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

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11   5   Partha Talukdar[1], Sue E. Hartley[2], Anthony J Travis[1], Adam H. Price[1], Gareth J. Norton[1]*
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14   7   Affiliations:
15
16   8   [1] School of Biological Sciences, University of Aberdeen, Aberdeen, AB24 3UU, UK.
17
18   9   [2] York Environmental Sustainability Institute, University of York, York, YO10 5DD, UK.
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21   11
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23
24   12   *Corresponding author:
25
26   13   Mail: partha.talukdar@abdn.ac.uk
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28   14   Tel: +44 (0)1224 272409
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35 Genotypic differences in shoot silicon content and the impact on grain arsenic accumulation

36 in rice

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38 Partha Talukdar[1], Sue E. Hartley[2], Anthony J Travis[1], Adam H. Price[1], Gareth J. Norton[1*]

39

40 Affiliations:

41 [1] School of Biological Sciences, University of Aberdeen, Aberdeen, AB24 3UU, UK.

42 [2] York Environmental Sustainability Institute, University of York, York, YO10 5DD, UK.

43 *Corresponding author: partha.talukdar@abdn.ac.uk

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45

46 **Abstract:**

47 Silicon in rice has been demonstrated to be involved in resistance to lodging, tolerance to

48 both drought and salinity, and also enhances resistance to pests and diseases. The aim of this

49 study was to determine the range of silicon content in a set of rice (*Oryza sativa* L.)

50 accessions, and to determine if the natural variation of shoot silicon is linked to the

51 previously identified silicon transporters (*Lsi* genes). Silicon content was determined in 50

52 field-grown accessions, representing all sub-populations of rice, with all accessions being

53 genotyped with 700K SNPs. SNPs within 10 kb of the *Lsi* genes were examined to determine

54 if any were significantly linked with the phenotypic variation. An XRF method of silicon

55 determination compared favourably with digestion and colorimetric analysis. There were

56 significant genotypic differences in shoot silicon ranging from $16.5 - 42.4$ mg g^{-1} of plant dry

57 weight, there was no significant difference between the rice sub-populations. Plants with

58 different alleles for SNPs representing *Lsi2* and *Lsi3* were significantly different for shoot

59 silicon content. Shoot silicon correlated negatively with grain arsenic in the *tropical* and

60 *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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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

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

116 leads to increased mobilisation of soil arsenic in the form of arsenite, which causes

117 anaerobically-grown rice to accumulate more arsenic through silicon transporters (*Ma* et al.,

118 2008; *Xu* et al., 2008; *Carey* et al., 2010). Silicon fertilisation has also been shown to

119 decrease shoot and grain arsenic indicating that silicon could play an important role in

120 decreasing total arsenic uptake in rice (*Li* et al., 2009; *Seyfferth and Ferdorf*, 2012).

121 This study was designed to address four questions all related to the process of silicon and

122 arsenic accumulation in rice: How does the cultivation method affect silicon distribution in

123 different organs of rice plants? Are there significant genotypic differences in shoot silicon

124 concentration across a diverse panel of rice related to the 5 different sub-populations of rice?

125 Is there a relationship between natural variation in shoot silicon and arsenic content in rice?

126 Can natural variation in shoot silicon be linked to known silicon transporters in rice? The

127 results provide a deeper understanding of the natural variation in silicon content across rice

128 accessions   and   its   relationship   to   arsenic   accumulation   in   rice   grains.

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 Italica 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.

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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 (H_2O_2, > 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

178 left overnight. The tubes were vortexed again after overnight extraction, then heated at 123°C

179 under a pressure of 0.15 MPa for 1 h. Samples were kept at room temperature for 2 h then

180 vortexed, followed by addition of 18.5 mL of ddH_2O. Prior to analysis, samples were diluted

181 1:5 with Milli-Q water. Silicon content was measured using an FIA spectrophotometer

182 (Tecator FIAstar 5010) a wavelength of 410 nm (*Carneiro* et al*.,* 2007; *Norton* et al*.,* 2010a;

183 *Norton* et al., 2010b).

2.4 Analysis of rice shoot silicon by P-XRF

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185 A total of 1.5 g of dried shoot material for each rice accession was sub-sampled at random

186 and powdered using a ball mill (Retsch, MM200, Germany). To perform the analysis of shoot

187 silicon by P-XRF, 19 accessions were selected at random from the 50 accessions for which

188 shoot silicon had been determined by FIA. For P-XRF analysis, 0.7 g of homogeneous

189 powder sample was compressed into 13 mm diameter pellets using a manual hydraulic press

190 with a 13mm die at a pressure of 10 tons (Specac, Orpington, United Kingdom). Shoot silicon

191 content (% of silicon dry shoot weight) was measured using a commercial P-XRF instrument

192 (Niton XL3t900 GOLDD analyzer: Thermo Scientific Winchester, UK), calibrated using Si-

193 spiked synthetic methyl cellulose and validated using Certified Reference Materials of NCS

194 DC73349 'Bush branches and leaves' obtained from the China National Analysis Center for

195 Iron and Steel, as described in *Reidinger* et al., (2012). The mean value of samples for each

196 accession was used for correlation analysis between P-XRF and FIA measurements.

2.5 Relationship between silicon and arsenic content in rice

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198 The plant material used in this study was previously examined for grain arsenic content

199 (*Norton* et al., 2012) which provided an opportunity to examine the relationship between

200 shoot silicon and grain arsenic in rice. The relationship between shoot silicon (log

201 transformed) and grain arsenic (log transformed) was investigated for the 50 rice accessions

202 based on accession means.

2.6 Single Marker Analysis

204 The accessions used in this study have been genotyped using a high-density SNP chip

205 (*McCouch* et al., 2016). SNPs for the accessions were extracted using PLINK (*Purcell* et al.,

206 2007). SNPs were extracted from 10 kb upstream of the start codon to 10 kb downstream of

207 the stop codon of the *Lsi1, Lsi2, Lsi3*, and *Lsi*6 loci. SNPs were excluded from the analysis if

208 they were invariant or if minor alleles were present in less than 5% of the accessions. The

209 RDP1 population has a high degree of stratification by rice sub-population (*Zhao* et al., 2011;

210 *McCouch* et al., 2016). To overcome this stratification, sub-population assignment was used

211 (based on the 700 K SNP data; *McCouch* et al., 2016) as a factor in a two-way ANOVA, with

212 SNP base call as the other factor. The two-way ANOVA was used to determine if the

213 phenotype for the accession was significantly different for each SNP tested.

2.7 Sequence alignments

215 Based on the result achieved from the single-marker analysis the sequence diversity of *Lsi2*

216 and *Lsi3* were investigated for 5 cultivars using BAM files produced after aligning sequence

217 reads against Nipponbare version 7 reference genome. The genome sequences of the cultivars

218 used in this study have been previously published (*Kawahara* et al., 2013; *Cardoso* et al.,

219 2014; *Schatz* et al., 2014). The accessions were from the following sub-populations; 2 *indica*

220 accessions (IR64 and Bala), 1 *aus* accession (DJ123) and 2 *tropical japonica* accessions

221 (Azucena and Nipponbare).The genomic DNA sequence was visualised using the IGV

222 (https://www.broadinstitute.org/igv/) to identify the difference of genomic DNA sequence

223 within *Lsi2 and Lsi3* in 5 cultivars (*Thorvaldsdóttir* et al., 2013; *Robinson* et al., 2011). Using

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.

3 Results

3.1 Shoot silicon content in different organs of rice plants

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

3.2 Genotypic difference in shoot silicon content of rice

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

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

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*).

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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280 23.1 mg g^{-1}. Both SNPs group the accessions in a similar way, the only difference was more

281 missing SNP information for SNP-3.438416 (Fig. 6).

282 A total of 20 SNPs from the SNP database are within 10 kb upstream and downstream of the

283 *Lsi3* gene (selected SNPs for the rice accessions are presented in supplementary Table 3).

284 Shoot silicon concentration for accessions with the different alleles for one of these SNPs was

285 significantly different. SNP- 10.21340470 is located 5299 bp prior to the start codon, and

286 revealed a significant difference between the G and A polymorphism ($P = 0.016$), where the

287 mean silicon content of accessions with the G allele was 28.4 mg g^{-1} while the mean silicon

288 content of the accessions with the A allele was 35.6 mg g^{-1} (Fig. 6).

289 There were 20 SNPs and 19 SNPs observed within 10 kb of *Lsi1* and *Lsi6* respectively.

290 However, at each of these SNPs the different alleles were not significantly different for shoot

291 silicon concentration.

292 To explore further, the sequence alignments of *Lsi2* and *Lsi3* were performed using available

293 high-quality genome sequences. The accessions used were Nipponbare, Azucena, IR64, Bala,

294 and DJ123 which are from the *tropical japonica, tropical japonica, indica, indica* and *aus*

295 rice subgroups respectively. From the sequence analyses of *Lsi2* and *Lsi3* a number of

296 polymorphisms within the genes were identified. For *Lsi2*, there was a synonymous SNP

297 substitution within the first exon, where DJ123 has "C" allele while the other 4 accessions

298 have "T" allele (Supplementary Figure 3). For *Lsi3*, 4 SNPs were detected in exons and 6

299 SNPs in introns (Supplementary Figure 4). There was only one non-synonymous SNP

300 observed in the first exon of *Lsi3* where DJ123 and Bala have "T" allele and other accessions

301 have "A" allele. The available 3000 rice genome sequence data indicates that this

302 polymorphism between "A" and "T" in *Lsi3* is associated with the *aus* sub-population in rice

303 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

4 Discussion

308

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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333   flooded plants in tissue associated with flowering and seed production significantly higher

334   than the rest. Since this reproductive tissue was removed from the field samples used in this

335   study we can be reassured that a mean value obtained from straw will be a good estimate of

336   the tissue concentration of the majority of rice plant.

337   Fifty accessions from 5 different sub-populations (10 accessions from each sub-population)

338   were selected at random to examine the difference of shoot silicon content in rice and this

339   revealed highly significant differences of shoot silicon content. A genotypic difference in

340   shoot silicon content across a wide group of accessions has been observed previously (*Deren*,

341   2001; *Norton* et al., 2010a). The 2.6-fold difference of shoot silicon content in this study is

342   similar to the previous 2.2 fold range detected for genotypic differences of shoot silicon

343   content in rice (*Norton* et al., 2010a). However, the maximum value observed in our study is

344   slightly lower than that detected previously (42.4 mg g^{-1} in this study, 60 mg g^{-1} (*Deren*,

345   2001), 61 mg g^{-1} (*Norton* et al*.,* 2010a).

346   The plant material used for determination of shoot silicon content in the 50 rice accessions

347   was grown in flooded, irrigated conditions (*Norton* et al*.,* 2012). Previous studies estimated

348   that 27% - 44% of the silicon taken up by rice plants is supplied by irrigation, while the

349   remaining percentage must be supplied by soil constituents (*Desplanques* et al*.,* 2006;

350   *Klotzbücher* et al., 2015). All the accessions tested in this study had a silicon content below

351   50 mg g^{-1} which, according to *Dobermann and Fairhurst* (2000), is below the critical level of

352   mineral deficiency for rice production. The low shoot silicon content (16.5 mg g^{-1} to 42.4 mg

353   g^{-1}) observed in this study may be due to removal of rice straw from the paddy field, which is

354   common practice in Bangladesh, and has been shown to contribute to lower shoot silicon in

355   the subsequent rice crop (*Seyfferth* et al., 2013). Future work should focus on linking the

356   flooded and non-flooded pot based experiment and the removal of straw at the field scale to

357   establish the importance of water management and field management on silicon accumulation

358   in field grown rice.

359   Several studies have demonstrated that the *Japonica* sub-species of rice have higher shoot

360   silicon than *Indicas* (*Winslow*, 1992; *Winslow* et al., 1997; *Ma* et al., 2007a). These studies

361   may have been limited by the number of accessions that were screened. For example, *Ma* et

362   al., (2007b) only screened two rice accessions to examine the genotypic difference in silicon

363   uptake of rice. To improve the current understanding of silicon biology in rice, we

364   investigated field grown shoot samples of 50 rice accessions across 5 sub-populations. Within

365   the material tested in this study the data suggests that the natural variation observed in shoot

366   silicon is not governed by genetic differences between rice sub-populations, but rather is

367   largely due to the genetic differences within individual sub-groups.

368   Data on more than 50 accessions would have opened the opportunity to conduct genome-

369   wide association (GWA) mapping where 200 accessions is considered a lower limit.

370   However, the FIA colorimetric method for the determination of silicon in rice shoots proved

371   not to be high throughput.   However, in addition to the FIA method, a sub-set of samples

372   were also analysed by P-XRF. The two different methods were strongly correlated, but not

373   perfectly, and indicated that values for silicon content in samples measured by FIA were

374   slightly higher than those measured by P-XRF. The observation that both methods provide

375   comparable results highlights the conclusion that P-XRF can be used for silicon analysis to

376   detect and measure genotypic differences across populations, instead of the more laborious

377   and time-consuming alkali digestion method. Furthermore, a second advantage of P-XRF is

378   that it is a non-destructive method. This would make it much more suitable for future GWA

379   mapping studies.

380   The plant material used in this study was previously used to examine the variation of shoot

381   and grain arsenic (*Norton* et al., 2012). The comparison of shoot silicon and grain arsenic in

382 this study is in agreement with previous studies where, in general, plants that had high shoot

383 silicon also had lower grain arsenic (*Seyfferth and Ferdorf*, 2012; *Norton* et al., 2012; *Norton*

384 et al., 2013). However, this study also adds more insight by taking into consideration the sub-

385 population structure of rice cultivars. The correlation between shoot silicon and grain arsenic

386 was sub-population specific. A strong relationship in between shoot silicon and grain arsenic

387 was observed in *temperate japonica* and a weaker one in *tropical japonica,* but was not

388 observed in *indica*, *aus* or *aromatic*. This important observation suggests that the genetic

389 regulation of arsenic content in rice grain is different in *temperate* and *tropical japonicas*

390 compared to the other rice sub-populations, implying that the silicon-transport-linked

391 pathway implicated for arsenic accumulation (*Ma* et al., 2007b; *Norton* et al., 2012) may be

392 less relevant in the *other* sub-populations.

393 The accessions used in the study have been genotyped using a 700K SNP chip (*McCouch* et

394 al., 2016). Single-marker analysis was used to test the candidacy of the known transporters of

395 silica in rice. The study indicated that two SNPs within 10 kb of *Lsi2* and one within 10 kb

396 of *Lsi3* were involved in contributing to the natural variation of shoot silicon accumulation in

397 rice (Fig. 6). The *Lsi2* gene has been shown to be pivotal for transport of silicon and

398 inorganic arsenic in studies conducted with mutants and transgenic plants (*Ma* et al., 2006;

399 *Ma* et al., 2007b; *Yamaji* et al*.,* 2008; *Mitani-Ueno* et al., 2011; *Yamaji* et al*.,* 2015). The

400 identification of differences in shoot silicon and the link with three SNPs close to the genes

401 further suggest that *Lsi2* and *Lsi3* are excellent candidate genes to explain the natural

402 variation observed in shoot silicon content of rice. When looking at the sequencing variation

403 of a number of diverse cultivars (which have been sequenced to a high depth) it is evident

404 that there is only a small number of polymorphisms within the genes (Supplementary figures

405 3 and 4). The highly conserved sequence for *Lsi2* may be due to its importance function for

406 silicon accumulation in rice. However, the accessions screened in this study are likely to have

407   greater sequence variation than the cultivars for which high quality sequence is available, and

408   therefore there may be greater sequence variation for *Lsi2* (and the other *Lsi* genes) than that

409   is represented in the 5 accessions reported here. A focus for future study will be to expand

410   sequence information to more accessions to more fully explore sequence variation associated

411   with the polymorphic SNPs presented in figure 6.

412

413   5 Conclusion

414   This study has demonstrated strong genotypic differences in shoot silicon in a diverse

415   collection of rice cultivars, showing that there is potential to breed rice with increased silicon

416   content that could improve resistance to both biotic and abiotic stresses in rice, which would

417   help to maintain crop yields. The identification of significant SNPs linked with the shoot

418   silicon phenotype within 10 kb of known silicon transporters warrants further study to

419   investigate the impact of different alleles of these genes on silicon and arsenic accumulation

420   in rice. Furthermore, the XRF method of silicon determination could be applied to GWA

421   mapping studies that might reveal further candidate genes for silicon content in rice.

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

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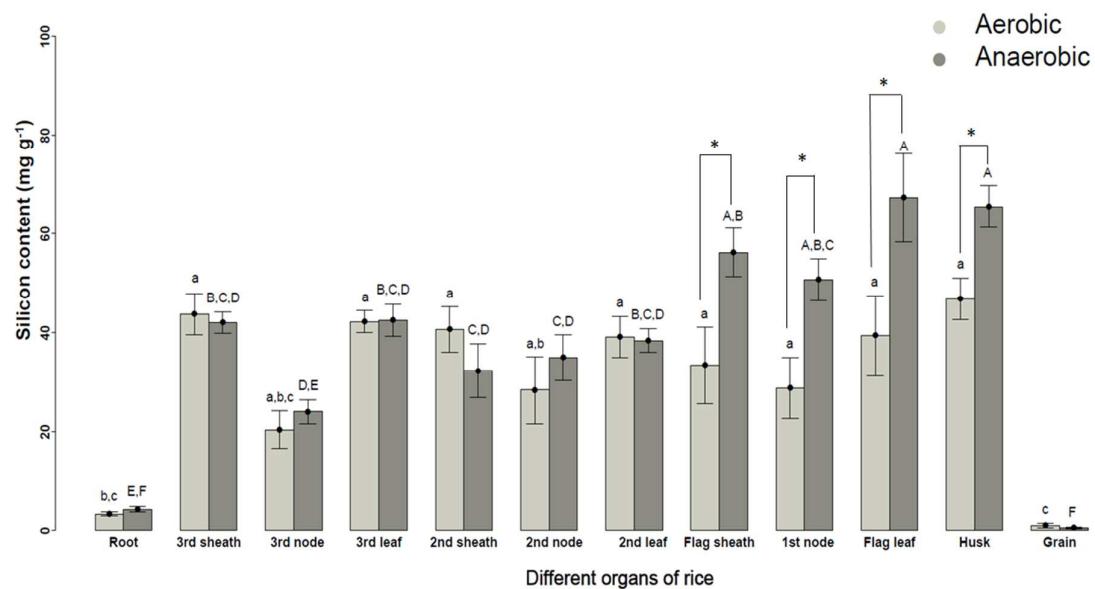
653 **Figure 2:** Mean shoot silicon (mg g^{-1}) of 50 rice accessions determined by FIA. Different
654 symbols refer to the accessions belonging to the different sub-populations; circle = *aus*,
655 square = *indica*, cross = *aromatic*, triangle = *tropical japonica*, upside down triangle =
656 *temperate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

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

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

664 **Figure 5:** Correlation between shoot silicon (mg g^{-1}) and grain arsenic (µg kg^{-1}) in ARO=
665 *aromatic*, AUS = *au*s, IND = *indica*, TEJ = *temperate japonica* and TRJ = *tropical japonica*
666 subpopulations.

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

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675   **Figure 1**
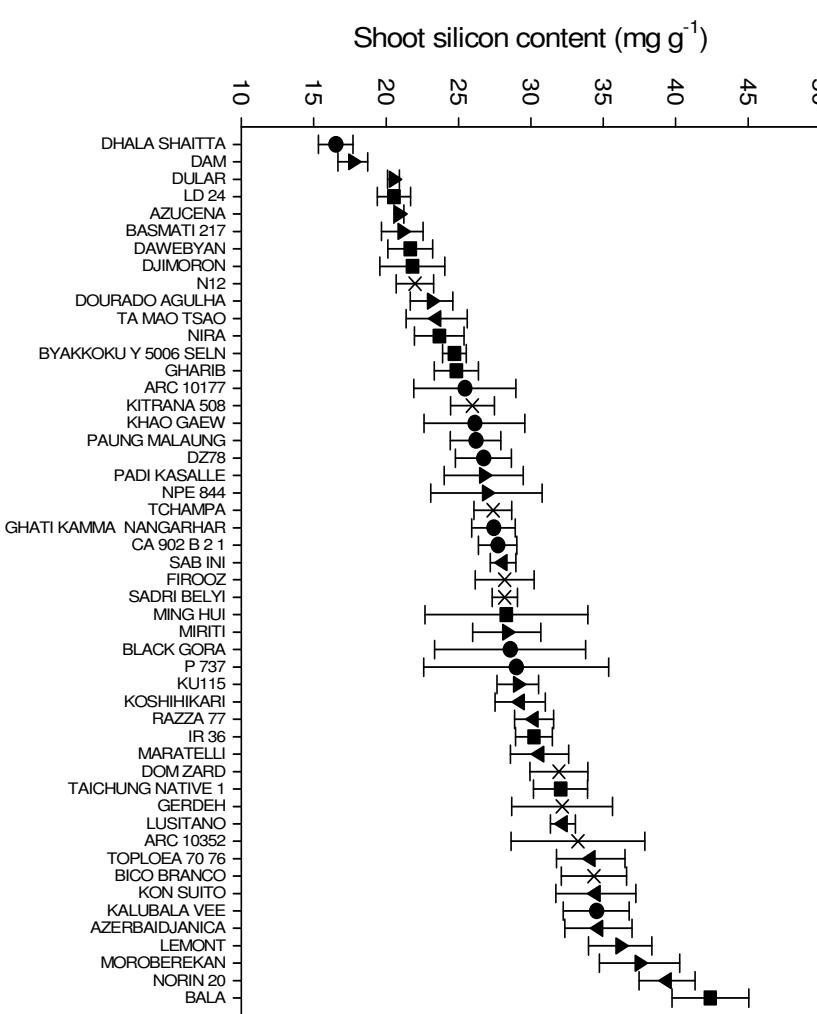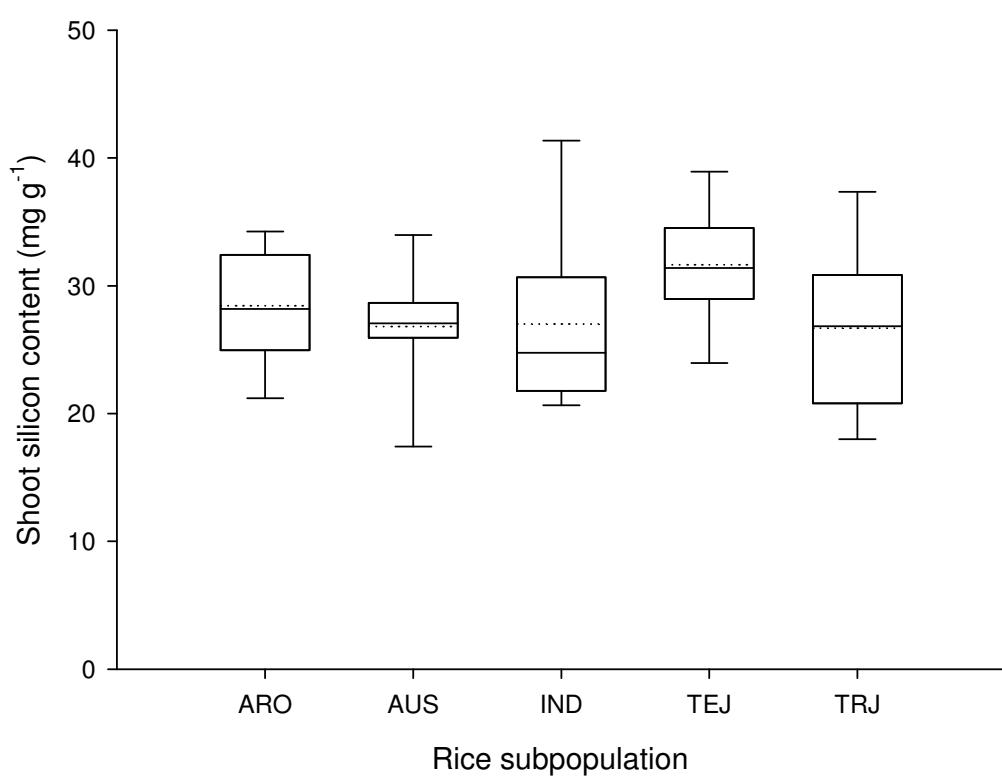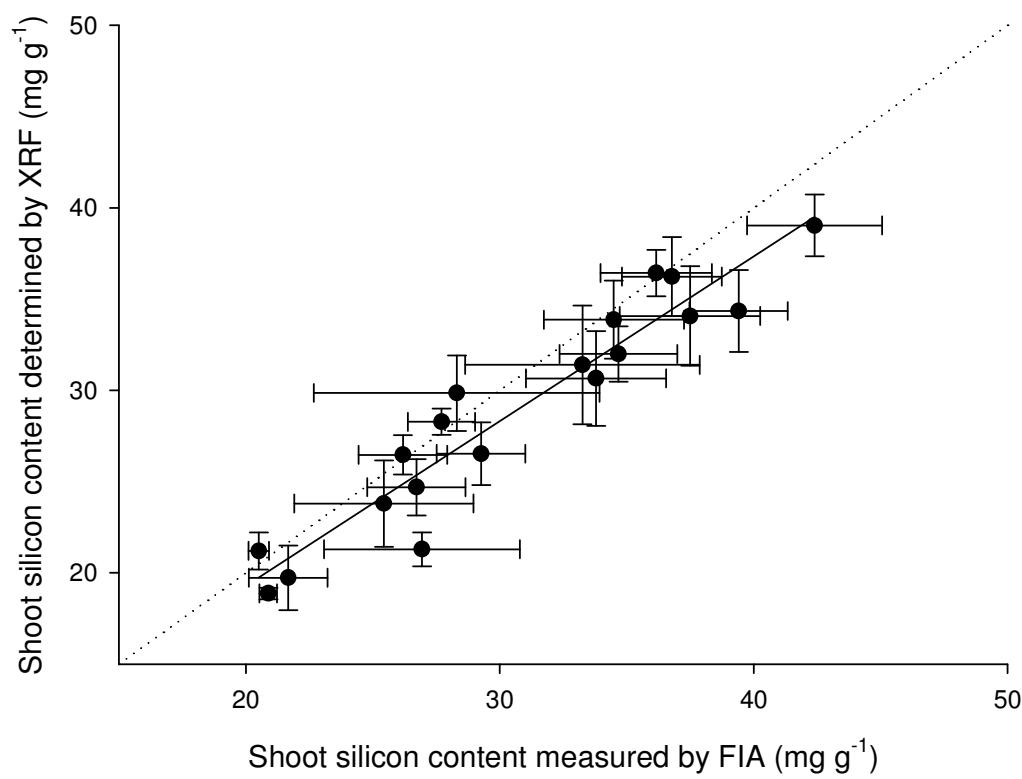
Figure 2

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682   **Figure 3**

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685   **Figure 4**
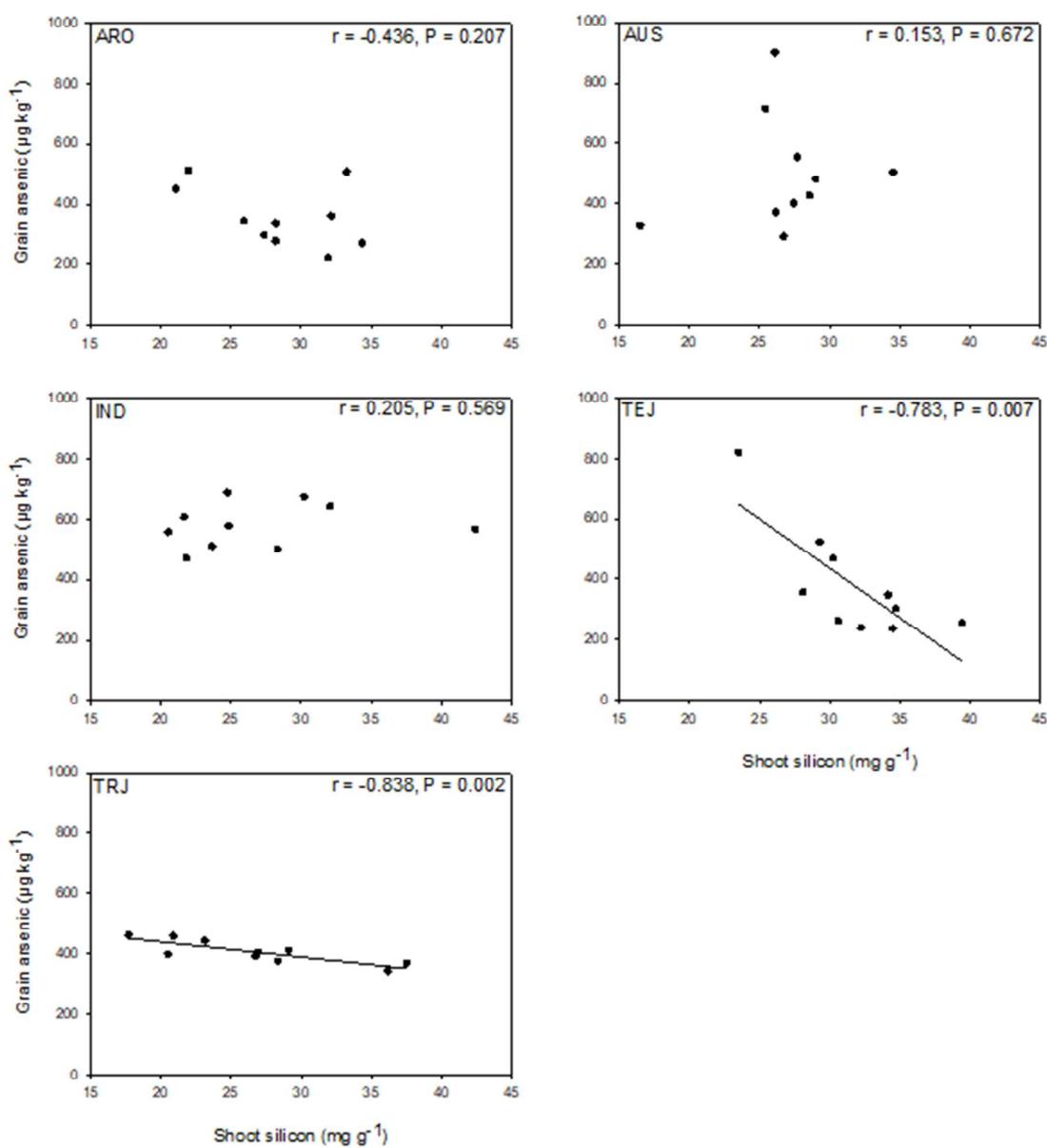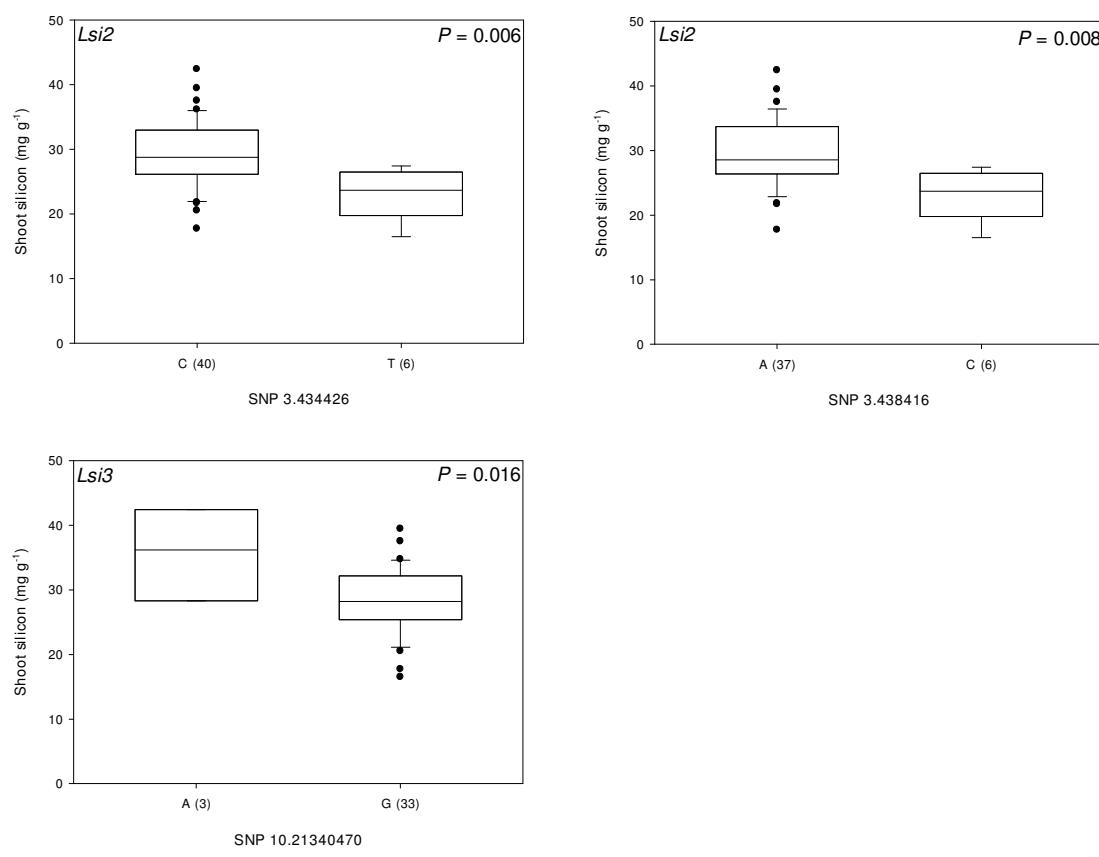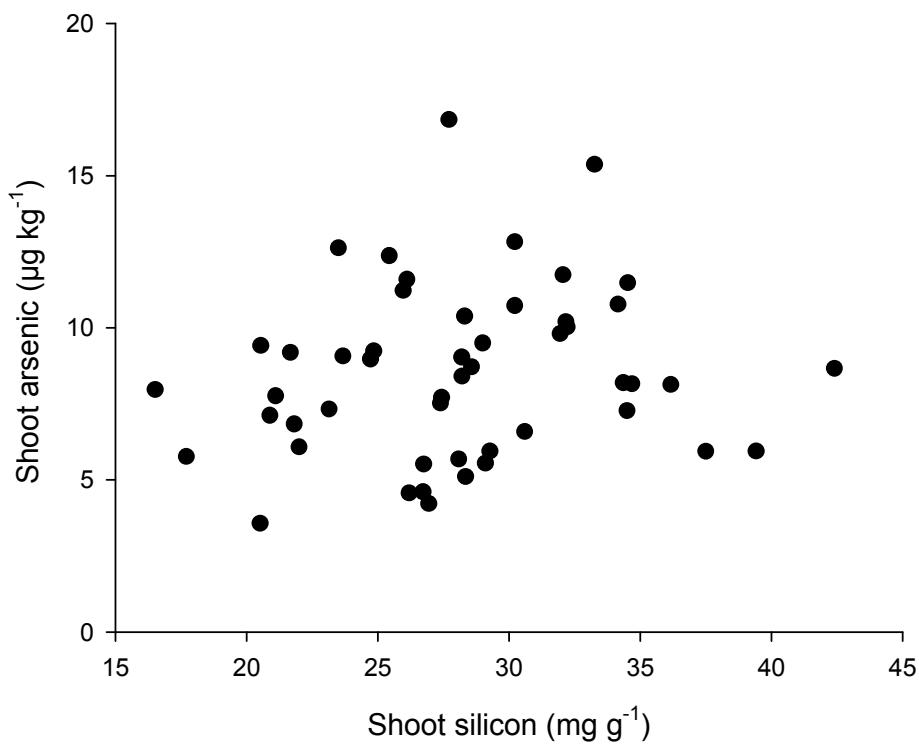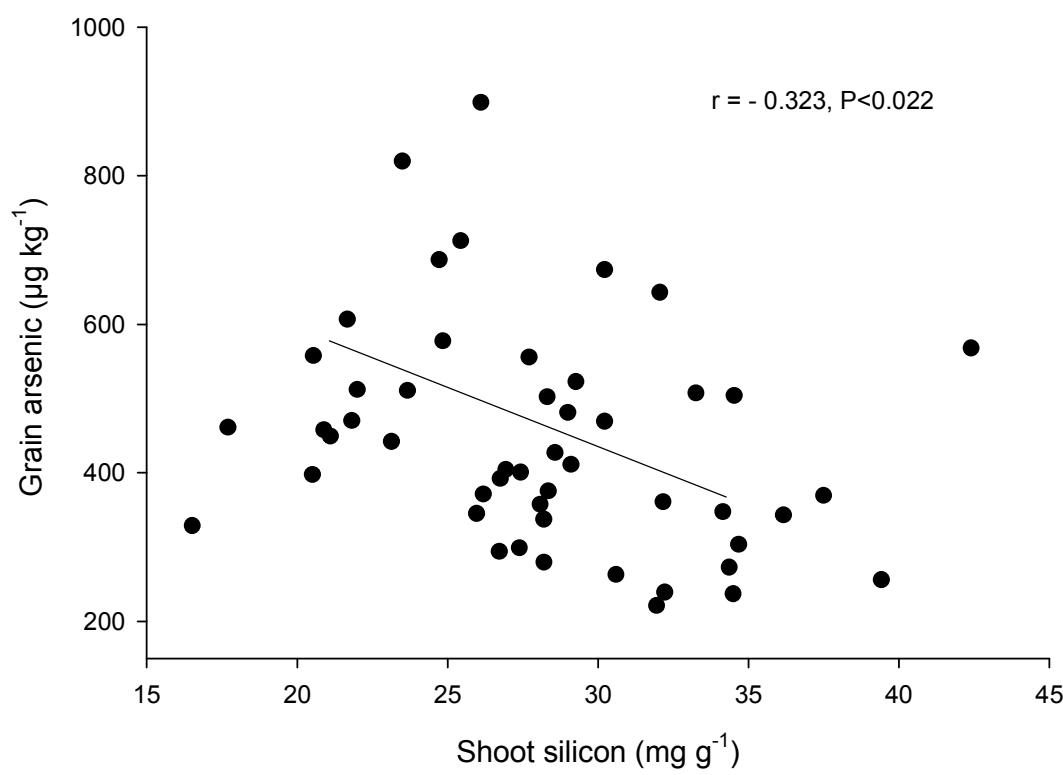
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687   **Figure 5**

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692   **Figure 6**

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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.

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

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3   *Lsi2* (LOC_Os03g01700)
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10   Bala        AGCATCAGCAGCGCATGCACTAGCAGCTTAGCTACTGCGCGCGTACCAGAGAGAGATCAT
11   IR64        AGCATCAGCAGCGCATGCACTAGCAGCTTAGCTACTGCGCGCGTACCAGAGAGAGATCAT
12   DJ123       AGCATCAGCAGCGCATGCACTAGCAGCTTAGCTACTGCGCGCGTACCAGAGAGAGATCAT
13   Nipponbare  AGCATCAGCAGCGCATGCACTAGCAGCTTAGCTACTGCGCGCGTACCAGAGAGAGATCAT
14   Azucena     AGCATCAGCAGCGCATGCACTAGCAGCTTAGCTACTGCGCGCGTACCAGAGAGAGATCAT
15               ************************************************************
16   Bala        CAGCTCAAGTAGCTAAGCTAGCTATAATCTGCTAGCTAGCTCGATCAGACACTTAATTAC
17   IR64        CAGCTCAAGTAGCTAAGCTAGCTATAATCTGCTAGCTAGCTCGATCAGACACTTAATTAC
18   DJ123       CAGCTCAAGTAGCTAAGCTAGCTATAATCTGCTAGCTAGCTCGATCAGACACTTAATTAC
19   Nipponbare  CAGCTCAAGTAGCTAAGCTAGCTATAATCTGCTAGCTAGCTCGATCAGACACTTAATTAC
20   Azucena     CAGCTCAAGTAGCTAAGCTAGCTATAATCTGCTAGCTAGCTCGATCAGACACTTAATTAC
21               ************************************************************
22   Bala        CTGCTAGGTGGTGGTCGATCGAAGAAGAAGAAGATGAGTGAGCTTGCGTCGGCGCCCAAG
23   IR64        CTGCTAGGTGGTGGTCGATCGAAGAAGAAGAAGATGAGTGAGCTTGCGTCGGCGCCCAAG
24   DJ123       CTGCTAGGTGGTGGTCGATCGAAGAAGAAGAAGATGAGTGAGCTTGCGTCGGCGCCCAAG
25   Nipponbare  CTGCTAGGTGGTGGTCGATCGAAGAAGAAGAAGATGAGTGAGCTTGCGTCGGCGCCCAAG
     Azucena     CTGCTAGGTGGTGGTCGATCGAAGAAGAAGAAGATGAGTGAGCTTGCGTCGGCGCCCAAG
26               ************************************************************
27   Bala        GTGGCGCTTGGATCGATCGCGTTCGCGGTGTTCTGGATGATGGCGGTGTTCCCGTCGGTG
28   IR64        GTGGCGCTTGGATCGATCGCGTTCGCGGTGTTCTGGATGATGGCGGTGTTCCCGTCGGTG
29   DJ123       GTGGCGCTTGGATCGATCGCGTTCGCGGTGTTCTGGATGATGGCGGTGTTCCCGTCGGTG
30   Nipponbare  GTGGCGCTTGGATCGATCGCGTTCGCGGTGTTCTGGATGATGGCGGTGTTCCCGTCGGTG
     Azucena     GTGGCGCTTGGATCGATCGCGTTCGCGGTGTTCTGGATGATGGCGGTGTTCCCGTCGGTG
31               ************************************************************
32   Bala        CCGTTCCTGCCGATCGGGCGGACGGCGGGGTCGCTGCTGAGCGCGGTGCTGATGGTGATA
33   IR64        CCGTTCCTGCCGATCGGGCGGACGGCGGGGTCGCTGCTGAGCGCGGTGCTGATGGTGATA
34   DJ123       CCGTTCCTGCCGATCGGGCGGACGGCGGGGTCGCTGCTGAGCGCGGTGCTGATGGTGATA
35   Nipponbare  CCGTTCCTGCCGATCGGGCGGACGGCGGGGTCGCTGCTGAGCGCGGTGCTGATGGTGATA
36   Azucena     CCGTTCCTGCCGATCGGGCGGACGGCGGGGTCGCTGCTGAGCGCGGTGCTGATGGTGATA
37               ************************************************************
38   Bala        TTCCACGTGATCAGCCCCGACGACGCGTACGCCTCCATCGACCTCCCAATCCTGGGCCTC
39   IR64        TTCCACGTGATCAGCCCCGACGACGCGTACGCCTCCATCGACCTCCCAATCCTGGGCCTC
40   DJ123       TTCCACGTGATCAGCCCCGACGACGCGTACGCCTCCATCGACCTCCCAATCCTGGGCCTC
41   Nipponbare  TTCCACGTGATCAGCCCCGACGACGCGTACGCCTCCATCGACCTCCCAATCCTGGGCCTC
42   Azucena     TTCCACGTGATCAGCCCCGACGACGCGTACGCCTCCATCGACCTCCCAATCCTGGGCCTC
43               ************************************************************
44   Bala        CTCTTCGCCACCATGGTGGTGGGCAGCTACCTCCGGAACGCCGGGATGTTCAAGCACCTG
45   IR64        CTCTTCGCCACCATGGTGGTGGGCAGCTACCTCCGGAACGCCGGGATGTTCAAGCACCTG
46   DJ123       CTCTTCGCCACCATGGTGGTGGGCAGCTACCTCCGGAACGCCGGGATGTTCAAGCACCTG
47   Nipponbare  CTCTTCGCCACCATGGTGGTGGGCAGCTACCTCCGGAACGCCGGGATGTTCAAGCACCTG
     Azucena     CTCTTCGCCACCATGGTGGTGGGCAGCTACCTCCGGAACGCCGGGATGTTCAAGCACCTG
48               ************************************************************
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51   Bala        GGGCGTCTGCTGGCGTGGAAGAGCCAGGGCGGGCGCGACCTCATGTGCCGCGTCTGCGTC
52   IR64        GGGCGTCTGCTGGCGTGGAAGAGCCAGGGCGGGCGCGACCTCATGTGCCGCGTCTGCGTC
53   DJ123       GGGCGTCTGCTGGCGTGGAAGAGCCAGGGCGGGCGCGACCTCATGTGCCGCGTCTGCGTC
54   Nipponbare  GGGCGTCTGCTGGCGTGGAAGAGCCAGGGCGGGCGCGACCTCATGTGCCGCGTCTGCGTC
     Azucena     GGGCGTCTGCTGGCGTGGAAGAGCCAGGGCGGGCGCGACCTCATGTGCCGCGTCTGCGTC
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3　　　Bala　　　　　GTCACCGCCCTCGCCAGCGCCCTCTTCACCAACGACACCTGCTGCGTCGTCCTCACCGAG
4　　　IR64　　　　　GTCACCGCCCTCGCCAGCGCCCTCTTCACCAACGACACCTGCTGCGTCGTCCTCACCGAG
5　　　DJ123　　　　GTCACCGCCCTCGCCAGCGCCCTCTTCACCAACGACACCTGCTGCGTCGTCCTCACCGAG
6　　　Nipponbare　GTCACCGCCCTCGCCAGCGCCCTCTTCACCAACGACACCTGCTGCGTCGTCCTCACCGAG
7　　　Azucena　　　GTCACCGCCCTCGCCAGCGCCCTCTTCACCAACGACACCTGCTGCGTCGTCCTCACCGAG
8　　　　　　　　　　**

9　　　Bala　　　　　TTCGTCCTCGAGCTCGCCGCCGAGCGCAACCTCCCCGCCAAGCCCTTCCTCCTCGCCCTC
10　　　IR64　　　　　TTCGTCCTCGAGCTCGCCGCCGAGCGCAACCTCCCCGCCAAGCCCTTCCTCCTCGCCCTC
11　　　DJ123　　　　TTCGTCCTCGAGCTCGCCGCCGAGCGCAACCTCCCCGCCAAGCCCTTCCTCCTCGCCCTC
12　　　Nipponbare　TTCGTCCTCGAGCTCGCCGCCGAGCGCAACCTCCCCGCCAAGCCCTTCCTCCTCGCCCTC
13　　　Azucena　　　TTCGTCCTCGAGCTCGCCGCCGAGCGCAACCTCCCCGCCAAGCCCTTCCTCCTCGCCCTC
14　　　　　　　　　　**

15　　　Bala　　　　　GCCTCCAGCGCCAACATCGGCTCCGCCGCCACCCCCATCGGCAACCCCCAGAACCTGGTC
16　　　IR64　　　　　GCCTCCAGCGCCAACATCGGCTCCGCCGCCACCCCCATCGGCAACCCCCAGAACCTGGTC
17　　　DJ123　　　　GCCTCCAGCGCCAACATCGGCTCCGCCGCCACCCCCATCGGCAACCCCCAGAACCTGGTC
18　　　Nipponbare　GCCTCCAGCGCCAACATCGGCTCCGCCGCCACCCCCATCGGCAACCCCCAGAACCTGGTC
19　　　Azucena　　　GCCTCCAGCGCCAACATCGGCTCCGCCGCCACCCCCATCGGCAACCCCCAGAACCTGGTC
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20　　　Bala　　　　　ATCGCCTTCAATAGCAAGATCACCTTCCCCAAGTTCCTCATGGGAATCCTCCCGGCCATG
21　　　IR64　　　　　ATCGCCTTCAATAGCAAGATCACCTTCCCCAAGTTCCTCATGGGAATCCTCCCGGCCATG
22　　　DJ123　　　　ATCGCCTTCAACAGCAAGATCACCTTCCCCAAGTTCCTCATGGGAATCCTCCCGGCCATG
23　　　Nipponbare　ATCGCCTTCAATAGCAAGATCACCTTCCCCAAGTTCCTCATGGGAATCCTCCCGGCCATG
24　　　Azucena　　　ATCGCCTTCAATAGCAAGATCACCTTCCCCAAGTTCCTCATGGGAATCCTCCCGGCCATG
25　　　　　　　　　　********** * **

26　　　Bala　　　　　CTCGTCGGGATGGCCGTCAACATGGTCATGCTGCTCTGCATGTACTGGAGGGAGCTGGGC
27　　　IR64　　　　　CTCGTCGGGATGGCCGTCAACATGGTCATGCTGCTCTGCATGTACTGGAGGGAGCTGGGC
28　　　DJ123　　　　CTCGTCGGGATGGCCGTCAACATGGTCATGCTGCTCTGCATGTACTGGAGGGAGCTGGGC
29　　　Nipponbare　CTCGTCGGGATGGCCGTCAACATGGTCATGCTGCTCTGCATGTACTGGAGGGAGCTGGGC
30　　　Azucena　　　CTCGTCGGGATGGCCGTCAACATGGTCATGCTGCTCTGCATGTACTGGAGGGAGCTGGGC
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31　　　Bala　　　　　GGAGGGGCCGAGCTCAGCGTCGACGGCAAGCAGATGGAGGCGGTGGAGGAAGGCAGGTCG
32　　　IR64　　　　　GGAGGGGCCGAGCTCAGCGTCGACGGCAAGCAGATGGAGGCGGTGGAGGAAGGCAGGTCG
33　　　DJ123　　　　GGAGGGGCCGAGCTCAGCGTCGACGGCAAGCAGATGGAGGCGGTGGAGGAAGGCAGGTCG
34　　　Nipponbare　GGAGGGGCCGAGCTCAGCGTCGACGGCAAGCAGATGGAGGCGGTGGAGGAAGGCAGGTCG
35　　　Azucena　　　GGAGGGGCCGAGCTCAGCGTCGACGGCAAGCAGATGGAGGCGGTGGAGGAAGGCAGGTCG
36　　　　　　　　　　**

37　　　Bala　　　　　CCGGCGTCGGCCAAGAGCACGCCGCAGCTGAACGGCAACGGCAACACGATGATGTCGCTG
38　　　IR64　　　　　CCGGCGTCGGCCAAGAGCACGCCGCAGCTGAACGGCAACGGCAACACGATGATGTCGCTG
39　　　DJ123　　　　CCGGCGTCGGCCAAGAGCACGCCGCAGCTGAACGGCAACGGCAACACGATGATGTCGCTG
40　　　Nipponbare　CCGGCGTCGGCCAAGAGCACGCCGCAGCTGAACGGCAACGGCAACACGATGATGTCGCTG
41　　　Azucena　　　CCGGCGTCGGCCAAGAGCACGCCGCAGCTGAACGGCAACGGCAACACGATGATGTCGCTG
42　　　　　　　　　　**

43　　　Bala　　　　　GAGATGTCGGAGAACATAACGACCAAGCACCCATGGTTCATGCAGTGCACGGAGGCGCGG
44　　　IR64　　　　　GAGATGTCGGAGAACATAACGACCAAGCACCCATGGTTCATGCAGTGCACGGAGGCGCGG
45　　　DJ123　　　　GAGATGTCGGAGAACATAACGACCAAGCACCCATGGTTCATGCAGTGCACGGAGGCGCGG
46　　　Nipponbare　GAGATGTCGGAGAACATAACGACCAAGCACCCATGGTTCATGCAGTGCACGGAGGCGCGG
47　　　Azucena　　　GAGATGTCGGAGAACATAACGACCAAGCACCCATGGTTCATGCAGTGCACGGAGGCGCGG
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48　　　Bala　　　　　CGGAAGCTGTTCCTCAAGAGCTTCGCGTACGTGGTGACGGTGGGGATGGTGGTGGCCTAC
49　　　IR64　　　　　CGGAAGCTGTTCCTCAAGAGCTTCGCGTACGTGGTGACGGTGGGGATGGTGGTGGCCTAC
50　　　DJ123　　　　CGGAAGCTGTTCCTCAAGAGCTTCGCGTACGTGGTGACGGTGGGGATGGTGGTGGCCTAC
51　　　Nipponbare　CGGAAGCTGTTCCTCAAGAGCTTCGCGTACGTGGTGACGGTGGGGATGGTGGTGGCCTAC
52　　　Azucena　　　CGGAAGCTGTTCCTCAAGAGCTTCGCGTACGTGGTGACGGTGGGGATGGTGGTGGCCTAC
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Bala          ATGGTGGGGCTCAACATGTCGTGGACGGCCATCACCACGGCGCTGGCGCTGGTGGTGGTC
IR64          ATGGTGGGGCTCAACATGTCGTGGACGGCCATCACCACGGCGCTGGCGCTGGTGGTGGTC
DJ123         ATGGTGGGGCTCAACATGTCGTGGACGGCCATCACCACGGCGCTGGCGCTGGTGGTGGTC
Nipponbare    ATGGTGGGGCTCAACATGTCGTGGACGGCCATCACCACGGCGCTGGCGCTGGTGGTGGTC
Azucena       ATGGTGGGGCTCAACATGTCGTGGACGGCCATCACCACGGCGCTGGCGCTGGTGGTGGTC
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Bala          GACTTCCGCGACGCCGAGCCGTGCCTGGACACCGTGTCCTACTCGCTGCTCGTCTTCTTC
IR64          GACTTCCGCGACGCCGAGCCGTGCCTGGACACCGTGTCCTACTCGCTGCTCGTCTTCTTC
DJ123         GACTTCCGCGACGCCGAGCCGTGCCTGGACACCGTGTCCTACTCGCTGCTCGTCTTCTTC
Nipponbare    GACTTCCGCGACGCCGAGCCGTGCCTGGACACCGTGTCCTACTCGCTGCTCGTCTTCTTC
Azucena       GACTTCCGCGACGCCGAGCCGTGCCTGGACACCGTGTCCTACTCGCTGCTCGTCTTCTTC
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Bala          TCCGGGATGTTCATCACCGTCAGCGGCTTCAACAAGACGGGCCTCCCGGGAGCCATCTGG
IR64          TCCGGGATGTTCATCACCGTCAGCGGCTTCAACAAGACGGGCCTCCCGGGAGCCATCTGG
DJ123         TCCGGGATGTTCATCACCGTCAGCGGCTTCAACAAGACGGGCCTCCCGGGAGCCATCTGG
Nipponbare    TCCGGGATGTTCATCACCGTCAGCGGCTTCAACAAGACGGGCCTCCCGGGAGCCATCTGG
Azucena       TCCGGGATGTTCATCACCGTCAGCGGCTTCAACAAGACGGGCCTCCCGGGAGCCATCTGG
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Bala          GACTTCATGGCCCCCTACTCCAAGGTCAACAGCGTCGGCGGCATCTCCGTCCTCTCCGTC
IR64          GACTTCATGGCCCCCTACTCCAAGGTCAACAGCGTCGGCGGCATCTCCGTCCTCTCCGTC
DJ123         GACTTCATGGCCCCCTACTCCAAGGTCAACAGCGTCGGCGGCATCTCCGTCCTCTCCGTC
Nipponbare    GACTTCATGGCCCCCTACTCCAAGGTCAACAGCGTCGGCGGCATCTCCGTCCTCTCCGTC
Azucena       GACTTCATGGCCCCCTACTCCAAGGTCAACAGCGTCGGCGGCATCTCCGTCCTCTCCGTC
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Bala          ATCATCCTCCTCCTCTCCAACCTCGCATCAAACGTACCAACGGGTAATTATACATTCTAT
IR64          ATCATCCTCCTCCTCTCCAACCTCGCATCAAACGTACCAACGGGTAATTATACATTCTAT
DJ123         ATCATCCTCCTCCTCTCCAACCTCGCATCAAACGTACCAACGGGTAATTATACATTCTAT
Nipponbare    ATCATCCTCCTCCTCTCCAACCTCGCATCAAACGTACCAACGGGTAATTATACATTCTAT
Azucena       ATCATCCTCCTCCTCTCCAACCTCGCATCAAACGTACCAACGGGTAATTATACATTCTAT
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Bala          TAATCCAGAATTAATGTATACTACTCCATATAAGTATAACTAATTAACATACAAAAATAT
IR64          TAATCCAGAATTAATGTATACTACTCCATATAAGTATAACTAATTAACATACAAAAATAT
DJ123         TAATCCAGAATTAATGTATACTACTCCATATAAGTATAACTAATTAACATACAAAAATAT
Nipponbare    TAATCCAGAATTAATGTATACTACTCCATATAAGTATAACTAATTAACATACAAAAATAT
Azucena       TAATCCAGAATTAATGTATACTACTCCATATAAGTATAACTAATTAACATACAAAAATAT
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Bala          ATATGCGCATGCATGCAGTGCTTCTTATGGGTGATGAGGTGGCGAAGGCGGCGGCGCTGA
IR64          ATATGCGCATGCATGCAGTGCTTCTTATGGGTGATGAGGTGGCGAAGGCGGCGGCGCTGA
DJ123         ATATGCGCATGCATGCAGTGCTTCTTATGGGTGATGAGGTGGCGAAGGCGGCGGCGCTGA
Nipponbare    ATATGCGCATGCATGCAGTGCTTCTTATGGGTGATGAGGTGGCGAAGGCGGCGGCGCTGA
Azucena       ATATGCGCATGCATGCAGTGCTTCTTATGGGTGATGAGGTGGCGAAGGCGGCGGCGCTGA
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Bala          TATCGCCGGCGGCGGTGACGACGTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGG
IR64          TATCGCCGGCGGCGGTGACGACGTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGG
DJ123         TATCGCCGGCGGCGGTGACGACGTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGG
Nipponbare    TATCGCCGGCGGCGGTGACGACGTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGG
Azucena       TATCGCCGGCGGCGGTGACGACGTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGG
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Bala          GGAACCTGTCGCTGCTGGGGTCGGCGGCGAACCTGATAGTGTGCGAGCAGGCGAGGAGGG
IR64          GGAACCTGTCGCTGCTGGGGTCGGCGGCGAACCTGATAGTGTGCGAGCAGGCGAGGAGGG
DJ123         GGAACCTGTCGCTGCTGGGGTCGGCGGCGAACCTGATAGTGTGCGAGCAGGCGAGGAGGG
Nipponbare    GGAACCTGTCGCTGCTGGGGTCGGCGGCGAACCTGATAGTGTGCGAGCAGGCGAGGAGGG
Azucena       GGAACCTGTCGCTGCTGGGGTCGGCGGCGAACCTGATAGTGTGCGAGCAGGCGAGGAGGG
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3   Bala   **CGCCCAGGAACGCCTACGACCTCACCTTCTGGCAGCACATCGTCTTCGGCGTCCCATCCA**
4   IR64   **CGCCCAGGAACGCCTACGACCTCACCTTCTGGCAGCACATCGTCTTCGGCGTCCCATCCA**
5   DJ123   **CGCCCAGGAACGCCTACGACCTCACCTTCTGGCAGCACATCGTCTTCGGCGTCCCATCCA**
6   Nipponbare   **CGCCCAGGAACGCCTACGACCTCACCTTCTGGCAGCACATCGTCTTCGGCGTCCCATCCA**
7   Azucena   **CGCCCAGGAACGCCTACGACCTCACCTTCTGGCAGCACATCGTCTTCGGCGTCCCATCCA**
                                  ****

8   Bala   **CCCTCATCGTCACCGCCGTCGGCATACCCCTCATCGGCAAGATCTGA**TCTCATCTCATCG
9   IR64   **CCCTCATCGTCACCGCCGTCGGCATACCCCTCATCGGCAAGATCTGA**TCTCATCTCATCG
10   DJ123   **CCCTCATCGTCACCGCCGTCGGCATACCCCTCATCGGCAAGATCTGA**TCTCATCTCATCG
11   Nipponbare   **CCCTCATCGTCACCGCCGTCGGCATACCCCTCATCGGCAAGATCTGA**TCTCATCTCATCG
12   Azucena   **CCCTCATCGTCACCGCCGTCGGCATACCCCTCATCGGCAAGATCTGA**TCTCATCTCATCG
                                  ****

13
14   Bala   ACCCATCCAAATTAATTAATTATGAGATCGACAAACATCCAAGCTTGCTAGGCTCGTCGT
15   IR64   ACCCATCCAAATTAATTAATTATGAGATCGACAAACATCCAAGCTTGCTAGGCTCGTCGT
16   DJ123   ACCCATCCAAATTAATTAATTATGAGATCGACAAACATCCAAGCTTGCTAGGCTCGTCGT
17   Nipponbare   ACCCATCCAAATTAATTAATTATGAGATCGACAAACATCCAAGCTTGCTAGGCTCGTCGT
18   Azucena   ACCCATCCAAATTAATTAATTATGAGATCGACAAACATCCAAGCTTGCTAGGCTCGTCGT
                                  ****

19
20   Bala   CGTCGTCGTCGACCACCGTACCATATATATGCATGCATGCCACGCACGTATATATATGCT
21   IR64   CGTCGTCGTCGACCACCGTACCATATATATGCATGCATGCCACGCACGTATATATATGCT
22   DJ123   CGTCGTCGTCGACCACCGTACCATATATATGCATGCATGCCACGCACGTATATATATGCT
23   Nipponbare   CGTCGTCGTCGACCACCGTACCATATATATGCATGCATGCCACGCACGTATATATATGCT
24   Azucena   CGTCGTCGTCGACCACCGTACCATATATATGCATGCATGCCACGCACGTATATATATGCT
                                  ****

25   Bala   CCTCAACCTCAAGTCAATTAATTAAGAGAATGGATGAATGAATCAATGTTGTGTATGATT
26   IR64   CCTCAACCTCAAGTCAATTAATTAAGAGAATGGATGAATGAATCAATGTTGTGTATGATT
27   DJ123   CCTCAACCTCAAGTCAATTAATTAAGAGAATGGATGAATGAATCAATGTTGTGTATGATT
28   Nipponbare   CCTCAACCTCAAGTCAATTAATTAAGAGAATGGATGAATGAATCAATGTTGTGTATGATT
29   Azucena   CCTCAACCTCAAGTCAATTAATTAAGAGAATGGATGAATGAATCAATGTTGTGTATGATT
                                  ****

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31   Bala   TCCTTTTTGTTTTTGTTTTACCCACCGTATATGTGCTGTGTGGTGTATGGCCGGCGAGCT
32   IR64   TCCTTTTTGTTTTTGTTTTACCCACCGTATATGTGCTGTGTGGTGTATGGCCGGCGAGCT
33   DJ123   TCCTTTTTGTTTTTGTTTTACCCACCGTATATGTGCTGTGTGGTGTATGGCCGGCGAGCT
34   Nipponbare   TCCTTTTTGTTTTTGTTTTACCCACCGTATATGTGCTGTGTGGTGTATGGCCGGCGAGCT
35   Azucena   TCCTTTTTGTTTTTGTTTTACCCACCGTATATGTGCTGTGTGGTGTATGGCCGGCGAGCT
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36   Bala   TGAAATTGAATTGGCCTTGCTTGCATGCATGCATGGTGATCAGATCAGCTCAGCTAGCTA
37   IR64   TGAAATTGAATTGGCCTTGCTTGCATGCATGCATGGTGATCAGATCAGCTCAGCTAGCTA
38   DJ123   TGAAATTGAATTGGCCTTGCTTGCATGCATGCATGGTGATCAGATCAGCTCAGCTAGCTA
39   Nipponbare   TGAAATTGAATTGGCCTTGCTTGCATGCATGCATGGTGATCAGATCAGCTCAGCTAGCTA
40   Azucena   TGAAATTGAATTGGCCTTGCTTGCATGCATGCATGGTGATCAGATCAGCTCAGCTAGCTA
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42   Bala   GCTAAATCTTGCTTAATTTTATCAAGTGTCAATCATGATGAGAAGAGAGGGAACTAGATG
43   IR64   GCTAAATCTTGCTTAATTTTATCAAGTGTCAATCATGATGAGAAGAGAGGGAACTAGATG
44   DJ123   GCTAAATCTTGCTTAATTTTATCAAGTGTCAATCATGATGAGAAGAGAGGGAACTAGATG
45   Nipponbare   GCTAAATCTTGCTTAATTTTATCAAGTGTCAATCATGATGAGAAGAGAGGGAACTAGATG
46   Azucena   GCTAAATCTTGCTTAATTTTATCAAGTGTCAATCATGATGAGAAGAGAGGGAACTAGATG
                                  ****

47   Bala   GAGCTAGCTAGCTAGCAACACATGCAGTGTGTGCTAAGCAGTGCACTCTGCATGCTTAAA
48   IR64   GAGCTAGCTAGCTAGCAACACATGCAGTGTGTGCTAAGCAGTGCACTCTGCATGCTTAAA
49   DJ123   GAGCTAGCTAGCTAGCAACACATGCAGTGTGTGCTAAGCAGTGCACTCTGCATGCTTAAA
50   Nipponbare   GAGCTAGCTAGCTAGCAACACATGCAGTGTGTGCTAAGCAGTGCACTCTGCATGCTTAAA
51   Azucena   GAGCTAGCTAGCTAGCAACACATGCAGTGTGTGCTAAGCAGTGCACTCTGCATGCTTAAA
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Bala         TTTGCTTGCTTAATTCAACATCGTATTACATGTATGTTGATATGTGCATGTTCCTCAAAA
IR64         TTTGCTTGCTTAATTCAACATCGTATTACATGTATGTTGATATGTGCATGTTCCTCAAAA
DJ123        TTTGCTTGCTTAATTCAACATCGTATTACATGTATGTTGATATGTGCATGTTCCTCAAAA
Nipponbare   TTTGCTTGCTTAATTCAACATCGTATTACATGTATGTTGATATGTGCATGTTCCTCAAAA
Azucena      TTTGCTTGCTTAATTCAACATCGTATTACATGTATGTTGATATGTGCATGTTCCTCAAAA
             ************************************************************

Bala         TACATGGAAAAAAAATGTTTCTACACATCTCAATCGTATAGAGTATGTAGCATGTCTTGT
IR64         TACATGGAAAAAAAATGTTTCTACACATCTCAATCGTATAGAGTATGTAGCATGTCTTGT
DJ123        TACATGGAAAAAAAATGTTTCTACACATCTCAATCGTATAGAGTATGTAGCATGTCTTGT
Nipponbare   TACATGGAAAAAAAATGTTTCTACACATCTCAATCGTATAGAGTATGTAGCATGTCTTGT
Azucena      TACATGGAAAAAAAATGTTTCTACACATCTCAATCGTATAGAGTATGTAGCATGTCTTGT
             ************************************************************

Bala         TCAAACAAATATTTGTAGCCCTCTCTCTCTCTCTCTCTTTAGGTTTATTAATTAATTAGT
IR64         TCAAACAAATATTTGTAGCCCTCTCTCTCTCTCTCTCTTTAGGTTTATTAATTAATTAGT
DJ123        TCAAACAAATATTTGTAGCCCTCTCTCTCTCTCTCTCTTTAGGTTTATTAATTAATTAGT
Nipponbare   TCAAACAAATATTTGTAGCCCTCTCTCTCTCTCTCTCTTTAGGTTTATTAATTAATTAGT
Azucena      TCAAACAAATATTTGTAGCCCTCTCTCTCTCTCTCTCTTTAGGTTTATTAATTAATTAGT
             ************************************************************

Bala         CTGTTCAATGTCACCTCAGTTCATAGAGATGCTATACTTAATTAATTGCTCCATGTATGC
IR64         CTGTTCAATGTCACCTCAGTTCATAGAGATGCTATACTTAATTAATTGCTCCATGTATGC
DJ123        CTGTTCAATGTCACCTCAGTTCATAGAGATGCTATACTTAATTAATTGCTCCATGTATGC
Nipponbare   CTGTTCAATGTCACCTCAGTTCATAGAGATGCTATACTTAATTAATTGCTCCATGTATGC
Azucena      CTGTTCAATGTCACCTCAGTTCATAGAGATGCTATACTTAATTAATTGCTCCATGTATGC
             ************************************************************

Bala         AGAGTTGTACTACCTTAAAATATCAGTATGCATGCTCTTGTTGCCATTCCAAAAGAACAA
IR64         AGAGTTGTACTACCTTAAAATATCAGTATGCATGCTCTTGTTGCCATTCCAAAAGAACAA
DJ123        AGAGTTGTACTACCTTAAAATATCAGTATGCATGCTCTTGTTGCCATTCCAAAAGAACAA
Nipponbare   AGAGTTGTACTACCTTAAAATATCAGTATGCATGCTCTTGTTGCCATTCCAAAAGAACAA
Azucena      AGAGTTGTACTACCTTAAAATATCAGTATGCATGCTCTTGTTGCCATTCCAAAAGAACAA
             ************************************************************

Bala         A
IR64         A
DJ123        A
Nipponbare   A
Azucena      A
             *
```

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         GAGGCGGCCATGGCGCTGGCGTCGCTGCCGAAGGTGGTGATGGGGTCGGTGGCGTTCGGT
IR64         GAGGCGGCCATGGCGCTGGCGTCGCTGCCGAAGGTGGTGATGGGGTCGGTGGCGTTCGGT
DJ123        GAGGCGGCCATGGCGCTGGCGTCGCTGCCGAAGGTGGTGATGGGGTCGGTGGCGTTCGGT
Nipponbare   GAGGCGGCCATGGCGCTGGCGTCGCTGCCGAAGGTGGTGATGGGGTCGGTGGCGTTCGGT
Azucena      GAGGCGGCCATGGCGCTGGCGTCGCTGCCGAAGGTGGTGATGGGGTCGGTGGCGTTCGGT
             ************************************************************

Bala         GTGTTCTGGATGCTGGCGGTGTTCCCGTCGGTGCCGTTCCTGCCGATCGGGCGGACGGCG
IR64         GTGTTCTGGATGCTGGCGGTGTTCCCGTCGGTGCCGTTCCTGCCGATCGGGCGGACGGCG
DJ123        GTGTTCTGGATGCTGGCGGTGTTCCCGTCGGTGCCGTTCCTGCCGATCGGGCGGACGGCG
Nipponbare   GTGTTCTGGATGCTGGCGGTGTTCCCGTCGGTGCCGTTCCTGCCGATCGGGCGGACGGCG
Azucena      GTGTTCTGGATGCTGGCGGTGTTCCCGTCGGTGCCGTTCCTGCCGATCGGGCGGACGGCG
             ************************************************************

Bala         GGGGCGCTGCTGGGCGCGGTGCTGATGATCGTGTTCCACGTGATCAGCGCCGACGACGCC
IR64         GGGGCGCTGCTGGGCGCGGTGCTGATGATCGTGTTCCACGTGATCAGCGCCGACGACGCC
DJ123        GGGGCGCTGCTGGGCGCGGTGCTGATGATCGTGTTCCACGTGATCAGCGCCGACGACGCC
Nipponbare   GGGGCGCTGCTGGGCGCGGTGCTGATGATCGTGTTCCACGTGATCAGCGCCGACGACGCC
Azucena      GGGGCGCTGCTGGGCGCGGTGCTGATGATCGTGTTCCACGTGATCAGCGCCGACGACGCC
             ************************************************************

Bala         TACGCCTCCATCGACCTCCCCATCCTGGGCCTCCTCTTCGCCACCATGGTCGTCGGCGGC
IR64         TACGCCTCCATCGACCTCCCCATCCTGGGCCTCCTCTTCGCCACCATGGTCGTCGGCGGC
DJ123        TACGCCTCCATCGACCTCCCCATCCTGGGCCTCCTCTTCGCCACCATGGTCGTCGGCGGC
Nipponbare   TACGCCTCCATCGACCTCCCCATCCTGGGCCTCCTCTTCGCCACCATGGTCGTCGGCGGC
Azucena      TACGCCTCCATCGACCTCCCCATCCTGGGCCTCCTCTTCGCCACCATGGTCGTCGGCGGC
             ************************************************************

Bala         TACCTCAAGAACGCCGGCATGTTCAGGCACCTGGGCCGGCTGCTGGCGTGGCGGAGCCAG
IR64         TACCTCAAGAACGCCGGCATGTTCAGGCACCTGGGCCGGCTGCTGGCGTGGCGGAGCCAG
DJ123        TACCTCAAGAACGCCGGCATGTTCAGGCACCTGGGCCGGCTGCTGGCGTGGCGGAGCCAG
Nipponbare   TACCTCAAGAACGCCGGCATGTTCAGGCACCTGGGCCGGCTGCTGGCGTGGCGGAGCCAG
Azucena      TACCTCAAGAACGCCGGCATGTTCAGGCACCTGGGCCGGCTGCTGGCGTGGCGGAGCCAG
             ************************************************************

Bala         GGCGGGCGCGACCTCATGTGCCGCGTCTGCGTCGTCACCGCGCTCGCCAGCGCGCTCTTC
IR64         GGCGGGCGCGACCTCATGTGCCGCGTCTGCGTCGTCACCGCGCTCGCCAGCGCGCTCTTC
DJ123        GGCGGGCGCGACCTCATGTGCCGCGTCTGCGTCGTCACCGCGCTCGCCAGCGCGCTCTTC
Nipponbare   GGCGGGCGCGACCTCATGTGCCGCGTCTGCGTCGTCACCGCGCTCGCCAGCGCGCTCTTC
Azucena      GGCGGGCGCGACCTCATGTGCCGCGTCTGCGTCGTCACCGCGCTCGCCAGCGCGCTCTTC
             ************************************************************

Bala         ACCAACGACACCTGCTGCGTCGTCCTCACCGAGTTCGTGCTCGAGCTCGCCGCCGAGCGC
IR64         ACCAACGACACCTGCTGCGTCGTCCTCACCGAGTTCGTGCTCGAGCTCGCCGCCGAGCGC
DJ123        ACCAACGACACCTGCTGCGTCGTCCTCACCGAGTTCGTGCTCGAGCTCGCCGCCGAGCGC
Nipponbare   ACCAACGACACCTGCTGCGTCGTCCTCACCGAGTTCGTGCTCGAGCTCGCCGCCGAGCGC
Azucena      ACCAACGACACCTGCTGCGTCGTCCTCACCGAGTTCGTGCTCGAGCTCGCCGCCGAGCGC
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3   Bala   AACCTCCCGGCGAAGCCGTTCCTGCTCGCGCTCGCCACCAGCGCCAACATCGGCTCCAGC
4   IR64   AACCTCCCGGCGAAGCCGTTCCTGCTCGCGCTCGCCACCAGCGCCAACATCGGCTCCAGC
     DJ123   AACCTCCCGGCGAAGCCGTTCCTGCTCGCGCTCGCCACCAGCGCCAACATCGGCTCCAGC
5   Nipponbare AACCTCCCGGCGAAGCCGTTCCTGCTCGCGCTCGCCACCAGCGCCAACATCGGCTCCAGC
6   Azucena   AACCTCCCGGCGAAGCCGTTCCTGCTCGCGCTCGCCACCAGCGCCAACATCGGCTCCAGC
7   **
8   Bala   GCGACGCCCATCGGCAACCCGCAGAACCTGGTCATCGCCTTCAACAGCAAGATCTCCTTT
9   IR64   GCGACGCCCATCGGCAACCCGCAGAACCTGGTCATCGCCTTCAACAGCAAGATCTCCTTT
10   DJ123   GCGACGCCCATCGGCAACCCGCAGAACCTGGTCATCGCCTTCAACAGCAAGATCTCCTTT
11   Nipponbare GCGACGCCCATCGGCAACCCGCAGAACCTGGTCATCGCCTTCAACAGCAAGATCTCCTTT
12   Azucena   GCGACGCCCATCGGCAACCCGCAGAACCTGGTCATCGCCTTCAACAGCAAGATCTCCTTT
13   **
14   Bala   ATTTCCTTCCTCCTCGGCATCCTCCCCGCCATGCTCGCCGGCATGGGGATCAACATGCTC
15   IR64   ATTTCCTTCCTCCTCGGCATCCTCCCCGCCATGCTCGCCGGCATGGGGATCAACATGCTC
16   DJ123   ATTTCCTTCCTCCTCGGCATCCTCCCCGCCATGCTCGCCGGCATGGGGATCAACATGCTC
17   Nipponbare ATTTCCTTCCTCCTCGGCATCCTCCCCGCCATGCTCGCCGGCATGGGGATCAACATGCTC
18   Azucena   ATTTCCTTCCTCCTCGGCATCCTCCCCGCCATGCTCGCCGGCATGGGGATCAACATGCTC
19   **
20   Bala   ATGCTGCTCTGCATGTACTGGAAGGAGCTCGACGGCGGCGCCTGCAGCCCCGACGAGGTC
21   IR64   ATGCTGCTCTGCATGTACTGGAAGGAGCTCGACGGCGGCGCCTGCAGCCCCGACGAGGTC
22   DJ123   ATGCTGCTCTGCATGTACTGGAAGGAGCTCGACGGCGGCGCCTGCAGCCCCGACGAGGTC
23   Nipponbare ATGCTGCTCTGCATGTACTGGAAGGAGCTCGACGGCGGCGCCTGCAGCCCCGACGAGGTC
     Azucena   ATGCTGCTCTGCATGTACTGGAAGGAGCTCGACGGCGGCGCCTGCAGCCCCGACGAGGTC
24   **
25   Bala   GCCGCCGGCAAGCAGATGGAGGCCATCGAGGAGGGCCGCCGCACGGCGCTCAACAACAAC
26   IR64   GCCGCCGGCAAGCAGATGGAGGCCATCGAGGAGGGCCGCCGCACGGCGCTCAACAACAAC
27   DJ123   GCCGCCGGCAAGCAGATGGAGGCCATCGAGGAGGGCCGCCGCACGGCGCTCAACAACAAC
28   Nipponbare GCCGCCGGCAAGCAGATGGAGGCCATCGAGGAGGGCCGCCGCACGGCGCTCAACAACAAC
29   Azucena   GCCGCCGGCAAGCAGATGGAGGCCATCGAGGAGGGCCGCCGCACGGCGCTCAACAACAAC
30   **
31   Bala   AAGAAAGACGACGGCGACGCCGCCACCCCCGCATCGCCGGAGGACGACGACGGCGGCGAC
32   IR64   AAGAAAGACGACGGCGACGCCGCCACCCCCGCATCGCCGGAGGACGACGACGGCGGCGAC
33   DJ123   AAGAAAGACGACGGCGACGCCGCCACCCCCGCATCGCCGGAGGACGACGACGGCGGCGAC
34   Nipponbare AAGAAAGACGACGGCGACGCCGCCACCCCCGCATCGCCGGAGGACGACGACGGCGGCGAC
     Azucena   AAGAAAGACGACGGCGACGCCGCCACCCCCGCATCGCCGGAGGACGACGACGGCGGCGAC
35   **
36   Bala   GCCGAGTCGATGATGTCGGAGAACATCTCGACGAAGCACCGGTGGTTCATGCAGTGCTCG
37   IR64   GCCGAGTCGATGATGTCGGAGAACATCTCGACGAAGCACCGGTGGTTCATGCAGTGCTCG
38   DJ123   GCCGAGTCGATGATGTCGGAGAACATCTCGACGAAGCACCGGTGGTTCATGCAGTGCTCG
39   Nipponbare GCCGAGTCGATGATGTCGGAGAACATCTCGACGAAGCACCGGTGGTTCATGCAGTGCTCG
40   Azucena   GCCGAGTCGATGATGTCGGAGAACATCTCGACGAAGCACCGGTGGTTCATGCAGTGCTCG
41   **
42   Bala   GAGCACCGGCGGAAGCTATTCCTCAAGTGCTTCGCGTACGTGGTCACCGTCGGCATGCTC
43   IR64   GAGCACCGGCGGAAGCTATTCCTCAAGAGCTTCGCGTACGTGGTCACCGTCGGCATGCTC
     DJ123   GAGCACCGGCGGAAGCTATTCCTCAAGTGCTTCGCGTACGTGGTCACCGTCGGCATGCTC
44   Nipponbare GAGCACCGGCGGAAGCTGTTCCTCAAGAGCTTCGCGTACGTGGTCACCGTCGGCATGCTC
45   Azucena   GAGCACCGGCGGAAGCTGTTCCTCAAGAGCTTCGCGTACGTGGTCACCGTCGGCATGCTC
46   *****************.*********.*.******************************
47   Bala   GTCGCCTACATGCTCGGCCTCAACATGTCGTGGACCGCCATCACCACCGCCATCGCCCTC
48   IR64   GTCGCCTACATGCTCGGCCTCAACATGTCGTGGACCGCCATCACCACCGCCATCGCCCTC
49   DJ123   GTCGCCTACATGCTCGGCCTCAACATGTCGTGGACCGCCATCACGACCGCCATCGCCCTC
50   Nipponbare GTCGCCTACATGCTCGGCCTCAACATGTCGTGGACCGCCATCACGACCGCCATCGCCCTC
51   Azucena   GTCGCCTACATGCTCGGCCTCAACATGTCGTGGACCGCCATCACGACCGCCATCGCCCTC
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2
3   Bala   **GTCGTCGTCGACTTCCGCGACGCCGAGCCATGCCTCGACAAG**GTAACCCATATCCGTCCA
4   IR64   **GTCGTCGTCGACTTCCGCGACGCCGAGCCATGCCTCGACAAG**GTAACCCATATCCGTCCA
5   DJ123   **GTCGTCGTCGACTTCCGCGACGCCGAGCCATGCCTCGACAAG**GTAACCCATATCCGTCCA
6   Nipponbare **GTCGTCGTCGACTTCCGCGACGCCGAGCCATGCCTCGACAAG**GTAACCCATATCCGTCCA
7   Azucena   **GTCGTCGTCGACTTCCGCGACGCCGAGCCATGCCTCGACAAG**GTAACCCATATCCGTCCA
                    ****

8   Bala   AATCACTATAACTCGAGGCCACACGAGCAAGTACACGTGGCGGCCATGCGGACAAGAAGC
9   IR64   AATCACTATAACTCTAGGCCACACGAGCAAGTACACGTGGCGGCCATGCGGACAAGAAGC
10   DJ123   AATCACTATAACTCTAGGCCACACGAGCAAGTACACGTGGCGGCCATGCGGACAAGAAGC
11   Nipponbare AATCACTATAACTCTAGGCCACACGAGCAAGTACACGTGGCGGCCATGCGGACAAGAAGC
12   Azucena   AATCACTATAACTCTAGGCCACACGAGCAAGTACACGTGGCGGCCATGCGGACAAGAAGC
                    *************** **
13
14   Bala   AATATGTGAACTGGGTGCCACGTGTGCGTGGATGTGAATGGATGATGCAG**GTGTCGTACT**
15   IR64   AATATGTGAACTGGGTGCCACGTGTGCGTGGATGTGAATGGATGATGCAG**GTGTCGTACT**
16   DJ123   AATATGTGAACTGGGTGCCACGTGTGCGTGGATGTGAATGGATGATGCAG**GTGTCGTACT**
17   Nipponbare AATATGTGAACTGGGTGCCACGTGTGCGTGGATGTGAATGGATGATGCAG**GTGTCGTACT**
18   Azucena   AATATGTGAACTGGGTGCCACGTGTGCGTGGATGTGAATGGATGATGCAG**GTGTCGTACT**
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19   Bala   **CGCTGCTGGTGTTCTTCTCGGGGATGTTCGTGACGGTGAGCGGA**TTCAACAAGACGGGGC
20   IR64   **CGCTGCTGGTGTTCTTCTCGGGGATGTTCGTGACGGTGAGCGGA**TTCAACAAGACGGGGC
21   DJ123   **CGCTGCTGGTGTTCTTCTCGGGGATGTTCGTGACGGTGAGCGGG**TTCAACAAGACGGGGC
22   Nipponbare **CGCTGCTGGTGTTCTTCTCGGGGATGTTCGTGACGGTGAGCGGG**TTCAACAAGACGGGGC
23   Azucena   **CGCTGCTGGTGTTCTTCTCGGGGATGTTCGTGACGGTGAGCGGG**TTCAACAAGACGGGGC
                    ** ***************
24
25   Bala   **TACCCGGGGCCATCTGGAACGTGATGGCGCCCTACTCAAAGATCAACCACGTCACCGGCG**
26   IR64   **TACCCGGGGCCATCTGGAACGTGATGGCGCCCTACTCAAAGATCAACCACGTCACCGGCG**
27   DJ123   **TACCCGGGGCCATCTGGAACGTGATGGCGCCCTACTCAAAGATCAACCACGTCACCGGCG**
28   Nipponbare **TACCCGGGGCCATCTGGAACGTGATGGCGCCCTACTCAAAGATCAACCACGTCACCGGCG**
29   Azucena   **TACCCGGGGCCATCTGGAACGTGATGGCGCCCTACTCAAAGATCAACCACGTCACCGGCG**
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30
31   Bala   **TCACCGTCCTCTCCGTCATCATCCTCCTCCTCTCCAACCTCGCCTCCAACGTCCCCACCG**
32   IR64   **TCACCGTCCTCTCCGTCATCATCCTCCTCCTCTCCAACCTCGCCTCCAACGTCCCCACCG**
33   DJ123   **TCACCGTCCTCTCCGTCATCATCCTCCTCCTCTCCAACCTCGCCTCCAACGTCCCCACCG**
34   Nipponbare **TCACCGTCCTCTCCGTCATCATCCTCCTCCTCTCCAACCTCGCCTCCAACGTCCCCACCG**
35   Azucena   **TCACCGTCCTCTCCGTCATCATCCTCCTCCTCTCCAACCTCGCCTCCAACGTCCCCACCG**
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36   Bala   GTACGTACTTACTCACAACCTTTCTCGCACCATGCTATTGCTCGTACCCAATGTGTCCCG
37   IR64   GTACGTACTTACTCACAACCTTTCTCGCACCATGCTATTGCTAGTACCCAATGTGTCCCG
38   DJ123   GTACGTACTTACTCACAACCTTTCTCGCACCATGCTATTGCTAGTACCCAATGTGTCCCG
39   Nipponbare GTACGTACTTACTCACAACCTTTCTCGCACCATGCTATTGCTAGTACCCAATGTGTCCCG
40   Azucena   GTACGTACTTACTCACAACCTTTCTCGCACCATGCTATTGCTAGTACCCAATGTGTCCCG
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41
42   Bala   ACTCACCCAGCACTAACATTTGTACTATACAGTACATCAGTTTCTTTTAATACATGATAT
43   IR64   ACTCACCCAGCACTAACATTTGTACTATACAGTACATCAGTTTCTTTTAATACATGATAT
44   DJ123   ACTCACCCAGCACTAACATTTGTACTGTACAGTACATCAGTTTCTTTTAATACATGATAT
45   Nipponbare ACTCACCCAGCACTAACATTTGTACTGTACAGTACATCAGTTTCTTTTAATACATGATAT
46   Azucena   ACTCACCCAGCACTAACATTTGTACTGTACAGTACATCAGTTTCTTTTAATACATGATAT
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47
48   Bala   CTGACTTTTTTTTATCGATGAATGGATCTTATTCAATTTCTTTTGTAAATATAAAAAGAT
49   IR64   CTGACTTTTTTTTATCGATGAATGGATCTTATTCAATTTCTTTTGTAAATATAAAAAGAT
50   DJ123   CTGACTTTTTTTTATCGATGAATGGATCTTATTCAATTTCTTTTGTAAATATAAAAAGAT
51   Nipponbare CTGACTTTTTTTTATCGATGAATGGATCTTATTCAATTTCTTTTGTAAATATAAAAAGAT
             Azucena   CTGACTTTTTTTTATCGATGAATGGATCTTATTCAATTTCTTTTGTAAATATAAAAAGAT
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Bala         TAAATCAGACAATAAATCAAGTCAAAATAAAATAAATGTAATTATATTTTTTAATAAGAT
IR64         TAAATCAGACAATAAATCAAGTCAAAATAAAATAAATGTAATTATATTTTTTAATAAGAT
DJ123        TAAATCAGACAATAAATCAAGTCAAAATAAAATAAATGTAATTATATTTTTTAATAAGAT
Nipponbare   TAAATCAGACAATAAATCAAGTCAAAATAAAATAAATGTAATTATATTTTTTAATAAGAT
Azucena      TAAATCAGACAATAAATCAAGTCAAAATAAAATAAATGTAATTATATTTTTTAATAAGAT
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Bala         GAATGATTATTAAAAGAAAAAAAATTTTTTTGTCTCTCATAATTAAAAAAGAACAACTCA
IR64         GAATGATTATTAAAAGAAAAAAAATTTTTTTGTCTCTCATAATTAAAAAAGAACAACTCA
DJ123        GAATGATTATTAAAAGAAAAAATTTTTTTTGTCTCTCATAATTAAAAAAGAACAACTCA
Nipponbare   GAATGATTATTAAAAGAAAAAATTTTTTTTGTCTCTCATAATTAAAAAAGAACAACTCA
Azucena      GAATGATTATTAAAAGAAAATTTTTTTTTTGTCTCTCATAATTAAAAAAGAACAACTCA
             ********************    ************************************
Bala         TTTTTTACATGTTATTTGAGTGGTAATTGTAAATTAATTTATATATGTGGGTGTAG**TGTT**
IR64         TTTTTTACATGTTATTTGAGTGGTAATTGTAAATTAATTTATATATGTGGGTGTAG**TGTT**
DJ123        TTTTTTACATGTTATTTGAGTGGTAATTGTAAATTAATTTATATATGTGGGTGTAG**TGTT**
Nipponbare   TTTTTTACATGTTATTTGAGTGGTAATTGTAAATTAATTTATATATGTGGGTGTAG**TGTT**
Azucena      TTTTTTACATGTTATTTGAGTGGTAATTGTAAATTAATTTATATATGTGGGTGTAG**TGTT**
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Bala         **GTTGATGGGGGATGAGGTGGCGGCGGCGGCGGCGACGATATCACCGGCGGCGGTGACGCG**
IR64         **GTTGATGGGGGATGAGGTGGCGGCGGCGGCGGCGACGATATCACCGGCGGCGGTGACGCG**
DJ123        **GTTGATGGGGGATGAGGTGGCGGCGGCGGCGGCGACGATATCACCGGCGGCGGTGACGCG**
Nipponbare   **GTTGATGGGGGATGAGGTGGCGGCGGCGGCGGCGACGATATCACCGGCGGCGGTGACGCG**
Azucena      **GTTGATGGGGGATGAGGTGGCGGCGGCGGCGGCGACGATATCACCGGCGGCGGTGACGCG**
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Bala         **GTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGGGGAACCTGTCGCTGCTGGGGTC**
IR64         **GTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGGGGAACCTGTCGCTGCTGGGGTC**
DJ123        **GTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGGGGAACCTGTCGCTGCTGGGGTC**
Nipponbare   **GTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGGGGAACCTGTCGCTGCTGGGGTC**
Azucena      **GTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGGGGAACCTGTCGCTGCTGGGGTC**
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Bala         **GGCGGCGAACCTGATCGTGTGCGAGCAGGCGAGGCGGGCGACGCGCAACGCCTACGACCT**
IR64         **GGCGGCGAACCTGATCGTGTGCGAGCAGGCGAGGCGGGCGACGCGCAACGCCTACGACCT**
DJ123        **GGCGGCGAACCTGATCGTGTGCGAGCAGGCGAGGCGGGCGACGCGCAACGCCTACGACCT**
Nipponbare   **GGCGGCGAACCTGATCGTGTGCGAGCAGGCGAGGCGGGCGACGCGCAACGCCTACGACCT**
Azucena      **GGCGGCGAACCTGATCGTGTGCGAGCAGGCGAGGCGGGCGACGCGCAACGCCTACGACCT**
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Bala         **CACCTTCTGGAACCACGTCATCTTCGGACTCCCATCCACCCTCGTCGTCACCGCCATCGG**
IR64         **CACCTTCTGGAACCACGTCATCTTCGGACTCCCATCCACCCTCGTCGTCACCGCCATCGG**
DJ123        **CACCTTCTGGAACCACGTCATCTTCGGACTCCCATCCACCCTCGTCGTCACCGCCATCGG**
Nipponbare   **CACCTTCTGGAACCACGTCATCTTCGGACTCCCATCCACCCTCGTCGTCACCGCCATCGG**
Azucena      **CACCTTCTGGAACCACGTCATCTTCGGACTCCCATCCACCCTCGTCGTCACCGCCATCGG**
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Bala         **CATCCCTCTCATCGGCAAGATCAACATCTAG**TCGTCATTAAATTAATTAATTAATATAAT
IR64         **CATCCCTCTCATCGGCAAGATCAACATCTAG**TCGTCATTAAATTAATTAATTAATATAAT
DJ123        **CATCCCTCTCATCGGCAAGATCAACATCTAG**TCGTCATTAAATTAATTAATTAATATAAT
Nipponbare   **CATCCCTCTCATCGGCAAGATCAACATCTAG**TATTCATTAAATTAATTAATTAATATAAT
Azucena      **CATCCCTCTCATCGGCAAGATCAACATCTAG**TATTCATTAAATTAATTAATTAATATAAT
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Bala         CCGGCTAGCTACTATCTACAACGATCAGAAGAAGGCAGAATGATCGAGCATGCAAGAATT
IR64         CCGGCTAGCTACTATCTACAACGATCAGAAGAAGGCAGAATGATCGAGCATGCAAGAATT
DJ123        CCGGCTAGCTACTATCTACAACGATCAGAAGAAGGCAGAATGATCGAGCATGCAAGAATT
Nipponbare   CCGGCTAGCTACTATCTACAACGATCAGAAGAAGGCAGAATGATCGAGCATGCAAGAATT
Azucena      CCGGCTAGCTACTATCTACAACGATCAGAAGAAGGCAGAATGATCGAGCATGCAAGAATT
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Bala         CATGCAAATTACAAGGGATGTGATATGATCATATTAATCCAAGGAAGCTAGTATGATGAT
IR64         CATGCAAATTACAAGGGATGTGATATGATCATATTAATCCAAGGAAGCTAGTATGATGAT
DJ123        CATGCAAATTACAAGGGATGTGATATGATCATATTAATCCAAGGAAGCTAGTATGATGAT
Nipponbare   CATGCAAATTACAAGGGATGTGATATGATCATATTAATCCAAGGAAGCTAGTATGATGAT
Azucena      CATGCAAATTACAAGGGATGTGATATGATCATATTAATCCAAGGAAGCTAGTATGATGAT
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Bala        TAAGTTTGTGATCAAGCCGTACTGTGTGCATGCCAATGCATGCATGTGTTCAAGTGTACA
IR64        TAAGTTTGTGATCAAGCCGTACTATGTGCATGCCAATGCATGCATGTGTTCAAGTGTACA
DJ123       TAAGTTTGTGATCAAGCCGTACTATGTGCATGCCAATGCATGCATGTGTTCAAGTGTACA
Nipponbare  TAAGTTTGTGATCAAGCCGTACTATGTGCATGCCAATGCATGCATGTGTTCAAGTGTACA
Azucena     TAAGTTTGTGATCAAGCCGTACTATGTGCATGCCAATGCATGCATGTGTTCAAGTGTACA
            ***********************  ***********************************

Bala        CTATGCTTGGTCTCAAGTGGATCAATTATATTGGTGCCATGCTCTGGGATAATTAGTTAA
IR64        CTATGCTTGGTCTCAAGTGGATCAATTATATTGGTGCCATGCTCTGGGATAATTAGTTAA
DJ123       CTATGCTTGGTCTCAAGTGGATCAATTATATTGGTGCCATGCTCTGGGATAATTAGTTAA
Nipponbare  CTATGCTTGGTCTCAAGTGGATCAATTATATTGGTGCCATGCTCTGGGATAATTAGTTAA
Azucena     CTATGCTTGGTCTCAAGTGGATCAATTATATTGGTGCCATGCTCTGGGATAATTAGTTAA
            ************************************************************

Bala        TTAGTACTTATGATAAGCTAGAGAGGAGGATGCATTATATTGGTGCATGGTAATAAGGCA
IR64        TTAGTACTTATGATAAGCTAGAGAGGAGGATGCATTATATTGGTGCATGGTAATAAGGCA
DJ123       TTAGTACTTATGATAAGCTAGAGAGGAGGATGCATTATATTGGTGCATGGTAATAAGGCA
Nipponbare  TTAGTACTTATGATAAGCTAGAGAGGAGGATGCATTATATTGGTGCATGGTAATAAGGCA
Azucena     TTAGTACTTATGATAAGCTAGAGAGGAGGATGCATTATATTGGTGCATGGTAATAAGGCA
            ************************************************************

Bala        AATGAACAGCAGAATGCATGTATATATGTACTGTATCCCTGTTGCCAGCTGGTCTCTGTT
IR64        AATGAACAGCAGAATGCATGTATATATGTACTGTATCCCTGTTGCCAGCTGGTCTCTGTT
DJ123       AATGAACAGCAGAATGCATGTATATATGTACTGTATCCCTGTTGCCAGCTGGTCTCTGTT
Nipponbare  AATGAACAGCAGAATGCATGTATATATGTACTGTATCCCTGTTGCCAGCTGGTCTCTGTT
Azucena     AATGAACAGCAGAATGCATGTATATATGTACTGTATCCCTGTTGCCAGCTGGTCTCTGTT
            ************************************************************

Bala        TGAATGAAGTTGATAGCAACAAAGCTAGCACTAGCGAATGAGCTAGTGACTCAAGTACGC
IR64        TGAATGAAGTTGATAGCAACAAAGCTAGCACTAGCGAATGAGCTAGTGACTCAAGTACGC
DJ123       TGAATGAAGTTGATAGCAACAAAGCTAGCACTAGCGAATGAGCTAGTGACTCAAGTACGC
Nipponbare  TGAATGAAGTTGATAGCAACAAAGCTAGCACTAGCGAATGAGCTAGTGACTCAAGTACGC
Azucena     TGAATGAAGTTGATAGCAACAAAGCTAGCACTAGCGAATGAGCTAGTGACTCAAGTACGC
            ************************************************************

Bala        ATGCCGGATTACCTCATTGCTCCAAACTTGCAATCAGTTTTGAACTTTTAATAAAGTTAA
IR64        ATGCCGGATTACCTCATTGCTCCAAACTTGCAATCAGTTTTGAACTTTTAATAAAGTTAA
DJ123       ATGCCGGATTACCTCATTGCTCCAAACTTGCAATCAGTTTTGAACTTTTAATAAAGTTAA
Nipponbare  ATGCCGGATTACCTCATTGCTCCAAACTTGCAATCAGTTTTGAACTTTTAATAAAGTTAA
Azucena     ATGCCGGATTACCTCATTGCTCCAAACTTGCAATCAGTTTTGAACTTTTAATAAAGTTAA
            ************************************************************

Bala        TTTGGCTTTGTGCACTTGTCAATTTGCTCCCTCTTTGTTTAACCGATCTTGCCTAGTGCG
IR64        TTTGGCTTTGTGCACTTGTCAATTTGCTCCCTCTTTGTTTAACCGATCTTGCCTAGTGCG
DJ123       TTTGGCTTTGTGCACTTGTCAATTTGCTCCCTCTTTGTTTAACCGATCTTGCCTAGTGCG
Nipponbare  TTTGGCTTTGTGCACTTGTCAATTTGCTCCCTCTTTGTTTAACCGATCTTGCCTAGTGCG
Azucena     TTTGGCTTTGTGCACTTGTCAATTTGCTCCCTCTTTGTTTAACCGATCTTGCCTAGTGCG
            ************************************************************

Bala        TACGTAGCTAGATTGCACATTTAAG
IR64        TACGTAGCTAGATTGCACATTTAAG
DJ123       TACGTAGCTAGATTGCACATTTAAG
Nipponbare  TACGTAGCTAGATTGCACATTTAAG
Azucena     TACGTAGCTAGATTGCACATTTAAG
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Supplementary figure 4. Genomic sequence alignment of *Lsi3*. Regions highlighted in bold are predicted exon regions based on the Rice Genome Annotation Project – release 7 sequence available at http://rice.plantbiology.msu.edu/.