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

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5 3 in rice
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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
29
30 48 both drought and salinity, and also enhances resistance to pests and diseases. The aim of this
31
32 49 study was to determine the range of silicon content in a set of rice (*Oryza sativa* L.)
33
34 50 accessions, and to determine if the natural variation of shoot silicon is linked to the
35
36 51 previously identified silicon transporters (*Lsi* genes). Silicon content was determined in 50
37
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⁻¹ 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⁻¹ (Deren, 2001) and 28 to 61 mg g⁻¹ (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
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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
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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.*,
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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
38
39 108 molecular species dimethylarsinic acid (DMA), and monomethylarsonic acid (MMA) as well
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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.*,
6
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).

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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
25
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, 3rd sheath,
145 3rd node, 3rd leaf, 2nd sheath, 2nd node, 2nd leaf, flag sheath, 1st 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 2nd 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[®], NY). To digest the sample, 0.6 mL of hydrogen
175 peroxide (H₂O₂, > 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 ddH₂O. Prior to analysis, samples were diluted
8
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;*
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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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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
24
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
30
31 191 content (% of silicon dry shoot weight) was measured using a commercial P-XRF instrument
32
33 192 (Niton XL3t900 GOLDD analyzer: Thermo Scientific Winchester, UK), calibrated using Si-
34
35 193 spiked synthetic methyl cellulose and validated using Certified Reference Materials of NCS
36
37 194 DC73349 ‘Bush branches and leaves’ obtained from the China National Analysis Center for
38
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**

46
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
15
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
19
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
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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
13
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, 1st 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 mg g^{-1}), while the lowest was in the grain (3.5 mg g^{-1}).
240 For plants grown under flooded conditions: The highest silicon content was observed in the
241 flag leaf (67.3 mg g^{-1}) and the lowest was in the grains (4.4 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 mg g^{-1} , and the lowest mean shoot silicon was observed in Dhala Shita (16.5 mg g^{-1}) The
248 highest mean shoot silicon was observed in Bala (42.4 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 mg g^{-1} while the mean silicon
276 content of the accessions with the T allele was 23.1 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 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⁻¹. 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
13
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⁻¹ while the mean silicon
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20 288 content of the accessions with the A allele was 35.6 mg g⁻¹ (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
37
38 296 polymorphisms within the genes were identified. For *Lsi2*, there was a synonymous SNP
39
40 297 substitution within the first exon, where DJ123 has “C” allele while the other 4 accessions
41
42 298 have “T” allele (Supplementary Figure 3). For *Lsi3*, 4 SNPs were detected in exons and 6
43
44 299 SNPs in introns (Supplementary Figure 4). There was only one non-synonymous SNP
45
46 300 observed in the first exon of *Lsi3* where DJ123 and Bala have “T” allele and other accessions
47
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, 1st
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, 2nd 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, 1st 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⁻¹ in this study, 60 mg g⁻¹ (*Deren,*
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28 345 2001), 61 mg g⁻¹ (*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⁻¹ 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⁻¹ to 42.4 mg
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45 353 g⁻¹) 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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16 17 18 428 **References**

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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 (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 (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 10th and 90th 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 (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.

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28 667 **Figure 6:** Variation in shoot silicon (mg g^{-1}) between different SNPs within 10 kb of *Lsi2* and
29 668 *Lsi3*

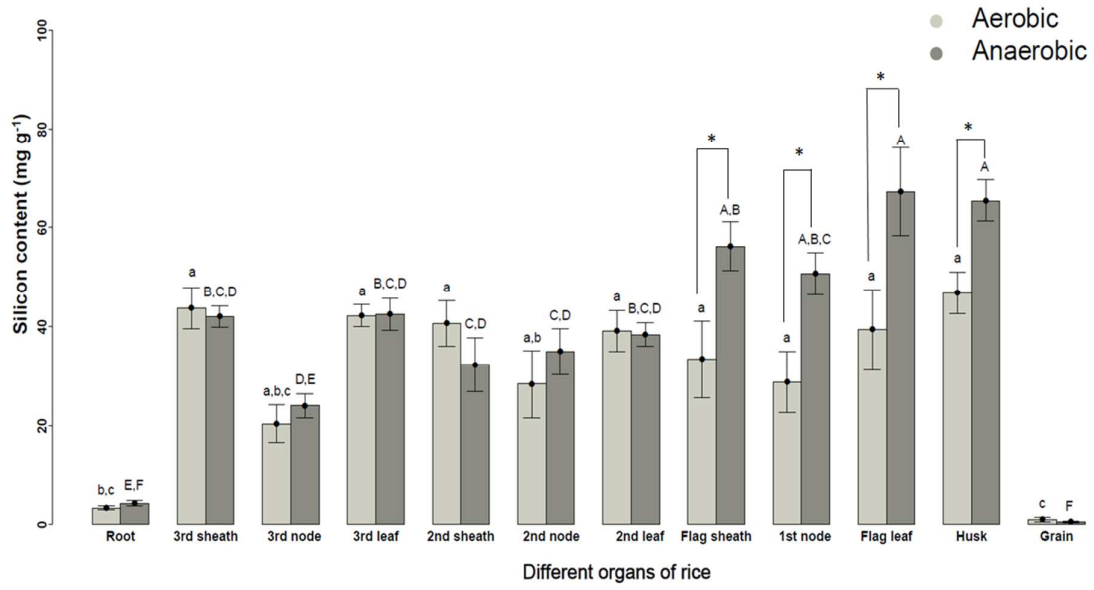
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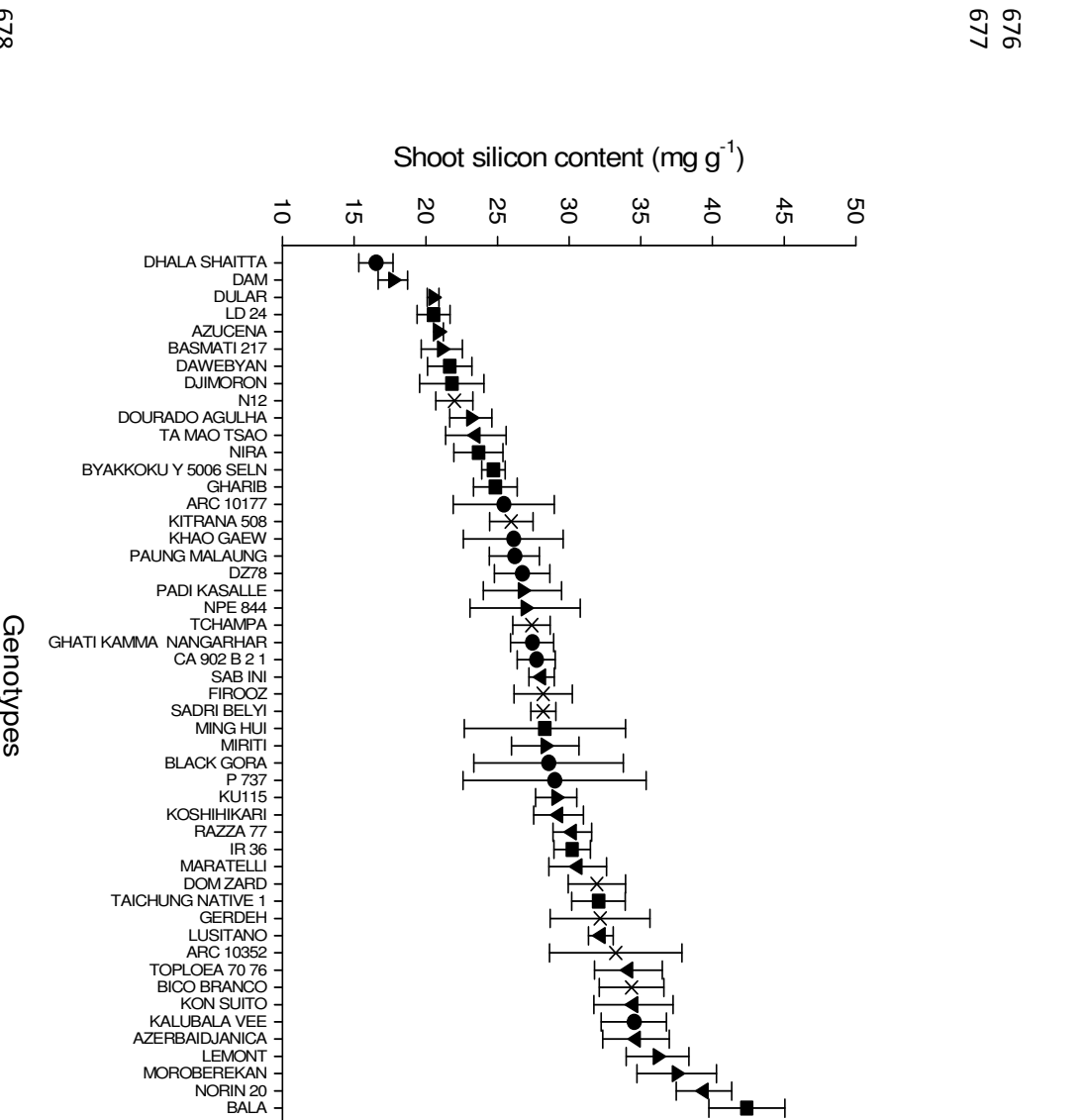
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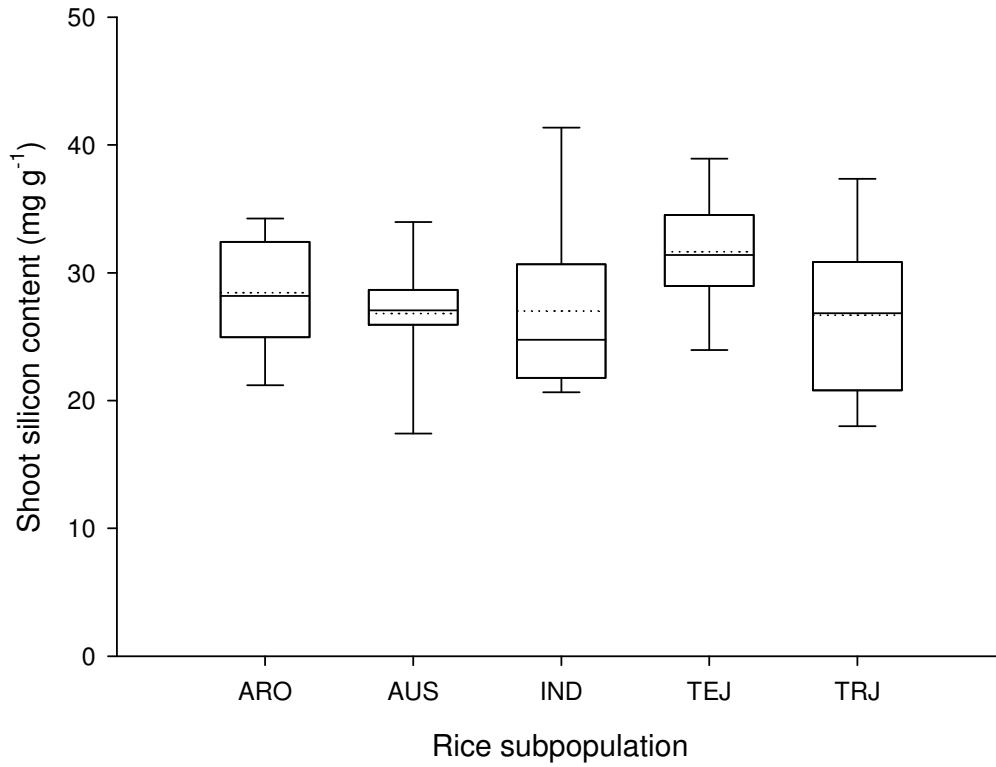


679 **Figure 2**

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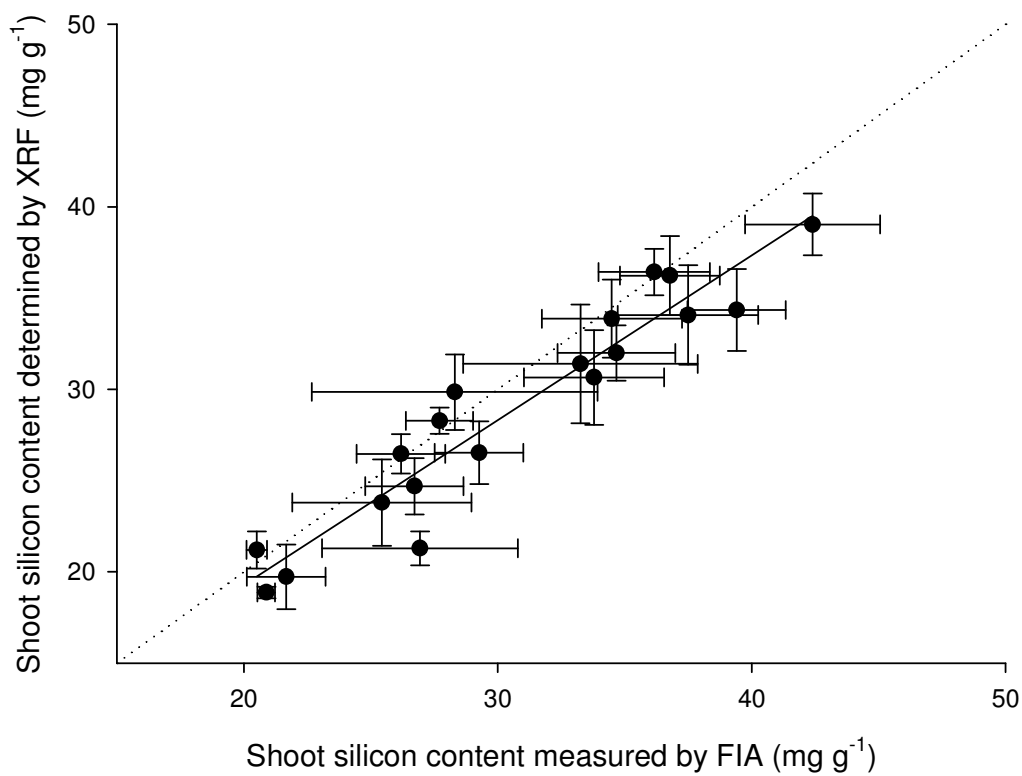


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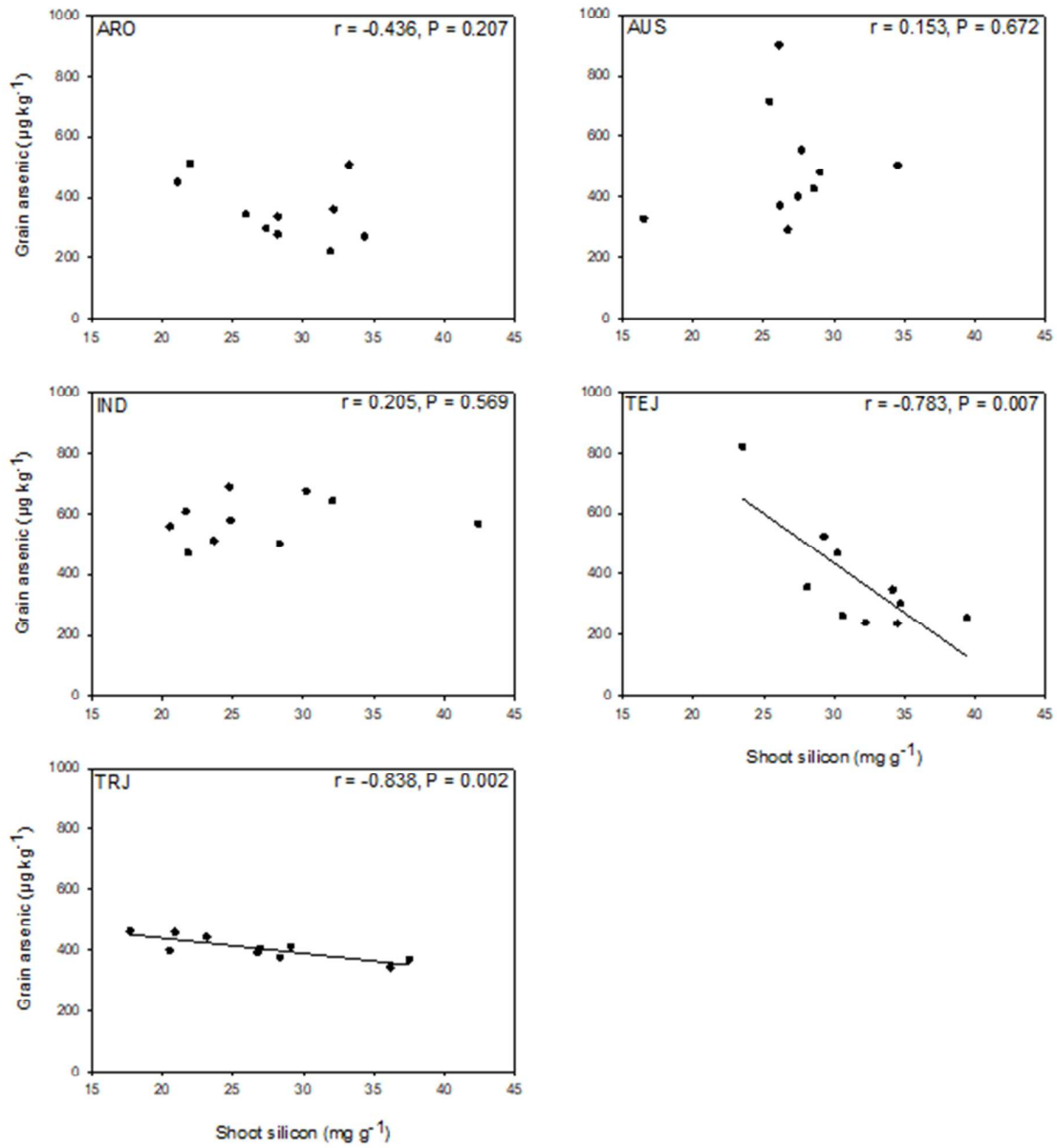
682 **Figure 3**

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685 **Figure 4**

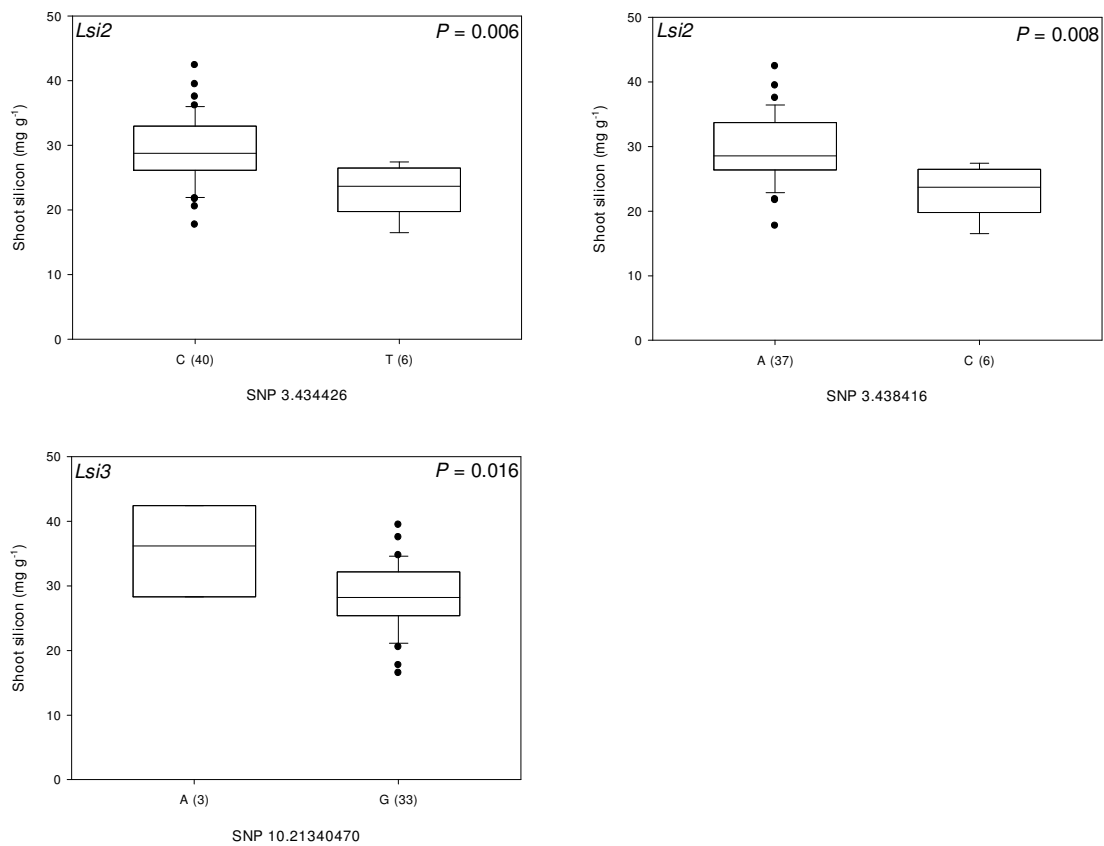


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687 **Figure 5**

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692 **Figure 6**

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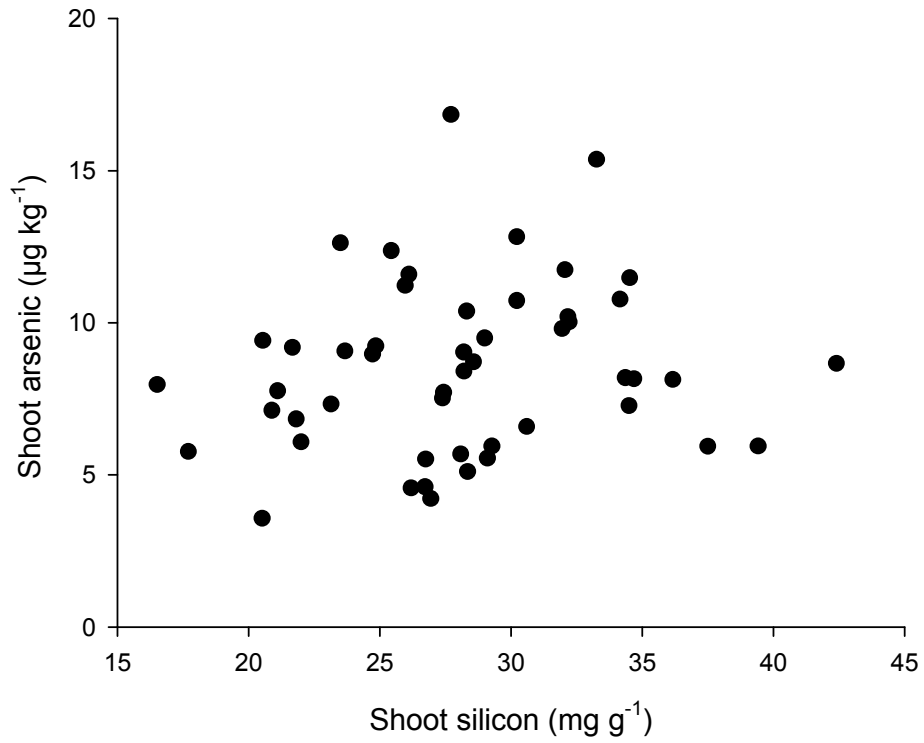
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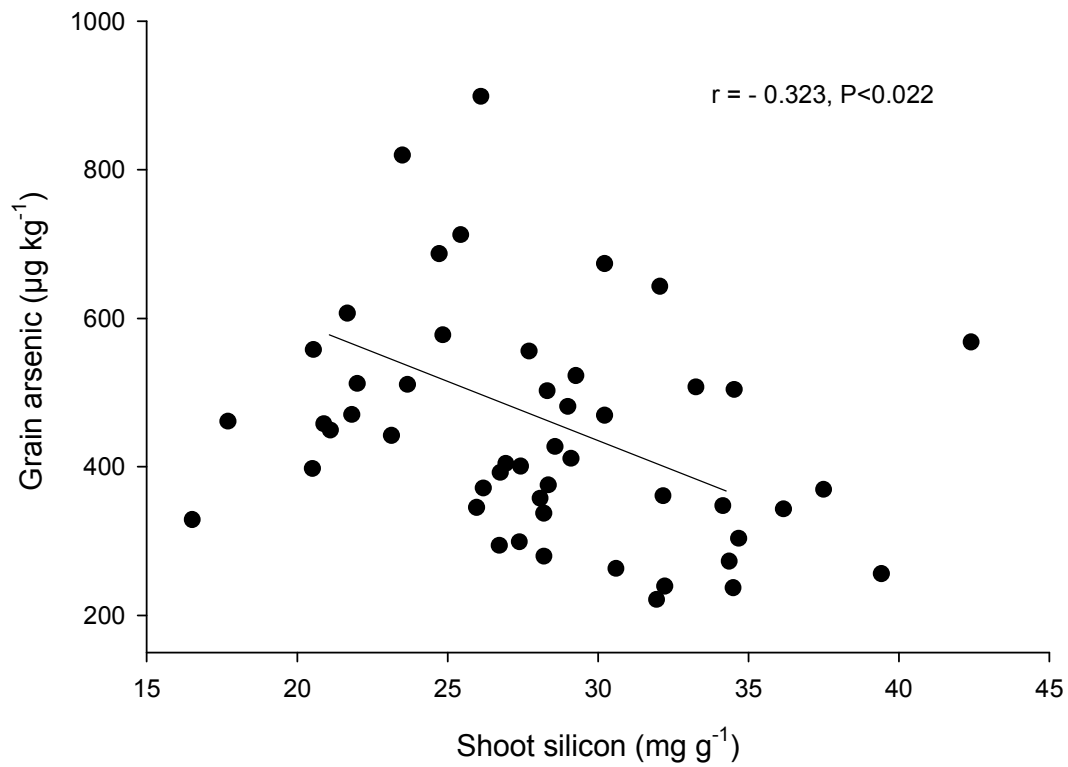
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3 706 **Supplementary Table 1:** Selected genotype from RDP1 for shoot silicon analysis

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⁻¹) and shoot arsenic (µg kg⁻¹) within 50 accessions of RDP1.



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

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 AAGATGAGTGAGCTTGCGTCGGCGCCAAG
23	IR64	CTGCTAGGTGGTGGTCGATCGAAGAAGAAG AAGATGAGTGAGCTTGCGTCGGCGCCAAG
24	DJ123	CTGCTAGGTGGTGGTCGATCGAAGAAGAAG AAGATGAGTGAGCTTGCGTCGGCGCCAAG
25	Nipponbare	CTGCTAGGTGGTGGTCGATCGAAGAAGAAG AAGATGAGTGAGCTTGCGTCGGCGCCAAG
26	Azucena	CTGCTAGGTGGTGGTCGATCGAAGAAGAAG AAGATGAGTGAGCTTGCGTCGGCGCCAAG
27		*****
28	Bala	GTGGCGCTTGATCGATCGCGTTCGCGGTGTTCTGGATGATGGCGGTGTTCCCGTCGGTG
29	IR64	GTGGCGCTTGATCGATCGCGTTCGCGGTGTTCTGGATGATGGCGGTGTTCCCGTCGGTG
30	DJ123	GTGGCGCTTGATCGATCGCGTTCGCGGTGTTCTGGATGATGGCGGTGTTCCCGTCGGTG
31	Nipponbare	GTGGCGCTTGATCGATCGCGTTCGCGGTGTTCTGGATGATGGCGGTGTTCCCGTCGGTG
32	Azucena	GTGGCGCTTGATCGATCGCGTTCGCGGTGTTCTGGATGATGGCGGTGTTCCCGTCGGTG
33		*****
34	Bala	CCGTTCTGCCGATCGGGCGGACGGCGGGGTCGCTGCTGAGCGCGGTGCTGATGGTGATA
35	IR64	CCGTTCTGCCGATCGGGCGGACGGCGGGGTCGCTGCTGAGCGCGGTGCTGATGGTGATA
36	DJ123	CCGTTCTGCCGATCGGGCGGACGGCGGGGTCGCTGCTGAGCGCGGTGCTGATGGTGATA
37	Nipponbare	CCGTTCTGCCGATCGGGCGGACGGCGGGGTCGCTGCTGAGCGCGGTGCTGATGGTGATA
38	Azucena	CCGTTCTGCCGATCGGGCGGACGGCGGGGTCGCTGCTGAGCGCGGTGCTGATGGTGATA
39		*****
40	Bala	TTCACGTGATCAGCCCCGACGACGCGTACGCCTCCATCGACCTCCCAATCCTGGGCCTC
41	IR64	TTCACGTGATCAGCCCCGACGACGCGTACGCCTCCATCGACCTCCCAATCCTGGGCCTC
42	DJ123	TTCACGTGATCAGCCCCGACGACGCGTACGCCTCCATCGACCTCCCAATCCTGGGCCTC
43	Nipponbare	TTCACGTGATCAGCCCCGACGACGCGTACGCCTCCATCGACCTCCCAATCCTGGGCCTC
44	Azucena	TTCACGTGATCAGCCCCGACGACGCGTACGCCTCCATCGACCTCCCAATCCTGGGCCTC
45		*****
46	Bala	CTCTTCGCCACCATGGTGGTGGGCAGCTACCTCCGGAACGCCGGGATGTTCAAGCACCTG
47	IR64	CTCTTCGCCACCATGGTGGTGGGCAGCTACCTCCGGAACGCCGGGATGTTCAAGCACCTG
48	DJ123	CTCTTCGCCACCATGGTGGTGGGCAGCTACCTCCGGAACGCCGGGATGTTCAAGCACCTG
49	Nipponbare	CTCTTCGCCACCATGGTGGTGGGCAGCTACCTCCGGAACGCCGGGATGTTCAAGCACCTG
50	Azucena	CTCTTCGCCACCATGGTGGTGGGCAGCTACCTCCGGAACGCCGGGATGTTCAAGCACCTG
51		*****
52	Bala	GGGCGTCTGCTGGCGTGGAAGAGCCAGGGCGGGCGGACCTCATGTGCCGCTCTGCGTC
53	IR64	GGGCGTCTGCTGGCGTGGAAGAGCCAGGGCGGGCGGACCTCATGTGCCGCTCTGCGTC
54	DJ123	GGGCGTCTGCTGGCGTGGAAGAGCCAGGGCGGGCGGACCTCATGTGCCGCTCTGCGTC
55	Nipponbare	GGGCGTCTGCTGGCGTGGAAGAGCCAGGGCGGGCGGACCTCATGTGCCGCTCTGCGTC
56	Azucena	GGGCGTCTGCTGGCGTGGAAGAGCCAGGGCGGGCGGACCTCATGTGCCGCTCTGCGTC
57		*****

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4 Bala **GTCACCGCCCTCGCCAGCGCCCTCTTCACCAACGACACCTGCTGCGTCTCCTCACCAG**
5 IR64 **GTCACCGCCCTCGCCAGCGCCCTCTTCACCAACGACACCTGCTGCGTCTCCTCACCAG**
6 DJ123 **GTCACCGCCCTCGCCAGCGCCCTCTTCACCAACGACACCTGCTGCGTCTCCTCACCAG**
7 Nipponbare **GTCACCGCCCTCGCCAGCGCCCTCTTCACCAACGACACCTGCTGCGTCTCCTCACCAG**
8 Azucena **GTCACCGCCCTCGCCAGCGCCCTCTTCACCAACGACACCTGCTGCGTCTCCTCACCAG**
9 *****
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11 Bala **TTCGTCTCGAGCTCGCCGCGGAGCGCAACCTCCCCGCAAGCCCTTCTCTCGCCCTC**
12 IR64 **TTCGTCTCGAGCTCGCCGCGGAGCGCAACCTCCCCGCAAGCCCTTCTCTCGCCCTC**
13 DJ123 **TTCGTCTCGAGCTCGCCGCGGAGCGCAACCTCCCCGCAAGCCCTTCTCTCGCCCTC**
14 Nipponbare **TTCGTCTCGAGCTCGCCGCGGAGCGCAACCTCCCCGCAAGCCCTTCTCTCGCCCTC**
15 Azucena **TTCGTCTCGAGCTCGCCGCGGAGCGCAACCTCCCCGCAAGCCCTTCTCTCGCCCTC**
16 *****
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18 Bala **GCCTCCAGCGCCAACATCGGCTCCGCCGCCACCCCATCGGCAACCCCCAGAACCTGGTC**
19 IR64 **GCCTCCAGCGCCAACATCGGCTCCGCCGCCACCCCATCGGCAACCCCCAGAACCTGGTC**
20 DJ123 **GCCTCCAGCGCCAACATCGGCTCCGCCGCCACCCCATCGGCAACCCCCAGAACCTGGTC**
21 Nipponbare **GCCTCCAGCGCCAACATCGGCTCCGCCGCCACCCCATCGGCAACCCCCAGAACCTGGTC**
22 Azucena **GCCTCCAGCGCCAACATCGGCTCCGCCGCCACCCCATCGGCAACCCCCAGAACCTGGTC**
23 *****
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25 Bala **ATCGCCTTCAATAGCAAGATCACCTTCCCAAGTTCTCATGGGAATCTCCCGGCCATG**
26 IR64 **ATCGCCTTCAATAGCAAGATCACCTTCCCAAGTTCTCATGGGAATCTCCCGGCCATG**
27 DJ123 **ATCGCCTTCAATAGCAAGATCACCTTCCCAAGTTCTCATGGGAATCTCCCGGCCATG**
28 Nipponbare **ATCGCCTTCAATAGCAAGATCACCTTCCCAAGTTCTCATGGGAATCTCCCGGCCATG**
29 Azucena **ATCGCCTTCAATAGCAAGATCACCTTCCCAAGTTCTCATGGGAATCTCCCGGCCATG**
30 *****
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32 Bala **CTCGTCGGGATGGCCGTCAACATGGTTCATGCTGCTCTGCATGTACTGGAGGGAGCTGGGC**
33 IR64 **CTCGTCGGGATGGCCGTCAACATGGTTCATGCTGCTCTGCATGTACTGGAGGGAGCTGGGC**
34 DJ123 **CTCGTCGGGATGGCCGTCAACATGGTTCATGCTGCTCTGCATGTACTGGAGGGAGCTGGGC**
35 Nipponbare **CTCGTCGGGATGGCCGTCAACATGGTTCATGCTGCTCTGCATGTACTGGAGGGAGCTGGGC**
36 Azucena **CTCGTCGGGATGGCCGTCAACATGGTTCATGCTGCTCTGCATGTACTGGAGGGAGCTGGGC**
37 *****
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39 Bala **GGAGGGGCCGAGCTCAGCGTCGACGGCAAGCAGATGGAGGCGGTGGAGGAAGGCAGGTTCG**
40 IR64 **GGAGGGGCCGAGCTCAGCGTCGACGGCAAGCAGATGGAGGCGGTGGAGGAAGGCAGGTTCG**
41 DJ123 **GGAGGGGCCGAGCTCAGCGTCGACGGCAAGCAGATGGAGGCGGTGGAGGAAGGCAGGTTCG**
42 Nipponbare **GGAGGGGCCGAGCTCAGCGTCGACGGCAAGCAGATGGAGGCGGTGGAGGAAGGCAGGTTCG**
43 Azucena **GGAGGGGCCGAGCTCAGCGTCGACGGCAAGCAGATGGAGGCGGTGGAGGAAGGCAGGTTCG**
44 *****
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46 Bala **CCGGCGTCGGCCAAGAGCACGCCGAGCTGAACGGCAACGGCAACACGATGATGTCGCTG**
47 IR64 **CCGGCGTCGGCCAAGAGCACGCCGAGCTGAACGGCAACGGCAACACGATGATGTCGCTG**
48 DJ123 **CCGGCGTCGGCCAAGAGCACGCCGAGCTGAACGGCAACGGCAACACGATGATGTCGCTG**
49 Nipponbare **CCGGCGTCGGCCAAGAGCACGCCGAGCTGAACGGCAACGGCAACACGATGATGTCGCTG**
50 Azucena **CCGGCGTCGGCCAAGAGCACGCCGAGCTGAACGGCAACGGCAACACGATGATGTCGCTG**
51 *****
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53 Bala **GAGATGTCGGAGAACATAACGACCAAGCACCCATGGTTCATGCAGTGCACGGAGGCGCGG**
54 IR64 **GAGATGTCGGAGAACATAACGACCAAGCACCCATGGTTCATGCAGTGCACGGAGGCGCGG**
55 DJ123 **GAGATGTCGGAGAACATAACGACCAAGCACCCATGGTTCATGCAGTGCACGGAGGCGCGG**
56 Nipponbare **GAGATGTCGGAGAACATAACGACCAAGCACCCATGGTTCATGCAGTGCACGGAGGCGCGG**
57 Azucena **GAGATGTCGGAGAACATAACGACCAAGCACCCATGGTTCATGCAGTGCACGGAGGCGCGG**
58 *****
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60 Bala **CGGAAGCTGTTCTCAAGAGCTTCGCGTACGTGGTGACGGTGGGGATGGTGGTGGCCTAC**
CGGAAGCTGTTCTCAAGAGCTTCGCGTACGTGGTGACGGTGGGGATGGTGGTGGCCTAC
CGGAAGCTGTTCTCAAGAGCTTCGCGTACGTGGTGACGGTGGGGATGGTGGTGGCCTAC
CGGAAGCTGTTCTCAAGAGCTTCGCGTACGTGGTGACGGTGGGGATGGTGGTGGCCTAC

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Bala	ATGGTGGGGCTCAACATGTCGTGGACGGCCATCACCACGGCGCTGGCGCTGGTGGTGGTC
IR64	ATGGTGGGGCTCAACATGTCGTGGACGGCCATCACCACGGCGCTGGCGCTGGTGGTGGTC
DJ123	ATGGTGGGGCTCAACATGTCGTGGACGGCCATCACCACGGCGCTGGCGCTGGTGGTGGTC
Nipponbare	ATGGTGGGGCTCAACATGTCGTGGACGGCCATCACCACGGCGCTGGCGCTGGTGGTGGTC
Azucena	ATGGTGGGGCTCAACATGTCGTGGACGGCCATCACCACGGCGCTGGCGCTGGTGGTGGTC *****
Bala	GACTTCCGCGACGCCGAGCCGTGCCTGGACACCGTGTCTACTCGCTGCTCGTCTTCTTC
IR64	GACTTCCGCGACGCCGAGCCGTGCCTGGACACCGTGTCTACTCGCTGCTCGTCTTCTTC
DJ123	GACTTCCGCGACGCCGAGCCGTGCCTGGACACCGTGTCTACTCGCTGCTCGTCTTCTTC
Nipponbare	GACTTCCGCGACGCCGAGCCGTGCCTGGACACCGTGTCTACTCGCTGCTCGTCTTCTTC
Azucena	GACTTCCGCGACGCCGAGCCGTGCCTGGACACCGTGTCTACTCGCTGCTCGTCTTCTTC *****
Bala	TCCGGGATGTTTCATCACCGTCAGCGGCTTCAACAAGACGGGCCTCCCGGGAGCCATCTGG
IR64	TCCGGGATGTTTCATCACCGTCAGCGGCTTCAACAAGACGGGCCTCCCGGGAGCCATCTGG
DJ123	TCCGGGATGTTTCATCACCGTCAGCGGCTTCAACAAGACGGGCCTCCCGGGAGCCATCTGG
Nipponbare	TCCGGGATGTTTCATCACCGTCAGCGGCTTCAACAAGACGGGCCTCCCGGGAGCCATCTGG
Azucena	TCCGGGATGTTTCATCACCGTCAGCGGCTTCAACAAGACGGGCCTCCCGGGAGCCATCTGG *****
Bala	GACTTCATGGCCCCCTACTCCAAGGTCAACAGCGTCGGCGGCATCTCCGTCCTCTCCGTC
IR64	GACTTCATGGCCCCCTACTCCAAGGTCAACAGCGTCGGCGGCATCTCCGTCCTCTCCGTC
DJ123	GACTTCATGGCCCCCTACTCCAAGGTCAACAGCGTCGGCGGCATCTCCGTCCTCTCCGTC
Nipponbare	GACTTCATGGCCCCCTACTCCAAGGTCAACAGCGTCGGCGGCATCTCCGTCCTCTCCGTC
Azucena	GACTTCATGGCCCCCTACTCCAAGGTCAACAGCGTCGGCGGCATCTCCGTCCTCTCCGTC *****
Bala	ATCATCTCTCCTCTCCAACCTCGCATCAAACGTACCAACGGTAATTATACATTCTAT
IR64	ATCATCTCTCCTCTCCAACCTCGCATCAAACGTACCAACGGTAATTATACATTCTAT
DJ123	ATCATCTCTCCTCTCCAACCTCGCATCAAACGTACCAACGGTAATTATACATTCTAT
Nipponbare	ATCATCTCTCCTCTCCAACCTCGCATCAAACGTACCAACGGTAATTATACATTCTAT
Azucena	ATCATCTCTCCTCTCCAACCTCGCATCAAACGTACCAACGGTAATTATACATTCTAT *****
Bala	TAATCCAGAATTAATGTATACTACTCCATATAAGTATAACTAATTAACATACAAAAATAT
IR64	TAATCCAGAATTAATGTATACTACTCCATATAAGTATAACTAATTAACATACAAAAATAT
DJ123	TAATCCAGAATTAATGTATACTACTCCATATAAGTATAACTAATTAACATACAAAAATAT
Nipponbare	TAATCCAGAATTAATGTATACTACTCCATATAAGTATAACTAATTAACATACAAAAATAT
Azucena	TAATCCAGAATTAATGTATACTACTCCATATAAGTATAACTAATTAACATACAAAAATAT *****
Bala	ATATGCGCATGCATGCAGTGCCTTCTTATGGGTGATGAGGTGGCGAAGCGGGCGGCTGA
IR64	ATATGCGCATGCATGCAGTGCCTTCTTATGGGTGATGAGGTGGCGAAGCGGGCGGCTGA
DJ123	ATATGCGCATGCATGCAGTGCCTTCTTATGGGTGATGAGGTGGCGAAGCGGGCGGCTGA
Nipponbare	ATATGCGCATGCATGCAGTGCCTTCTTATGGGTGATGAGGTGGCGAAGCGGGCGGCTGA
Azucena	ATATGCGCATGCATGCAGTGCCTTCTTATGGGTGATGAGGTGGCGAAGCGGGCGGCTGA *****
Bala	TATCGCCGGCGGGTGACGACGTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGG
IR64	TATCGCCGGCGGGTGACGACGTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGG
DJ123	TATCGCCGGCGGGTGACGACGTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGG
Nipponbare	TATCGCCGGCGGGTGACGACGTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGG
Azucena	TATCGCCGGCGGGTGACGACGTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGG *****
Bala	GGAACCTGTCGCTGCTGGGGTCCGGCGGCAACCTGATAGTGTGCGAGCAGGCGAGGAGGG
IR64	GGAACCTGTCGCTGCTGGGGTCCGGCGGCAACCTGATAGTGTGCGAGCAGGCGAGGAGGG
DJ123	GGAACCTGTCGCTGCTGGGGTCCGGCGGCAACCTGATAGTGTGCGAGCAGGCGAGGAGGG
Nipponbare	GGAACCTGTCGCTGCTGGGGTCCGGCGGCAACCTGATAGTGTGCGAGCAGGCGAGGAGGG
Azucena	GGAACCTGTCGCTGCTGGGGTCCGGCGGCAACCTGATAGTGTGCGAGCAGGCGAGGAGGG *****

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3 Bala CGCCAGGAACGCCTACGACCTCACCTTCTGGCAGCACATCGTCTTCGGCGTCCCATCCA
4 IR64 CGCCAGGAACGCCTACGACCTCACCTTCTGGCAGCACATCGTCTTCGGCGTCCCATCCA
5 DJ123 CGCCAGGAACGCCTACGACCTCACCTTCTGGCAGCACATCGTCTTCGGCGTCCCATCCA
6 Nipponbare CGCCAGGAACGCCTACGACCTCACCTTCTGGCAGCACATCGTCTTCGGCGTCCCATCCA
7 Azucena CGCCAGGAACGCCTACGACCTCACCTTCTGGCAGCACATCGTCTTCGGCGTCCCATCCA
8 *****
9 Bala CCCTCATCGTCACCGCCGTCGGCATACCCCTCATCGGCAAGATCTGATCTCATCTCATCG
10 IR64 CCCTCATCGTCACCGCCGTCGGCATACCCCTCATCGGCAAGATCTGATCTCATCTCATCG
11 DJ123 CCCTCATCGTCACCGCCGTCGGCATACCCCTCATCGGCAAGATCTGATCTCATCTCATCG
12 Nipponbare CCCTCATCGTCACCGCCGTCGGCATACCCCTCATCGGCAAGATCTGATCTCATCTCATCG
13 Azucena CCCTCATCGTCACCGCCGTCGGCATACCCCTCATCGGCAAGATCTGATCTCATCTCATCG
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 GCTAAATCTTGCTTAATTTTATCAAGTGTCAATCATGATGAGAAGAGAGGGAACCTAGATG
46 IR64 GCTAAATCTTGCTTAATTTTATCAAGTGTCAATCATGATGAGAAGAGAGGGAACCTAGATG
47 DJ123 GCTAAATCTTGCTTAATTTTATCAAGTGTCAATCATGATGAGAAGAGAGGGAACCTAGATG
48 Nipponbare GCTAAATCTTGCTTAATTTTATCAAGTGTCAATCATGATGAGAAGAGAGGGAACCTAGATG
49 Azucena GCTAAATCTTGCTTAATTTTATCAAGTGTCAATCATGATGAGAAGAGAGGGAACCTAGATG
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 *****
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28 Bala AGAGTTGTACTACCTTAAAATATCAGTATGCATGCTCTTGTGGCCATTCCAAAAGAACAA
29 IR64 AGAGTTGTACTACCTTAAAATATCAGTATGCATGCTCTTGTGGCCATTCCAAAAGAACAA
30 DJ123 AGAGTTGTACTACCTTAAAATATCAGTATGCATGCTCTTGTGGCCATTCCAAAAGAACAA
31 Nipponbare AGAGTTGTACTACCTTAAAATATCAGTATGCATGCTCTTGTGGCCATTCCAAAAGAACAA
32 Azucena AGAGTTGTACTACCTTAAAATATCAGTATGCATGCTCTTGTGGCCATTCCAAAAGAACAA
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)

8 Bala ACTCTCATCGCCACCACCACCTCCCACTCCAAGACGCTAGCTGCATTGTAGTGAGAGAGA
 9 IR64 ACTCTCATCGCCACCACCACCTCCCACTCCAAGACGCTAGCTGCATTGTAGTGAGAGAGA
 10 DJ123 ACTCTCATCGCCACCACCACCTCCCACTCCAAGACGCTAGCTGCATTGTAGTGAGAGAGA
 11 Nipponbare ACTCTCATCGCCACCACCACCTCCCACTCCAAGACGCTAGCTGCATTGTAGTGAGAGAGA
 12 Azucena ACTCTCATCGCCACCACCACCTCCCACTCCAAGACGCTAGCTGCATTGTAGTGAGAGAGA

14 Bala GAGGCGGCCATGGCGCTGGCGTCGCTGCCGAAGGTGGTGGTGGGGTCGGTGGCGTTCGGT
 15 IR64 GAGGCGGCCATGGCGCTGGCGTCGCTGCCGAAGGTGGTGGTGGGGTCGGTGGCGTTCGGT
 16 DJ123 GAGGCGGCCATGGCGCTGGCGTCGCTGCCGAAGGTGGTGGTGGGGTCGGTGGCGTTCGGT
 17 Nipponbare GAGGCGGCCATGGCGCTGGCGTCGCTGCCGAAGGTGGTGGTGGGGTCGGTGGCGTTCGGT
 18 Azucena GAGGCGGCCATGGCGCTGGCGTCGCTGCCGAAGGTGGTGGTGGGGTCGGTGGCGTTCGGT

19 Bala GTGTTCTGGATGCTGGCGGTGTTCCCGTCGGTGCCGTTCTGCGGATCGGGCGGACGGCG
 20 IR64 GTGTTCTGGATGCTGGCGGTGTTCCCGTCGGTGCCGTTCTGCGGATCGGGCGGACGGCG
 21 DJ123 GTGTTCTGGATGCTGGCGGTGTTCCCGTCGGTGCCGTTCTGCGGATCGGGCGGACGGCG
 22 Nipponbare GTGTTCTGGATGCTGGCGGTGTTCCCGTCGGTGCCGTTCTGCGGATCGGGCGGACGGCG
 23 Azucena GTGTTCTGGATGCTGGCGGTGTTCCCGTCGGTGCCGTTCTGCGGATCGGGCGGACGGCG

25 Bala GGGGCGCTGCTGGGCGCGGTGCTGATGATCGTGTTCACGATGATCAGCGCCGACGACGCC
 26 IR64 GGGGCGCTGCTGGGCGCGGTGCTGATGATCGTGTTCACGATGATCAGCGCCGACGACGCC
 27 DJ123 GGGGCGCTGCTGGGCGCGGTGCTGATGATCGTGTTCACGATGATCAGCGCCGACGACGCC
 28 Nipponbare GGGGCGCTGCTGGGCGCGGTGCTGATGATCGTGTTCACGATGATCAGCGCCGACGACGCC
 29 Azucena GGGGCGCTGCTGGGCGCGGTGCTGATGATCGTGTTCACGATGATCAGCGCCGACGACGCC

31 Bala TACGCCTCCATCGACCTCCCCATCCTGGGCTCCTCTTCGCCACCATGGTCGTGGCGGC
 32 IR64 TACGCCTCCATCGACCTCCCCATCCTGGGCTCCTCTTCGCCACCATGGTCGTGGCGGC
 33 DJ123 TACGCCTCCATCGACCTCCCCATCCTGGGCTCCTCTTCGCCACCATGGTCGTGGCGGC
 34 Nipponbare TACGCCTCCATCGACCTCCCCATCCTGGGCTCCTCTTCGCCACCATGGTCGTGGCGGC
 35 Azucena TACGCCTCCATCGACCTCCCCATCCTGGGCTCCTCTTCGCCACCATGGTCGTGGCGGC

36 Bala TACCTCAAGAACGCCGGCATGTTACGGCACCTGGGCGGCTGCTGGCGTGGCGGAGCCAG
 37 IR64 TACCTCAAGAACGCCGGCATGTTACGGCACCTGGGCGGCTGCTGGCGTGGCGGAGCCAG
 38 DJ123 TACCTCAAGAACGCCGGCATGTTACGGCACCTGGGCGGCTGCTGGCGTGGCGGAGCCAG
 39 Nipponbare TACCTCAAGAACGCCGGCATGTTACGGCACCTGGGCGGCTGCTGGCGTGGCGGAGCCAG
 40 Azucena TACCTCAAGAACGCCGGCATGTTACGGCACCTGGGCGGCTGCTGGCGTGGCGGAGCCAG

42 Bala GGGGGGCGGACCTCATGTGCCGCTCTGCGTCGTCACCGGCTCGCCAGCGCGCTCTTC
 43 IR64 GGGGGGCGGACCTCATGTGCCGCTCTGCGTCGTCACCGGCTCGCCAGCGCGCTCTTC
 44 DJ123 GGGGGGCGGACCTCATGTGCCGCTCTGCGTCGTCACCGGCTCGCCAGCGCGCTCTTC
 45 Nipponbare GGGGGGCGGACCTCATGTGCCGCTCTGCGTCGTCACCGGCTCGCCAGCGCGCTCTTC
 46 Azucena GGGGGGCGGACCTCATGTGCCGCTCTGCGTCGTCACCGGCTCGCCAGCGCGCTCTTC

47 Bala ACCAACGACACCTGCTGCGTCGTCCTCACCGAGTTCGTGCTCGAGCTCGCCGCGGAGCGC
 48 IR64 ACCAACGACACCTGCTGCGTCGTCCTCACCGAGTTCGTGCTCGAGCTCGCCGCGGAGCGC
 49 DJ123 ACCAACGACACCTGCTGCGTCGTCCTCACCGAGTTCGTGCTCGAGCTCGCCGCGGAGCGC
 50 Nipponbare ACCAACGACACCTGCTGCGTCGTCCTCACCGAGTTCGTGCTCGAGCTCGCCGCGGAGCGC
 51 Azucena ACCAACGACACCTGCTGCGTCGTCCTCACCGAGTTCGTGCTCGAGCTCGCCGCGGAGCGC

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Bala AACCTCCCGCGAAGCCGTTCCCTGCTCGCGCTCGCCACCAGCGCCAACATCGGCTCCAGC
 IR64 AACCTCCCGCGAAGCCGTTCCCTGCTCGCGCTCGCCACCAGCGCCAACATCGGCTCCAGC
 DJ123 AACCTCCCGCGAAGCCGTTCCCTGCTCGCGCTCGCCACCAGCGCCAACATCGGCTCCAGC
 Nipponbare AACCTCCCGCGAAGCCGTTCCCTGCTCGCGCTCGCCACCAGCGCCAACATCGGCTCCAGC
 Azucena AACCTCCCGCGAAGCCGTTCCCTGCTCGCGCTCGCCACCAGCGCCAACATCGGCTCCAGC

Bala GCGACGCCCATCGGCAACCCGCGAGAACCCTGGTCATCGCCTTCAACAGCAAGATCTCCTTT
 IR64 GCGACGCCCATCGGCAACCCGCGAGAACCCTGGTCATCGCCTTCAACAGCAAGATCTCCTTT
 DJ123 GCGACGCCCATCGGCAACCCGCGAGAACCCTGGTCATCGCCTTCAACAGCAAGATCTCCTTT
 Nipponbare GCGACGCCCATCGGCAACCCGCGAGAACCCTGGTCATCGCCTTCAACAGCAAGATCTCCTTT
 Azucena GCGACGCCCATCGGCAACCCGCGAGAACCCTGGTCATCGCCTTCAACAGCAAGATCTCCTTT

Bala ATTTCCCTTCCCTCCTCGGCATCCTCCCCGCCATGCTCGCCGGCATGGGGATCAACATGCTC
 IR64 ATTTCCCTTCCCTCCTCGGCATCCTCCCCGCCATGCTCGCCGGCATGGGGATCAACATGCTC
 DJ123 ATTTCCCTTCCCTCCTCGGCATCCTCCCCGCCATGCTCGCCGGCATGGGGATCAACATGCTC
 Nipponbare ATTTCCCTTCCCTCCTCGGCATCCTCCCCGCCATGCTCGCCGGCATGGGGATCAACATGCTC
 Azucena ATTTCCCTTCCCTCCTCGGCATCCTCCCCGCCATGCTCGCCGGCATGGGGATCAACATGCTC

Bala ATGCTGCTCTGCATGTACTGGAAGGAGCTCGACGGCGGCGCCTGCAGCCCCGACGAGGTC
 IR64 ATGCTGCTCTGCATGTACTGGAAGGAGCTCGACGGCGGCGCCTGCAGCCCCGACGAGGTC
 DJ123 ATGCTGCTCTGCATGTACTGGAAGGAGCTCGACGGCGGCGCCTGCAGCCCCGACGAGGTC
 Nipponbare ATGCTGCTCTGCATGTACTGGAAGGAGCTCGACGGCGGCGCCTGCAGCCCCGACGAGGTC
 Azucena ATGCTGCTCTGCATGTACTGGAAGGAGCTCGACGGCGGCGCCTGCAGCCCCGACGAGGTC

Bala GCCGCCGGCAAGCAGATGGAGGCCATCGAGGAGGGCCGCCGACGGCGCTCAACAACAAC
 IR64 GCCGCCGGCAAGCAGATGGAGGCCATCGAGGAGGGCCGCCGACGGCGCTCAACAACAAC
 DJ123 GCCGCCGGCAAGCAGATGGAGGCCATCGAGGAGGGCCGCCGACGGCGCTCAACAACAAC
 Nipponbare GCCGCCGGCAAGCAGATGGAGGCCATCGAGGAGGGCCGCCGACGGCGCTCAACAACAAC
 Azucena GCCGCCGGCAAGCAGATGGAGGCCATCGAGGAGGGCCGCCGACGGCGCTCAACAACAAC

Bala AAGAAAGACGACGGCGACGCCGCCACCCCGCATCGCCGGAGGACGACGACGGCGGCGAC
 IR64 AAGAAAGACGACGGCGACGCCGCCACCCCGCATCGCCGGAGGACGACGACGGCGGCGAC
 DJ123 AAGAAAGACGACGGCGACGCCGCCACCCCGCATCGCCGGAGGACGACGACGGCGGCGAC
 Nipponbare AAGAAAGACGACGGCGACGCCGCCACCCCGCATCGCCGGAGGACGACGACGGCGGCGAC
 Azucena AAGAAAGACGACGGCGACGCCGCCACCCCGCATCGCCGGAGGACGACGACGGCGGCGAC

Bala GCCGAGTCGATGATGTCGGAGAACATCTCGACGAAGCACCGGTGGTTTCATGCAGTGCTCG
 IR64 GCCGAGTCGATGATGTCGGAGAACATCTCGACGAAGCACCGGTGGTTTCATGCAGTGCTCG
 DJ123 GCCGAGTCGATGATGTCGGAGAACATCTCGACGAAGCACCGGTGGTTTCATGCAGTGCTCG
 Nipponbare GCCGAGTCGATGATGTCGGAGAACATCTCGACGAAGCACCGGTGGTTTCATGCAGTGCTCG
 Azucena GCCGAGTCGATGATGTCGGAGAACATCTCGACGAAGCACCGGTGGTTTCATGCAGTGCTCG

Bala GAGCACCGGCGGAAGCTATTCCCTCAAGTCTTCGCGTACGTGGTCACCGTCGGCATGCTC
 IR64 GAGCACCGGCGGAAGCTATTCCCTCAAGTCTTCGCGTACGTGGTCACCGTCGGCATGCTC
 DJ123 GAGCACCGGCGGAAGCTATTCCCTCAAGTCTTCGCGTACGTGGTCACCGTCGGCATGCTC
 Nipponbare GAGCACCGGCGGAAGCTATTCCCTCAAGTCTTCGCGTACGTGGTCACCGTCGGCATGCTC
 Azucena GAGCACCGGCGGAAGCTATTCCCTCAAGTCTTCGCGTACGTGGTCACCGTCGGCATGCTC

Bala GTCGCCTACATGCTCGGCCTCAACATGTCGTGGACCGCCATCACACCACCACCATCGCCCTC
 IR64 GTCGCCTACATGCTCGGCCTCAACATGTCGTGGACCGCCATCACACCACCACCATCGCCCTC
 DJ123 GTCGCCTACATGCTCGGCCTCAACATGTCGTGGACCGCCATCACACCACCACCATCGCCCTC
 Nipponbare GTCGCCTACATGCTCGGCCTCAACATGTCGTGGACCGCCATCACACCACCACCATCGCCCTC
 Azucena GTCGCCTACATGCTCGGCCTCAACATGTCGTGGACCGCCATCACACCACCACCATCGCCCTC

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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 **CGTGCTGGTGTTCCTCTCGGGATGTTTCGTGACGGTGAGCGGATTCAACAAGACGGGGC**
22 IR64 **CGTGCTGGTGTTCCTCTCGGGATGTTTCGTGACGGTGAGCGGATTCAACAAGACGGGGC**
23 DJ123 **CGTGCTGGTGTTCCTCTCGGGATGTTTCGTGACGGTGAGCGGATTCAACAAGACGGGGC**
24 Nipponbare **CGTGCTGGTGTTCCTCTCGGGATGTTTCGTGACGGTGAGCGGATTCAACAAGACGGGGC**
25 Azucena **CGTGCTGGTGTTCCTCTCGGGATGTTTCGTGACGGTGAGCGGATTCAACAAGACGGGGC**
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**
56 *****

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Bala TAAATCAGACAATAAATCAAGTCAAATAAAAATAAATGTAATTATATTTTTTAATAAGAT
 IR64 TAAATCAGACAATAAATCAAGTCAAATAAAAATAAATGTAATTATATTTTTTAATAAGAT
 DJ123 TAAATCAGACAATAAATCAAGTCAAATAAAAATAAATGTAATTATATTTTTTAATAAGAT
 Nipponbare TAAATCAGACAATAAATCAAGTCAAATAAAAATAAATGTAATTATATTTTTTAATAAGAT
 Azucena TAAATCAGACAATAAATCAAGTCAAATAAAAATAAATGTAATTATATTTTTTAATAAGAT

Bala GAATGATTATTAAGAAAAAATTTTTTTGTCTCTCATAATTAAGAAAAAACAACCTCA
 IR64 GAATGATTATTAAGAAAAAATTTTTTTGTCTCTCATAATTAAGAAAAAACAACCTCA
 DJ123 GAATGATTATTAAGAAAAAATTTTTTTGTCTCTCATAATTAAGAAAAAACAACCTCA
 Nipponbare GAATGATTATTAAGAAAAAATTTTTTTGTCTCTCATAATTAAGAAAAAACAACCTCA
 Azucena GAATGATTATTAAGAAAAAATTTTTTTGTCTCTCATAATTAAGAAAAAACAACCTCA

Bala TTTTTTACATGTTATTTGAGTGGAATTTGTAATTAATTTATATATGTGGGTGTAGT**GT**
 IR64 TTTTTTACATGTTATTTGAGTGGAATTTGTAATTAATTTATATATGTGGGTGTAGT**GT**
 DJ123 TTTTTTACATGTTATTTGAGTGGAATTTGTAATTAATTTATATATGTGGGTGTAGT**GT**
 Nipponbare TTTTTTACATGTTATTTGAGTGGAATTTGTAATTAATTTATATATGTGGGTGTAGT**GT**
 Azucena TTTTTTACATGTTATTTGAGTGGAATTTGTAATTAATTTATATATGTGGGTGTAGT**GT**

Bala **GTTGATGGGGGATGAGGTGGCGCGGGCGGGCGGCGACGATATCACCGGGCGGGT**GACGCG
 IR64 **GTTGATGGGGGATGAGGTGGCGCGGGCGGGCGGCGACGATATCACCGGGCGGGT**GACGCG
 DJ123 **GTTGATGGGGGATGAGGTGGCGCGGGCGGGCGGCGACGATATCACCGGGCGGGT**GACGCG
 Nipponbare **GTTGATGGGGGATGAGGTGGCGCGGGCGGGCGGCGACGATATCACCGGGCGGGT**GACGCG
 Azucena **GTTGATGGGGGATGAGGTGGCGCGGGCGGGCGGCGACGATATCACCGGGCGGGT**GACGCG

Bala **GTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGGGGAACCTGTCGCTGCTGGGGTC**
 IR64 **GTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGGGGAACCTGTCGCTGCTGGGGTC**
 DJ123 **GTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGGGGAACCTGTCGCTGCTGGGGTC**
 Nipponbare **GTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGGGGAACCTGTCGCTGCTGGGGTC**
 Azucena **GTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGGGGAACCTGTCGCTGCTGGGGTC**

Bala **GGCGGCGAACCTGATCGTGTGCGAGCAGGCGAGGCGGGCGACGCGCAACGCCTACGACCT**
 IR64 **GGCGGCGAACCTGATCGTGTGCGAGCAGGCGAGGCGGGCGACGCGCAACGCCTACGACCT**
 DJ123 **GGCGGCGAACCTGATCGTGTGCGAGCAGGCGAGGCGGGCGACGCGCAACGCCTACGACCT**
 Nipponbare **GGCGGCGAACCTGATCGTGTGCGAGCAGGCGAGGCGGGCGACGCGCAACGCCTACGACCT**
 Azucena **GGCGGCGAACCTGATCGTGTGCGAGCAGGCGAGGCGGGCGACGCGCAACGCCTACGACCT**

Bala **CACCTTCTGGAACCAGTCATCTTCGGACTCCCATCCACCCTCGTTCGTCACCGCCATCGG**
 IR64 **CACCTTCTGGAACCAGTCATCTTCGGACTCCCATCCACCCTCGTTCGTCACCGCCATCGG**
 DJ123 **CACCTTCTGGAACCAGTCATCTTCGGACTCCCATCCACCCTCGTTCGTCACCGCCATCGG**
 Nipponbare **CACCTTCTGGAACCAGTCATCTTCGGACTCCCATCCACCCTCGTTCGTCACCGCCATCGG**
 Azucena **CACCTTCTGGAACCAGTCATCTTCGGACTCCCATCCACCCTCGTTCGTCACCGCCATCGG**

Bala **CATCCCTCTCATCGGCAAGATCAACATCTAGT**CGTCATTAAATTAATTAATTAATATAAT
 IR64 **CATCCCTCTCATCGGCAAGATCAACATCTAGT**CGTCATTAAATTAATTAATTAATATAAT
 DJ123 **CATCCCTCTCATCGGCAAGATCAACATCTAGT**CGTCATTAAATTAATTAATTAATATAAT
 Nipponbare **CATCCCTCTCATCGGCAAGATCAACATCTAGT**CGTCATTAAATTAATTAATTAATATAAT
 Azucena **CATCCCTCTCATCGGCAAGATCAACATCTAGT**CGTCATTAAATTAATTAATTAATATAAT

Bala CCGGCTAGCTACTATCTACAACGATCAGAAGAAGGCAGAATGATCGAGCATGCAAGAATT
 IR64 CCGGCTAGCTACTATCTACAACGATCAGAAGAAGGCAGAATGATCGAGCATGCAAGAATT
 DJ123 CCGGCTAGCTACTATCTACAACGATCAGAAGAAGGCAGAATGATCGAGCATGCAAGAATT
 Nipponbare CCGGCTAGCTACTATCTACAACGATCAGAAGAAGGCAGAATGATCGAGCATGCAAGAATT
 Azucena CCGGCTAGCTACTATCTACAACGATCAGAAGAAGGCAGAATGATCGAGCATGCAAGAATT

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**
9 *****
10
11 Bala CTATGCTTGGTCTCAAGTGGATCAATTATATTGGTGCCATGCTCTGGGATAAATTAGTTAA
12 IR64 CTATGCTTGGTCTCAAGTGGATCAATTATATTGGTGCCATGCTCTGGGATAAATTAGTTAA
13 DJ123 CTATGCTTGGTCTCAAGTGGATCAATTATATTGGTGCCATGCTCTGGGATAAATTAGTTAA
14 Nipponbare CTATGCTTGGTCTCAAGTGGATCAATTATATTGGTGCCATGCTCTGGGATAAATTAGTTAA
15 Azucena CTATGCTTGGTCTCAAGTGGATCAATTATATTGGTGCCATGCTCTGGGATAAATTAGTTAA
16 *****
17
18 Bala TTAGTACTTATGATAAGCTAGAGAGGAGGATGCATTATATTGGTGCATGGTAATAAGGCA
19 IR64 TTAGTACTTATGATAAGCTAGAGAGGAGGATGCATTATATTGGTGCATGGTAATAAGGCA
20 DJ123 TTAGTACTTATGATAAGCTAGAGAGGAGGATGCATTATATTGGTGCATGGTAATAAGGCA
21 Nipponbare TTAGTACTTATGATAAGCTAGAGAGGAGGATGCATTATATTGGTGCATGGTAATAAGGCA
22 Azucena TTAGTACTTATGATAAGCTAGAGAGGAGGATGCATTATATTGGTGCATGGTAATAAGGCA
23 *****
24
25 Bala AATGAACAGCAGAATGCATGTATATATGTACTGTATCCCTGTTGCCAGCTGGTCTCTGTT
26 IR64 AATGAACAGCAGAATGCATGTATATATGTACTGTATCCCTGTTGCCAGCTGGTCTCTGTT
27 DJ123 AATGAACAGCAGAATGCATGTATATATGTACTGTATCCCTGTTGCCAGCTGGTCTCTGTT
28 Nipponbare AATGAACAGCAGAATGCATGTATATATGTACTGTATCCCTGTTGCCAGCTGGTCTCTGTT
29 Azucena AATGAACAGCAGAATGCATGTATATATGTACTGTATCCCTGTTGCCAGCTGGTCTCTGTT
30 *****
31
32 Bala TGAATGAAGTTGATAGCAACAAAGCTAGCACTAGCGAATGAGCTAGTGACTCAAGTACGC
33 IR64 TGAATGAAGTTGATAGCAACAAAGCTAGCACTAGCGAATGAGCTAGTGACTCAAGTACGC
34 DJ123 TGAATGAAGTTGATAGCAACAAAGCTAGCACTAGCGAATGAGCTAGTGACTCAAGTACGC
35 Nipponbare TGAATGAAGTTGATAGCAACAAAGCTAGCACTAGCGAATGAGCTAGTGACTCAAGTACGC
36 Azucena TGAATGAAGTTGATAGCAACAAAGCTAGCACTAGCGAATGAGCTAGTGACTCAAGTACGC
37 *****
38
39 Bala ATGCCGGATTACCTCATTTGCTCCAAACTTGCAATCAGTTTTGAACTTTAAATAAAGTTAA
40 IR64 ATGCCGGATTACCTCATTTGCTCCAAACTTGCAATCAGTTTTGAACTTTAAATAAAGTTAA
41 DJ123 ATGCCGGATTACCTCATTTGCTCCAAACTTGCAATCAGTTTTGAACTTTAAATAAAGTTAA
42 Nipponbare ATGCCGGATTACCTCATTTGCTCCAAACTTGCAATCAGTTTTGAACTTTAAATAAAGTTAA
43 Azucena ATGCCGGATTACCTCATTTGCTCCAAACTTGCAATCAGTTTTGAACTTTAAATAAAGTTAA
44 *****
45
46 Bala TTTGGCTTTGTGCACCTTGTCATTTGCTCCCTCTTTGTTTAAACCGATCTTGCCTAGTGCG
47 IR64 TTTGGCTTTGTGCACCTTGTCATTTGCTCCCTCTTTGTTTAAACCGATCTTGCCTAGTGCG
48 DJ123 TTTGGCTTTGTGCACCTTGTCATTTGCTCCCTCTTTGTTTAAACCGATCTTGCCTAGTGCG
49 Nipponbare TTTGGCTTTGTGCACCTTGTCATTTGCTCCCTCTTTGTTTAAACCGATCTTGCCTAGTGCG
50 Azucena TTTGGCTTTGTGCACCTTGTCATTTGCTCCCTCTTTGTTTAAACCGATCTTGCCTAGTGCG
51 *****
52
53 Bala TACGTAGCTAGATTGCACATTTAAG
54 IR64 TACGTAGCTAGATTGCACATTTAAG
55 DJ123 TACGTAGCTAGATTGCACATTTAAG
56 Nipponbare TACGTAGCTAGATTGCACATTTAAG
57 Azucena TACGTAGCTAGATTGCACATTTAAG
58 *****

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/>.