATCGCCCTC  
56 \*\*\*\*\*

1  
2  
3 Bala **GTCGTCGTCGACTTCCGCGACGCCGAGCCATGCCTCGACAAGGTAACCCATATCCGTCCA**  
4 IR64 **GTCGTCGTCGACTTCCGCGACGCCGAGCCATGCCTCGACAAGGTAACCCATATCCGTCCA**  
5 DJ123 **GTCGTCGTCGACTTCCGCGACGCCGAGCCATGCCTCGACAAGGTAACCCATATCCGTCCA**  
6 Nipponbare **GTCGTCGTCGACTTCCGCGACGCCGAGCCATGCCTCGACAAGGTAACCCATATCCGTCCA**  
7 Azucena **GTCGTCGTCGACTTCCGCGACGCCGAGCCATGCCTCGACAAGGTAACCCATATCCGTCCA**  
8 \*\*\*\*\*  
9 Bala AATCACTATAACTCGAGGCCACACGAGCAAGTACACGTGGCGGCCATGCGGACAAGAAGC  
10 IR64 AATCACTATAACTCTAGGCCACACGAGCAAGTACACGTGGCGGCCATGCGGACAAGAAGC  
11 DJ123 AATCACTATAACTCTAGGCCACACGAGCAAGTACACGTGGCGGCCATGCGGACAAGAAGC  
12 Nipponbare AATCACTATAACTCTAGGCCACACGAGCAAGTACACGTGGCGGCCATGCGGACAAGAAGC  
13 Azucena AATCACTATAACTCTAGGCCACACGAGCAAGTACACGTGGCGGCCATGCGGACAAGAAGC  
14 \*\*\*\*\*  
15 Bala AATATGTGAAGTGGGTGCCACGTGTGCGTGGATGTGAATGGATGATGCAG**GTGTCGTA**  
16 IR64 AATATGTGAAGTGGGTGCCACGTGTGCGTGGATGTGAATGGATGATGCAG**GTGTCGTA**  
17 DJ123 AATATGTGAAGTGGGTGCCACGTGTGCGTGGATGTGAATGGATGATGCAG**GTGTCGTA**  
18 Nipponbare AATATGTGAAGTGGGTGCCACGTGTGCGTGGATGTGAATGGATGATGCAG**GTGTCGTA**  
19 Azucena AATATGTGAAGTGGGTGCCACGTGTGCGTGGATGTGAATGGATGATGCAG**GTGTCGTA**  
20 \*\*\*\*\*  
21 Bala **CGTGCTGGTGTTCCTCTCGGGGATGTTTCGTGACGGTGAGCGGATTCAACAAGACGGGGC**  
22 IR64 **CGTGCTGGTGTTCCTCTCGGGGATGTTTCGTGACGGTGAGCGGATTCAACAAGACGGGGC**  
23 DJ123 **CGTGCTGGTGTTCCTCTCGGGGATGTTTCGTGACGGTGAGCGGATTCAACAAGACGGGGC**  
24 Nipponbare **CGTGCTGGTGTTCCTCTCGGGGATGTTTCGTGACGGTGAGCGGATTCAACAAGACGGGGC**  
25 Azucena **CGTGCTGGTGTTCCTCTCGGGGATGTTTCGTGACGGTGAGCGGATTCAACAAGACGGGGC**  
26 \*\*\*\*\*  
27 Bala **TACCCGGGGCCATCTGGAACGTGATGGCGCCCTACTCAAAGATCAACCACGTACC**  
28 IR64 **TACCCGGGGCCATCTGGAACGTGATGGCGCCCTACTCAAAGATCAACCACGTACC**  
29 DJ123 **TACCCGGGGCCATCTGGAACGTGATGGCGCCCTACTCAAAGATCAACCACGTACC**  
30 Nipponbare **TACCCGGGGCCATCTGGAACGTGATGGCGCCCTACTCAAAGATCAACCACGTACC**  
31 Azucena **TACCCGGGGCCATCTGGAACGTGATGGCGCCCTACTCAAAGATCAACCACGTACC**  
32 \*\*\*\*\*  
33 Bala **TCACCGTCCCTCTCCGTATCATCTCCTCCTCTCCAACCTCGCCTCCAACGTCCCACCG**  
34 IR64 **TCACCGTCCCTCTCCGTATCATCTCCTCCTCCTCTCCAACCTCGCCTCCAACGTCCCACCG**  
35 DJ123 **TCACCGTCCCTCTCCGTATCATCTCCTCCTCCTCTCCAACCTCGCCTCCAACGTCCCACCG**  
36 Nipponbare **TCACCGTCCCTCTCCGTATCATCTCCTCCTCCTCTCCAACCTCGCCTCCAACGTCCCACCG**  
37 Azucena **TCACCGTCCCTCTCCGTATCATCTCCTCCTCCTCTCCAACCTCGCCTCCAACGTCCCACCG**  
38 \*\*\*\*\*  
39 Bala **GTACGTACTTACTCACAACCTTCTCGCACCATGCTATTGCTAGTACCCAATGTGTCCCG**  
40 IR64 **GTACGTACTTACTCACAACCTTCTCGCACCATGCTATTGCTAGTACCCAATGTGTCCCG**  
41 DJ123 **GTACGTACTTACTCACAACCTTCTCGCACCATGCTATTGCTAGTACCCAATGTGTCCCG**  
42 Nipponbare **GTACGTACTTACTCACAACCTTCTCGCACCATGCTATTGCTAGTACCCAATGTGTCCCG**  
43 Azucena **GTACGTACTTACTCACAACCTTCTCGCACCATGCTATTGCTAGTACCCAATGTGTCCCG**  
44 \*\*\*\*\*  
45 Bala **ACTCACCAGCACTAACATTTGTACTATACAGTACATCAGTTTCTTTTAATACATGATAT**  
46 IR64 **ACTCACCAGCACTAACATTTGTACTATACAGTACATCAGTTTCTTTTAATACATGATAT**  
47 DJ123 **ACTCACCAGCACTAACATTTGTACTATACAGTACATCAGTTTCTTTTAATACATGATAT**  
48 Nipponbare **ACTCACCAGCACTAACATTTGTACTATACAGTACATCAGTTTCTTTTAATACATGATAT**  
49 Azucena **ACTCACCAGCACTAACATTTGTACTATACAGTACATCAGTTTCTTTTAATACATGATAT**  
50 \*\*\*\*\*  
51 Bala **CTGACTTTTTTTTATCGATGAATGGATCTTATTCAATTTCTTTTGTAATATAAAAAGAT**  
52 IR64 **CTGACTTTTTTTTATCGATGAATGGATCTTATTCAATTTCTTTTGTAATATAAAAAGAT**  
53 DJ123 **CTGACTTTTTTTTATCGATGAATGGATCTTATTCAATTTCTTTTGTAATATAAAAAGAT**  
54 Nipponbare **CTGACTTTTTTTTATCGATGAATGGATCTTATTCAATTTCTTTTGTAATATAAAAAGAT**  
55 Azucena **CTGACTTTTTTTTATCGATGAATGGATCTTATTCAATTTCTTTTGTAATATAAAAAGAT**  
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Bala TAAATCAGACAATAAATCAAGTCAAATAAAAATAAATGTAATTATATTTTTTAATAAGAT  
 IR64 TAAATCAGACAATAAATCAAGTCAAATAAAAATAAATGTAATTATATTTTTTAATAAGAT  
 DJ123 TAAATCAGACAATAAATCAAGTCAAATAAAAATAAATGTAATTATATTTTTTAATAAGAT  
 Nipponbare TAAATCAGACAATAAATCAAGTCAAATAAAAATAAATGTAATTATATTTTTTAATAAGAT  
 Azucena TAAATCAGACAATAAATCAAGTCAAATAAAAATAAATGTAATTATATTTTTTAATAAGAT  
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Bala GAATGATTATTAAGAAAAAATTTTTTTGTCTCTCATAATTAAGAAAAAACAACCTCA  
 IR64 GAATGATTATTAAGAAAAAATTTTTTTGTCTCTCATAATTAAGAAAAAACAACCTCA  
 DJ123 GAATGATTATTAAGAAAAAATTTTTTTGTCTCTCATAATTAAGAAAAAACAACCTCA  
 Nipponbare GAATGATTATTAAGAAAAAATTTTTTTGTCTCTCATAATTAAGAAAAAACAACCTCA  
 Azucena GAATGATTATTAAGAAAAAATTTTTTTGTCTCTCATAATTAAGAAAAAACAACCTCA  
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Bala TTTTTTACATGTTATTTGAGTGGAATTTGTAATTAATTTATATATGTGGGTGTAGT**GT**  
 IR64 TTTTTTACATGTTATTTGAGTGGAATTTGTAATTAATTTATATATGTGGGTGTAGT**GT**  
 DJ123 TTTTTTACATGTTATTTGAGTGGAATTTGTAATTAATTTATATATGTGGGTGTAGT**GT**  
 Nipponbare TTTTTTACATGTTATTTGAGTGGAATTTGTAATTAATTTATATATGTGGGTGTAGT**GT**  
 Azucena TTTTTTACATGTTATTTGAGTGGAATTTGTAATTAATTTATATATGTGGGTGTAGT**GT**  
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Bala **GTTGATGGGGATGAGGTGGCGCGGGCGGGCGACGATATCACCGGGCGGGTACCGCG**  
 IR64 **GTTGATGGGGATGAGGTGGCGCGGGCGGGCGACGATATCACCGGGCGGGTACCGCG**  
 DJ123 **GTTGATGGGGATGAGGTGGCGCGGGCGGGCGACGATATCACCGGGCGGGTACCGCG**  
 Nipponbare **GTTGATGGGGATGAGGTGGCGCGGGCGGGCGACGATATCACCGGGCGGGTACCGCG**  
 Azucena **GTTGATGGGGATGAGGTGGCGCGGGCGGGCGACGATATCACCGGGCGGGTACCGCG**  
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Bala **GTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGGGGAACCTGTCGCTGCTGGGGTC**  
 IR64 **GTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGGGGAACCTGTCGCTGCTGGGGTC**  
 DJ123 **GTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGGGGAACCTGTCGCTGCTGGGGTC**  
 Nipponbare **GTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGGGGAACCTGTCGCTGCTGGGGTC**  
 Azucena **GTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGGGGAACCTGTCGCTGCTGGGGTC**  
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Bala **GGCGGCGAACCTGATCGTGTGCGAGCAGGCGAGGCGGGCGACGCGCAACGCCTACGACCT**  
 IR64 **GGCGGCGAACCTGATCGTGTGCGAGCAGGCGAGGCGGGCGACGCGCAACGCCTACGACCT**  
 DJ123 **GGCGGCGAACCTGATCGTGTGCGAGCAGGCGAGGCGGGCGACGCGCAACGCCTACGACCT**  
 Nipponbare **GGCGGCGAACCTGATCGTGTGCGAGCAGGCGAGGCGGGCGACGCGCAACGCCTACGACCT**  
 Azucena **GGCGGCGAACCTGATCGTGTGCGAGCAGGCGAGGCGGGCGACGCGCAACGCCTACGACCT**  
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Bala **CACCTTCTGGAACCAGTCATCTTCGGACTCCCATCCACCCTCGTCTGTCACCGCCATCGG**  
 IR64 **CACCTTCTGGAACCAGTCATCTTCGGACTCCCATCCACCCTCGTCTGTCACCGCCATCGG**  
 DJ123 **CACCTTCTGGAACCAGTCATCTTCGGACTCCCATCCACCCTCGTCTGTCACCGCCATCGG**  
 Nipponbare **CACCTTCTGGAACCAGTCATCTTCGGACTCCCATCCACCCTCGTCTGTCACCGCCATCGG**  
 Azucena **CACCTTCTGGAACCAGTCATCTTCGGACTCCCATCCACCCTCGTCTGTCACCGCCATCGG**  
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Bala **CATCCCTCTCATCGGCAAGATCAACATCTAGT**CGTCATTAAATTAATTAATTAATATAAT  
 IR64 **CATCCCTCTCATCGGCAAGATCAACATCTAGT**CGTCATTAAATTAATTAATTAATATAAT  
 DJ123 **CATCCCTCTCATCGGCAAGATCAACATCTAGT**CGTCATTAAATTAATTAATTAATATAAT  
 Nipponbare **CATCCCTCTCATCGGCAAGATCAACATCTAGT**ATTTCATTAAATTAATTAATTAATATAAT  
 Azucena **CATCCCTCTCATCGGCAAGATCAACATCTAGT**ATTTCATTAAATTAATTAATTAATATAAT  
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Bala CCGGCTAGCTACTATCTACAACGATCAGAAGAAGGCAGAATGATCGAGCATGCAAGAATT  
 IR64 CCGGCTAGCTACTATCTACAACGATCAGAAGAAGGCAGAATGATCGAGCATGCAAGAATT  
 DJ123 CCGGCTAGCTACTATCTACAACGATCAGAAGAAGGCAGAATGATCGAGCATGCAAGAATT  
 Nipponbare CCGGCTAGCTACTATCTACAACGATCAGAAGAAGGCAGAATGATCGAGCATGCAAGAATT  
 Azucena CCGGCTAGCTACTATCTACAACGATCAGAAGAAGGCAGAATGATCGAGCATGCAAGAATT  
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Bala CATGCAAATTACAAGGGATGTGATATGATCATATTAATCCAAGGAAGCTAGTATGATGAT  
 IR64 CATGCAAATTACAAGGGATGTGATATGATCATATTAATCCAAGGAAGCTAGTATGATGAT  
 DJ123 CATGCAAATTACAAGGGATGTGATATGATCATATTAATCCAAGGAAGCTAGTATGATGAT  
 Nipponbare CATGCAAATTACAAGGGATGTGATATGATCATATTAATCCAAGGAAGCTAGTATGATGAT  
 Azucena CATGCAAATTACAAGGGATGTGATATGATCATATTAATCCAAGGAAGCTAGTATGATGAT  
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4 Bala TAAGTTTGTGATCAAGCCGTACT**GTGTGCATGCCAATGCATGCATGTGTTCAAGTGTACA**  
5 IR64 TAAGTTTGTGATCAAGCCGTACT**GTGTGCATGCCAATGCATGCATGTGTTCAAGTGTACA**  
6 DJ123 TAAGTTTGTGATCAAGCCGTACT**GTGTGCATGCCAATGCATGCATGTGTTCAAGTGTACA**  
7 Nipponbare TAAGTTTGTGATCAAGCCGTACT**GTGTGCATGCCAATGCATGCATGTGTTCAAGTGTACA**  
8 Azucena TAAGTTTGTGATCAAGCCGTACT**GTGTGCATGCCAATGCATGCATGTGTTCAAGTGTACA**  
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11 Bala CTATGCTTGGTCTCAAGTGGATCAATTATATTGGTGCCATGCTCTGGGATAAATTAGTTAA  
12 IR64 CTATGCTTGGTCTCAAGTGGATCAATTATATTGGTGCCATGCTCTGGGATAAATTAGTTAA  
13 DJ123 CTATGCTTGGTCTCAAGTGGATCAATTATATTGGTGCCATGCTCTGGGATAAATTAGTTAA  
14 Nipponbare CTATGCTTGGTCTCAAGTGGATCAATTATATTGGTGCCATGCTCTGGGATAAATTAGTTAA  
15 Azucena CTATGCTTGGTCTCAAGTGGATCAATTATATTGGTGCCATGCTCTGGGATAAATTAGTTAA  
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18 Bala TTAGTACTTATGATAAGCTAGAGAGGAGGATGCATTATATTGGTGCATGGTAATAAGGCA  
19 IR64 TTAGTACTTATGATAAGCTAGAGAGGAGGATGCATTATATTGGTGCATGGTAATAAGGCA  
20 DJ123 TTAGTACTTATGATAAGCTAGAGAGGAGGATGCATTATATTGGTGCATGGTAATAAGGCA  
21 Nipponbare TTAGTACTTATGATAAGCTAGAGAGGAGGATGCATTATATTGGTGCATGGTAATAAGGCA  
22 Azucena TTAGTACTTATGATAAGCTAGAGAGGAGGATGCATTATATTGGTGCATGGTAATAAGGCA  
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25 Bala AATGAACAGCAGAATGCATGTATATATGTACTGTATCCCTGTTGCCAGCTGGTCTCTGTT  
26 IR64 AATGAACAGCAGAATGCATGTATATATGTACTGTATCCCTGTTGCCAGCTGGTCTCTGTT  
27 DJ123 AATGAACAGCAGAATGCATGTATATATGTACTGTATCCCTGTTGCCAGCTGGTCTCTGTT  
28 Nipponbare AATGAACAGCAGAATGCATGTATATATGTACTGTATCCCTGTTGCCAGCTGGTCTCTGTT  
29 Azucena AATGAACAGCAGAATGCATGTATATATGTACTGTATCCCTGTTGCCAGCTGGTCTCTGTT  
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32 Bala TGAATGAAGTTGATAGCAACAAAGCTAGCACTAGCGAATGAGCTAGTGACTCAAGTACGC  
33 IR64 TGAATGAAGTTGATAGCAACAAAGCTAGCACTAGCGAATGAGCTAGTGACTCAAGTACGC  
34 DJ123 TGAATGAAGTTGATAGCAACAAAGCTAGCACTAGCGAATGAGCTAGTGACTCAAGTACGC  
35 Nipponbare TGAATGAAGTTGATAGCAACAAAGCTAGCACTAGCGAATGAGCTAGTGACTCAAGTACGC  
36 Azucena TGAATGAAGTTGATAGCAACAAAGCTAGCACTAGCGAATGAGCTAGTGACTCAAGTACGC  
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39 Bala ATGCCGATTACCTCATTTGCTCCAAACTTGCAATCAGTTTTGAACTTTTAATAAAGTTAA  
40 IR64 ATGCCGATTACCTCATTTGCTCCAAACTTGCAATCAGTTTTGAACTTTTAATAAAGTTAA  
41 DJ123 ATGCCGATTACCTCATTTGCTCCAAACTTGCAATCAGTTTTGAACTTTTAATAAAGTTAA  
42 Nipponbare ATGCCGATTACCTCATTTGCTCCAAACTTGCAATCAGTTTTGAACTTTTAATAAAGTTAA  
43 Azucena ATGCCGATTACCTCATTTGCTCCAAACTTGCAATCAGTTTTGAACTTTTAATAAAGTTAA  
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46 Bala TTTGGCTTTGTGCACCTTGTCATTTGCTCCCTCTTTGTTTAAACCGATCTTGCCTAGTGCG  
47 IR64 TTTGGCTTTGTGCACCTTGTCATTTGCTCCCTCTTTGTTTAAACCGATCTTGCCTAGTGCG  
48 DJ123 TTTGGCTTTGTGCACCTTGTCATTTGCTCCCTCTTTGTTTAAACCGATCTTGCCTAGTGCG  
49 Nipponbare TTTGGCTTTGTGCACCTTGTCATTTGCTCCCTCTTTGTTTAAACCGATCTTGCCTAGTGCG  
50 Azucena TTTGGCTTTGTGCACCTTGTCATTTGCTCCCTCTTTGTTTAAACCGATCTTGCCTAGTGCG  
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53 Bala TACGTAGCTAGATTGCACATTTAAG  
54 IR64 TACGTAGCTAGATTGCACATTTAAG  
55 DJ123 TACGTAGCTAGATTGCACATTTAAG  
56 Nipponbare TACGTAGCTAGATTGCACATTTAAG  
57 Azucena TACGTAGCTAGATTGCACATTTAAG  
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Supplementary figure 4. Genomic sequence alignment of *Lsi3*. Regions highlighted in bold are predicted exon regions based on the Rice Genome Annotation Project – release 7 sequence available at <http://rice.plantbiology.msu.edu/>.