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

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1 Title Page:
2 Genotypic differences in shoot silicon content and the impact on grain arsenic accumulation
3 in rice
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16 Table: 3 Supplementary tables
17 Figures:6 figures in the main text and 4 supplementary figures
18 Short running tile: Genotypic differences of shoot silicon in rice.

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

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Abstract:

Silicon in rice has been demonstrated to be involved in resistance to lodging, tolerance to both drought and salinity, and also enhances resistance to pests and diseases. The aim of this study was to determine the range of silicon content in a set of rice (*Oryza sativa* L.) accessions, and to determine if the natural variation of shoot silicon is linked to the previously identified silicon transporters (*Lsi* genes). Silicon content was determined in 50 field-grown accessions, representing all sub-populations of rice, with all accessions being genotyped with 700K SNPs. SNPs within 10 kb of the *Lsi* genes were examined to determine if any were significantly linked with the phenotypic variation. An XRF method of silicon determination compared favourably with digestion and colorimetric analysis. There were significant genotypic differences in shoot silicon ranging from 16.5 – 42.4 mg g⁻¹ of plant dry weight, there was no significant difference between the rice sub-populations. Plants with different alleles for SNPs representing *Lsi2* and *Lsi3* were significantly different for shoot silicon content. Shoot silicon correlated negatively with grain arsenic in the *tropical* and *temperate japonica* sub-population, suggesting that accessions with high shoot silicon have

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61 reduced grain arsenic. This study indicates that alleles for *Lsi* genes are excellent candidate
62 genes for further study to explain the natural variation of shoot silicon in rice.

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64 **Key words:** arsenic, natural variation, rice, silicon, XRF

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1 Introduction

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

Genetically rice can be classified into two major sub-species, *Japonica* and *Indica* (Chang, 2003) and these have been further classified into 5 sub-populations; *indica*, *aus*, (both *Indica* sub-species) *tropical japonica*, *temperate japonica*, and *aromatic* (all three *Japonica* sub-species) (Garris et al., 2005; Zhao et al., 2011). Several previous studies indicate that there are differences in shoot silicon content between the *Indica* and *Japonica* sub-species of rice. Deren et al., (1992) showed that *Japonica* sub-species usually have a higher silicon content than *Indica* rice varieties, based on screening 10 accessions in the greenhouse and 18 under field conditions. A study conducted by Winslow (1992) revealed that African upland *Japonica* rice accessions had 50 to 100% higher silicon content in mature flag leaves than Asian upland *Indica* accessions. In addition to the differences at the subspecies level several studies have looked at genotypic differences in silicon content, showing ranges of 41 to 60 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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91 observed that silicon uptake by the root and the concentration silicon present in the shoot are
92 both higher in *Japonica* than *Indica* rice accessions, which they attributed to differences in
93 the expression of silicon transporter genes.

94 Two types of silicon transporters have been identified in rice to date. A gene
95 (LOC_Os02g51110) identified for silicic acid influx in rice is classified as an aquaporin
96 (Low silicon 1 or *Lsi1*) which is a member of the nodulin 26-like intrinsic protein (OsNIP2;
97 1) group of aquaporins (*Ma et al.*, 2006; *Ma et al.*, 2008). A homologue of *Lsi1*, known as
98 *Lsi6* (LOC_Os06g12310; OsNIP2; 2), responsible for shoot and husk silicon distribution in
99 rice is also classified as an aquaporin (*Yamaji et al.*, 2008). The efflux of silicic acid through
100 the plasma membrane protein known as low silicon 2 (*Lsi2*; LOC_Os03g01700) is an energy
101 dependent process in rice (*Ma et al.*, 2007b). A homologue of *Lsi2*, known as *Lsi3*
102 (LOC_Os10g39980), is also an energy dependent active transporter involved in regulating
103 shoot silicon accumulation in rice (*Yamaji et al.*, 2015).

104 It has been shown that arsenic, classified as a class one carcinogen, can be transported
105 through silicon transporters in rice (*Ma et al.*, 2008; *Zhao et al.*, 2010; *Mitani-Ueno et al.*,
106 2011). There are two different forms of arsenic present in rice: organic arsenic and inorganic
107 arsenic (*Williams et al.*, 2005). Organic arsenic is found in rice in two main types of
108 molecular species dimethylarsinic acid (DMA), and monomethylarsonic acid (MMA) as well
109 as tetramethylarsonium (*Williams et al.*, 2005; *Hansen et al.*, 2011). Inorganic arsenic is
110 found in rice as two molecular species; arsenate and arsenite (*Abedin et al.*, 2002; *Williams et*
111 *al.*, 2005). Arsenate is an analogue of phosphate and is taken up via phosphate transporters
112 while arsenite is taken up by silicic acid transporters in rice (*Abedin et al.*, 2002; *Ma et al.*,
113 2008). It has been shown that the silicon transporters *Lsi1*, *Lsi2* and *Lsi6* are also arsenic
114 transporters, using a combination of mutants and transgenic lines (*Ma et al.*, 2008; *Zhao et*
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
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9 119 decrease shoot and grain arsenic indicating that silicon could play an important role in
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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?
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22 125 Is there a relationship between natural variation in shoot silicon and arsenic content in rice?
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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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2 Materials and Methods

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

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

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

2.2 Genotypic differences in shoot silicon content of rice

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

2.3 Analysis of rice shoot silicon by FIA

Plant material and certified reference material (CRM) were prepared for silicon analysis as described by Carneiro et al., (2007). A total of 1.5 g shoot material from each sample was sub-sampled at random and powderised using a ball mill (Retsch, MM200, Germany). From the powderised plant material, a sub-sample of 20 mg was weighed into a 50 mL polyethylene centrifuge tube (CORNING®, NY). To digest the sample, 0.6 mL of hydrogen peroxide (H₂O₂, > 30% W/V, Fisher Scientific) and 1.5 mL of sodium hydroxide (NaOH, solutions 50%, Fluka) were added and the samples were then vortexed (mixed using a vortex mixer). The samples were heated for 1 hour at 90°C in a water bath, then vortexed again and

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left overnight. The tubes were vortexed again after overnight extraction, then heated at 123°C under a pressure of 0.15 MPa for 1 h. Samples were kept at room temperature for 2 h then vortexed, followed by addition of 18.5 mL of ddH₂O. Prior to analysis, samples were diluted 1:5 with Milli-Q water. Silicon content was measured using an FIA spectrophotometer (Tecator FIAstar 5010) a wavelength of 410 nm (Carneiro et al., 2007; Norton et al., 2010a; Norton et al., 2010b).

2.4 Analysis of rice shoot silicon by P-XRF

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

2.5 Relationship between silicon and arsenic content in rice

The plant material used in this study was previously examined for grain arsenic content (Norton et al., 2012) which provided an opportunity to examine the relationship between shoot silicon and grain arsenic in rice. The relationship between shoot silicon (log

transformed) and grain arsenic (log transformed) was investigated for the 50 rice accessions based on accession means.

2.6 Single Marker Analysis

The accessions used in this study have been genotyped using a high-density SNP chip (McCouch et al., 2016). SNPs for the accessions were extracted using PLINK (Purcell et al., 2007). SNPs were extracted from 10 kb upstream of the start codon to 10 kb downstream of the stop codon of the *Lsi1*, *Lsi2*, *Lsi3*, and *Lsi6* loci. SNPs were excluded from the analysis if they were invariant or if minor alleles were present in less than 5% of the accessions. The RDP1 population has a high degree of stratification by rice sub-population (Zhao et al., 2011; McCouch et al., 2016). To overcome this stratification, sub-population assignment was used (based on the 700 K SNP data; McCouch et al., 2016) as a factor in a two-way ANOVA, with SNP base call as the other factor. The two-way ANOVA was used to determine if the phenotype for the accession was significantly different for each SNP tested.

2.7 Sequence alignments

Based on the result achieved from the single-marker analysis the sequence diversity of *Lsi2* and *Lsi3* were investigated for 5 cultivars using BAM files produced after aligning sequence reads against Nipponbare version 7 reference genome. The genome sequences of the cultivars used in this study have been previously published (Kawahara et al., 2013; Cardoso et al., 2014; Schatz et al., 2014). The accessions were from the following sub-populations; 2 *indica* accessions (IR64 and Bala), 1 *aus* accession (DJ123) and 2 *tropical japonica* accessions (Azucena and Nipponbare). The genomic DNA sequence was visualised using the IGV (<https://www.broadinstitute.org/igv/>) to identify the difference of genomic DNA sequence within *Lsi2* and *Lsi3* in 5 cultivars (Thorvaldsdóttir et al., 2013; Robinson et al., 2011). Using

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224 Clustal Omega the DNA sequences of 5 cultivars were aligned for *Lsi2* and *Lsi3* separately
225 and showed in supplementary figure 3 and 4 respectively (Sievers *et al.*, 2011).

226 **2.8 Statistical analysis**

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

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

23.1 mg g⁻¹. Both SNPs group the accessions in a similar way, the only difference was more missing SNP information for SNP-3.438416 (Fig. 6).

A total of 20 SNPs from the SNP database are within 10 kb upstream and downstream of the *Lsi3* gene (selected SNPs for the rice accessions are presented in supplementary Table 3). Shoot silicon concentration for accessions with the different alleles for one of these SNPs was significantly different. SNP- 10.21340470 is located 5299 bp prior to the start codon, and revealed a significant difference between the G and A polymorphism ($P = 0.016$), where the mean silicon content of accessions with the G allele was 28.4 mg g⁻¹ while the mean silicon content of the accessions with the A allele was 35.6 mg g⁻¹ (Fig. 6).

There were 20 SNPs and 19 SNPs observed within 10 kb of *Lsi1* and *Lsi6* respectively. However, at each of these SNPs the different alleles were not significantly different for shoot silicon concentration.

To explore further, the sequence alignments of *Lsi2* and *Lsi3* were performed using available high-quality genome sequences. The accessions used were Nipponbare, Azucena, IR64, Bala, and DJ123 which are from the *tropical japonica*, *tropical japonica*, *indica*, *indica* and *aus* rice subgroups respectively. From the sequence analyses of *Lsi2* and *Lsi3* a number of polymorphisms within the genes were identified. For *Lsi2*, there was a synonymous SNP substitution within the first exon, where DJ123 has “C” allele while the other 4 accessions have “T” allele (Supplementary Figure 3). For *Lsi3*, 4 SNPs were detected in exons and 6 SNPs in introns (Supplementary Figure 4). There was only one non-synonymous SNP observed in the first exon of *Lsi3* where DJ123 and Bala have “T” allele and other accessions have “A” allele. The available 3000 rice genome sequence data indicates that this polymorphism between “A” and “T” in *Lsi3* is associated with the *aus* sub-population in rice where 15 accessions have “A” allele and 184 accessions have “T” allele (Alexandrov et al.,

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304 2015). This non-synonymous polymorphism between “A” and “T” in *Lsi3* with the “T” allele
305 is very rarely observed in *indica* and *japonica* subpopulations of rice in 3000 rice genome
306 sequence data (Alexandrov et al., 2015).
307

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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2
3 333 flooded plants in tissue associated with flowering and seed production significantly higher
4
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
8
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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23 342 similar to the previous 2.2 fold range detected for genotypic differences of shoot silicon
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25 343 content in rice (*Norton et al.*, 2010a). However, the maximum value observed in our study is
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27 344 slightly lower than that detected previously (42.4 mg g⁻¹ in this study, 60 mg g⁻¹ (*Deren*,
28
29 345 2001), 61 mg g⁻¹ (*Norton et al.*, 2010a).
30
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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25 368 Data on more than 50 accessions would have opened the opportunity to conduct genome-
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27 369 wide association (GWA) mapping where 200 accessions is considered a lower limit.
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29 370 However, the FIA colorimetric method for the determination of silicon in rice shoots proved
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31 371 not to be high throughput. However, in addition to the FIA method, a sub-set of samples
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33 372 were also analysed by P-XRF. The two different methods were strongly correlated, but not
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35 373 perfectly, and indicated that values for silicon content in samples measured by FIA were
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37 374 slightly higher than those measured by P-XRF. The observation that both methods provide
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39 375 comparable results highlights the conclusion that P-XRF can be used for silicon analysis to
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41 376 detect and measure genotypic differences across populations, instead of the more laborious
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43 377 and time-consuming alkali digestion method. Furthermore, a second advantage of P-XRF is
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45 378 that it is a non-destructive method. This would make it much more suitable for future GWA
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47 379 mapping studies.
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49 380 The plant material used in this study was previously used to examine the variation of shoot
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51 381 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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greater sequence variation than the cultivars for which high quality sequence is available, and therefore there may be greater sequence variation for *Lsi2* (and the other *Lsi* genes) than that is represented in the 5 accessions reported here. A focus for future study will be to expand sequence information to more accessions to more fully explore sequence variation associated with the polymorphic SNPs presented in figure 6.

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413 **5 Conclusion**

This study has demonstrated strong genotypic differences in shoot silicon in a diverse collection of rice cultivars, showing that there is potential to breed rice with increased silicon content that could improve resistance to both biotic and abiotic stresses in rice, which would help to maintain crop yields. The identification of significant SNPs linked with the shoot silicon phenotype within 10 kb of known silicon transporters warrants further study to investigate the impact of different alleles of these genes on silicon and arsenic accumulation in rice. Furthermore, the XRF method of silicon determination could be applied to GWA mapping studies that might reveal further candidate genes for silicon content in rice.

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Figure 1: Silicon in different organs of rice (bars are the mean of four replicates and error bars represent standard error of the mean). Letters above the columns (upper-case = anaerobic and lower case = aerobic) indicate statistically significant differences in silicon content of different plant organs using Tukey's test in two conditions. *denotes a significant difference between the two treatments for that plant organ.

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

Figure 3: Shoot silicon (mg g^{-1}) content of 50 accessions in 5 different sub-populations of rice. ARO = *aromatic*, AUS = *aus*, IND = *indica*, TEJ = *temperate japonica* and TRJ = *tropical japonica*. The edges of each box show the upper and lower quantile and the bold line in the box shows the median value and the dotted line the mean value. The whiskers are the 10th and 90th percentiles.

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

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

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

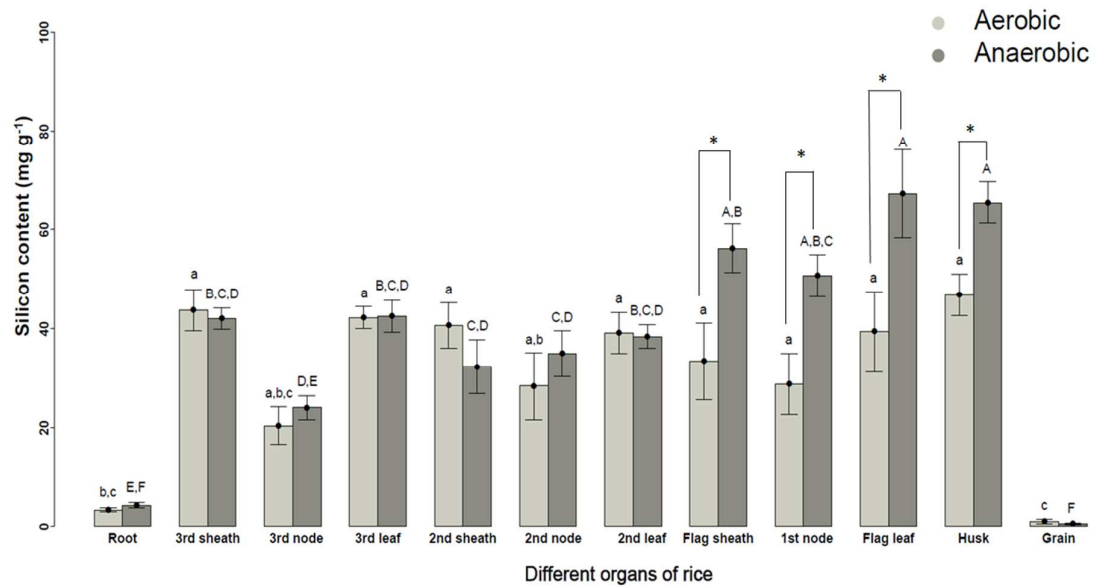


Figure 1

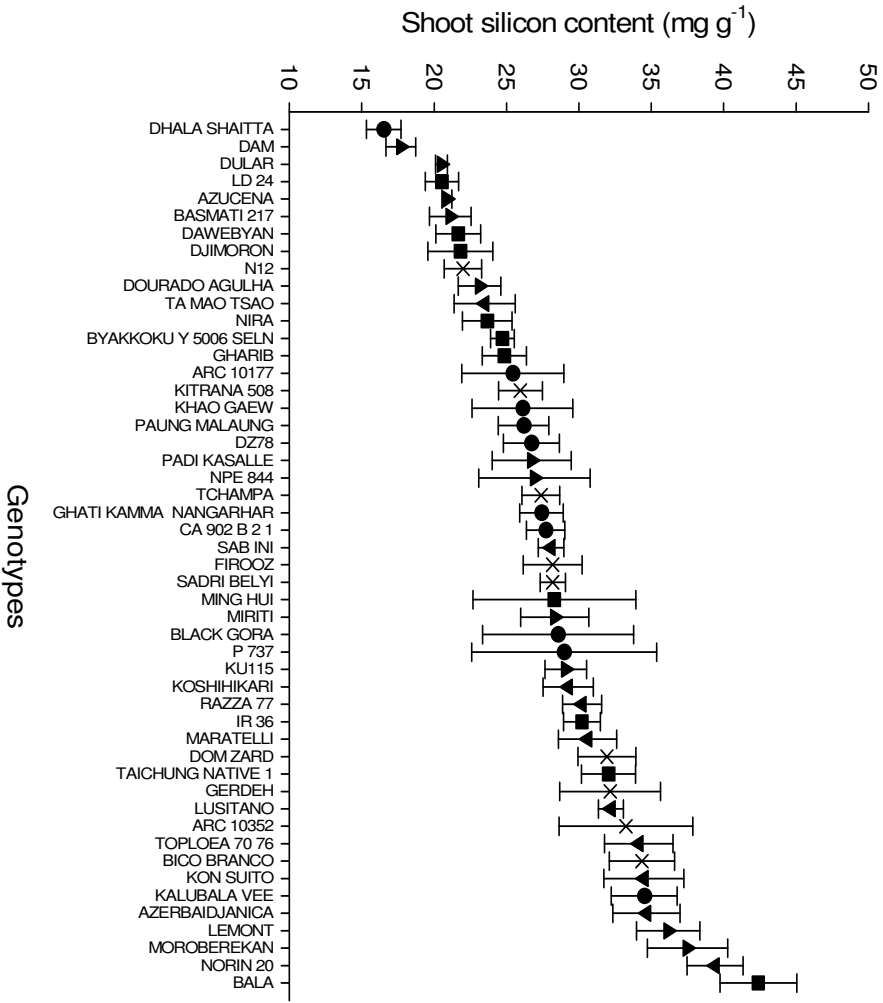


Figure 2

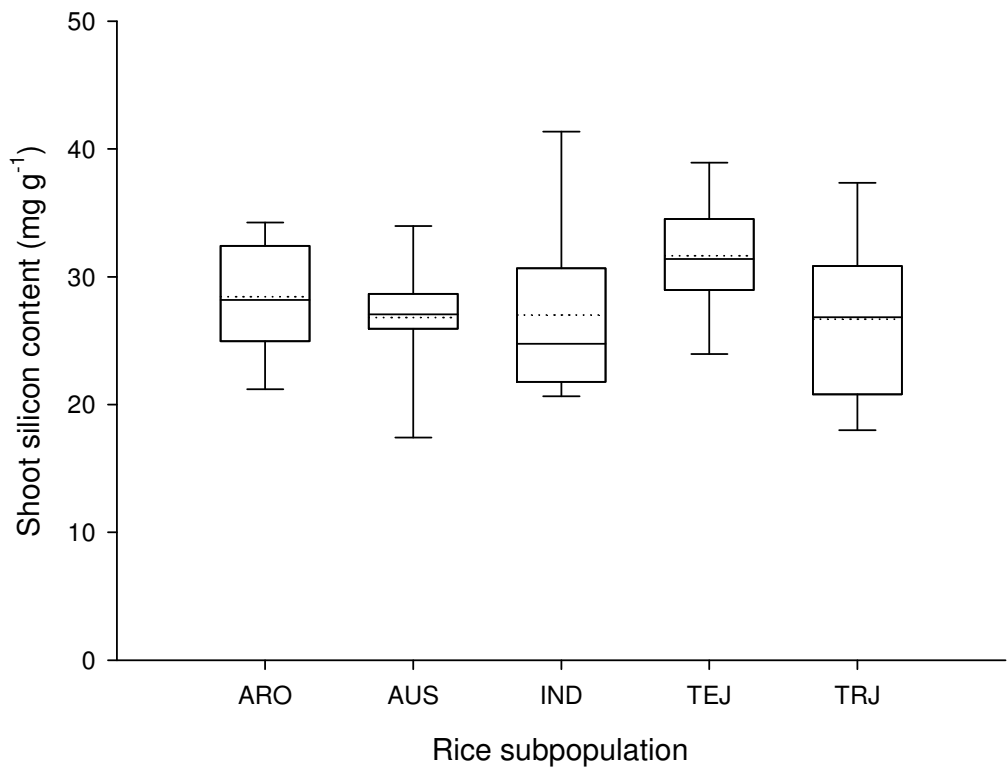
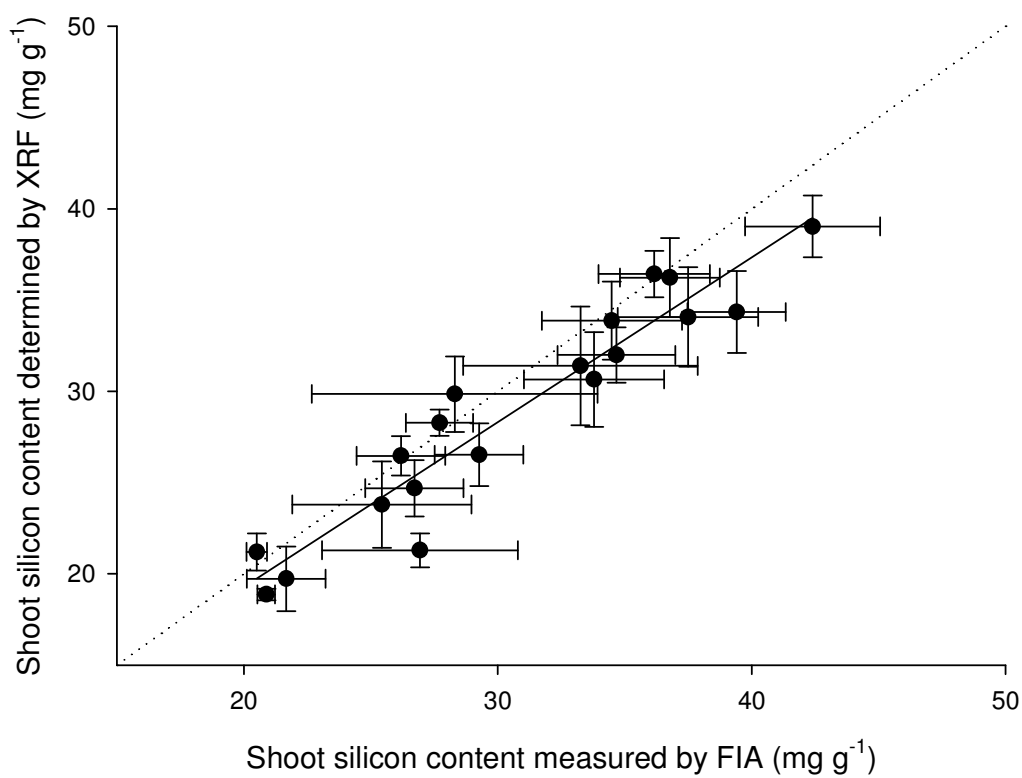


Figure 3



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685 **Figure 4**

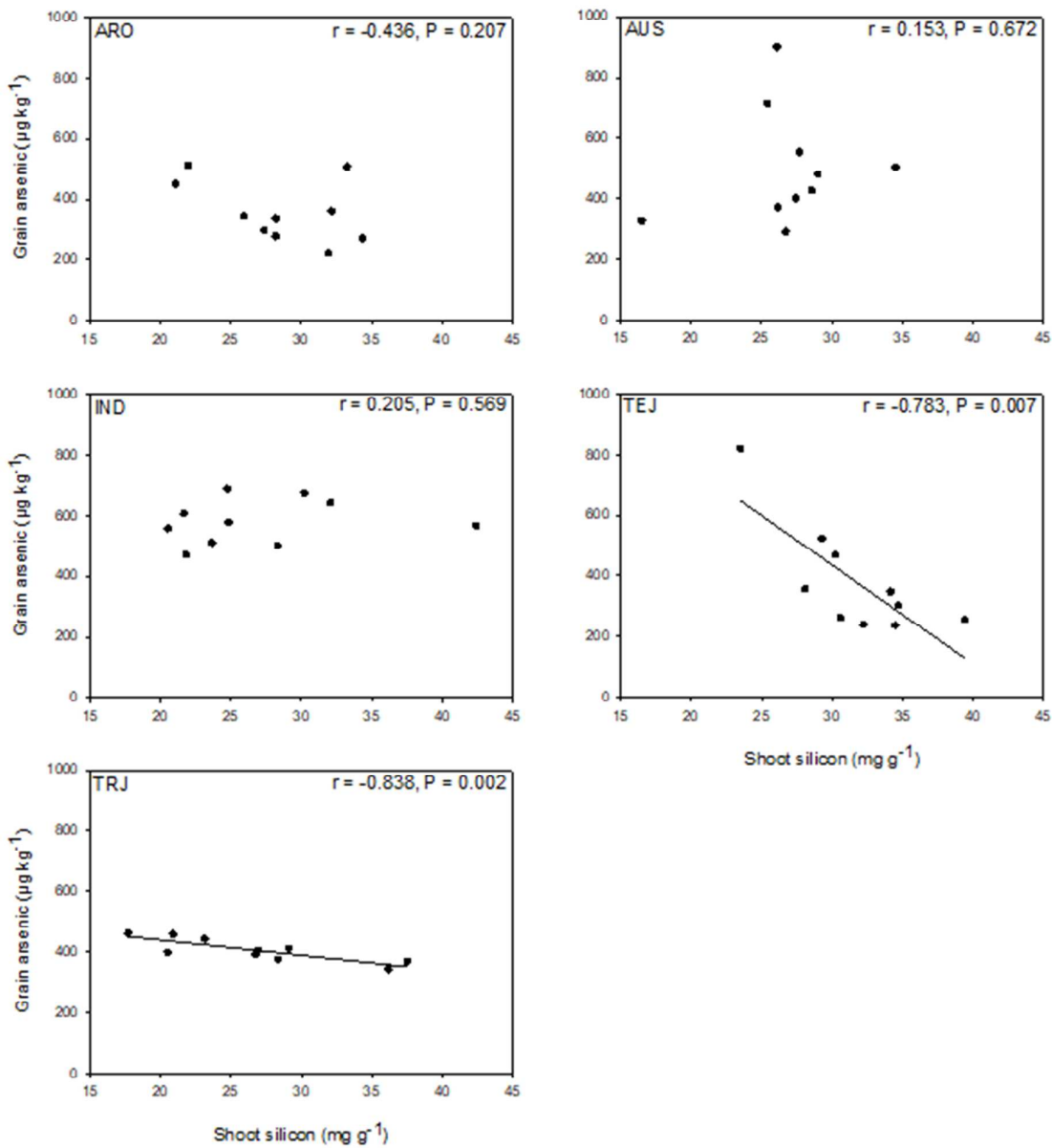
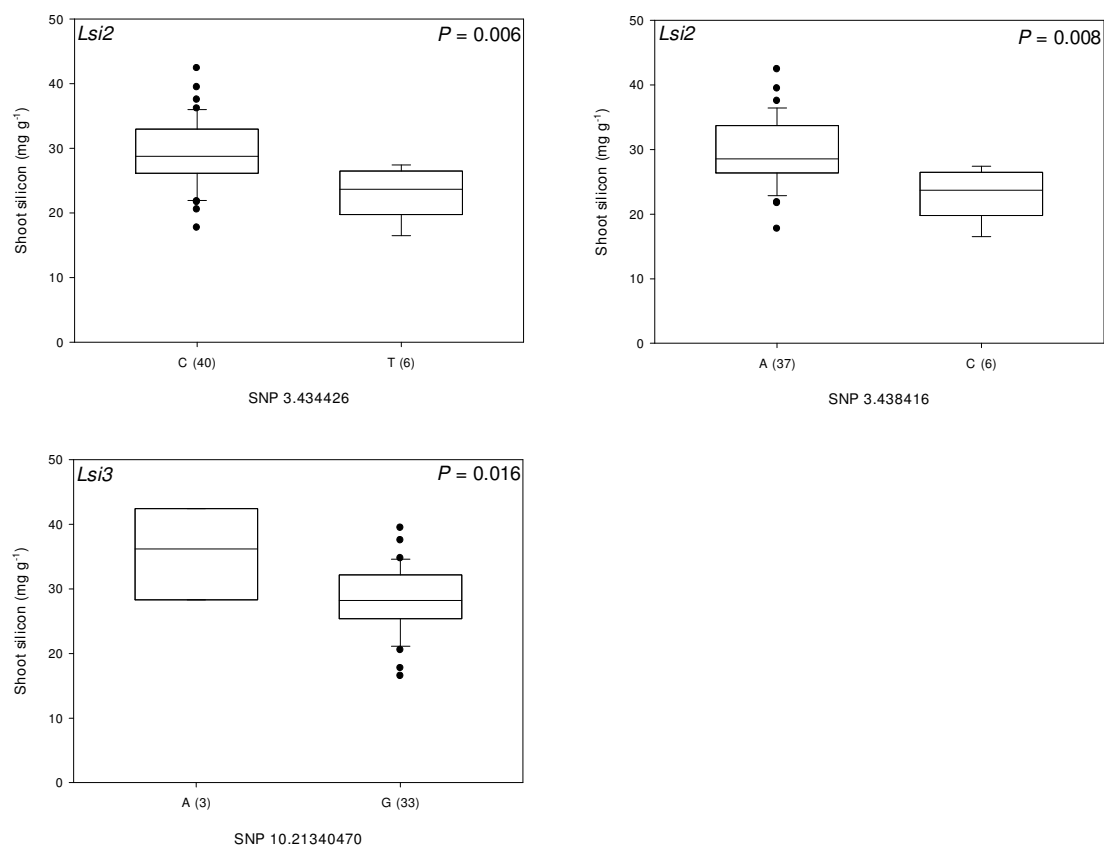


Figure 5

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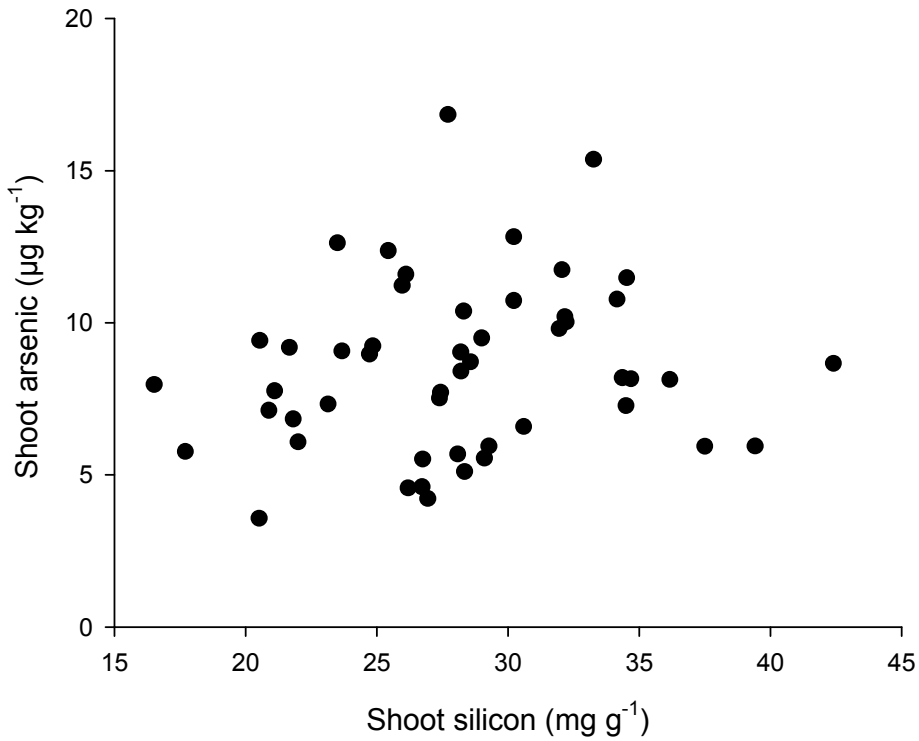
**Figure 6**

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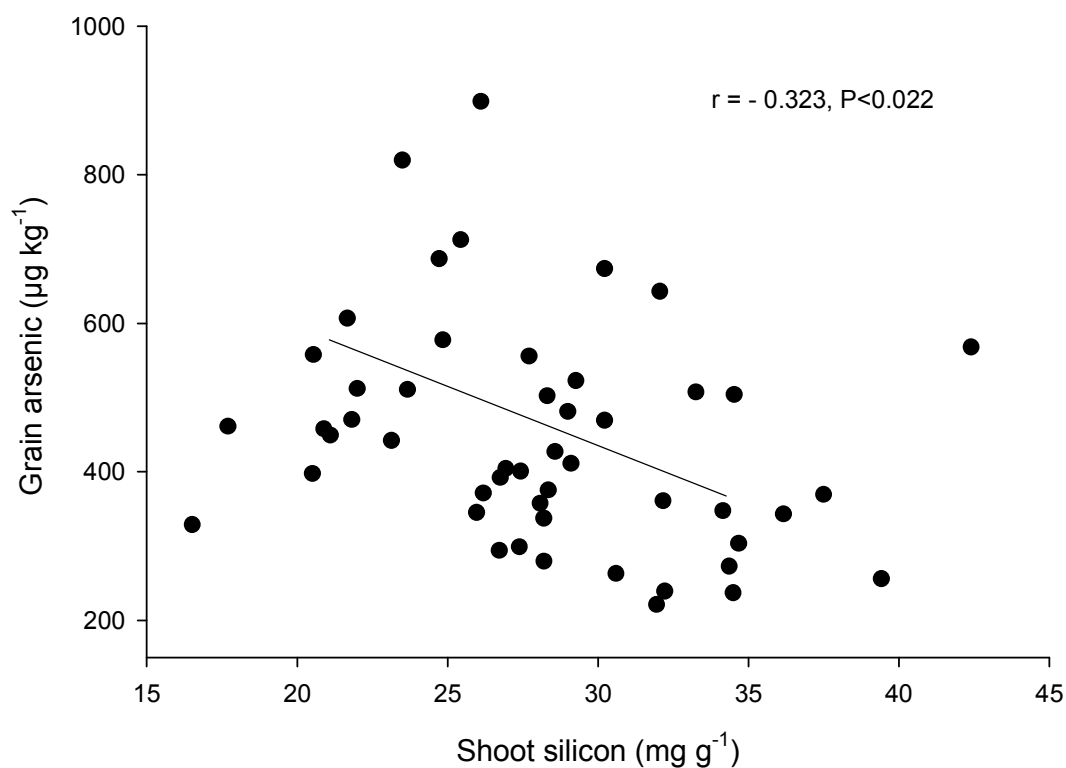
706 **Supplementary Table 1:** Selected genotype from RDP1 for shoot silicon analysis

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

712 **Supplementary Table 3:** SNPs with 10 kb (upstream and downstream) of *Lsi3*. SNPs data
713 taken from the High-density rice array (HDRA, 700k SNPs data) available at
714 <http://ricediversity.org/data/index.cfm>. After each SNP name (in brackets) is the location of
715 that SNP aligned to the Rice Genome Annotation Project – release 7 sequence available at
716 <http://rice.plantbiology.msu.edu/>



Supplementary figure 1: Correlation between shoot silicon (mg g⁻¹) and shoot arsenic (µg kg⁻¹) within 50 accessions of RDP1.



Supplementary figure 2: Correlation between shoot silicon (mg g⁻¹) and grain arsenic (μg kg⁻¹) within 50 accessions of RDP1.

Lsi2 (LOC_Os03g01700)

		<div>5'←-----→3'</div>
Bala	AGCATCAGCAGCGCATGCACTAGCAGCTTAGCTACTGCGCGCGTACCAGAGAGAGATCAT	
IR64	AGCATCAGCAGCGCATGCACTAGCAGCTTAGCTACTGCGCGCGTACCAGAGAGAGATCAT	
DJ123	AGCATCAGCAGCGCATGCACTAGCAGCTTAGCTACTGCGCGCGTACCAGAGAGAGATCAT	
Nipponbare	AGCATCAGCAGCGCATGCACTAGCAGCTTAGCTACTGCGCGCGTACCAGAGAGAGATCAT	
Azucena	AGCATCAGCAGCGCATGCACTAGCAGCTTAGCTACTGCGCGCGTACCAGAGAGAGATCA	*****
Bala	CAGCTCAAGTAGCTAAGCTAGCTATAATCTGCTAGCTAGCTCGATCAGACACTTAATTAC	
IR64	CAGCTCAAGTAGCTAAGCTAGCTATAATCTGCTAGCTAGCTCGATCAGACACTTAATTAC	
DJ123	CAGCTCAAGTAGCTAAGCTAGCTATAATCTGCTAGCTAGCTCGATCAGACACTTAATTAC	
Nipponbare	CAGCTCAAGTAGCTAAGCTAGCTATAATCTGCTAGCTAGCTCGATCAGACACTTAATTAC	
Azucena	CAGCTCAAGTAGCTAAGCTAGCTATAATCTGCTAGCTAGCTCGATCAGACACTTAATTAC	*****
Bala	CTGCTAGGTGGTGGTCGATCGAAGAAGAAG AAGATGAGTGAGCTTGCGTCGGCGCCCAAG	
IR64	CTGCTAGGTGGTGGTCGATCGAAGAAGAAG AAGATGAGTGAGCTTGCGTCGGCGCCCAAG	
DJ123	CTGCTAGGTGGTGGTCGATCGAAGAAGAAG AAGATGAGTGAGCTTGCGTCGGCGCCCAAG	
Nipponbare	CTGCTAGGTGGTGGTCGATCGAAGAAGAAG AAGATGAGTGAGCTTGCGTCGGCGCCCAAG	
Azucena	CTGCTAGGTGGTGGTCGATCGAAGAAGAAG AAGATGAGTGAGCTTGCGTCGGCGCCCAAG	*****
Bala	GTGGCGCTTGGATCGATCGCGTTTCGCGGTGTTCTGGATGATGGCGGTGTTCCCGTCGGTG	
IR64	GTGGCGCTTGGATCGATCGCGTTTCGCGGTGTTCTGGATGATGGCGGTGTTCCCGTCGGTG	
DJ123	GTGGCGCTTGGATCGATCGCGTTTCGCGGTGTTCTGGATGATGGCGGTGTTCCCGTCGGTG	
Nipponbare	GTGGCGCTTGGATCGATCGCGTTTCGCGGTGTTCTGGATGATGGCGGTGTTCCCGTCGGTG	
Azucena	GTGGCGCTTGGATCGATCGCGTTTCGCGGTGTTCTGGATGATGGCGGTGTTCCCGTCGGTG	*****
Bala	CCGTTCTCTGCCGATCGGGCGGACGGCGGGGTTCGCTGCTGAGCGCGGTGCTGATGGTGATA	
IR64	CCGTTCTCTGCCGATCGGGCGGACGGCGGGGTTCGCTGCTGAGCGCGGTGCTGATGGTGATA	
DJ123	CCGTTCTCTGCCGATCGGGCGGACGGCGGGGTTCGCTGCTGAGCGCGGTGCTGATGGTGATA	
Nipponbare	CCGTTCTCTGCCGATCGGGCGGACGGCGGGGTTCGCTGCTGAGCGCGGTGCTGATGGTGATA	
Azucena	CCGTTCTCTGCCGATCGGGCGGACGGCGGGGTTCGCTGCTGAGCGCGGTGCTGATGGTGATA	*****
Bala	TTCCACGTGATCAGCCCCGACGACGCGTACGCCTCCATCGACCTCCCAATCCTGGGCCTC	
IR64	TTCCACGTGATCAGCCCCGACGACGCGTACGCCTCCATCGACCTCCCAATCCTGGGCCTC	
DJ123	TTCCACGTGATCAGCCCCGACGACGCGTACGCCTCCATCGACCTCCCAATCCTGGGCCTC	
Nipponbare	TTCCACGTGATCAGCCCCGACGACGCGTACGCCTCCATCGACCTCCCAATCCTGGGCCTC	
Azucena	TTCCACGTGATCAGCCCCGACGACGCGTACGCCTCCATCGACCTCCCAATCCTGGGCCTC	*****
Bala	CTCTTCGCCACCATGGTGGTGGGCAGCTACCTCCGGAACGCCGGGATGTTCAAGCACCTG	
IR64	CTCTTCGCCACCATGGTGGTGGGCAGCTACCTCCGGAACGCCGGGATGTTCAAGCACCTG	
DJ123	CTCTTCGCCACCATGGTGGTGGGCAGCTACCTCCGGAACGCCGGGATGTTCAAGCACCTG	
Nipponbare	CTCTTCGCCACCATGGTGGTGGGCAGCTACCTCCGGAACGCCGGGATGTTCAAGCACCTG	
Azucena	CTCTTCGCCACCATGGTGGTGGGCAGCTACCTCCGGAACGCCGGGATGTTCAAGCACCTG	*****
Bala	GGGCGTCTGCTGGCGTGGAAGAGCCAGGGCGGGCGCGACCTCATGTGCCGCGTCTGCGTC	
IR64	GGGCGTCTGCTGGCGTGGAAGAGCCAGGGCGGGCGCGACCTCATGTGCCGCGTCTGCGTC	
DJ123	GGGCGTCTGCTGGCGTGGAAGAGCCAGGGCGGGCGCGACCTCATGTGCCGCGTCTGCGTC	
Nipponbare	GGGCGTCTGCTGGCGTGGAAGAGCCAGGGCGGGCGCGACCTCATGTGCCGCGTCTGCGTC	
Azucena	GGGCGTCTGCTGGCGTGGAAGAGCCAGGGCGGGCGCGACCTCATGTGCCGCGTCTGCGTC	*****

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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	TCCGGGATGTTTCATCACCCTCAGCGGCTTCAACAAGACGGGCCTCCCGGGAGCCATCTGG
16	IR64	TCCGGGATGTTTCATCACCCTCAGCGGCTTCAACAAGACGGGCCTCCCGGGAGCCATCTGG
17	DJ123	TCCGGGATGTTTCATCACCCTCAGCGGCTTCAACAAGACGGGCCTCCCGGGAGCCATCTGG
18	Nipponbare	TCCGGGATGTTTCATCACCCTCAGCGGCTTCAACAAGACGGGCCTCCCGGGAGCCATCTGG
19	Azucena	TCCGGGATGTTTCATCACCCTCAGCGGCTTCAACAAGACGGGCCTCCCGGGAGCCATCTGG
20		*****
21	Bala	GACTTCATGGCCCCCTACTCCAAGGTCAACAGCGTCGGCGGCATCTCCGTCTCTCCGTC
22	IR64	GACTTCATGGCCCCCTACTCCAAGGTCAACAGCGTCGGCGGCATCTCCGTCTCTCCGTC
23	DJ123	GACTTCATGGCCCCCTACTCCAAGGTCAACAGCGTCGGCGGCATCTCCGTCTCTCCGTC
24	Nipponbare	GACTTCATGGCCCCCTACTCCAAGGTCAACAGCGTCGGCGGCATCTCCGTCTCTCCGTC
25	Azucena	GACTTCATGGCCCCCTACTCCAAGGTCAACAGCGTCGGCGGCATCTCCGTCTCTCCGTC
26		*****
27	Bala	ATCATCTCTCCTCTCCAACCTCGCATCAAACGTACCAACGGGTAATTATACATTCTAT
28	IR64	ATCATCTCTCCTCTCCAACCTCGCATCAAACGTACCAACGGGTAATTATACATTCTAT
29	DJ123	ATCATCTCTCCTCTCCAACCTCGCATCAAACGTACCAACGGGTAATTATACATTCTAT
30	Nipponbare	ATCATCTCTCCTCTCCAACCTCGCATCAAACGTACCAACGGGTAATTATACATTCTAT
31	Azucena	ATCATCTCTCCTCTCCAACCTCGCATCAAACGTACCAACGGGTAATTATACATTCTAT
32		*****
33	Bala	TAATCCAGAATTAATGTATACTACTCCATATAAGTATAACTAATTAACATACAAAAATAT
34	IR64	TAATCCAGAATTAATGTATACTACTCCATATAAGTATAACTAATTAACATACAAAAATAT
35	DJ123	TAATCCAGAATTAATGTATACTACTCCATATAAGTATAACTAATTAACATACAAAAATAT
36	Nipponbare	TAATCCAGAATTAATGTATACTACTCCATATAAGTATAACTAATTAACATACAAAAATAT
37	Azucena	TAATCCAGAATTAATGTATACTACTCCATATAAGTATAACTAATTAACATACAAAAATAT
38		*****
39	Bala	ATATGCGCATGCATGCAGTGCTTCTTATGGGTGATGAGGTGGCGAAGGCGGCGCGCTGA
40	IR64	ATATGCGCATGCATGCAGTGCTTCTTATGGGTGATGAGGTGGCGAAGGCGGCGCGCTGA
41	DJ123	ATATGCGCATGCATGCAGTGCTTCTTATGGGTGATGAGGTGGCGAAGGCGGCGCGCTGA
42	Nipponbare	ATATGCGCATGCATGCAGTGCTTCTTATGGGTGATGAGGTGGCGAAGGCGGCGCGCTGA
43	Azucena	ATATGCGCATGCATGCAGTGCTTCTTATGGGTGATGAGGTGGCGAAGGCGGCGCGCTGA
44		*****
45	Bala	TATCGCCGGCGGCGGTGACGACGTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGG
46	IR64	TATCGCCGGCGGCGGTGACGACGTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGG
47	DJ123	TATCGCCGGCGGCGGTGACGACGTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGG
48	Nipponbare	TATCGCCGGCGGCGGTGACGACGTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGG
49	Azucena	TATCGCCGGCGGCGGTGACGACGTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGG
50		*****
51	Bala	GGAACCTGTCGCTGCTGGGGTCGGCGGCGAACCTGATAGTGTGCGAGCAGGCGAGGAGGG
52	IR64	GGAACCTGTCGCTGCTGGGGTCGGCGGCGAACCTGATAGTGTGCGAGCAGGCGAGGAGGG
53	DJ123	GGAACCTGTCGCTGCTGGGGTCGGCGGCGAACCTGATAGTGTGCGAGCAGGCGAGGAGGG
54	Nipponbare	GGAACCTGTCGCTGCTGGGGTCGGCGGCGAACCTGATAGTGTGCGAGCAGGCGAGGAGGG
55	Azucena	GGAACCTGTCGCTGCTGGGGTCGGCGGCGAACCTGATAGTGTGCGAGCAGGCGAGGAGGG
56		*****

Bala	CGCCCAGGAACGCCTACGACCTCACCTTCTGGCAGCACATCGTCTTCGGCGTCCCATCCA
IR64	CGCCCAGGAACGCCTACGACCTCACCTTCTGGCAGCACATCGTCTTCGGCGTCCCATCCA
DJ123	CGCCCAGGAACGCCTACGACCTCACCTTCTGGCAGCACATCGTCTTCGGCGTCCCATCCA
Nipponbare	CGCCCAGGAACGCCTACGACCTCACCTTCTGGCAGCACATCGTCTTCGGCGTCCCATCCA
Azucena	CGCCCAGGAACGCCTACGACCTCACCTTCTGGCAGCACATCGTCTTCGGCGTCCCATCCA

Bala	CCCTCATCGTCACCGCCGTCGGCATACCCCTCATCGGCAAGATCTGATCTCATCTCATCG
IR64	CCCTCATCGTCACCGCCGTCGGCATACCCCTCATCGGCAAGATCTGATCTCATCTCATCG
DJ123	CCCTCATCGTCACCGCCGTCGGCATACCCCTCATCGGCAAGATCTGATCTCATCTCATCG
Nipponbare	CCCTCATCGTCACCGCCGTCGGCATACCCCTCATCGGCAAGATCTGATCTCATCTCATCG
Azucena	CCCTCATCGTCACCGCCGTCGGCATACCCCTCATCGGCAAGATCTGATCTCATCTCATCG

Bala	ACCCATCCAAATTAATTAATTATGAGATCGACAAACATCCAAGCTTGCTAGGCTCGTCGT
IR64	ACCCATCCAAATTAATTAATTATGAGATCGACAAACATCCAAGCTTGCTAGGCTCGTCGT
DJ123	ACCCATCCAAATTAATTAATTATGAGATCGACAAACATCCAAGCTTGCTAGGCTCGTCGT
Nipponbare	ACCCATCCAAATTAATTAATTATGAGATCGACAAACATCCAAGCTTGCTAGGCTCGTCGT
Azucena	ACCCATCCAAATTAATTAATTATGAGATCGACAAACATCCAAGCTTGCTAGGCTCGTCGT

Bala	CGTCGTCGTCGACCACCGTACCATATATATGCATGCATGCCACGCACGTATATATATGCT
IR64	CGTCGTCGTCGACCACCGTACCATATATATGCATGCATGCCACGCACGTATATATATGCT
DJ123	CGTCGTCGTCGACCACCGTACCATATATATGCATGCATGCCACGCACGTATATATATGCT
Nipponbare	CGTCGTCGTCGACCACCGTACCATATATATGCATGCATGCCACGCACGTATATATATGCT
Azucena	CGTCGTCGTCGACCACCGTACCATATATATGCATGCATGCCACGCACGTATATATATGCT

Bala	CCTCAACCTCAAGTCAATTAATTAAGAGAATGGATGAATGAATCAATGTTGTGTATGATT
IR64	CCTCAACCTCAAGTCAATTAATTAAGAGAATGGATGAATGAATCAATGTTGTGTATGATT
DJ123	CCTCAACCTCAAGTCAATTAATTAAGAGAATGGATGAATGAATCAATGTTGTGTATGATT
Nipponbare	CCTCAACCTCAAGTCAATTAATTAAGAGAATGGATGAATGAATCAATGTTGTGTATGATT
Azucena	CCTCAACCTCAAGTCAATTAATTAAGAGAATGGATGAATGAATCAATGTTGTGTATGATT

Bala	TCCTTTTTGTTTTGTTTTTACCCACCGTATATGTGCTGTGTGGTGTATGGCCGGCGAGCT
IR64	TCCTTTTTGTTTTGTTTTTACCCACCGTATATGTGCTGTGTGGTGTATGGCCGGCGAGCT
DJ123	TCCTTTTTGTTTTGTTTTTACCCACCGTATATGTGCTGTGTGGTGTATGGCCGGCGAGCT
Nipponbare	TCCTTTTTGTTTTGTTTTTACCCACCGTATATGTGCTGTGTGGTGTATGGCCGGCGAGCT
Azucena	TCCTTTTTGTTTTGTTTTTACCCACCGTATATGTGCTGTGTGGTGTATGGCCGGCGAGCT

Bala	TGAAATTGAATTGGCCTTGCTTGCATGCATGCATGGTGATCAGATCAGCTCAGCTAGCTA
IR64	TGAAATTGAATTGGCCTTGCTTGCATGCATGCATGGTGATCAGATCAGCTCAGCTAGCTA
DJ123	TGAAATTGAATTGGCCTTGCTTGCATGCATGCATGGTGATCAGATCAGCTCAGCTAGCTA
Nipponbare	TGAAATTGAATTGGCCTTGCTTGCATGCATGCATGGTGATCAGATCAGCTCAGCTAGCTA
Azucena	TGAAATTGAATTGGCCTTGCTTGCATGCATGCATGGTGATCAGATCAGCTCAGCTAGCTA

Bala	GCTAAATCTTGCTTAATTTTATCAAGTGTCATCATGATGAGAAGAGAGGGAACCTAGATG
IR64	GCTAAATCTTGCTTAATTTTATCAAGTGTCATCATGATGAGAAGAGAGGGAACCTAGATG
DJ123	GCTAAATCTTGCTTAATTTTATCAAGTGTCATCATGATGAGAAGAGAGGGAACCTAGATG
Nipponbare	GCTAAATCTTGCTTAATTTTATCAAGTGTCATCATGATGAGAAGAGAGGGAACCTAGATG
Azucena	GCTAAATCTTGCTTAATTTTATCAAGTGTCATCATGATGAGAAGAGAGGGAACCTAGATG

Bala	GAGCTAGCTAGCTAGCAACACATGCAGTGTGTGCTAAGCAGTGCACTCTGCATGCTTAAA
IR64	GAGCTAGCTAGCTAGCAACACATGCAGTGTGTGCTAAGCAGTGCACTCTGCATGCTTAAA
DJ123	GAGCTAGCTAGCTAGCAACACATGCAGTGTGTGCTAAGCAGTGCACTCTGCATGCTTAAA
Nipponbare	GAGCTAGCTAGCTAGCAACACATGCAGTGTGTGCTAAGCAGTGCACTCTGCATGCTTAAA
Azucena	GAGCTAGCTAGCTAGCAACACATGCAGTGTGTGCTAAGCAGTGCACTCTGCATGCTTAAA

1		
2		
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	TCAAACAAATATTTGTAGCCCTCTCTCTCTCTCTCTCTTTAGGTTTATTAATTAATTAGT
16	IR64	TCAAACAAATATTTGTAGCCCTCTCTCTCTCTCTCTCTTTAGGTTTATTAATTAATTAGT
17	DJ123	TCAAACAAATATTTGTAGCCCTCTCTCTCTCTCTCTCTTTAGGTTTATTAATTAATTAGT
18	Nipponbare	TCAAACAAATATTTGTAGCCCTCTCTCTCTCTCTCTCTTTAGGTTTATTAATTAATTAGT
19	Azucena	TCAAACAAATATTTGTAGCCCTCTCTCTCTCTCTCTCTTTAGGTTTATTAATTAATTAGT
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
39		*

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

Bala	GAGGCGGCCATGGCGCTGGCGTCGCTGCCGAAGGTGGTGGTGGGGTCGGTGGCGTTCGGT
IR64	GAGGCGGCCATGGCGCTGGCGTCGCTGCCGAAGGTGGTGGTGGGGTCGGTGGCGTTCGGT
DJ123	GAGGCGGCCATGGCGCTGGCGTCGCTGCCGAAGGTGGTGGTGGGGTCGGTGGCGTTCGGT
Nipponbare	GAGGCGGCCATGGCGCTGGCGTCGCTGCCGAAGGTGGTGGTGGGGTCGGTGGCGTTCGGT
Azucena	GAGGCGGCCATGGCGCTGGCGTCGCTGCCGAAGGTGGTGGTGGGGTCGGTGGCGTTCGGT

Bala	GTGTTCTGGATGCTGGCGGTGTTCCCGTCGGTGCCGTTCTGCCGATCGGGCGGACGGCG
IR64	GTGTTCTGGATGCTGGCGGTGTTCCCGTCGGTGCCGTTCTGCCGATCGGGCGGACGGCG
DJ123	GTGTTCTGGATGCTGGCGGTGTTCCCGTCGGTGCCGTTCTGCCGATCGGGCGGACGGCG
Nipponbare	GTGTTCTGGATGCTGGCGGTGTTCCCGTCGGTGCCGTTCTGCCGATCGGGCGGACGGCG
Azucena	GTGTTCTGGATGCTGGCGGTGTTCCCGTCGGTGCCGTTCTGCCGATCGGGCGGACGGCG

Bala	GGGGCGCTGCTGGGCGCGGTGCTGATGATCGTGTTCACGTGATCAGCGCCGACGACGCC
IR64	GGGGCGCTGCTGGGCGCGGTGCTGATGATCGTGTTCACGTGATCAGCGCCGACGACGCC
DJ123	GGGGCGCTGCTGGGCGCGGTGCTGATGATCGTGTTCACGTGATCAGCGCCGACGACGCC
Nipponbare	GGGGCGCTGCTGGGCGCGGTGCTGATGATCGTGTTCACGTGATCAGCGCCGACGACGCC
Azucena	GGGGCGCTGCTGGGCGCGGTGCTGATGATCGTGTTCACGTGATCAGCGCCGACGACGCC

Bala	TACGCCTCCATCGACCTCCCCATCCTGGGCCTCCTCTTCGCCACCATGGTCGTCGGCGGC
IR64	TACGCCTCCATCGACCTCCCCATCCTGGGCCTCCTCTTCGCCACCATGGTCGTCGGCGGC
DJ123	TACGCCTCCATCGACCTCCCCATCCTGGGCCTCCTCTTCGCCACCATGGTCGTCGGCGGC
Nipponbare	TACGCCTCCATCGACCTCCCCATCCTGGGCCTCCTCTTCGCCACCATGGTCGTCGGCGGC
Azucena	TACGCCTCCATCGACCTCCCCATCCTGGGCCTCCTCTTCGCCACCATGGTCGTCGGCGGC

Bala	TACCTCAAGAACGCCGGCATGTTACGGCACCTGGGCCGGCTGCTGGCGTGGCGGAGCCAG
IR64	TACCTCAAGAACGCCGGCATGTTACGGCACCTGGGCCGGCTGCTGGCGTGGCGGAGCCAG
DJ123	TACCTCAAGAACGCCGGCATGTTACGGCACCTGGGCCGGCTGCTGGCGTGGCGGAGCCAG
Nipponbare	TACCTCAAGAACGCCGGCATGTTACGGCACCTGGGCCGGCTGCTGGCGTGGCGGAGCCAG
Azucena	TACCTCAAGAACGCCGGCATGTTACGGCACCTGGGCCGGCTGCTGGCGTGGCGGAGCCAG

Bala	GGCGGGCGCGACCTCATGTGCCGCGTCTGCGTCGTACCGCGCTCGCCAGCGCGCTCTTC
IR64	GGCGGGCGCGACCTCATGTGCCGCGTCTGCGTCGTACCGCGCTCGCCAGCGCGCTCTTC
DJ123	GGCGGGCGCGACCTCATGTGCCGCGTCTGCGTCGTACCGCGCTCGCCAGCGCGCTCTTC
Nipponbare	GGCGGGCGCGACCTCATGTGCCGCGTCTGCGTCGTACCGCGCTCGCCAGCGCGCTCTTC
Azucena	GGCGGGCGCGACCTCATGTGCCGCGTCTGCGTCGTACCGCGCTCGCCAGCGCGCTCTTC

Bala	ACCAACGACACCTGCTGCGTCGTCCTACCGAGTTCGTGCTCGAGCTCGCCGCCGAGCGC
IR64	ACCAACGACACCTGCTGCGTCGTCCTACCGAGTTCGTGCTCGAGCTCGCCGCCGAGCGC
DJ123	ACCAACGACACCTGCTGCGTCGTCCTACCGAGTTCGTGCTCGAGCTCGCCGCCGAGCGC
Nipponbare	ACCAACGACACCTGCTGCGTCGTCCTACCGAGTTCGTGCTCGAGCTCGCCGCCGAGCGC
Azucena	ACCAACGACACCTGCTGCGTCGTCCTACCGAGTTCGTGCTCGAGCTCGCCGCCGAGCGC

1		
2		
3	Bala	AACCTCCCGGCGAAGCCGTTCCCTGCTCGCGCTCGCCACCAGCGCCAACATCGGCTCCAGC
4	IR64	AACCTCCCGGCGAAGCCGTTCCCTGCTCGCGCTCGCCACCAGCGCCAACATCGGCTCCAGC
5	DJ123	AACCTCCCGGCGAAGCCGTTCCCTGCTCGCGCTCGCCACCAGCGCCAACATCGGCTCCAGC
6	Nipponbare	AACCTCCCGGCGAAGCCGTTCCCTGCTCGCGCTCGCCACCAGCGCCAACATCGGCTCCAGC
7	Azucena	AACCTCCCGGCGAAGCCGTTCCCTGCTCGCGCTCGCCACCAGCGCCAACATCGGCTCCAGC
8		*****
9	Bala	GCGACGCCCATCGGCAACCCGCGAGAACCCTGGTCATCGCCTTCAACAGCAAGATCTCCTTT
10	IR64	GCGACGCCCATCGGCAACCCGCGAGAACCCTGGTCATCGCCTTCAACAGCAAGATCTCCTTT
11	DJ123	GCGACGCCCATCGGCAACCCGCGAGAACCCTGGTCATCGCCTTCAACAGCAAGATCTCCTTT
12	Nipponbare	GCGACGCCCATCGGCAACCCGCGAGAACCCTGGTCATCGCCTTCAACAGCAAGATCTCCTTT
13	Azucena	GCGACGCCCATCGGCAACCCGCGAGAACCCTGGTCATCGCCTTCAACAGCAAGATCTCCTTT
14		*****
15	Bala	ATTTCCCTTCCTCCTCGGCATCCTCCCCGCCATGCTCGCCGGCATGGGGATCAACATGCTC
16	IR64	ATTTCCCTTCCTCCTCGGCATCCTCCCCGCCATGCTCGCCGGCATGGGGATCAACATGCTC
17	DJ123	ATTTCCCTTCCTCCTCGGCATCCTCCCCGCCATGCTCGCCGGCATGGGGATCAACATGCTC
18	Nipponbare	ATTTCCCTTCCTCCTCGGCATCCTCCCCGCCATGCTCGCCGGCATGGGGATCAACATGCTC
19	Azucena	ATTTCCCTTCCTCCTCGGCATCCTCCCCGCCATGCTCGCCGGCATGGGGATCAACATGCTC
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	GTCGCCTACATGCTCGGCCTCAACATGTCGTGGACCGCCATCACGACCGCCATCGCCCTC
52	IR64	GTCGCCTACATGCTCGGCCTCAACATGTCGTGGACCGCCATCACGACCGCCATCGCCCTC
53	DJ123	GTCGCCTACATGCTCGGCCTCAACATGTCGTGGACCGCCATCACGACCGCCATCGCCCTC
54	Nipponbare	GTCGCCTACATGCTCGGCCTCAACATGTCGTGGACCGCCATCACGACCGCCATCGCCCTC
55	Azucena	GTCGCCTACATGCTCGGCCTCAACATGTCGTGGACCGCCATCACGACCGCCATCGCCCTC
56		*****

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Bala	TAAATCAGACAATAAATCAAGTCAAAATAAAATAAATGTAATTATATTTTTTTAATAAGAT
IR64	TAAATCAGACAATAAATCAAGTCAAAATAAAATAAATGTAATTATATTTTTTTAATAAGAT
DJ123	TAAATCAGACAATAAATCAAGTCAAAATAAAATAAATGTAATTATATTTTTTTAATAAGAT
Nipponbare	TAAATCAGACAATAAATCAAGTCAAAATAAAATAAATGTAATTATATTTTTTTAATAAGAT
Azucena	TAAATCAGACAATAAATCAAGTCAAAATAAAATAAATGTAATTATATTTTTTTAATAAGAT

Bala	GAATGATTATTAAGAAAAAATTTTTTTGTCTCTCATAATTAAGAAAGAACACTCA
IR64	GAATGATTATTAAGAAAAAATTTTTTTGTCTCTCATAATTAAGAAAGAACACTCA
DJ123	GAATGATTATTAAGAAAAAATTTTTTTGTCTCTCATAATTAAGAAAGAACACTCA
Nipponbare	GAATGATTATTAAGAAAAAATTTTTTTGTCTCTCATAATTAAGAAAGAACACTCA
Azucena	GAATGATTATTAAGAAAAAATTTTTTTGTCTCTCATAATTAAGAAAGAACACTCA

Bala	TTTTTTACATGTTATTTGAGTGGAATTGTAAATTAATTTATATATGTGGGTGTAGTGT
IR64	TTTTTTACATGTTATTTGAGTGGAATTGTAAATTAATTTATATATGTGGGTGTAGTGT
DJ123	TTTTTTACATGTTATTTGAGTGGAATTGTAAATTAATTTATATATGTGGGTGTAGTGT
Nipponbare	TTTTTTACATGTTATTTGAGTGGAATTGTAAATTAATTTATATATGTGGGTGTAGTGT
Azucena	TTTTTTACATGTTATTTGAGTGGAATTGTAAATTAATTTATATATGTGGGTGTAGTGT

Bala	GTTGATGGGGGATGAGGTGGCGCGCGCGCGCGCGACGATATCACCGCGCGCGGTGACGCG
IR64	GTTGATGGGGGATGAGGTGGCGCGCGCGCGCGCGACGATATCACCGCGCGCGGTGACGCG
DJ123	GTTGATGGGGGATGAGGTGGCGCGCGCGCGCGCGACGATATCACCGCGCGCGGTGACGCG
Nipponbare	GTTGATGGGGGATGAGGTGGCGCGCGCGCGCGCGACGATATCACCGCGCGCGGTGACGCG
Azucena	GTTGATGGGGGATGAGGTGGCGCGCGCGCGCGCGACGATATCACCGCGCGCGGTGACGCG

Bala	GTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGGGGAACCTGTCGCTGCTGGGGTC
IR64	GTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGGGGAACCTGTCGCTGCTGGGGTC
DJ123	GTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGGGGAACCTGTCGCTGCTGGGGTC
Nipponbare	GTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGGGGAACCTGTCGCTGCTGGGGTC
Azucena	GTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGGGGAACCTGTCGCTGCTGGGGTC

Bala	GGCGGCGAACCTGATCGTGTGCGAGCAGGCGAGGCGGGCGACGCGCAACGCCTACGACCT
IR64	GGCGGCGAACCTGATCGTGTGCGAGCAGGCGAGGCGGGCGACGCGCAACGCCTACGACCT
DJ123	GGCGGCGAACCTGATCGTGTGCGAGCAGGCGAGGCGGGCGACGCGCAACGCCTACGACCT
Nipponbare	GGCGGCGAACCTGATCGTGTGCGAGCAGGCGAGGCGGGCGACGCGCAACGCCTACGACCT
Azucena	GGCGGCGAACCTGATCGTGTGCGAGCAGGCGAGGCGGGCGACGCGCAACGCCTACGACCT

Bala	CACCTTCTGGAACACGTCATCTTCGGACTCCCATCCACCCTCGTCGTCACCGCCATCGG
IR64	CACCTTCTGGAACACGTCATCTTCGGACTCCCATCCACCCTCGTCGTCACCGCCATCGG
DJ123	CACCTTCTGGAACACGTCATCTTCGGACTCCCATCCACCCTCGTCGTCACCGCCATCGG
Nipponbare	CACCTTCTGGAACACGTCATCTTCGGACTCCCATCCACCCTCGTCGTCACCGCCATCGG
Azucena	CACCTTCTGGAACACGTCATCTTCGGACTCCCATCCACCCTCGTCGTCACCGCCATCGG

Bala	CATCCCTCTCATCGGCAAGATCAACATCTAGTCGTCATTAAATTAATTAATTAATATAAT
IR64	CATCCCTCTCATCGGCAAGATCAACATCTAGTCGTCATTAAATTAATTAATTAATATAAT
DJ123	CATCCCTCTCATCGGCAAGATCAACATCTAGTCGTCATTAAATTAATTAATTAATATAAT
Nipponbare	CATCCCTCTCATCGGCAAGATCAACATCTAGTCGTCATTAAATTAATTAATTAATATAAT
Azucena	CATCCCTCTCATCGGCAAGATCAACATCTAGTCGTCATTAAATTAATTAATTAATATAAT

Bala	CCGGCTAGCTACTATCTACAACGATCAGAAGAAGGCAGAATGATCGAGCATGCAAGAATT
IR64	CCGGCTAGCTACTATCTACAACGATCAGAAGAAGGCAGAATGATCGAGCATGCAAGAATT
DJ123	CCGGCTAGCTACTATCTACAACGATCAGAAGAAGGCAGAATGATCGAGCATGCAAGAATT
Nipponbare	CCGGCTAGCTACTATCTACAACGATCAGAAGAAGGCAGAATGATCGAGCATGCAAGAATT
Azucena	CCGGCTAGCTACTATCTACAACGATCAGAAGAAGGCAGAATGATCGAGCATGCAAGAATT

Bala	CATGCAAATTACAAGGGATGTGATATGATCATATTAATCCAAGGAAGCTAGTATGATGAT
IR64	CATGCAAATTACAAGGGATGTGATATGATCATATTAATCCAAGGAAGCTAGTATGATGAT
DJ123	CATGCAAATTACAAGGGATGTGATATGATCATATTAATCCAAGGAAGCTAGTATGATGAT
Nipponbare	CATGCAAATTACAAGGGATGTGATATGATCATATTAATCCAAGGAAGCTAGTATGATGAT
Azucena	CATGCAAATTACAAGGGATGTGATATGATCATATTAATCCAAGGAAGCTAGTATGATGAT

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1
2
3
4 Bala TAAGTTTGTGATCAAGCCGTACTGTGTGCATGCCAATGCATGCATGTGTTCAAGTGTA
5 IR64 TAAGTTTGTGATCAAGCCGTACTGTGTGCATGCCAATGCATGCATGTGTTCAAGTGTA
6 DJ123 TAAGTTTGTGATCAAGCCGTACTGTGTGCATGCCAATGCATGCATGTGTTCAAGTGTA
7 Nipponbare TAAGTTTGTGATCAAGCCGTACTGTGTGCATGCCAATGCATGCATGTGTTCAAGTGTA
8 Azucena TAAGTTTGTGATCAAGCCGTACTGTGTGCATGCCAATGCATGCATGTGTTCAAGTGTA
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 ATGCCGGATTACCTCATTGCTCCAAACTTGCAATCAGTTTTGAACTTTAAATAAAGTTAA
40 IR64 ATGCCGGATTACCTCATTGCTCCAAACTTGCAATCAGTTTTGAACTTTAAATAAAGTTAA
41 DJ123 ATGCCGGATTACCTCATTGCTCCAAACTTGCAATCAGTTTTGAACTTTAAATAAAGTTAA
42 Nipponbare ATGCCGGATTACCTCATTGCTCCAAACTTGCAATCAGTTTTGAACTTTAAATAAAGTTAA
43 Azucena ATGCCGGATTACCTCATTGCTCCAAACTTGCAATCAGTTTTGAACTTTAAATAAAGTTAA
44 *****
45
46 Bala TTTGGCTTTGTGCACTTGTCAATTTGCTCCCTCTTTGTTTAAACCGATCTTGCCTAGTGCG
47 IR64 TTTGGCTTTGTGCACTTGTCAATTTGCTCCCTCTTTGTTTAAACCGATCTTGCCTAGTGCG
48 DJ123 TTTGGCTTTGTGCACTTGTCAATTTGCTCCCTCTTTGTTTAAACCGATCTTGCCTAGTGCG
49 Nipponbare TTTGGCTTTGTGCACTTGTCAATTTGCTCCCTCTTTGTTTAAACCGATCTTGCCTAGTGCG
50 Azucena TTTGGCTTTGTGCACTTGTCAATTTGCTCCCTCTTTGTTTAAACCGATCTTGCCTAGTGCG
51 *****
52
53 Bala TACGTAGCTAGATTGCACATTTAAG
54 IR64 TACGTAGCTAGATTGCACATTTAAG
55 DJ123 TACGTAGCTAGATTGCACATTTAAG
56 Nipponbare TACGTAGCTAGATTGCACATTTAAG
57 Azucena TACGTAGCTAGATTGCACATTTAAG
58 *****

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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/>.