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Weifeng Yang
South China Agricultural University
Liang Xiong
South China Agricultural University
Jiayan Liang
South China Agricultural University
Qingwen Hao
South China Agricultural University
Xin Luan
South China Agricultural University
Quanya Tan
South China Agricultural University
Shiwan Lin
South China Agricultural University
Haitao Zhu
South China Agricultural University
Guifu Liu
South China Agricultural University
Zupei Liu
South China Agricultural University
Suhong Bu
South China Agricultural University
Shaokui Wang
South China Agricultural University
Guiquan Zhang (gqzhang@scau.edu.cn)
South China Agricultural University https://orcid.org/0000-0003-3322-2753

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Substitution mapping of two closely linked QTLs controlling grain chalkiness and grain shape on rice chromosome 8

Weifeng Yang, Liang Xiong, Jiayan Liang, Qingwen Hao, Xin Luan, Quanya Tan, Shiwan Lin, Haitao Zhu, Guifu Liu, Zupei Liu, Suhong Bu, Shaokui Wang*, Guiquan Zhang*

Guangdong Provincial Key Laboratory of Plant Molecular Breeding, State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, South China Agricultural University, Guangzhou 510642, China.

*Correspondence: gqzhang@scau.edu.cn; shaokuiwang@scau.edu.cn

Abstract

Background: Rice varieties are required to have high yield and good grain quality. Grain chalkiness and grain shape are two important traits of rice grain quality. Low chalkiness slender grains are preferred by most rice consumers. Here, we dissected two closely linked quantitative trait loci (QTLs) controlling grain chalkiness and grain shape on rice chromosome 8 by substitution mapping.

Results: Two closely linked QTLs controlling grain chalkiness and grain shape were identified using single-segment substitution lines (SSSLs). The two QTLs were then dissected on rice chromosome 8 by secondary substitution mapping. *qPGC8.1* was located in an interval of 1382.6 kb and *qPGC8.2* was mapped in a 2057.1 kb region. The maximum distance of the two QTLs was 4.37 Mb and the space distance of two QTL intervals was 0.72 Mb. *qPGC8.1* controlled grain chalkiness and grain width. *qPGC8.2* was responsible for grain chalkiness and for grain length and grain width. The additive effects of *qPGC8.1* and *qPGC8.2* on grain chalkiness were not affected by heat stress.

Conclusions: Two closely linked QTLs *qPGC8.1* and *qPGC8.2* were dissected on rice chromosome 8. They controlled the phenotypes of grain chalkiness and grain shape. The two QTLs were insensitive to high temperature.

Key words: grain chalkiness, grain shape, quantitative trait locus, heat stress, substitution mapping, rice

Background

Rice is an important food crop in the world. Rice varieties with higher head rice yield, higher transparency and less chalkiness are more popular in the market (Sreenivasulu et al. 2015; Misra et al. 2019). With the increase of living standard, rice varieties are required to have both a higher grain yield and a better grain quality. Grain chalkiness not only affects grain appearance, but also has adverse effects on milling and cooking performance. Chalkiness is a complex quantitative trait, which is easily affected by environments. In the early and middle stages of seed development, the occurrence of temperature stress will cause uneven seed filling and storage biosynthesis obstacles, leading to the formation of chalkiness (Masutomi et al. 2015; Sreenivasulu et al. 2015; Morita et al. 2016). The chalkiness of rice varieties varied greatly. It was found that the proportion of chalky grains of newly developed varieties was higher than that of the old modern varieties, and the proportion of chalky grains of the hybrid varieties was higher than that of other modern varieties (Laborte et al. 2015). Therefore, high-yielding varieties usually have higher chalkiness levels (Misra et al. 2019).

Percentage of grain chalkiness (PGC) is a quantitative index of grain chalkiness controlled by quantitative trait loci (QTLs) (Sreenivasulu et al. 2015; Misra et al. 2019). More than one hundred of QTLs controlling chalkiness were reported in rice genome (Sreenivasulu et al. 2015; Yang et al. 2021). Many QTLs for chalkiness were detected on chromosomes 5, 6 and 8 in different populations and environments (He et al. 1999; Tan et al. 2000; Wan et al. 2005; Hao et al. 2009; Liu et al. 2011; Chen et al. 2011; Guo et al. 2011; Liu et al. 2012; Li et al. 2014; Peng et al. 2014; Zhao et al. 2015; Chen et al. 2016; Gao et al. 2016; Yun et al. 2016; Zhao et al. 2016; Wang et al. 2017; Zhu et al. 2018a; Misra et al. 2019; Misra et al. 2020). On chromosome 8, the hotspot region of the QTLs for chalkiness was located on the long arm (Li et al. 2003; Wan et al. 2005; Hao et al. 2009; Guo et al. 2011; Liu et al. 2012; Gao et al. 2016; Zhao et al. 2016; Wang et al. 2017). Although a larger number of the chalk QTLs were detected on the hotspot chromosome region, only one of the QTLs was usually detected in a population. Therefore, it is not clear how many chalk QTLs there are in the hotspot chromosome region.

Grain chalkiness is a complex trait and easily affected by other genetic factors. Several fine-mapped target genes and GWAS loci have been found to influence chalkiness, but many have

either low or moderate effect (Gong et al. 2017; Wang et al. 2017; Quero et al. 2018 Misra et al. 2019). Although 11 GWAS loci for chalky grain rate were identified, the GWAS loci could only explain a small part of the phenotypic variation (Gong et al. 2017). Grain chalkiness and grain shape are two important traits for rice grain quality. Grain width was positively correlated with chalkiness (Zhao et al. 2015). It was found that some QTLs controlling grain width were overlapped with chalk QTLs (Wang et al. 2017). Misra et al. (2020) reported 78 chalk QTLs, but only three QTL regions overlapped with known grain width genes. The phenotypic variation for chalkiness showed a weak positive correlation with grain width. They believed that the key genes of grain width are not causal factors of chalkiness. Therefore, the genetic architecture of grain chalkiness and grain shape of rice remains unclear.

Substitution mapping is a powerful tool to detect QTLs in complex traits (Tan et al. 2021; Yang et al. 2021). Like near-isogenic lines (NILs), single-segment substitution lines (SSSLs) carry only one substitution segment from donors in the recipient genetic background (Zhang et al. 2004; Keurentjes et al. 2007). We have developed a library of 2360 SSSLs, which were derived from 43 donors of 7 species of rice AA genome in the genetic background of Huajingxian 74 (HJX74), an *indica* elite variety in southern China (Zhang et al. 2004; Xi et al. 2006; Zhang 2019). These SSSLs were widely used to detect QTLs for complex traits, to clone QTLs of agronomic importance and to mine alleles of different functions (Zeng et al. 2006; Teng et al. 2012; Wang et al. 2012; Zhang et al. 2012; Zhu et al. 2014; Yang et al. 2016; Zhou et al. 2017; Zhu et al. 2018b; Fang et al. 2019; Sui et al. 2019; Tan et al. 2020; Tan et al. 2021; Yang et al. 2021). Recently, the SSSLs were used to detect QTLs controlling stigma exsertion rate (SER) of rice. Two pairs of tightly linked QTLs for SER, *qSER-2a* and *qSER-2b* on chromosome 2 and *qSER-3a* and *qSER-3b* on chromosome 3, were dissected by substitution mapping (Tan et al. 2021). In previous study, we fine-mapped two QTLs *qPGC9* and *qPGC11* for grain chalkiness on rice chromosomes 9 and 11, which were sensitive to high temperature (Yang et al. 2021). In the present study, two closely linked QTLs for grain chalkiness on chromosome 8, *qPGC8.1* and *qPGC8.2*, were detected. The two QTLs were showed effect on grain chalkiness and grain shape, and insensitive to high temperature. Dissection of the two closely linked QTLs laid a foundation for revealing the genetic architecture of grain chalkiness and the relationship between grain chalkiness and grain shape in rice.

Results

Grain chalkiness in SSSLs

Two SSSLs 15-08 and 03-08 with lower grain chalkiness were selected from the HJX74-SSSL library (Fig. 1a). The SSSLs were used to investigate grain chalkiness in consecutive 6 cropping seasons from the first cropping season (FCS) of 2017 to the second cropping season (SCS) of 2019. On average, the PGC of 15-08 and 03-08 was 11.9% and 10.3% respectively, which were significantly lower than 21.2% of recipient HJX74 (Fig. 1b and Additional file 1: Table S1). It meant that the two SSSLs each carried a QTL for PGC on their substitution segments.

The substitution segments of 15-08 and 03-08 were surveyed by densifying molecular markers (Additional file 1: Table S2). The estimated length of substitution segments was 2726.7 kb in 15-08 and 4974.3 kb in 03-08. The two substitution segments overlapped in a 553.7 kb interval (Fig. 1c and Additional file 1: Table S3).

Eight agronomic traits of 15-08 and 03-08 were investigated. Most traits of the SSSLs had no significant difference with HJX74 (Fig. 1a and Additional file 1: Table S4). However, the grain shapes of 15-08 and 03-08 were significantly different with HJX74. For 15-08, the grain width was significantly narrower than that of HJX74, the former was 2.62 mm and 2.45 mm and the later was 2.69 mm and 2.64 mm in SCS of 2018 and in FCS of 2019, respectively. Compared with HJX74, 03-08 showed significantly longer in grain length and significantly narrower in grain width. In SCS of 2018 and in FCS of 2019, 03-08 had 9.14 mm and 8.88 mm in grain length and 2.55 mm and 2.40 mm in grain width, respectively (Additional file 1: Table S4). It is noted that *GW8* is outside the substitution segment of 03-08 (Fig. 1c). Therefore, the difference of grain shape between 03-08 and HJX74 was not controlled by *GW8* gene.

Substitution mapping of *qPGC8.1*

To map the QTL for grain chalkiness on the substitution segment of 15-08, the SSSL was used to develop secondary SSSLs or NILs. Four NILs were developed from an $F_{2:3}$ population derived from the cross of HJX74/15-08. The four NILs were then investigated for grain chalkiness. PGC levels of two NILs, NIL15-08-26 and NIL15-08-43, were as low as 15-08, while those of other two NILs, NIL15-08-4 and NIL15-08-14, were as high as HJX74. Substitution segments of the two NILs with low PGC overlapped in the region between markers RM4815 and RM23137, while substitution segments of other two NILs with high PGC located outside the region. These results indicated that the QTL for

grain chalkiness, *qPGC8.1*, was located in the region between markers RM4815 and RM23137 with the estimated interval length of 1382.6 kb (Fig. 2a-b).

Using RM8264 marker in *qPGC8.1* interval, Chi-square test was performed in 86 individuals of F_2 population. The results showed that the segregation ratio of the three marker genotypes was 1:2:1 (χ^2 = 0.51 < $\chi^2_{0.01, 2}$ = 9.21). The effect of heterozygous genotype (*qPGC8.1*/*qpgc8.1*) was significantly higher than that of dominant homozygous genotype (*qPGC8.1*/*qPGC8.1*) and significantly lower than that of recessive homozygous genotype (*qpgc8.1*/*qpgc8.1*). The result showed that *qPGC8.1* was incomplete dominance (Fig. 2c).

Like 15-08, the four NILs showed no-significant difference with HJX74 in grain length (Fig. 2d). However, the grain width segregated in the NILs. Two NILs, NIL15-08-26 and NIL15-08-43, with low chalkiness showed narrower in grain width as 15-08, while other two NILs, NIL15-08-4 and NIL15-08-14, with high chalkiness were wider in grain width as HJX74, the former was 2.56 mm and 2.58 mm and the later was 2.66 mm and 2.67 mm, respectively (Fig. 2e). The results indicated that *qPGC8.1* controlled grain width besides grain chalkiness.

Substitution mapping of *qPGC8.2*

To map the QTL for grain chalkiness on the substitution segment of 03-08, the SSSL was used to develop secondary SSSLs or NILs. Five NILs were developed from an $F_{2:3}$ population derived from the cross of HJX74/03-08. The five NILs were then investigated for grain chalkiness. PGC levels of three NILs, NIL03-08-9, NIL03-08-15 and NIL03-08-19, were as low as 03-08, while those of other two NILs, NIL03-08-14 and NIL03-08-55, were as high as HJX74. Substitution segments of the three NILs with low PGC overlapped in the region between markers SNP8M54 and SNP8M28, while substitution segments of other two NILs with high PGC located outside the region. These results indicated that the QTL for grain chalkiness, *qPGC8.2*, was located in the region between markers SNP8M54 and SNP8M28 with the estimated interval length of 2057.1 kb (Fig. 3a-b).

In the F_2 population of 100 individuals, segregation ratio of three marker genotypes of RM210 in *qPGC8.2* region was 1:2:1 (χ^2 = 1.34 < $\chi^2_{0.01, 2}$ = 9.21). The effect of heterozygous genotype (*qPGC8.2*/*qpgc8.2*) was significantly different from that of the homozygous genotypes (*qPGC8.2*/*qPGC8.2* and *qpgc8.2*/*qpgc8.2*). The result showed that *qPGC8.2* was incomplete dominance (Fig. 3c).

In substitution mapping, NILs segregated in grain length and grain width. Three NILs, NIL03-08-9, NIL03-08-15 and NIL03-08-19, with low chalkiness showed longer in grain length and narrower in grain width as 03-08, while other two NILs, NIL03-08-14 and NIL03-08-55, with high chalkiness had shorter in grain length and wider in grain width as HJX74. The three NILs with low chalkiness were 8.75 mm, 8.91 mm and 9.02 mm in grain length and 2.58 mm, 2.54 mm and 2.62 mm in grain width, while the two NILs with high chalkiness were 8.46 mm and 8.48 mm in grain length and 2.66 mm and 2.65 mm in grain width, respectively (Fig. 3d-e). The results indicated that *qPGC8.2* controlled grain shape besides grain chalkiness.

It was noted that *qPGC8.2* was closely linked with *qPGC8.1* on chromosome 8. The maximum distance of the two QTL location was 4.37 Mb, from markers RM4815 to SNP8M28. The space distance between the two QTL regions was 0.72 Mb, from markers RM23137 to SNP8M54 (Fig. 1c, Fig. 2b and Fig. 3b).

Effects of different cropping seasons on grain chalkiness

The grain chalkiness was tested in two cropping seasons per year. During flowering to harvest of rice, the day and night temperatures of FCS and SCS were very different. In 2017-2019, the average values of maximum, minimum and mean temperatures were 32.1℃, 25.9℃ and 29.0℃ in FCS, and 28.6℃, 21.0℃ and 24.9℃ in SCS, respectively. The mean temperature in SCS was 4.1℃ lower than that in FCS (Additional file 1: Table S5).

The grain chalkiness was lower in SCS than that in FCS in all lines. Percentage of chalky grains (PCG) and PGC were significantly different in all lines. Percentage of chalk area (PCA) was significantly different in 15-08, and no significantly different in 03-08 and HJX74. The PGC of HJX74, 15-08 and 03-08 was 25.8%, 15.9% and 13.6% in FCS, and 16.6%, 7.8% and 7.1% in SCS, respectively (Fig. 4). It is obvious that high temperature had great influence on PGC of HJX74 and SSSLs during seed filling period, mainly through the influence on PCG.

Additive effects of *qPGC8.1* and *qPGC8.2* on grain chalkiness

According to the estimation of chalkiness phenotype in 2017-2019, the additive effects of *qPGC8.1* and *qPGC8.2* had no significant difference between SCS and FCS. For *qPGC8.1*, the additive effects on PGC were -9.9% in FCS and -8.8% in SCS. For *qPGC8.2*, the additive effects on PGC in FCS and SCS

were -12.3% and -9.5%, respectively (Fig. 5). The results showed that *qPGC8.1* and *qPGC8.2* were insensitive to high temperature of FCS.

Discussion

Dissection of two closely linkage QTLs *qPGC8.1* and *qPGC8.2* on chromosome 8

Grain chalkiness is a complex polygenic quantitative trait (Sreenivasulu et al. 2015). More than one hundred of QTLs for the chalkiness traits were reported across all 12 chromosomes of rice genome (Sreenivasulu et al. 2015). On chromosome 8, many QTLs for chalkiness were located on the long arm in different populations and environments. In the hotspot region, however, only one chalk QTL was detected in each population (Li et al. 2003; Wan et al. 2005; Hao et al. 2009; Guo et al. 2011; Liu et al. 2012; Gao et al. 2016; Zhao et al. 2016; Wang et al. 2017). In the present study, we detected two closely linked QTLs, *qPGC8.1* and *qPGC8.2*, on the region of 19.01-23.38 Mb of chromosome 8. The maximum distance of the two QTL location was 4.37 Mb and the space distance between the two QTL regions was 0.72 Mb (Fig. 1c, Fig. 2b and Fig. 3b). Dissection of the two closely linked QTLs showed that the hotspot region of chromosome 8 contained multiple QTLs for grain chalkiness. The results revealed the genetic architecture of grain chalkiness on the hotspot region of chromosome 8.

Recently, two pairs of tightly linked QTLs controlling SER, *qSER-2a* and *qSER-2b* on chromosome 2 and *qSER-3a* and *qSER-3b* on chromosome 3, were dissected by substitution mapping. On chromosome 2, two linkage QTLs, *qSER-2a* and *qSER-2b*, were located in the region of 1288.0 kb, and were respectively delimited to the intervals of 234.9 kb and 214.3 kb. On chromosome 3, two QTLs, *qSER-3a* and *qSER-3b*, were detected in the region of 3575.5 kb and were narrowed down to 319.1 kb and 637.3 kb, respectively (Tan et al. 2021). Together, those results indicated that substitution mapping using SSSLs is a powerful tool for dissection of closely linked QTLs for complex traits.

Relationship of grain chalkiness and grain shape

Grain chalkiness and grain shape are two important traits for rice grain quality, which relationship is complicated. It was found that grain width had a negative pleiotropic effect on grain chalkiness (Zhao et al. 2015). However, grain chalkiness was weakly positively correlated with phenotypic variation of grain width, and the key genes affecting grain width were not the cause of chalkiness (Misra et al. 2020). It was also found that although several fine-mapped target genes and GWAS loci have been

found to influence chalkiness, many have either low or moderate effect (Gong et al. 2017; Wang et al. 2017; Quero et al. 2018; Misra et al. 2019). Recently, we detected two QTLs for grain chalkiness, *qPGC9* and *qPGC11*, by substitution mapping, and found that they had no effect on grain shape (Yang et al. 2021). Therefore, the relationship of grain chalkiness and grain shape in rice remains unclear. In the present study, two closely linked QTLs, *qPGC8.1* and *qPGC8.2*, on chromosome 8 showed effects on grain chalkiness and grain shape (Fig. 2 and Fig. 3). In addition, it is noted that the *fgr* gene for fragrance (Bradbury et al. 2005) is located not only on the substitution segment of 15-08 but also in the *qPGC8.1* mapped region, and the *GW8* gene controlling grain width (Wang et al. 2012) is located near the *qPGC8.2* mapped region although it is outside the substitution segment of 03-08 (Fig. 1c). These results indicated that the long arm of chromosome 8 may be a gene cluster area for grain development. It is unclear that each of the regions of *qPGC8.1* and *qPGC8.2* carries two different genes, which controlled grain chalkiness and grain shape respectively, or carries one gene controlling grain chalkiness with pleiotropic effects on grain shape. Therefore, the mapping of *qPGC8.1* and *qPGC8.2* laid a foundation for revealing the relationship between chalkiness and grain shape and its molecular mechanism.

Influence of high temperature on the effect of chalk QTLs

It is found that the high temperature during the seed filling stage caused uneven grain filling and resulted in chalk formation (Sreenivasulu et al. 2015; Masutomi et al. 2015; Morita et al. 2016; Ishimaru et al. 2019). In Guandong province of China, rice is planted in two cropping seasons per year. During the seed filling period, the air temperature of FCS is usually higher than that of SCS. In 2017-2019, the mean temperature of FCS was 4.1℃ higher than that of SCS (Additional file 1: Table S5). It led to grain chalkiness of all lines in FCS was significantly higher than that in SCS (Fig. 4). Obviously, the grain chalkiness of HJX74 and SSSLs was greatly affected by higher temperature during the seed filling period.

Some QTLs for chalkiness were detected under high temperature stress (Nevame et al. 2018). Kobayashi et al. (2007) detected three QTLs for chalkiness in *japonica* varieties under heat stress. Tabata et al. (2007) identified four QTLs for chalkiness in a RIL population derived from a cross between a heat stress-tolerant variety and a heat stress-sensitive variety. Wada et al. (2015) and Miyahara et al. (2017) identified a set of chalk QTLs under heat stress condition using a RIL

population derived from a cross between a heat-tolerant variety and a heat stress-sensitive variety. Recently, we mapped two QTLs for grain chalkiness, *qPGC9* and *qPGC11*, and found that the additive effects of *qPGC9* and *qPGC11* on chalkiness in SCS were almost twice of those in FCS. The additive effects of *qPGC9* and *qPGC11* on chalkiness were decreased by heat stress in FCS. Therefore, the *qPGC9* and *qPGC11* were sensitive to high temperature (Yang et al. 2021). In the present study, we found that although grain chalkiness of HJX74 and SSSLs was greatly affected by higher temperature during the seed filling period, the additive effects of *qPGC8.1* and *qPGC8.2* on PCG, PCA and PGC were no significant difference in different cropping seasons (Fig. 5). The results showed that the additive effects of *qPGC8.1* and *qPGC8.2* on grain chalkiness were not affected by the high temperature in FCS. It indicates that *qPGC8.1* and *qPGC8.2* were insensitive to high temperature of FCS, which reactions are different from *qPGC9* and *qPGC11*. Therefore, *qPGC8.1* and *qPGC8.2* will be useful for breeding of rice varieties with lower grain chalkiness under heat stress.

Conclusion

Two closely linked QTLs *qPGC8.1* and *qPGC8.2* controlling grain chalkiness and grain shape were located on chromosome 8. The effect of *qPGC8.1* and *qPGC8.2* was incomplete dominance. The additive effects of the two QTLs on grain chalkiness were no significant difference between FCS and SCS. The *qPGC8.1* and *qPGC8.2* were insensitive to the high temperature in FCS. The mapping of *qPGC8.1* and *qPGC8.2* laid a foundation for revealing the relationship between grain chalkiness and grain shape and its molecular mechanism. The two QTLs will be useful for breeding of rice varieties with lower grain chalkiness under heat stress.

Materials and methods

Rice materials and cropping seasons

Two SSSLs 03-08 and 15-08 with lower grain chalkiness were selected from the HJX74-SSSL library. The substitution segment of 03-08 was from the donor Zhong4188 and that of 15-08 was from the donor American Jasmine. The donors of both SSSLs are *indica* varieties. All rice materials were planted at the farm of South China Agricultural University, Guangzhou (23°07′N, 113°15′E) from 2017 to 2019. The materials were planted in two cropping seasons per year, the FCS from late February to middle July and the SCS from late July to middle November. Rice cultivation and controlling of

diseases and insect pests were common practices in southern China.

Measurement of grain chalkiness

The seeds of each line were harvested after full maturity. The dried seeds of 10 plants of each line were processed into milled rice, and 200 head rice of each plant were randomly selected for measurement of chalkiness (Yang et al. 2021). Images of the head rice were captured and the chalkiness parameters were measured by Microtek ScanWizard EZ scanner and rice quality analyzer SC-E software (Hangzhou Wanshen Detection Technology Co., Ltd., Hangzhou, China, www.wseen.com). PGC is the product of PCG in total grains multiplied by PCA per chalky grain.

Phenotyping of traits and statistical analysis

Heading date, plant height and panicle number per plant was investigated in the field. Grain traits were measured by the yield traits scorer (YTS), a rice phenotypic facility (Yang et al. 2014). The percentages were converted to the arcsine square root for statistical analysis. The student's *t*-test was used for comparison between two groups. Dunnett *t*-test was used to compare multiple groups with control group. Least significance range (LSR) was used for multiple range test among multiple groups (Duncan 1955). The data analysis and figure making were done by SPSS statistics 23.0 and OriginPro 9.0 (https://www.originlab.com).

Genotyping of molecular markers and substitution mapping

Molecular markers labeled "RM" were selected from online resources (https://archive.gramene.org/markers/). New markers used in this study were designed using the software of Primer Premier 5.0 (Lalitha 2000). The DNA samples were amplified by PCR method. The PCR products were separated by gel electrophoresis on 6% denatured PAGE and detected by the silver staining method (Tan et al. 2020). To develop secondary SSSLs or NILs, 03-08 and 15-08 were crossed with the recipient HJX74. The NILs were developed from $F_{2:3}$ populations derived from the crosses. Minimum length, maximum length and estimated length of a substitution segment were estimated as described by Tan (2020). When PGC showed significant difference between SSSL genotype and HJX74 genotype, a QTL for PGC was detected on the substitution segment of SSSL. When multiple substitution segments overlapped in NILs, the QTL was located in the overlapping region (Eshed and

Zamir, 1995; Tan et al. 2020). Additive effect of a QTL was defined as the phenotypic difference between SSSL and HJX74 (Zhou et al. 2020). QTLs were named followed the method of McCouch et al. (1997).

Abbreviations

FCS: First cropping season; HJX74: Huajingxian 74; NIL: Near-isogenic line; PCA: Percentage of chalky area; PCG: Percentage of chalky grain; PGC: Percentage of grain chalkiness; QTL: Quantitative trait locus; SCS: Second cropping season; SER: Stigma exsertion rate; SSSL: Single-segment substitution line.

Declarations

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Availability of data and materials

All data generated or analyzed in this study are included in this published article and its additional information files.

Authors' contributions

GZ and SW designed and supervised the works. WY performed most of the experiments and analyzed the experimental data and prepared the draft of manuscript. LX, JL, QH, XL, QT, SL, ZL, SB, HZ, and GL conducted a part of experiments. GZ analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable**.**

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figure legends

Fig. 1 Grain chalkiness in HJX74 and SSSLs. **a**, Plant types of HJX74 and SSSLs 15-08 and 03-08. Scale bar: 15 cm. **b**, Percentage of grain chalkiness (PGC) (%) in HJX74 and SSSLs. Data were presented as mean ± S.E. in six cropping seasons, one-way ANOVA, two-tailed, Dunnett *t*-test was used to test the differences, with HJX74 as control, ** represent $P \leq 0.01$. **c**, Chromosome locations of the two SSSLs. Physical distance (Mb) is shown as rulers on the right of chromosome. Black bars on the left of the chromosome 8 represent the estimated length of substitution segments in the SSSLs with their code on the left. The functional markers fgr and GW8 represent the loci of *fgr* gene for fragrance and *GW8* gene controlling grain width. *Chr.* chromosome, *Mb* megabase.

Fig. 2 Substitution mapping of *qPGC8.1* for grain chalkiness. **a**, The milled rice of the HJX74 and SSSL 15-08. Scale bar: 1 cm. **b**, Substitution mapping of *qPGC8.1*. The positions of substitution segments and the percentage of grain chalkiness (PGC) of 15-08 and its NILs are shown, with HJX74 as the control. The numbers under the chromosome indicate physical distance (Mb). White and black blocks represent the homozygous genotypes of HJX74 and 15-08, respectively. PGC (%) was a mean ± S.E. in two cropping seasons. **c**, PGC of three genotypes of *qPGC8.1* in an F_2 population.

qpgc8.1/*qpgc8.1* represents homozygous genotype of HJX74 (n = 24); *qPGC8.1*/*qpgc8.1* represents heterozygous genotype of HJX74/15-08 (n = 40); *qPGC8.1*/*qPGC8.1* represents homozygous genotype of 15-08 (n = 22). **d**, Grain length of 15-08 and its NILs. **e**, Grain width of 15-08 and its NILs. Significant difference analysis was by one-way ANOVA, Duncan, two-tailed. Values in the lines among different letters are different at 1% level of significance in **b** and at 5% level of significance in **c**, **d** and **e**.

Fig. 3 Substitution mapping of *qPGC8.2* for grain chalkiness. **a**, The milled rice of the HJX74 and SSSL 03-08. Scale bar: 1 cm. **b**, Substitution mapping of *qPGC8.2*. The positions of substitution segments and the percentage of grain chalkiness (PGC) of 03-08 and its NILs are shown, with HJX74 as the control. The numbers under the chromosome indicate physical distance (Mb). White and black blocks represent the homozygous genotypes of HJX74 and 03-08, respectively. PGC (%) was a mean ± S.E. in two cropping seasons. **c**, PGC of three genotypes of *qPGC8.2* in an F_2 population. *qpgc8.2*/*qpgc8.2* represents homozygous genotype of HJX74 (n = 23); *qPGC8.2*/*qpgc8.2* represents heterozygous genotype of HJX74/03-08 (n = 47); *qPGC8.2*/*qPGC8.2* represents homozygous genotype of 03-08 (n = 30). **d**, Grain length of 03-08 and its NILs. **e**, Grain width of 03-08 and its NILs. Significant difference analysis was by one-way ANOVA, Duncan, two-tailed. Values in the lines among different letters are different at 1% level of significance in **b** and at 5% level of significance in **c**, **d** and **e**.

Fig. 4 The difference of chalky traits between first cropping seasons (FCS) and second cropping seasons (SCS) in HJX74, 15-08 and 03-08. **a**, Percentage of chalky grain (PCG). **b**, Percentage of chalky area (PCA). **c**, Percentage of grain chalkiness (PGC). *, ** and *** indicate the difference at 0.05, 0.01 and 0.001 levels of significance, respectively. *NS* no significance.

Fig. 5 The additive effects of *qPGC8.1* and *qPGC8.2* on grain chalkiness in first cropping seasons (FCS) and second cropping seasons (SCS). **a**, Percentage of chalky grain (PCG). **b**, Percentage of chalky area (PCA). **c**, Percentage of grain chalkiness (PGC). *NS* no significance.

Supplementary Information

Additional file 1: Table S1. Phenotypes of chalky traits in SSSLs. **Table S2.** Markers developed to detect the substitution segments of SSSLs. **Table S3.** Substitution segments of SSSLs. **Table S4.** Phenotypes of agronomic traits in SSSLs. **Table S5.** Average temperatures for 30 days after flowering

541 of rice in two cropping seasons (XLSX 21 kb)

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Figures

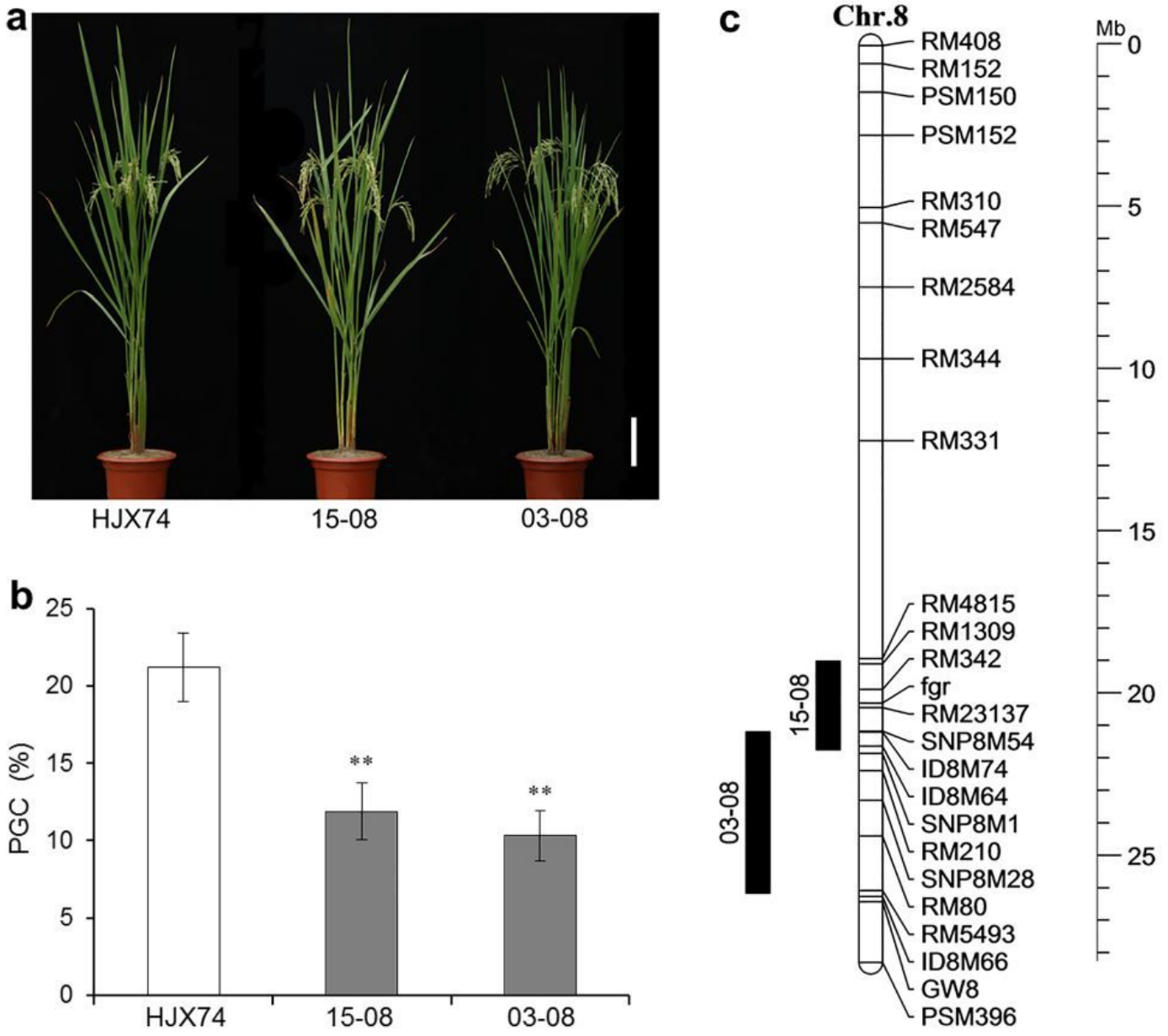

Figure 1

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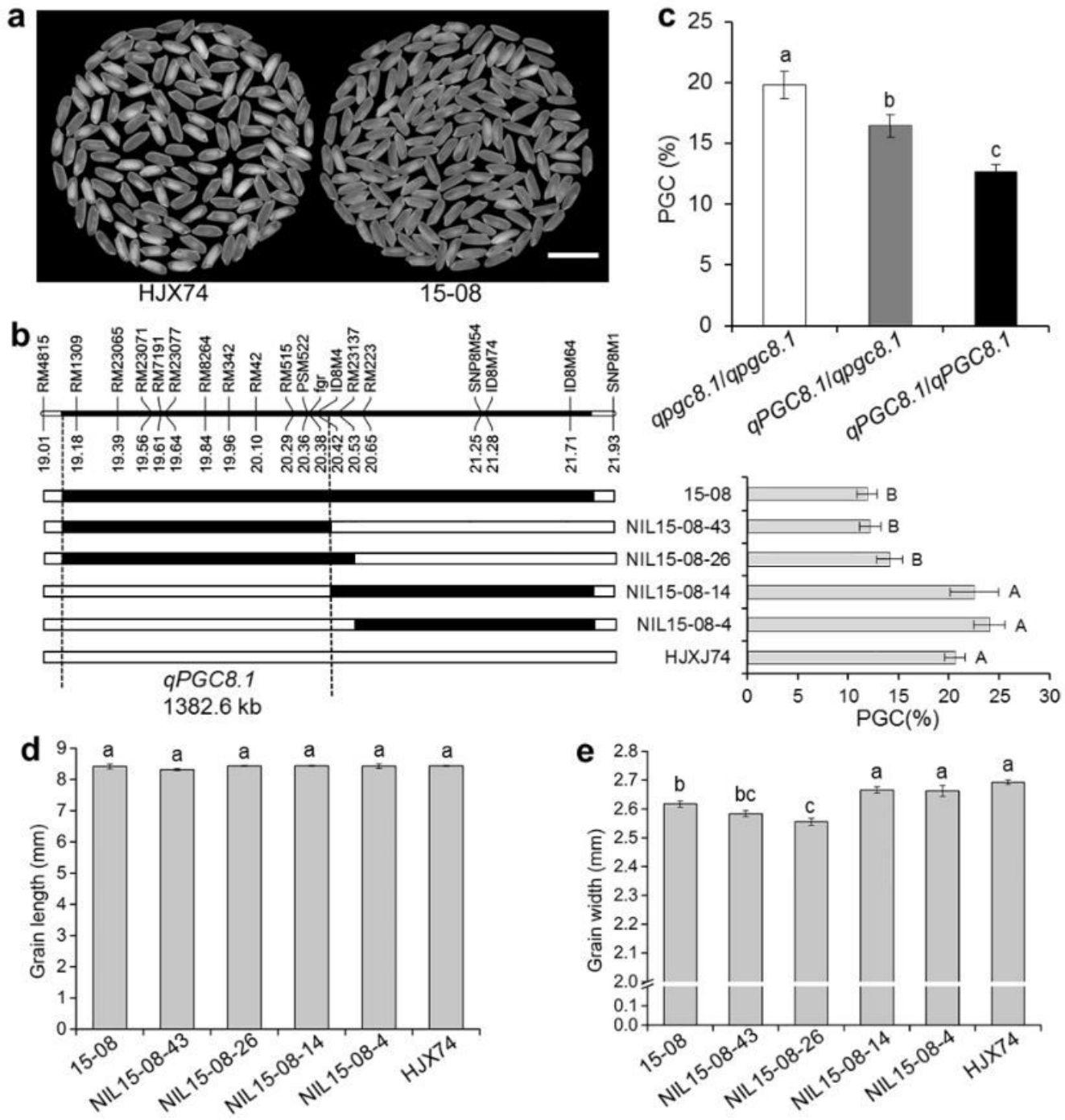

Figure 2

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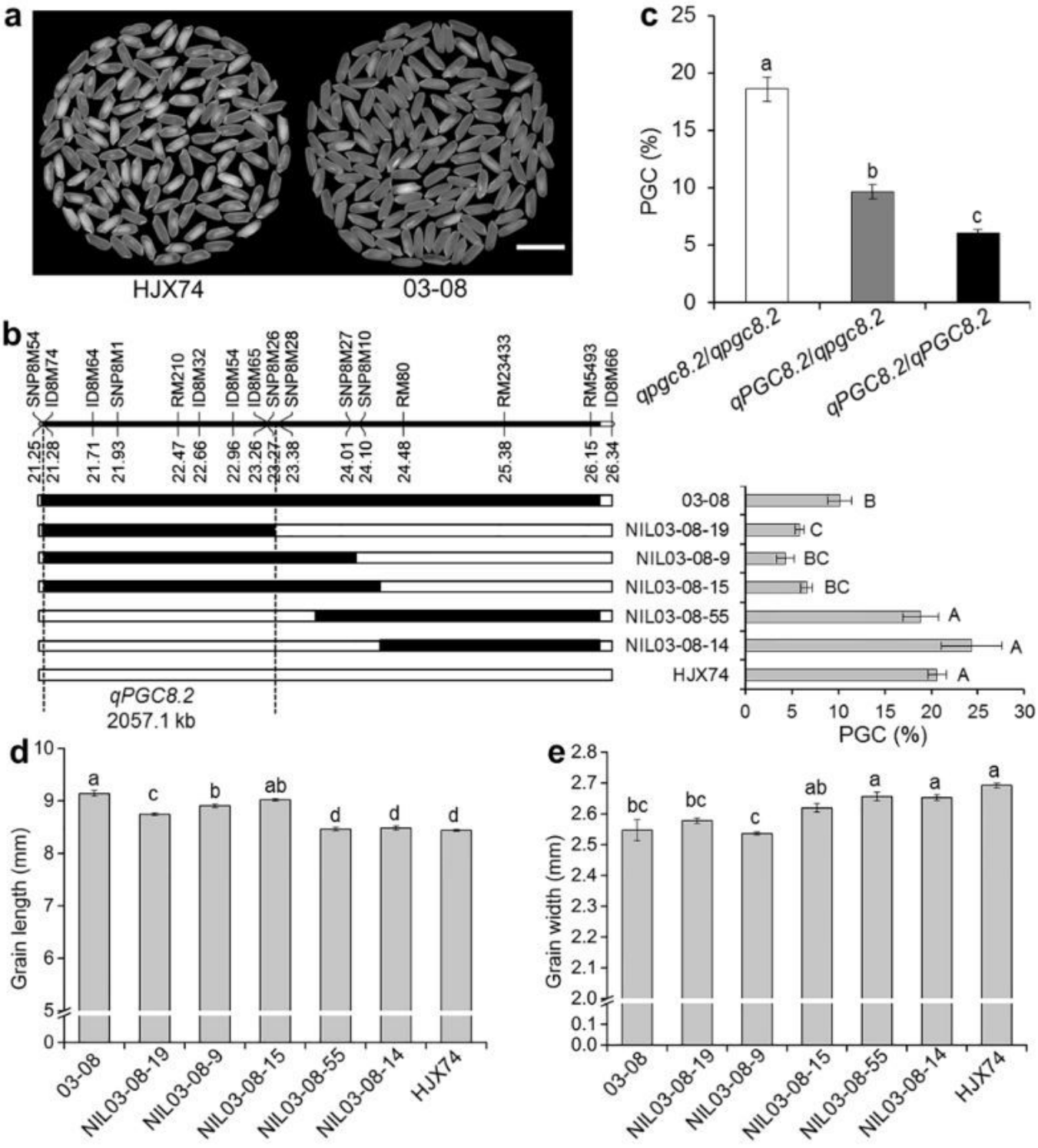

Figure 3

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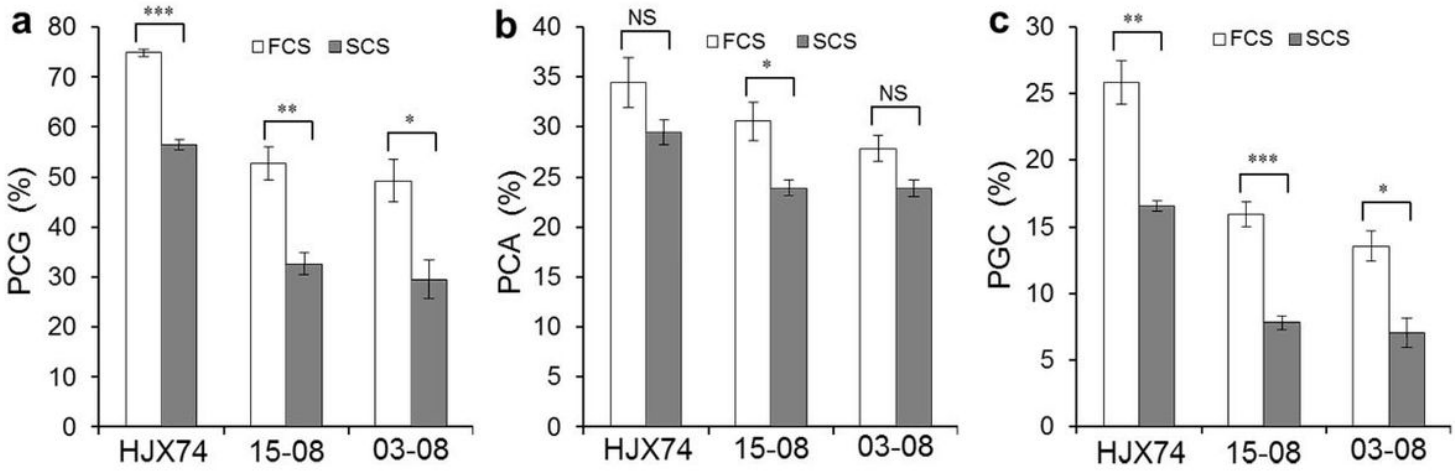

Figure 4

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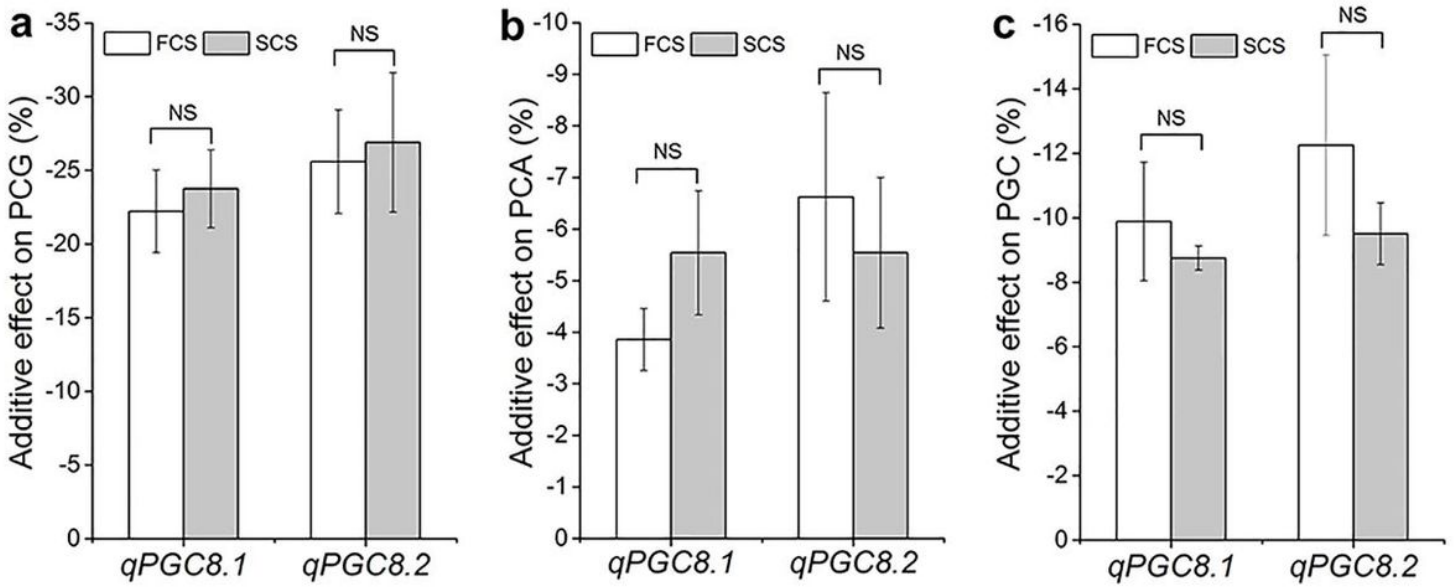

Figure 5

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