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

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

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

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

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

It has been shown that arsenic, classified as a class one carcinogen, can be transported through silicon transporters in rice (*Ma* et al., 2008; *Zhao* et al., 2010; *Mitani-Ueno* et al., 2011). There are two different forms of arsenic present in rice: organic arsenic and inorganic arsenic (*Williams* et al., 2005). Organic arsenic is found in rice in two main types of molecular species dimethylarsinic acid (DMA), and monomethylarsonic acid (MMA) as well as tetramethylarsonium (*Williams* et al., 2005; *Hansen* et al., 2011). Inorganic arsenic is found in rice as two molecular species; arsenate and arsenite (*Abedin* et al., 2002; *Williams* et al., 2005). Arsenate is an analogue of phosphate and is taken up via phosphate transporters while arsenite is taken up by silicic acid transporters in rice (*Abedin* et al., 2002; *Ma* et al., 2008). It has been shown that the silicon transporters *Lsi1*, *Lsi2* and *Lsi6* are also arsenic transporters, using a combination of mutants and transgenic lines (*Ma* et al., 2008; *Zhao* et al., 2010; *Mitani-Ueno* et al., 2011). Several studies indicate that anaerobic rice cultivation leads to increased mobilisation of soil arsenic in the form of arsenite, which causes anaerobically-grown rice to accumulate more arsenic through silicon transporters (*Ma* et al., 2008; *Xu* et al., 2008; *Carey* et al., 2010). Silicon fertilisation has also been shown to decrease shoot and grain arsenic indicating that silicon could play an important role in decreasing total arsenic uptake in rice (*Li* et al., 2009; *Seyfferth and Ferdorf*, 2012).

This study was designed to address four questions all related to the process of silicon and arsenic accumulation in rice: How does the cultivation method affect silicon distribution in different organs of rice plants? Are there significant genotypic differences in shoot silicon concentration across a diverse panel of rice related to the 5 different sub-populations of rice? Is there a relationship between natural variation in shoot silicon and arsenic content in rice? Can natural variation in shoot silicon be linked to known silicon transporters in rice? The results provide a deeper understanding of the natural variation in silicon content across rice 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.

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 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 ddH_2O. 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 *Lsi*6 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

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

2.8 Statistical analysis

Statistical significance was set at $P < 0.05$ for all analyses, which were performed using Minitab 16. The normality of distribution and homogeneity of variance of the data were tested prior to one or two-way analysis of variance (ANOVA), as appropriate. Pearson correlation analysis was used to investigate the relationship between measurements of shoot 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 significant and large positive correlation between the two methods (*r* = 0.95; *P* < 0.001; df = 18) (Fig 4).

3.3 Correlation between shoot silicon and grain arsenic in rice

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

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

A total of 10 SNPs from the SNP database are within 10 kb upstream and downstream of the *Lsi2* gene (selected SNPs for the rice accessions are presented in supplementary Table 2). Shoot silicon concentration for accessions with the different alleles for two of these SNPs was significantly different. SNP-3.434426 is located 2551 bp before the start codon, and revealed a significant difference between the C and T polymorphism (*P* = 0.006), where the mean silicon content of accessions with the C allele was 29.3 mg g^{-1} while the mean silicon content of the accessions with the T allele was 23.1 mg g^{-1}. SNP-3.438416 is located 6541 bp before the start codon and revealed a significant difference between the A and C polymorphism (*P* = 0.008), where the mean silicon content of the accessions with the A allele 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., 2015). This non-synonymous polymorphism between "A" and "T" in *Lsi3* with the "T" allele is very rarely observed in *indica* and *japonica* subpopulations of rice in 3000 rice genome sequence data (*Alexandrov* et al., 2015).

4 Discussion

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

Flooded conditions increased silicon content in the upper part of the plant (flag sheath, 1st node, flag leaf and husk) compared to the non-flooded conditions, which suggests that the uptake or translocation of shoot silicon into these plant organs might be controlled by different processes (compared to those determining silicon uptake in other tissues) which differ between aerobic and anaerobic conditions. It has been shown that silicon dissolution and bio-availability plays a significant role in the variation of silicon content in grasses (*Quigley* et al., 2017). Therefore, the difference in dissolved silicon in flooded and non-flooded conditions might affect the accumulation of silicon in the rice plants used in this study. It was also notable that there was no significant difference in silicon content in different tissues between the internodes (e.g. flag leaf, 2nd leaf) in non-flooded conditions but there was a significant difference between the silicon content of internodes under flooded conditions (Fig. 1). Previous studies have shown that transpiration is one of the most important factors responsible for higher *silicification* in plants and that transpirational flow is higher in anaerobic conditions than in aerobic ones (*Mitani-Ueno* et al., 2005; *Kato and Okami*, 2011; *Kumar* et al., 2017; *McLarnon* et al., 2017). Therefore, one potential explanation for increased silicon accumulation in the upper organs or developing organs of rice plants (e.g. flag sheath, 1st node, flag leaf and husk) grown in flooded soils is a higher transpirational flow in these plants. Importantly, the data presented in figure 1 shows that tissue silicon content is reasonably evenly distributed across tissues with only that from flooded plants in tissue associated with flowering and seed production significantly higher than the rest. Since this reproductive tissue was removed from the field samples used in this study we can be reassured that a mean value obtained from straw will be a good estimate of the tissue concentration of the majority of rice plant.

Fifty accessions from 5 different sub-populations (10 accessions from each sub-population) were selected at random to examine the difference of shoot silicon content in rice and this revealed highly significant differences of shoot silicon content. A genotypic difference in shoot silicon content across a wide group of accessions has been observed previously (*Deren*, 2001; *Norton* et al., 2010a). The 2.6-fold difference of shoot silicon content in this study is similar to the previous 2.2 fold range detected for genotypic differences of shoot silicon content in rice (*Norton* et al., 2010a). However, the maximum value observed in our study is slightly lower than that detected previously (42.4 mg g^{-1} in this study, 60 mg g^{-1} (*Deren*, 2001), 61 mg g^{-1} (*Norton* et al., 2010a).

The plant material used for determination of shoot silicon content in the 50 rice accessions was grown in flooded, irrigated conditions (*Norton* et al., 2012). Previous studies estimated that 27% - 44% of the silicon taken up by rice plants is supplied by irrigation, while the remaining percentage must be supplied by soil constituents (*Desplanques* et al., 2006; *Klotzbücher* et al., 2015). All the accessions tested in this study had a silicon content below 50 mg g^{-1} which, according to *Dobermann and Fairhurst* (2000), is below the critical level of mineral deficiency for rice production. The low shoot silicon content (16.5 mg g^{-1} to 42.4 mg g^{-1}) observed in this study may be due to removal of rice straw from the paddy field, which is common practice in Bangladesh, and has been shown to contribute to lower shoot silicon in the subsequent rice crop (*Seyfferth* et al., 2013). Future work should focus on linking the flooded and non-flooded pot based experiment and the removal of straw at the field scale to establish the importance of water management and field management on silicon accumulation in field grown rice.

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

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

The plant material used in this study was previously used to examine the variation of shoot and grain arsenic (*Norton* et al., 2012). The comparison of shoot silicon and grain arsenic in this study is in agreement with previous studies where, in general, plants that had high shoot silicon also had lower grain arsenic (*Seyfferth and Ferdorf*, 2012; *Norton* et al., 2012; *Norton* et al., 2013). However, this study also adds more insight by taking into consideration the sub-population structure of rice cultivars. The correlation between shoot silicon and grain arsenic was sub-population specific. A strong relationship in between shoot silicon and grain arsenic was observed in *temperate japonica* and a weaker one in *tropical japonica,* but was not observed in *indica*, *aus* or *aromatic*. This important observation suggests that the genetic regulation of arsenic content in rice grain is different in *temperate* and *tropical japonicas* compared to the other rice sub-populations, implying that the silicon-transport-linked pathway implicated for arsenic accumulation (*Ma* et al., 2007b; *Norton* et al., 2012) may be less relevant in the *other* sub-populations.

The accessions used in the study have been genotyped using a 700K SNP chip (*McCouch* et al., 2016). Single-marker analysis was used to test the candidacy of the known transporters of silica in rice. The study indicated that two SNPs within 10 kb of *Lsi2* and one within 10 kb of *Lsi3* were involved in contributing to the natural variation of shoot silicon accumulation in rice (Fig. 6). The *Lsi2* gene has been shown to be pivotal for transport of silicon and inorganic arsenic in studies conducted with mutants and transgenic plants (*Ma* et al., 2006; *Ma* et al., 2007b; *Yamaji* et al*.,* 2008; *Mitani-Ueno* et al., 2011; *Yamaji* et al*.,* 2015). The identification of differences in shoot silicon and the link with three SNPs close to the genes further suggest that *Lsi2* and *Lsi3* are excellent candidate genes to explain the natural variation observed in shoot silicon content of rice. When looking at the sequencing variation of a number of diverse cultivars (which have been sequenced to a high depth) it is evident that there is only a small number of polymorphisms within the genes (Supplementary figures 3 and 4). The highly conserved sequence for *Lsi2* may be due to its importance function for silicon accumulation in rice. However, the accessions screened in this study are likely to have 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.

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 = *au*s, 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 (µg kg^{-1}) in ARO= *aromatic*, AUS = *au*s, 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*

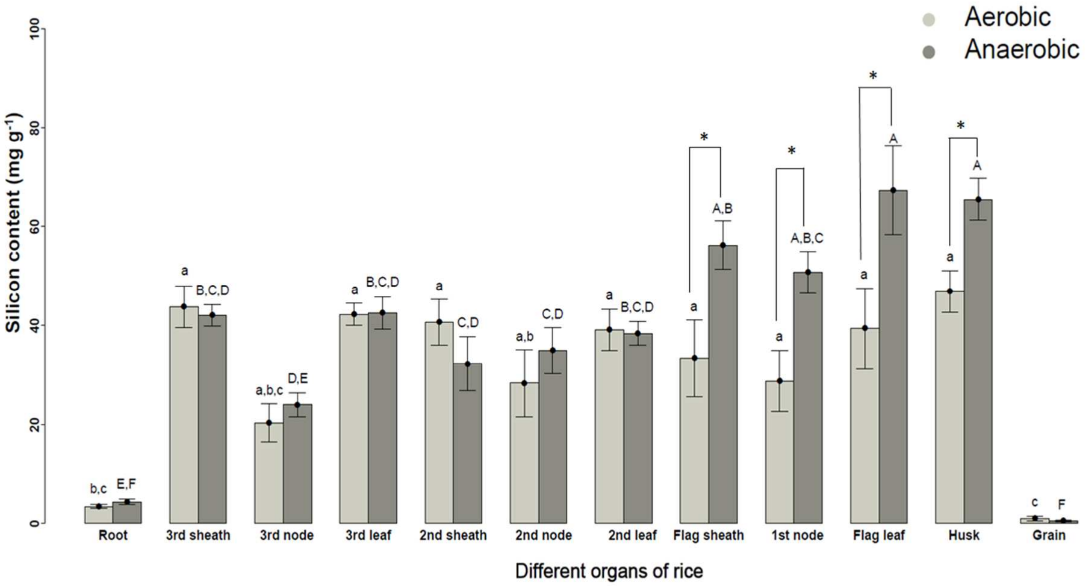

Aerobic
Anaerobic
Silicon content (mg g^{-1})
0
20
40
60
80
100
b,c
E,F
a
B,C,D
a,b,c
D,E
a
B,C,D
a
C,D
a,b
C,D
a
B,C,D
*
a
A,B
*
a
A,B,C
*
a
A
*
a
A
c
F
Root
3rd sheath
3rd node
3rd leaf
2nd sheath
2nd node
2nd leaf
Flag sheath
1st node
Flag leaf
Husk
Grain
Different organs of rice

Figure 1

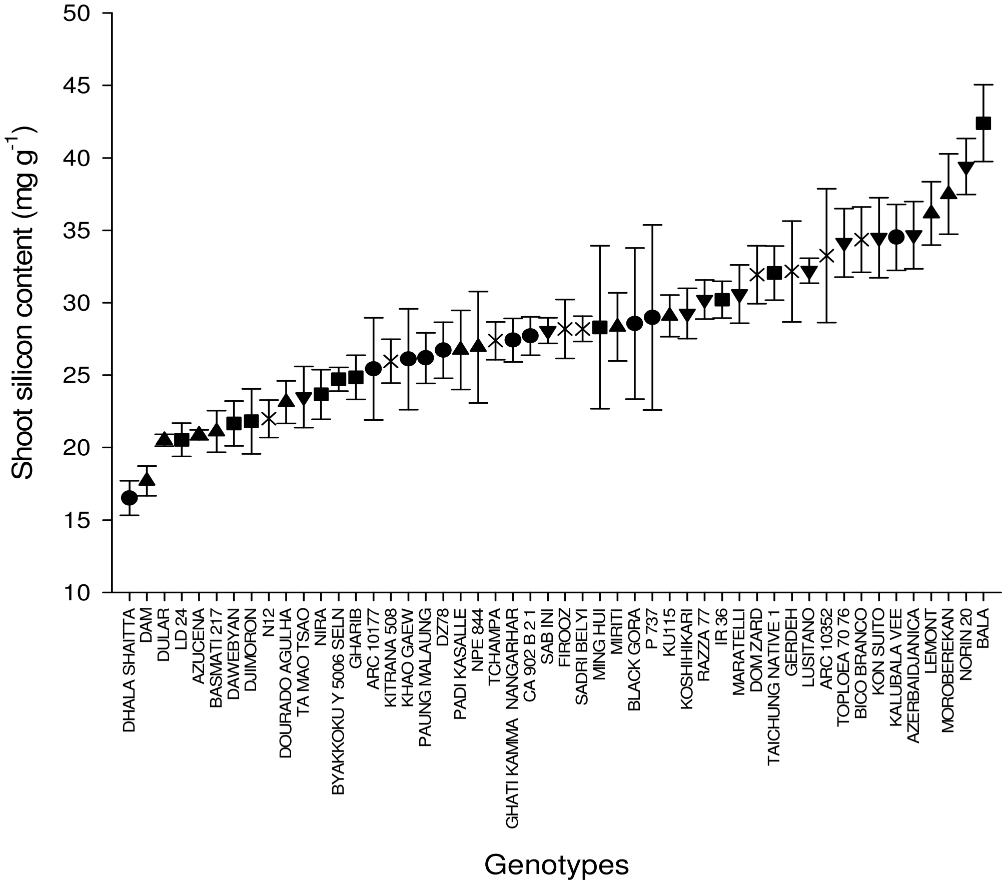

Shoot silicon content (mg g^{-1})
10
15
20
25
30
35
40
45
50
DHALA SHAITTA
DAM
DULAR
LD 24
AZUCENA
BASMATI 217
DAWEBYAN
DJIMORON
N12
DOURADO AGULHA
TA MAO TSAO
NIRA
BYAKKOKU Y 5006 SELN
GHARIB
ARC 10177
KITRANA 508
KHAO GAEW
PAUNG MALAUNG
DZ78
PADI KASALLE
NPE 844
TCHAMPA
GHATI KAMMA NANGARHAR
CA 902 B 2 1
SAB INI
FIROOZ
SADRI BELYI
MING HUI
MIRITI
BLACK GORA
P 737
KU115
KOSHIHIKARI
RAZZA 77
IR 36
MARATELLI
DOM ZARD
TAICHUNG NATIVE 1
GERDEH
LUSITANO
ARC 10352
TOPLOEA 70 76
BICO BRANCO
KON SUITO
KALUBALA VEE
AZERBAIDJANICA
LEMONT
MOROBEREKAN
NORIN 20
BALA
Genotypes

Figure 2

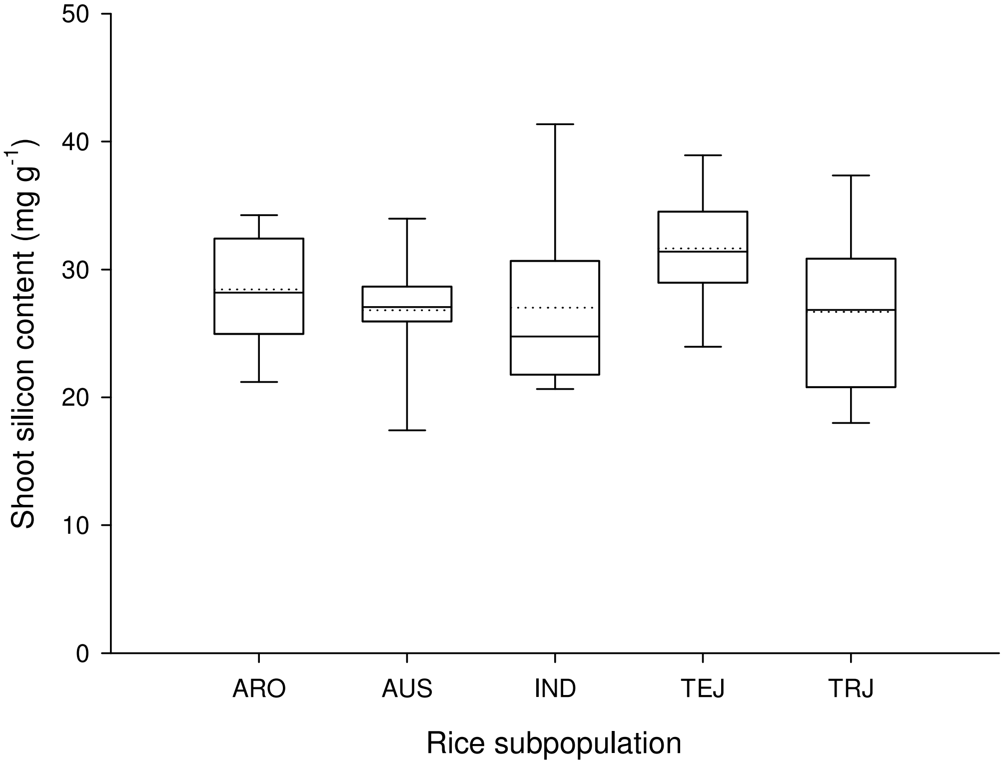
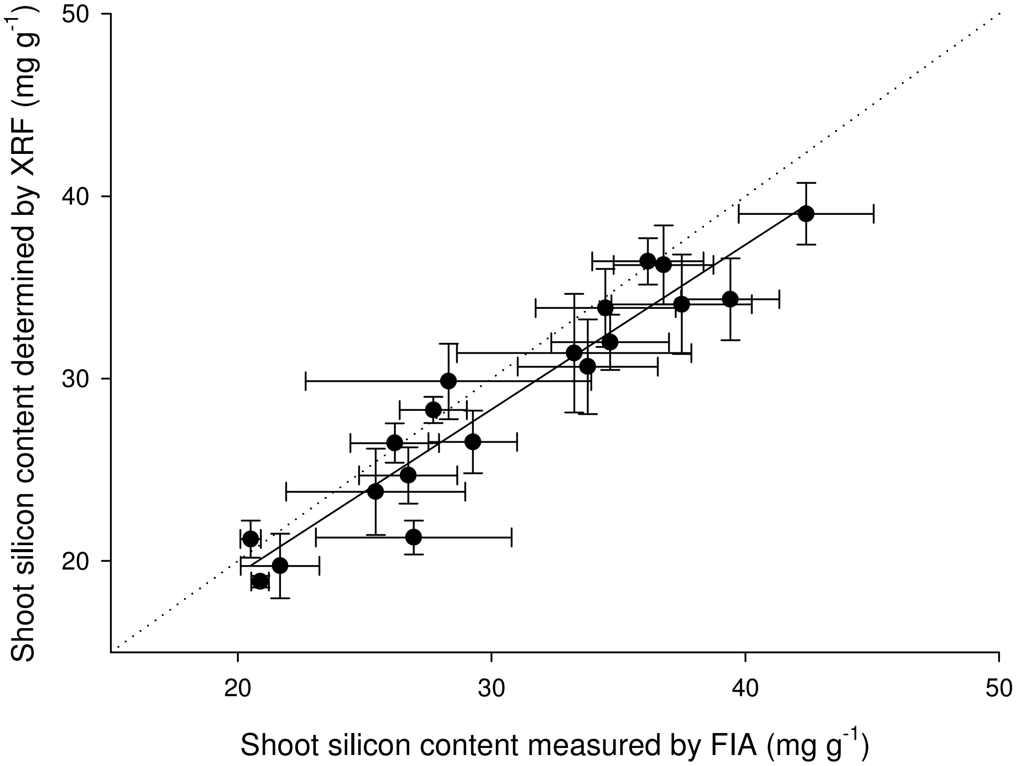

50
40
30
20
10
0
Shoot silicon content (mg g^{-1})
ARO
AUS
IND
TEJ
TRJ
Rice subpopulation

Figure 3



684

685 **Figure 4**

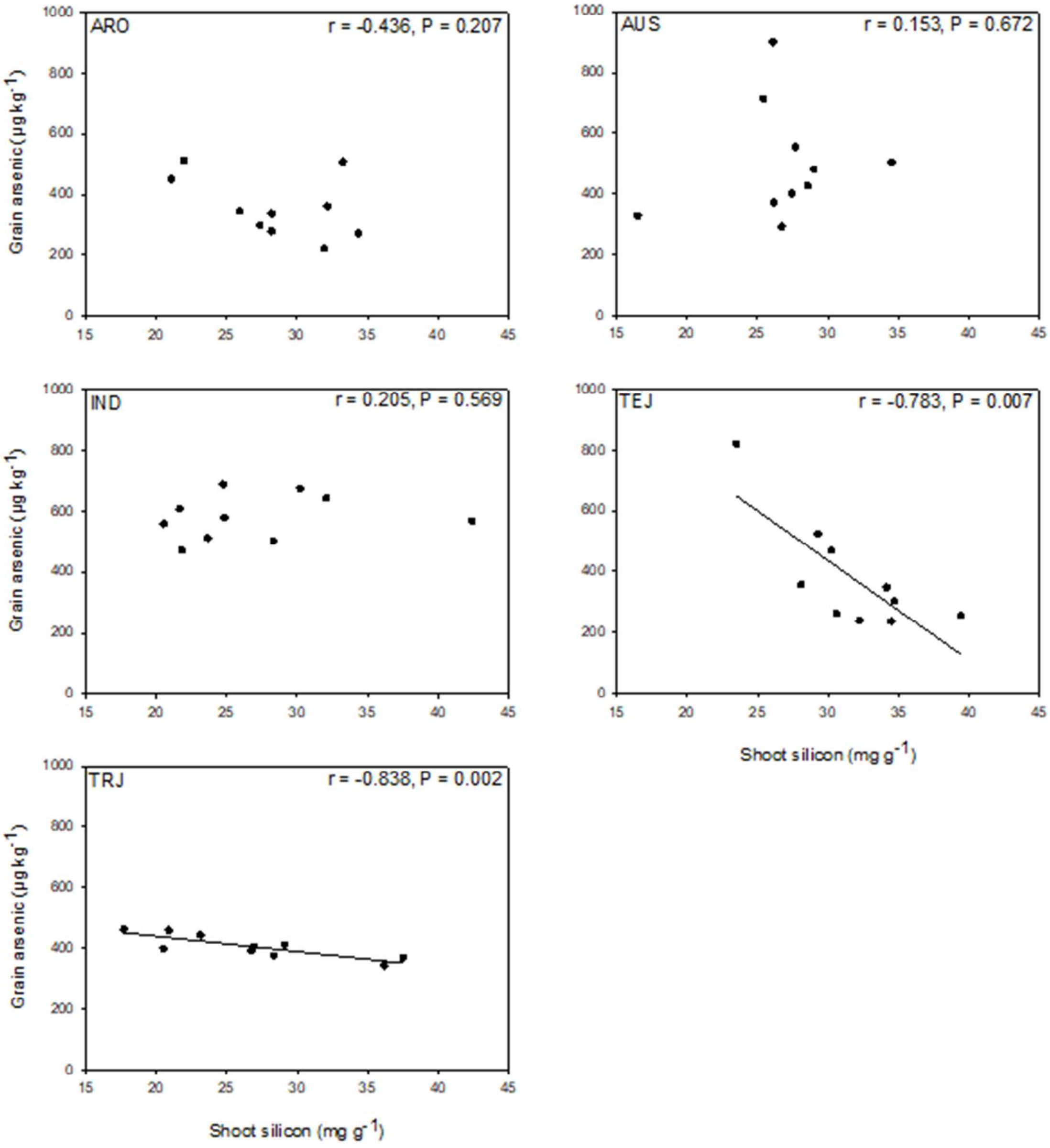

ARO
r = -0.436, P = 0.207
AUS
r = 0.153, P = 0.672
IND
r = 0.205, P = 0.569
TEJ
r = -0.783, P = 0.007
TRJ
r = -0.838, P = 0.002
Grain arsenic (µg kg-1)
Shoot silicon (mg g-1)
0
200
400
600
800
1000
15
20
25
30
35
40
45

Figure 5

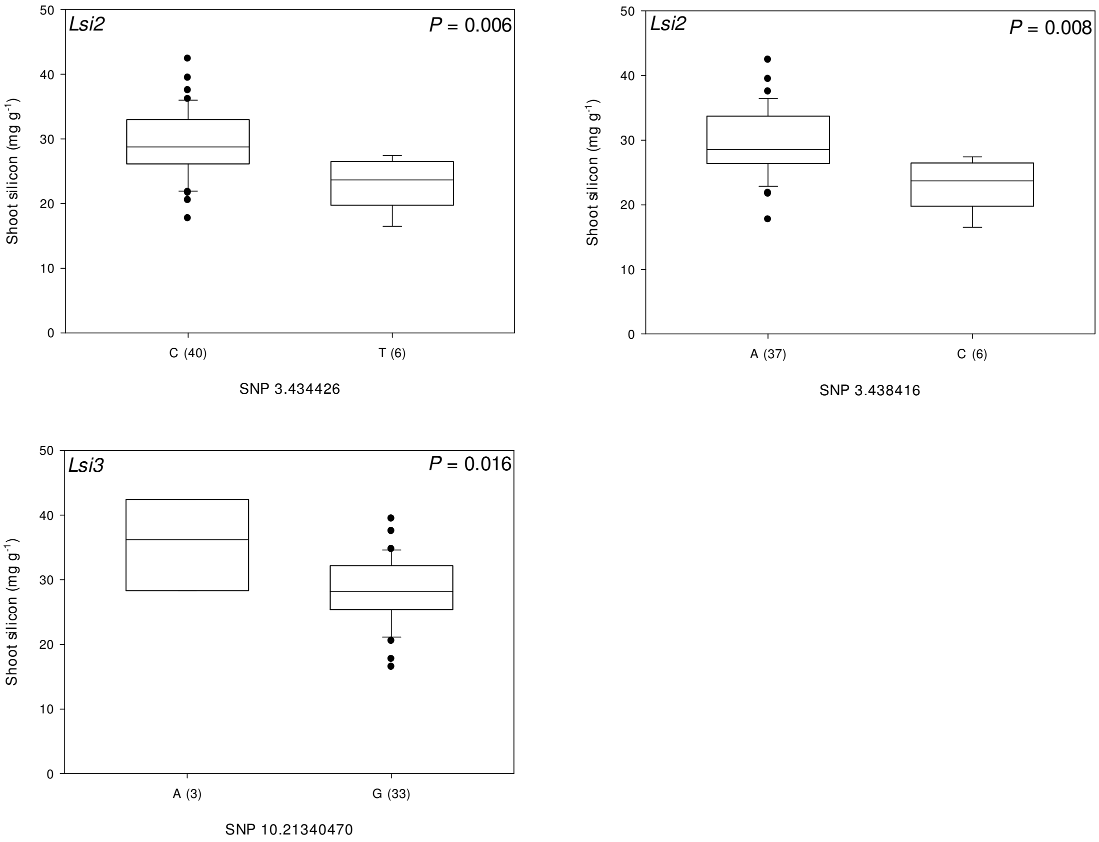
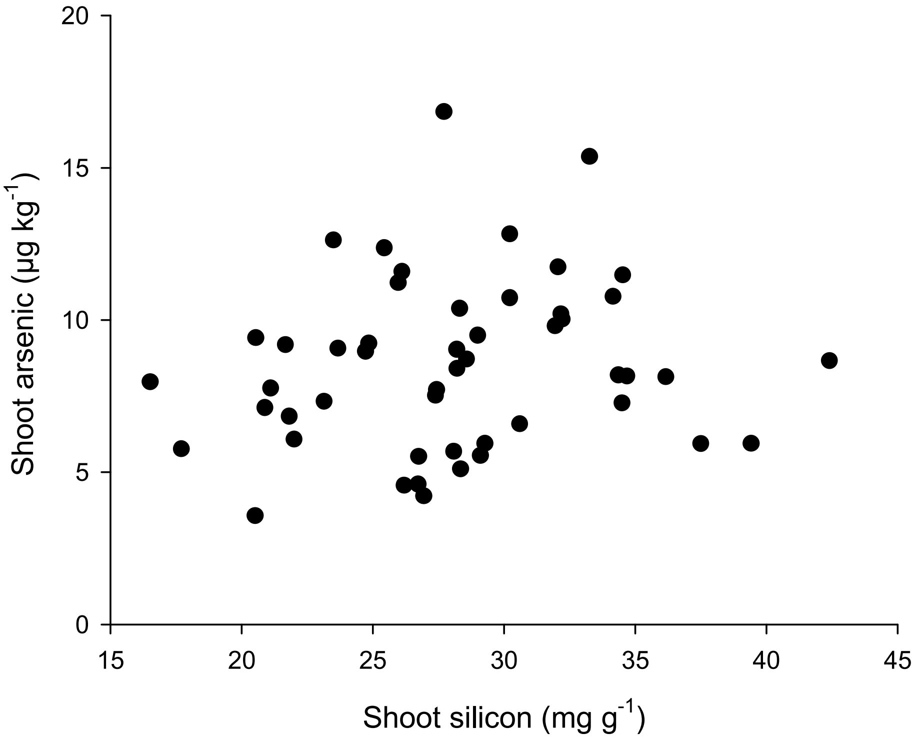

Lsi2
P = 0.006
Shoot silicon (mg g-1)
0
10
20
30
40
50
C (40)
T (6)
SNP 3.434426
Lsi2
P = 0.008
Shoot silicon (mg g-1)
0
10
20
30
40
50
A (37)
C (6)
SNP 3.438416
Lsi3
P = 0.016
Shoot silicon (mg g-1)
0
10
20
30
40
50
A (3)
G (33)
SNP 10.21340470

Figure 6

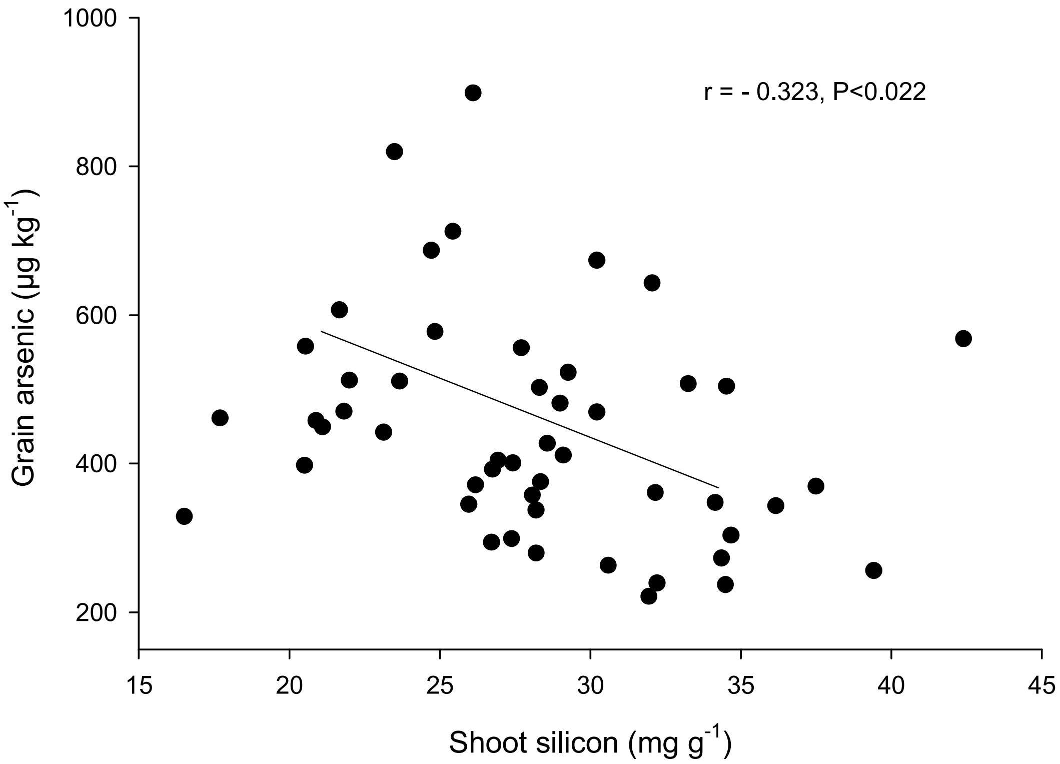
Supplementary Table 1: Selected genotype from RDP1 for shoot silicon analysis

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

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

```
Bala             AGCATCAGCAGCGCATGCACTAGCAGCTTAGCTACTGCGCGCGTACCAGAGAGAGATCAT
IR64             AGCATCAGCAGCGCATGCACTAGCAGCTTAGCTACTGCGCGCGTACCAGAGAGAGATCAT
DJ123            AGCATCAGCAGCGCATGCACTAGCAGCTTAGCTACTGCGCGCGTACCAGAGAGAGATCAT
Nipponbare       AGCATCAGCAGCGCATGCACTAGCAGCTTAGCTACTGCGCGCGTACCAGAGAGAGATCAT
Azucena          AGCATCAGCAGCGCATGCACTAGCAGCTTAGCTACTGCGCGCGTACCAGAGAGAGATCAT
                 ************************************************************

Bala             CAGCTCAAGTAGCTAAGCTAGCTATAATCTGCTAGCTAGCTCGATCAGACACTTAATTAC
IR64             CAGCTCAAGTAGCTAAGCTAGCTATAATCTGCTAGCTAGCTCGATCAGACACTTAATTAC
DJ123            CAGCTCAAGTAGCTAAGCTAGCTATAATCTGCTAGCTAGCTCGATCAGACACTTAATTAC
Nipponbare       CAGCTCAAGTAGCTAAGCTAGCTATAATCTGCTAGCTAGCTCGATCAGACACTTAATTAC
Azucena          CAGCTCAAGTAGCTAAGCTAGCTATAATCTGCTAGCTAGCTCGATCAGACACTTAATTAC
                 ************************************************************

Bala             CTGCTAGGTGGTGGTCGATCGAAGAAGAAGAAGATGAGTGAGCTTGCGTCGGCGCCCAAG
IR64             CTGCTAGGTGGTGGTCGATCGAAGAAGAAGAAGATGAGTGAGCTTGCGTCGGCGCCCAAG
DJ123            CTGCTAGGTGGTGGTCGATCGAAGAAGAAGAAGATGAGTGAGCTTGCGTCGGCGCCCAAG
Nipponbare       CTGCTAGGTGGTGGTCGATCGAAGAAGAAGAAGATGAGTGAGCTTGCGTCGGCGCCCAAG
Azucena          CTGCTAGGTGGTGGTCGATCGAAGAAGAAGAAGATGAGTGAGCTTGCGTCGGCGCCCAAG
                 ************************************************************

Bala             GTGGCGCTTGGATCGATCGCGTTCGCGGTGTTCTGGATGATGGCGGTGTTCCCGTCGGTG
IR64             GTGGCGCTTGGATCGATCGCGTTCGCGGTGTTCTGGATGATGGCGGTGTTCCCGTCGGTG
DJ123            GTGGCGCTTGGATCGATCGCGTTCGCGGTGTTCTGGATGATGGCGGTGTTCCCGTCGGTG
Nipponbare       GTGGCGCTTGGATCGATCGCGTTCGCGGTGTTCTGGATGATGGCGGTGTTCCCGTCGGTG
Azucena          GTGGCGCTTGGATCGATCGCGTTCGCGGTGTTCTGGATGATGGCGGTGTTCCCGTCGGTG
                 ************************************************************

Bala             CCGTTCCTGCCGATCGGGCGGACGGCGGGGTCGCTGCTGAGCGCGGTGCTGATGGTGATA
IR64             CCGTTCCTGCCGATCGGGCGGACGGCGGGGTCGCTGCTGAGCGCGGTGCTGATGGTGATA
DJ123            CCGTTCCTGCCGATCGGGCGGACGGCGGGGTCGCTGCTGAGCGCGGTGCTGATGGTGATA
Nipponbare       CCGTTCCTGCCGATCGGGCGGACGGCGGGGTCGCTGCTGAGCGCGGTGCTGATGGTGATA
Azucena          CCGTTCCTGCCGATCGGGCGGACGGCGGGGTCGCTGCTGAGCGCGGTGCTGATGGTGATA
                 ************************************************************

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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Bala             GTCACCGCCCTCGCCAGCGCCCTCTTCACCAACGACACCTGCTGCGTCGTCCTCACCGAG
IR64             GTCACCGCCCTCGCCAGCGCCCTCTTCACCAACGACACCTGCTGCGTCGTCCTCACCGAG
DJ123            GTCACCGCCCTCGCCAGCGCCCTCTTCACCAACGACACCTGCTGCGTCGTCCTCACCGAG
Nipponbare       GTCACCGCCCTCGCCAGCGCCCTCTTCACCAACGACACCTGCTGCGTCGTCCTCACCGAG
Azucena          GTCACCGCCCTCGCCAGCGCCCTCTTCACCAACGACACCTGCTGCGTCGTCCTCACCGAG
                 ************************************************************

Bala             TTCGTCCTCGAGCTCGCCGCCGAGCGCAACCTCCCCGCCAAGCCCTTCCTCCTCGCCCTC
IR64             TTCGTCCTCGAGCTCGCCGCCGAGCGCAACCTCCCCGCCAAGCCCTTCCTCCTCGCCCTC
DJ123            TTCGTCCTCGAGCTCGCCGCCGAGCGCAACCTCCCCGCCAAGCCCTTCCTCCTCGCCCTC
Nipponbare       TTCGTCCTCGAGCTCGCCGCCGAGCGCAACCTCCCCGCCAAGCCCTTCCTCCTCGCCCTC
Azucena          TTCGTCCTCGAGCTCGCCGCCGAGCGCAACCTCCCCGCCAAGCCCTTCCTCCTCGCCCTC
                 ************************************************************

Bala             GCCTCCAGCGCCAACATCGGCTCCGCCGCCACCCCCATCGGCAACCCCCAGAACCTGGTC
IR64             GCCTCCAGCGCCAACATCGGCTCCGCCGCCACCCCCATCGGCAACCCCCAGAACCTGGTC
DJ123            GCCTCCAGCGCCAACATCGGCTCCGCCGCCACCCCCATCGGCAACCCCCAGAACCTGGTC
Nipponbare       GCCTCCAGCGCCAACATCGGCTCCGCCGCCACCCCCATCGGCAACCCCCAGAACCTGGTC
Azucena          GCCTCCAGCGCCAACATCGGCTCCGCCGCCACCCCCATCGGCAACCCCCAGAACCTGGTC
                 ************************************************************

Bala             ATCGCCTTCAATAGCAAGATCACCTTCCCCAAGTTCCTCATGGGAATCCTCCCGGCCATG
IR64             ATCGCCTTCAATAGCAAGATCACCTTCCCCAAGTTCCTCATGGGAATCCTCCCGGCCATG
DJ123            ATCGCCTTCAACAGCAAGATCACCTTCCCCAAGTTCCTCATGGGAATCCTCCCGGCCATG
Nipponbare       ATCGCCTTCAATAGCAAGATCACCTTCCCCAAGTTCCTCATGGGAATCCTCCCGGCCATG
Azucena          ATCGCCTTCAATAGCAAGATCACCTTCCCCAAGTTCCTCATGGGAATCCTCCCGGCCATG
                 *********** ************************************************

Bala             CTCGTCGGGATGGCCGTCAACATGGTCATGCTGCTCTGCATGTACTGGAGGGAGCTGGGC
IR64             CTCGTCGGGATGGCCGTCAACATGGTCATGCTGCTCTGCATGTACTGGAGGGAGCTGGGC
DJ123            CTCGTCGGGATGGCCGTCAACATGGTCATGCTGCTCTGCATGTACTGGAGGGAGCTGGGC
Nipponbare       CTCGTCGGGATGGCCGTCAACATGGTCATGCTGCTCTGCATGTACTGGAGGGAGCTGGGC
Azucena          CTCGTCGGGATGGCCGTCAACATGGTCATGCTGCTCTGCATGTACTGGAGGGAGCTGGGC
                 ************************************************************

Bala             GGAGGGGCCGAGCTCAGCGTCGACGGCAAGCAGATGGAGGCGGTGGAGGAAGGCAGGTCG
IR64             GGAGGGGCCGAGCTCAGCGTCGACGGCAAGCAGATGGAGGCGGTGGAGGAAGGCAGGTCG
DJ123            GGAGGGGCCGAGCTCAGCGTCGACGGCAAGCAGATGGAGGCGGTGGAGGAAGGCAGGTCG
Nipponbare       GGAGGGGCCGAGCTCAGCGTCGACGGCAAGCAGATGGAGGCGGTGGAGGAAGGCAGGTCG
Azucena          GGAGGGGCCGAGCTCAGCGTCGACGGCAAGCAGATGGAGGCGGTGGAGGAAGGCAGGTCG
                 ************************************************************

Bala             CCGGCGTCGGCCAAGAGCACGCCGCAGCTGAACGGCAACGGCAACACGATGATGTCGCTG
IR64             CCGGCGTCGGCCAAGAGCACGCCGCAGCTGAACGGCAACGGCAACACGATGATGTCGCTG
DJ123            CCGGCGTCGGCCAAGAGCACGCCGCAGCTGAACGGCAACGGCAACACGATGATGTCGCTG
Nipponbare       CCGGCGTCGGCCAAGAGCACGCCGCAGCTGAACGGCAACGGCAACACGATGATGTCGCTG
Azucena          CCGGCGTCGGCCAAGAGCACGCCGCAGCTGAACGGCAACGGCAACACGATGATGTCGCTG
                 ************************************************************

Bala             GAGATGTCGGAGAACATAACGACCAAGCACCCATGGTTCATGCAGTGCACGGAGGCGCGG
IR64             GAGATGTCGGAGAACATAACGACCAAGCACCCATGGTTCATGCAGTGCACGGAGGCGCGG
DJ123            GAGATGTCGGAGAACATAACGACCAAGCACCCATGGTTCATGCAGTGCACGGAGGCGCGG
Nipponbare       GAGATGTCGGAGAACATAACGACCAAGCACCCATGGTTCATGCAGTGCACGGAGGCGCGG
Azucena          GAGATGTCGGAGAACATAACGACCAAGCACCCATGGTTCATGCAGTGCACGGAGGCGCGG
                 ************************************************************

Bala             CGGAAGCTGTTCCTCAAGAGCTTCGCGTACGTGGTGACGGTGGGGATGGTGGTGGCCTAC
IR64             CGGAAGCTGTTCCTCAAGAGCTTCGCGTACGTGGTGACGGTGGGGATGGTGGTGGCCTAC
DJ123            CGGAAGCTGTTCCTCAAGAGCTTCGCGTACGTGGTGACGGTGGGGATGGTGGTGGCCTAC
Nipponbare       CGGAAGCTGTTCCTCAAGAGCTTCGCGTACGTGGTGACGGTGGGGATGGTGGTGGCCTAC
Azucena          CGGAAGCTGTTCCTCAAGAGCTTCGCGTACGTGGTGACGGTGGGGATGGTGGTGGCCTAC
                 ************************************************************
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Bala             ATGGTGGGGCTCAACATGTCGTGGACGGCCATCACCACGGCGCTGGCGCTGGTGGTGGTC
IR64             ATGGTGGGGCTCAACATGTCGTGGACGGCCATCACCACGGCGCTGGCGCTGGTGGTGGTC
DJ123            ATGGTGGGGCTCAACATGTCGTGGACGGCCATCACCACGGCGCTGGCGCTGGTGGTGGTC
Nipponbare       ATGGTGGGGCTCAACATGTCGTGGACGGCCATCACCACGGCGCTGGCGCTGGTGGTGGTC
Azucena          ATGGTGGGGCTCAACATGTCGTGGACGGCCATCACCACGGCGCTGGCGCTGGTGGTGGTC
                 ************************************************************

Bala             GACTTCCGCGACGCCGAGCCGTGCCTGGACACCGTGTCCTACTCGCTGCTCGTCTTCTTC
IR64             GACTTCCGCGACGCCGAGCCGTGCCTGGACACCGTGTCCTACTCGCTGCTCGTCTTCTTC
DJ123            GACTTCCGCGACGCCGAGCCGTGCCTGGACACCGTGTCCTACTCGCTGCTCGTCTTCTTC
Nipponbare       GACTTCCGCGACGCCGAGCCGTGCCTGGACACCGTGTCCTACTCGCTGCTCGTCTTCTTC
Azucena          GACTTCCGCGACGCCGAGCCGTGCCTGGACACCGTGTCCTACTCGCTGCTCGTCTTCTTC
                 ************************************************************

Bala             TCCGGGATGTTCATCACCGTCAGCGGCTTCAACAAGACGGGCCTCCCGGGAGCCATCTGG
IR64             TCCGGGATGTTCATCACCGTCAGCGGCTTCAACAAGACGGGCCTCCCGGGAGCCATCTGG
DJ123            TCCGGGATGTTCATCACCGTCAGCGGCTTCAACAAGACGGGCCTCCCGGGAGCCATCTGG
Nipponbare       TCCGGGATGTTCATCACCGTCAGCGGCTTCAACAAGACGGGCCTCCCGGGAGCCATCTGG
Azucena          TCCGGGATGTTCATCACCGTCAGCGGCTTCAACAAGACGGGCCTCCCGGGAGCCATCTGG
                 ************************************************************

Bala             GACTTCATGGCCCCCTACTCCAAGGTCAACAGCGTCGGCGGCATCTCCGTCCTCTCCGTC
IR64             GACTTCATGGCCCCCTACTCCAAGGTCAACAGCGTCGGCGGCATCTCCGTCCTCTCCGTC
DJ123            GACTTCATGGCCCCCTACTCCAAGGTCAACAGCGTCGGCGGCATCTCCGTCCTCTCCGTC
Nipponbare       GACTTCATGGCCCCCTACTCCAAGGTCAACAGCGTCGGCGGCATCTCCGTCCTCTCCGTC
Azucena          GACTTCATGGCCCCCTACTCCAAGGTCAACAGCGTCGGCGGCATCTCCGTCCTCTCCGTC
                 ************************************************************

Bala             ATCATCCTCCTCCTCTCCAACCTCGCATCAAACGTACCAACGGGTAATTATACATTCTAT
IR64             ATCATCCTCCTCCTCTCCAACCTCGCATCAAACGTACCAACGGGTAATTATACATTCTAT
DJ123            ATCATCCTCCTCCTCTCCAACCTCGCATCAAACGTACCAACGGGTAATTATACATTCTAT
Nipponbare       ATCATCCTCCTCCTCTCCAACCTCGCATCAAACGTACCAACGGGTAATTATACATTCTAT
Azucena          ATCATCCTCCTCCTCTCCAACCTCGCATCAAACGTACCAACGGGTAATTATACATTCTAT
                 ************************************************************

Bala             TAATCCAGAATTAATGTATACTACTCCATATAAGTATAACTAATTAACATACAAAAATAT
IR64             TAATCCAGAATTAATGTATACTACTCCATATAAGTATAACTAATTAACATACAAAAATAT
DJ123            TAATCCAGAATTAATGTATACTACTCCATATAAGTATAACTAATTAACATACAAAAATAT
Nipponbare       TAATCCAGAATTAATGTATACTACTCCATATAAGTATAACTAATTAACATACAAAAATAT
Azucena          TAATCCAGAATTAATGTATACTACTCCATATAAGTATAACTAATTAACATACAAAAATAT
                 ************************************************************

Bala             ATATGCGCATGCATGCAGTGCTTCTTATGGGTGATGAGGTGGCGAAGGCGGCGGCGCTGA
IR64             ATATGCGCATGCATGCAGTGCTTCTTATGGGTGATGAGGTGGCGAAGGCGGCGGCGCTGA
DJ123            ATATGCGCATGCATGCAGTGCTTCTTATGGGTGATGAGGTGGCGAAGGCGGCGGCGCTGA
Nipponbare       ATATGCGCATGCATGCAGTGCTTCTTATGGGTGATGAGGTGGCGAAGGCGGCGGCGCTGA
Azucena          ATATGCGCATGCATGCAGTGCTTCTTATGGGTGATGAGGTGGCGAAGGCGGCGGCGCTGA
                 ************************************************************

Bala             TATCGCCGGCGGCGGTGACGACGTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGG
IR64             TATCGCCGGCGGCGGTGACGACGTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGG
DJ123            TATCGCCGGCGGCGGTGACGACGTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGG
Nipponbare       TATCGCCGGCGGCGGTGACGACGTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGG
Azucena          TATCGCCGGCGGCGGTGACGACGTCGTGGCTGCTGCTGGCGTGGGTGAGCACGGTGGCGG
                 ************************************************************

Bala             GGAACCTGTCGCTGCTGGGGTCGGCGGCGAACCTGATAGTGTGCGAGCAGGCGAGGAGGG
IR64             GGAACCTGTCGCTGCTGGGGTCGGCGGCGAACCTGATAGTGTGCGAGCAGGCGAGGAGGG
DJ123            GGAACCTGTCGCTGCTGGGGTCGGCGGCGAACCTGATAGTGTGCGAGCAGGCGAGGAGGG
Nipponbare       GGAACCTGTCGCTGCTGGGGTCGGCGGCGAACCTGATAGTGTGCGAGCAGGCGAGGAGGG
Azucena          GGAACCTGTCGCTGCTGGGGTCGGCGGCGAACCTGATAGTGTGCGAGCAGGCGAGGAGGG
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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             TCCTTTTTGTTTTTGTTTTACCCACCGTATATGTGCTGTGTGGTGTATGGCCGGCGAGCT
IR64             TCCTTTTTGTTTTTGTTTTACCCACCGTATATGTGCTGTGTGGTGTATGGCCGGCGAGCT
DJ123            TCCTTTTTGTTTTTGTTTTACCCACCGTATATGTGCTGTGTGGTGTATGGCCGGCGAGCT
Nipponbare       TCCTTTTTGTTTTTGTTTTACCCACCGTATATGTGCTGTGTGGTGTATGGCCGGCGAGCT
Azucena          TCCTTTTTGTTTTTGTTTTACCCACCGTATATGTGCTGTGTGGTGTATGGCCGGCGAGCT
                 ************************************************************

Bala             TGAAATTGAATTGGCCTTGCTTGCATGCATGCATGGTGATCAGATCAGCTCAGCTAGCTA
IR64             TGAAATTGAATTGGCCTTGCTTGCATGCATGCATGGTGATCAGATCAGCTCAGCTAGCTA
DJ123            TGAAATTGAATTGGCCTTGCTTGCATGCATGCATGGTGATCAGATCAGCTCAGCTAGCTA
Nipponbare       TGAAATTGAATTGGCCTTGCTTGCATGCATGCATGGTGATCAGATCAGCTCAGCTAGCTA
Azucena          TGAAATTGAATTGGCCTTGCTTGCATGCATGCATGGTGATCAGATCAGCTCAGCTAGCTA
                 ************************************************************

Bala             GCTAAATCTTGCTTAATTTTATCAAGTGTCAATCATGATGAGAAGAGAGGGAACTAGATG
IR64             GCTAAATCTTGCTTAATTTTATCAAGTGTCAATCATGATGAGAAGAGAGGGAACTAGATG
DJ123            GCTAAATCTTGCTTAATTTTATCAAGTGTCAATCATGATGAGAAGAGAGGGAACTAGATG
Nipponbare       GCTAAATCTTGCTTAATTTTATCAAGTGTCAATCATGATGAGAAGAGAGGGAACTAGATG
Azucena          GCTAAATCTTGCTTAATTTTATCAAGTGTCAATCATGATGAGAAGAGAGGGAACTAGATG
                 ************************************************************

Bala             GAGCTAGCTAGCTAGCAACACATGCAGTGTGTGCTAAGCAGTGCACTCTGCATGCTTAAA
IR64             GAGCTAGCTAGCTAGCAACACATGCAGTGTGTGCTAAGCAGTGCACTCTGCATGCTTAAA
DJ123            GAGCTAGCTAGCTAGCAACACATGCAGTGTGTGCTAAGCAGTGCACTCTGCATGCTTAAA
Nipponbare       GAGCTAGCTAGCTAGCAACACATGCAGTGTGTGCTAAGCAGTGCACTCTGCATGCTTAAA
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             TCAAACAAATATTTGTAGCCCTCTCTCTCTCTCTCTTTAGGTTTATTAATTAATTAGT
IR64             TCAAACAAATATTTGTAGCCCTCTCTCTCTCTCTCTTTAGGTTTATTAATTAATTAGT
DJ123            TCAAACAAATATTTGTAGCCCTCTCTCTCTCTCTCTTTAGGTTTATTAATTAATTAGT
Nipponbare       TCAAACAAATATTTGTAGCCCTCTCTCTCTCTCTCTTTAGGTTTATTAATTAATTAGT
Azucena          TCAAACAAATATTTGTAGCCCTCTCTCTCTCTCTCTTTAGGTTTATTAATTAATTAGT
                 ************************************************************

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
                 *
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Supplementary figure 3. Genomic sequence alignment of *Lsi2*. Regions highlighted in bold are predicted exon regions based on the Rice Genome Annotation Project – release 7 sequence available at http://rice.plantbiology.msu.edu/.

Lsi3 (LOC_Os10g39980)

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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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Bala              AACCTCCCGGCGAAGCCGTTCCTGCTCGCGCTCGCCACCAGCGCCAACATCGGCTCCAGC
IR64              AACCTCCCGGCGAAGCCGTTCCTGCTCGCGCTCGCCACCAGCGCCAACATCGGCTCCAGC
DJ123             AACCTCCCGGCGAAGCCGTTCCTGCTCGCGCTCGCCACCAGCGCCAACATCGGCTCCAGC
Nipponbare        AACCTCCCGGCGAAGCCGTTCCTGCTCGCGCTCGCCACCAGCGCCAACATCGGCTCCAGC
Azucena           AACCTCCCGGCGAAGCCGTTCCTGCTCGCGCTCGCCACCAGCGCCAACATCGGCTCCAGC
                  ************************************************************

Bala              GCGACGCCCATCGGCAACCCGCAGAACCTGGTCATCGCCTTCAACAGCAAGATCTCCTTT
IR64              GCGACGCCCATCGGCAACCCGCAGAACCTGGTCATCGCCTTCAACAGCAAGATCTCCTTT
DJ123             GCGACGCCCATCGGCAACCCGCAGAACCTGGTCATCGCCTTCAACAGCAAGATCTCCTTT
Nipponbare        GCGACGCCCATCGGCAACCCGCAGAACCTGGTCATCGCCTTCAACAGCAAGATCTCCTTT
Azucena           GCGACGCCCATCGGCAACCCGCAGAACCTGGTCATCGCCTTCAACAGCAAGATCTCCTTT
                  ************************************************************

Bala              ATTTCCTTCCTCCTCGGCATCCTCCCCGCCATGCTCGCCGGCATGGGGATCAACATGCTC
IR64              ATTTCCTTCCTCCTCGGCATCCTCCCCGCCATGCTCGCCGGCATGGGGATCAACATGCTC
DJ123             ATTTCCTTCCTCCTCGGCATCCTCCCCGCCATGCTCGCCGGCATGGGGATCAACATGCTC
Nipponbare        ATTTCCTTCCTCCTCGGCATCCTCCCCGCCATGCTCGCCGGCATGGGGATCAACATGCTC
Azucena           ATTTCCTTCCTCCTCGGCATCCTCCCCGCCATGCTCGCCGGCATGGGGATCAACATGCTC
                  ************************************************************

Bala              ATGCTGCTCTGCATGTACTGGAAGGAGCTCGACGGCGGCGCCTGCAGCCCCGACGAGGTC
IR64              ATGCTGCTCTGCATGTACTGGAAGGAGCTCGACGGCGGCGCCTGCAGCCCCGACGAGGTC
DJ123             ATGCTGCTCTGCATGTACTGGAAGGAGCTCGACGGCGGCGCCTGCAGCCCCGACGAGGTC
Nipponbare        ATGCTGCTCTGCATGTACTGGAAGGAGCTCGACGGCGGCGCCTGCAGCCCCGACGAGGTC
Azucena           ATGCTGCTCTGCATGTACTGGAAGGAGCTCGACGGCGGCGCCTGCAGCCCCGACGAGGTC
                  ************************************************************

Bala              GCCGCCGGCAAGCAGATGGAGGCCATCGAGGAGGGCCGCCGCACGGCGCTCAACAACAAC
IR64              GCCGCCGGCAAGCAGATGGAGGCCATCGAGGAGGGCCGCCGCACGGCGCTCAACAACAAC
DJ123             GCCGCCGGCAAGCAGATGGAGGCCATCGAGGAGGGCCGCCGCACGGCGCTCAACAACAAC
Nipponbare        GCCGCCGGCAAGCAGATGGAGGCCATCGAGGAGGGCCGCCGCACGGCGCTCAACAACAAC
Azucena           GCCGCCGGCAAGCAGATGGAGGCCATCGAGGAGGGCCGCCGCACGGCGCTCAACAACAAC
                  ************************************************************

Bala              AAGAAAGACGACGGCGACGCCGCCACCCCCGCATCGCCGGAGGACGACGACGGCGGCGAC
IR64              AAGAAAGACGACGGCGACGCCGCCACCCCCGCATCGCCGGAGGACGACGACGGCGGCGAC
DJ123             AAGAAAGACGACGGCGACGCCGCCACCCCCGCATCGCCGGAGGACGACGACGGCGGCGAC
Nipponbare        AAGAAAGACGACGGCGACGCCGCCACCCCCGCATCGCCGGAGGACGACGACGGCGGCGAC
Azucena           AAGAAAGACGACGGCGACGCCGCCACCCCCGCATCGCCGGAGGACGACGACGGCGGCGAC
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Bala              GCCGAGTCGATGATGTCGGAGAACATCTCGACGAAGCACCGGTGGTTCATGCAGTGCTCG
IR64              GCCGAGTCGATGATGTCGGAGAACATCTCGACGAAGCACCGGTGGTTCATGCAGTGCTCG
DJ123             GCCGAGTCGATGATGTCGGAGAACATCTCGACGAAGCACCGGTGGTTCATGCAGTGCTCG
Nipponbare        GCCGAGTCGATGATGTCGGAGAACATCTCGACGAAGCACCGGTGGTTCATGCAGTGCTCG
Azucena           GCCGAGTCGATGATGTCGGAGAACATCTCGACGAAGCACCGGTGGTTCATGCAGTGCTCG
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Bala              GAGCACCGGCGGAAGCTATTCCTCAAGTGCTTCGCGTACGTGGTCACCGTCGGCATGCTC
IR64              GAGCACCGGCGGAAGCTATTCCTCAAGAGCTTCGCGTACGTGGTCACCGTCGGCATGCTC
DJ123             GAGCACCGGCGGAAGCTATTCCTCAAGTGCTTCGCGTACGTGGTCACCGTCGGCATGCTC
Nipponbare        GAGCACCGGCGGAAGCTGTTCCTCAAGAGCTTCGCGTACGTGGTCACCGTCGGCATGCTC
Azucena           GAGCACCGGCGGAAGCTGTTCCTCAAGAGCTTCGCGTACGTGGTCACCGTCGGCATGCTC
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Bala              GTCGCCTACATGCTCGGCCTCAACATGTCGTGGACCGCCATCACCACCGCCATCGCCCTC
IR64              GTCGCCTACATGCTCGGCCTCAACATGTCGTGGACCGCCATCACCACCGCCATCGCCCTC
DJ123             GTCGCCTACATGCTCGGCCTCAACATGTCGTGGACCGCCATCACGACCGCCATCGCCCTC
Nipponbare        GTCGCCTACATGCTCGGCCTCAACATGTCGTGGACCGCCATCACGACCGCCATCGCCCTC
Azucena           GTCGCCTACATGCTCGGCCTCAACATGTCGTGGACCGCCATCACGACCGCCATCGCCCTC
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Bala            GTCGTCGTCGACTTCCGCGACGCCGAGCCATGCCTCGACAAGGTAACCCATATCCGTCCA
IR64            GTCGTCGTCGACTTCCGCGACGCCGAGCCATGCCTCGACAAGGTAACCCATATCCGTCCA
DJ123           GTCGTCGTCGACTTCCGCGACGCCGAGCCATGCCTCGACAAGGTAACCCATATCCGTCCA
Nipponbare      GTCGTCGTCGACTTCCGCGACGCCGAGCCATGCCTCGACAAGGTAACCCATATCCGTCCA
Azucena         GTCGTCGTCGACTTCCGCGACGCCGAGCCATGCCTCGACAAGGTAACCCATATCCGTCCA
                ************************************************************

Bala            AATCACTATAACTCGAGGCCACACGAGCAAGTACACGTGGCGGCCATGCGGACAAGAAGC
IR64            AATCACTATAACTCTAGGCCACACGAGCAAGTACACGTGGCGGCCATGCGGACAAGAAGC
DJ123           AATCACTATAACTCTAGGCCACACGAGCAAGTACACGTGGCGGCCATGCGGACAAGAAGC
Nipponbare      AATCACTATAACTCTAGGCCACACGAGCAAGTACACGTGGCGGCCATGCGGACAAGAAGC
Azucena         AATCACTATAACTCTAGGCCACACGAGCAAGTACACGTGGCGGCCATGCGGACAAGAAGC
                ************** *********************************************

Bala            AATATGTGAACTGGGTGCCACGTGTGCGTGGATGTGAATGGATGATGCAGGTGTCGTACT
IR64            AATATGTGAACTGGGTGCCACGTGTGCGTGGATGTGAATGGATGATGCAGGTGTCGTACT
DJ123           AATATGTGAACTGGGTGCCACGTGTGCGTGGATGTGAATGGATGATGCAGGTGTCGTACT
Nipponbare      AATATGTGAACTGGGTGCCACGTGTGCGTGGATGTGAATGGATGATGCAGGTGTCGTACT
Azucena         AATATGTGAACTGGGTGCCACGTGTGCGTGGATGTGAATGGATGATGCAGGTGTCGTACT
                ************************************************************

Bala            CGCTGCTGGTGTTCTTCTCGGGGATGTTCGTGACGGTGAGCGGATTCAACAAGACGGGGC
IR64            CGCTGCTGGTGTTCTTCTCGGGGATGTTCGTGACGGTGAGCGGATTCAACAAGACGGGGC
DJ123           CGCTGCTGGTGTTCTTCTCGGGGATGTTCGTGACGGTGAGCGGGTTCAACAAGACGGGGC
Nipponbare      CGCTGCTGGTGTTCTTCTCGGGGATGTTCGTGACGGTGAGCGGGTTCAACAAGACGGGGC
Azucena         CGCTGCTGGTGTTCTTCTCGGGGATGTTCGTGACGGTGAGCGGGTTCAACAAGACGGGGC
                ******************************************* ****************

Bala            TACCCGGGGCCATCTGGAACGTGATGGCGCCCTACTCAAAGATCAACCACGTCACCGGCG
IR64            TACCCGGGGCCATCTGGAACGTGATGGCGCCCTACTCAAAGATCAACCACGTCACCGGCG
DJ123           TACCCGGGGCCATCTGGAACGTGATGGCGCCCTACTCAAAGATCAACCACGTCACCGGCG
Nipponbare      TACCCGGGGCCATCTGGAACGTGATGGCGCCCTACTCAAAGATCAACCACGTCACCGGCG
Azucena         TACCCGGGGCCATCTGGAACGTGATGGCGCCCTACTCAAAGATCAACCACGTCACCGGCG
                ************************************************************

Bala            TCACCGTCCTCTCCGTCATCATCCTCCTCCTCTCCAACCTCGCCTCCAACGTCCCCACCG
IR64            TCACCGTCCTCTCCGTCATCATCCTCCTCCTCTCCAACCTCGCCTCCAACGTCCCCACCG
DJ123           TCACCGTCCTCTCCGTCATCATCCTCCTCCTCTCCAACCTCGCCTCCAACGTCCCCACCG
Nipponbare      TCACCGTCCTCTCCGTCATCATCCTCCTCCTCTCCAACCTCGCCTCCAACGTCCCCACCG
Azucena         TCACCGTCCTCTCCGTCATCATCCTCCTCCTCTCCAACCTCGCCTCCAACGTCCCCACCG
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Bala            GTACGTACTTACTCACAACCTTTCTCGCACCATGCTATTGCTCGTACCCAATGTGTCCCG
IR64            GTACGTACTTACTCACAACCTTTCTCGCACCATGCTATTGCTAGTACCCAATGTGTCCCG
DJ123           GTACGTACTTACTCACAACCTTTCTCGCACCATGCTATTGCTAGTACCCAATGTGTCCCG
Nipponbare      GTACGTACTTACTCACAACCTTTCTCGCACCATGCTATTGCTAGTACCCAATGTGTCCCG
Azucena         GTACGTACTTACTCACAACCTTTCTCGCACCATGCTATTGCTAGTACCCAATGTGTCCCG
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Bala            ACTCACCCAGCACTAACATTTGTACTATACAGTACATCAGTTTCTTTTAATACATGATAT
IR64            ACTCACCCAGCACTAACATTTGTACTATACAGTACATCAGTTTCTTTTAATACATGATAT
DJ123           ACTCACCCAGCACTAACATTTGTACTGTACAGTACATCAGTTTCTTTTAATACATGATAT
Nipponbare      ACTCACCCAGCACTAACATTTGTACTGTACAGTACATCAGTTTCTTTTAATACATGATAT
Azucena         ACTCACCCAGCACTAACATTTGTACTGTACAGTACATCAGTTTCTTTTAATACATGATAT
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Bala            CTGACTTTTTTTTATCGATGAATGGATCTTATTCAATTTCTTTTGTAAATATAAAAAGAT
IR64            CTGACTTTTTTTTATCGATGAATGGATCTTATTCAATTTCTTTTGTAAATATAAAAAGAT
DJ123           CTGACTTTTTTTTATCGATGAATGGATCTTATTCAATTTCTTTTGTAAATATAAAAAGAT
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             GAATGATTATTAAAAGAAAAAAATTTTTTTTGTCTCTCATAATTAAAAAAGAACAACTCA
Nipponbare        GAATGATTATTAAAAGAAAAAAATTTTTTTTGTCTCTCATAATTAAAAAAGAACAACTCA
Azucena           GAATGATTATTAAAAGAAAAATTTTTTTTTTGTCTCTCATAATTAAAAAAGAACAACTCA
                  *********************   ************************************
Bala              TTTTTTACATGTTATTTGAGTGGTAATTGTAAATTAATTTATATATGTGGGTGTAGTGTT
IR64              TTTTTTACATGTTATTTGAGTGGTAATTGTAAATTAATTTATATATGTGGGTGTAGTGTT
DJ123             TTTTTTACATGTTATTTGAGTGGTAATTGTAAATTAATTTATATATGTGGGTGTAGTGTT
Nipponbare        TTTTTTACATGTTATTTGAGTGGTAATTGTAAATTAATTTATATATGTGGGTGTAGTGTT
Azucena           TTTTTTACATGTTATTTGAGTGGTAATTGTAAATTAATTTATATATGTGGGTGTAGTGTT
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Bala              GTTGATGGGGGATGAGGTGGCGGCGGCGGCGGCGACGATATCACCGGCGGCGGTGACGCG
IR64              GTTGATGGGGGATGAGGTGGCGGCGGCGGCGGCGACGATATCACCGGCGGCGGTGACGCG
DJ123             GTTGATGGGGGATGAGGTGGCGGCGGCGGCGGCGACGATATCACCGGCGGCGGTGACGCG
Nipponbare        GTTGATGGGGGATGAGGTGGCGGCGGCGGCGGCGACGATATCACCGGCGGCGGTGACGCG
Azucena           GTTGATGGGGGATGAGGTGGCGGCGGCGGCGGCGACGATATCACCGGCGGCGGTGACGCG
                  ************************************************************

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

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              CATCCCTCTCATCGGCAAGATCAACATCTAGTCGTCATTAAATTAATTAATTAATATAAT
IR64              CATCCCTCTCATCGGCAAGATCAACATCTAGTCGTCATTAAATTAATTAATTAATATAAT
DJ123             CATCCCTCTCATCGGCAAGATCAACATCTAGTCGTCATTAAATTAATTAATTAATATAAT
Nipponbare        CATCCCTCTCATCGGCAAGATCAACATCTAGTATTCATTAAATTAATTAATTAATATAAT
Azucena           CATCCCTCTCATCGGCAAGATCAACATCTAGTATTCATTAAATTAATTAATTAATATAAT
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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
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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/.