

Identification QTLs and Effective Factors on Grain Appearance Quality in Rice

Hossein Nohtani¹, R. Amirifahlani², B.A. Fakheri³, A. Masomiasl⁴

¹M.Sc. Department of Plant Breeding and Biotechnology, Faculty of Agriculture, University of Zabol, Iran

^{2,4}Assistant Professor Department of Agronomy and Plant Breeding, Faculty of Agriculture, Yasouj University, Iran

³Associate Professor Department of Plant Breeding and Biotechnology, Faculty of Agriculture, University of Zabol, Iran

ABSTRACT

Rice (*Oryza sativa* L.), a staple cereal crop, feeds more than half of the world's population. Improvement of rice yield and grain quality is the major objective of rice breeding worldwide. Grain quality primarily includes grain appearance, milling, eating and cooking and nutrition qualities. Grain appearance quality mainly includes grain shape and chalkiness. Grain shape, described by grain length (GL), grain width (GW), grain thickness (GT) and grain length to width ratio (GLWR), contributes to grain weight and yield and has a great impact on the market values of rice grain products. Grain chalkiness is an important grain quality related to starch granules in the endosperm. A high percentage of grain chalkiness is a major problem because it diminishes grain quality in rice. Grain size is a major determinant of grain weight, one of the three components (number of panicles per plant, number of grains per panicle and grain weight) of grain yield. In breeding applications, grain size is usually evaluated by grain weight, which is positively correlated with several characters including grain length, grain width and grain thickness. Grain size is also a highly important quality trait in rice. Recently, multiple naturally occurring quantitative trait loci (QTLs) regulating grain length, grain shape and grain width have been cloned and studied.

Keywords : Rice, Grain Appearance Quality, Grain Length , Grain width, QTLs.

I. INTRODUCTION

Rice is one of the most important cereal crops worldwide, and is also the staple food of Asia. Grain size is one of the most important agronomic traits of grain yield. Grain size, which is specified by grain length, width and thickness. Several regulators of grain size have been identified in rice (Song et al., 2007; Shomura et al., 2008; Weng et al., 2008; Wang et al., 2012; Zhang et al., 2012), although the genetic and molecular mechanisms of these factors in grain size regulation are largely unknown. As the world population increases, food security largely depends on our ability to improve grain yield through crop breeding (Gupta et al., 2006). Thousand-grain weight (TGW), an important agronomic trait, is significantly associated with grain yield. This trait is primarily determined by grain width (GW), grain length (GL) and grain thickness (GT) (Dholakia et al., 2003), to which it is positively related (Brescghello and Sorrells, 2006; Sun et al., 2009). Grain

shape, described by grain length (GL), grain width (GW), grain thickness (GT) and grain length to width ratio (GLWR), contributes to grain weight and yield and has a great impact on the market values of rice grain products. (Unnevehr et al., 1992). Chalkiness, according to its location within the endosperm, can be divided into white belly, white core and white back in rice grain (Tan et al., 2000). Recently, multiple naturally occurring quantitative trait loci (QTLs) regulating grain size have been cloned and studied, providing insights into the molecular basis of the regulation of grain size (Shomura et al., 2008; Weng et al., 2008; Li et al., 2011; Qi et al., 2012; Wang et al., 2012; Zhang et al., 2012). Since rice grain length and breadth are quantitatively inherited, it is difficult for the breeders to efficiently improve grain appearance using conventional selection methods (Tan et al. 2000). It would be particularly helpful for enhancing breeding efficiency to use molecular markers closely linked to the genes or major quantitative trait loci (QTLs) for grain dimensions for selecting target

genotypes directly in the early generations of breeding cycle. Unlike other cereals, much of the rice produced in the world is cooked and consumed as whole kernel therefore grain shape and size are important quality parameters for consumer acceptance. A study was conducted by Suwannaporn and Linnemann (2008) on rice eating quality among consumers in different rice grain preference countries, which showed that rice texture, was the best discriminator. It could correctly predict consumers from non-specific grain preference, short grain preference and basmati preference at 63, 71 and 81%, respectively.

Th effect of High temperatures during grain filling on Grain appearance quality

High temperatures during grain filling often cause serious damage to the grain quality of rice (*Oryza sativa*L.) (Terashima et al., 2001; Lanning et al., 2011; Kondo et al., 2012), reducing the proportion of first-grade rice (Hasegawa et al. 2009) or milling quality (Lyman et al., 2013). One of the main reasons for the reduction is the decreased percentage of undamaged grains, which is due to increased proportions of chalky and unfilled grains. The occurrence of chalky grains under high temperatures is attributable mainly to the inhibition of starch accumulation (Morita, 2008; Morita and Nakano, 2011). Daily mean temperatures $>26^{\circ}\text{C}$ during the grain-filling period cause chalkiness in the grains of japonica cultivars (Morita 2008). The cumulative temperature above 26°C within 15 days after heading can be used as an index of the extent of chalky grains (Lur et al., 2009).

Grain Chalkiness

Chalkiness is a major concern in rice breeding because it is one of the key factors in determining quality and price. The chalky endosperm consists of loosely packed, round and large compound starch granules while the translucent endosperm comprises tightly packed, polyhedral and small single starch granules. The chalky grains show significantly different physicochemical, morphological, thermal, cooking and textural properties from translucent grains. Percentage of grains with chalkiness (PGWC) is one of the main indices of rice-determining appearance quality, which is easily determined. Many factors contribute to the formation of

chalkiness in the rice grain. Environmentally, rice grown at the higher temperature contains more chalky grains (Yoshioka. et al., 2007).

Overe expression of L-GalLDH in rice leads to reduced grain chalkiness

Ascorbic acid (Asc) is considered an excellent water-soluble antioxidant in plants and animals. It is widely associated with photosynthetic function and stress tolerance in plants and must be obtained regularly from dietary sources by animals, which cannot synthesize it (Gallie, 2013). The importance of endogenous Asc in plants has been proposed to be its function as a cofactor for many enzymes and as a regulator of cell division and growth, as well as in signal transduction (Pignocchi and Foyer, 2003). Several studies have been performed to investigate whether the overexpression of L-GalLDH would affect the accumulation of Asc. For example, it has been reported that overexpression of L-GalLDH leads to an increase of Asc content in tobacco suspension cells (Tokunaga et al., 2005). Ascorbic acid (Asc) is a major plant antioxidant that performs many functions in plants. L-Galactono-1,4-lactone dehydrogenase (L-GalLDH, EC1.3.2.3) is an enzyme that catalyzes the final step of Asc biosynthesis in plants. (Bartoli et al., 2000). the L-GalLDH-overexpressing transgenic rice, GO-2, which has constitutively higher leaf Asc content than wild-type (WT) plants, exhibits significantly reduced grain chalkiness.6

Fine mapping of grain length QTLs

Grain size and shape determine the appearance of rice and affect milling, cooking and eating qualities, and are therefore important quality traits in rice breeding programs (Luo and Lin, 1990). Redona and Mackill (1998) identified seven QTLs for grain length on chromosomes 2, 3, 4 and 7 that together explained 42% of the phenotypic variation. Xu et al. (2004) identified eight QTLs for grain length explaining 65.9% of the phenotypic variations, two of these with major effect were located on chromosomes 3 and 5. Wan et al. (2005) reported twenty two QTLs for grain dimension distributed over eight rice chromosomes. Thus, grain dimension QTLs have been identified on almost all the 12 rice chromosomes, but accurate chromosomal

locations have been determined only for a small number of major consistent QTLs.

QTLs for Grain width

GRAIN WIDTH 5 (GW5) is a major QTL for seed width on chromosome 5 (qSW5) (Wan et al., 2008; Weng et al., 2008; Shomura et al., 2008). A survey of GW5/qSW5 polymorphisms in various rice landraces has revealed that deletions in this gene may have played an important role in the selection of increased grain size from artificial and natural crossings during rice domestication (Shomura et al., 2008). The GW5/qSW5 gene encodes a nuclear protein of 144 amino acids with an arginine-rich domain. Because GW5/qSW5 physically interacts with polyubiquitin, it is likely to act as a regulator in the ubiquitin–proteasome pathway and regulates cell division of the outer glume of the rice spikelet (Wan et al., 2008; Weng et al., 2008; Shomura et al., 2008). GRAIN SIZE ON CHROMOSOME 5 (GS5) is a major QTL affecting grain width, grain filling, and grain weight (Li et al., 2011). It encodes a serine carboxy peptidase and functions as a positive regulator of grain size. Analysis of genomic DNA sequences and promoter swaps in transgenic plants reveals that nucleotide changes in three segments of the GS5 promoter seem to be responsible for the variations in grain width (Li et al., 2011). GRAIN WIDTH 8 (GW8) is a major QTL affecting grain width and grain yield from the cross between HXJ74 and Basmati385 (Wang et al., 2012), which encodes SQUAMOSA promoter binding protein-like 16, referred to OsSPL16, belonging to the protein family of SBP domain containing transcription factors. Six polymorphisms in the DNA sequence of OsSPL16 exist in the parents HXJ74 and Basmati 385. Among them, a 10-bp deletion in the promoter region has been shown to be responsible for the slender grain trait of Basmati 385 (Wang et al., 2012).

QTLs for Grain shape

Grain shape, a typical complex quantitative trait, is closely associated with grain weight and usually measured by grain length (GL), width (GW), thickness (GT) and length-to-width ratio (LWR) (Lin and Wu, 2003; Yoon et al., 2006). LWR is considered to be an

important measure of rice appearance quality since people's preferences for it are rather different in the rice-producing areas of the world (Wan et al., 2006, 2008). A total number of 24 QTLs were detected on seven different chromosomes. QTLs for GL, GW, GT and LWR explained 11.6%, 95.62%, 91.5% and 89.9% of total phenotypic variation, respectively. Based on QTL identified with relatively large phenotypic effects, some genes related to Kitagawa et al. (2010), grain shape have been isolated and cloned in rice, such as GW2 (Song et al., 2007), gw5/qSW5 (Shomura et al., 2008; Weng et al., 2008; Wan et al., 2008), GIF1/OsCIN2 (Wang et al., 2008, 2010), srs-3 (Tanabe et al., 2007), GS5 (Yu et al. 1997; Li et al. 2000, 2011), GS3 (Fan et al., 2006; Takano-Kai et al., 2009, 2011; Mao et al., 2010; Wang et al., 2011), qGW8/OsSPL16 (Wang et al., 2012a, b), SG1 (Nakagawa et al., 2012), and DEP1/qPE9-1 (Yan et al., 2007; Zhou et al., 2009; Huang et al., 2009; Yi et al., 2011; Taguchi-Shiobara et al., 2011; Sun et al., 2014). Positive regulator PGL1 and PGL2 were found in the network of grain shape genes (Heang and Sassa, 2012a, b), and the relationship of four grain shape genes GS3, GW2, gw5/qSW5, and GIF1 has been studied (Yan et al., 2011). However, the gene-to-trait pathway and relationship between genes identified by different characters are largely unknown (Huang et al., 2013).

II. REFERENCES

- [1] Bartoli CG, Pastori GM, Foyer CH. 2000. Ascorbate biosynthesis in mitochondria is linked to the electron transport chain between complexes III and IV, *Plant Physiol.* 123:335–344
- [2] Breseghello F, Sorrells ME. 2006. Association mapping of kernel size and milling quality in wheat (*Triticum aestivum* L.) cultivars. *Genetics* 172:1165–1177
- [3] Dholakia BB, Ammiraju JSS, Singh H, Lagu MD, Dhaliwal HS, Ranjekar PK, Gupta VS, Weber WE. 2003. Molecular marker analysis of kernel size and shape in bread wheat. *Plant Breed* 122:392–395
- [4] Fan C, Xing Y, Mao H, Lu T, Han B, Xu C, Li X, Zhang Q. 2006. GS3, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. *Theor Appl Genet* 112:1164–1171
- [5] Gallie DR 2013.. The role of L-ascorbic acid recycling in responding to environmental stress and in promoting plant growth, *J. Exp. Bot.* 64: 433–443.
- [6] Gupta PK, Rustgi S, Kumar N. 2006. Genetic and molecular basis of grain size and grain number and its

- relevance to grain productivity in higher plants. *Genome* 49:565–571
- [7] Hasegawa T, Kuwagata T, Nishimori M, Ishigooka Y, Murakami M, Yoshimoto M, Kondo M, Ishimaru T, Sawano S, Masaki Y, Matsuzaki H. 2009. Recent warming trends and rice growth and yield in Japan. In: Hasegawa T, Sakai H (eds) *Proceeding of the MARCO Symposium*. National Institute for AgroEnvironmental Sciences, Tsukuba, Japan, pp 44–51, 5–9.
- [8] Heang D, Sassa H. 2012a. Antagonistic actions of HLH/bHLH proteins are involved in grain length and weight in rice. *Plos One* 7(2):e31325
- [9] Heang D, Sassa H. 2012b. An atypical bHLH protein encoded by POSITIVE REGULATOR OF GRAIN LENGTH 2 is involved in controlling grain length and weight of rice through interaction with a typical bHLH protein APG. *Breed Sci* 62:133–141
- [10] Huang R, Jiang L, Zheng J, Wang T, Wang H, Huang Y, Hong Z. 2013. Genetic bases of rice grain shape: so many genes, so little known. *Trends Plant Sci* 18:218–226
- [11] Kitagawa K, Kurinami S, Oki K, Abe Y, Ando T, Kono I, Yano M, Kitano H, Iwasaki Y. 2010. A novel kinesin 13 protein regulating rice seed length. *Plant Cell Physiol* 51:1315–1329
- [12] Kondo M, Iwasawa N, Yoshida H, Nakagawa H, Ohno H, Nakazono K, Usui Y, Tokida T, Hasegawa T, Kuwagata T, Morita S, Nagta K. 2012. Factors influencing the appearance quality in rice under high temperature in 2010. *Jpn J Crop Sci* 81(Extra issue 1):120–121
- [13] Lanning SB, Siebenmorgen TJ, Counce PA, Ambardekar AA, Mauromoustakos 2011. Extreme nighttime air temperatures 2010 impact rice chalkiness and milling. *Field Crops Res* 124:132–136, doi.org/10.1016/j.fcr.2011.06.012
- [14] Li J, Yu S, Xu C, Tan Y, Gao Y, Li X, Zhang Q. 2000. Analyzing quantitative trait loci for yield using a vegetatively replicated F2 population from a cross between the parents of an elite rice hybrid. *Theor Appl Genet* 101:248–254
- [15] Li Y, Fan C, Xing Y, Jiang Y, Luo L, Sun L, Shao D, Cu C, Li X, Xiao J, He Y, Zhang Q. 2011. Natural variation in GS5 plays an important role in regulating grain size and yield in rice. *Nat Genet* 43:1266–1269
- [16] Lin LH, Wu WR. 2003. Mapping of QTLs underlying grain shape and grain weight in rice. *Mol. Plant Breed.* 1, 337e342
- [17] Luo YK, Lin RH. 1990. Quality and processing of rice in china. In: Xiong ZM, Cai HF (eds) *Rice in china*. China Agriculture Science and Technology Publisher, Beijing, pp 164–181
- [18] Lur HS, Wu YC, Chang SJ, Lao CL, Hsu CL, Kondo M. 2009. Effects of high temperature on yield and grain quality of rice in Taiwan. In: Hasegawa T, Sakai H (eds) *Proceeding of the MARCO Symposium*. National Institute for Agro-Environmental Sciences, Tsukuba, Japan, pp 38–43, 5–9.
- [19] Mao H, Sun S, Yao J, Wang C, Yu S, Xu C, Li X, Zhang Q. 2010. Linking differential domain functions of the GS3 protein to natural variation of grain size in rice. *Proc Nat Acad Sci* 107:19579–19584
- [20] Morita S, Nakano H. 2011. Nonstructural carbohydrate content in the stem at full heading contributes to high performance of ripening in heat-tolerant rice cultivar Nikomaru. *Crop Sci* 51:818–828. doi:10.2135/cropsci2010.06.0373
- [21] Morita S. 2008. Prospect for developing measures to prevent high-temperature damage to rice grain ripening. *Jpn J Crop Sci* 77:1–12
- [22] Nakagawa H, Tanaka A, Tanabata T, Ohtake M, Fujioka S, Nakamura H, Ichikawa H, Mori M. 2012. SHORT GRAINS decreases organ elongation and brassinosteroid response in rice. *Plant Physiol* 158:1208–1219
- [23] Pignocchi C, Foyer CH. 2003. Apoplastic ascorbate metabolism and its role in the regulation of cell signaling. *Curr. Opin. Plant Biol.* 6; 379–389.
- [24] Qi P, Lin Y S, Song X J, Shen J B, Huang W, Shan J X, Zhu M Z, Jiang L, Gao J P, Lin H X. 2012. The novel quantitative trait locus GL3.1 controls rice grain size and yield by regulating Cyclin-T1;3. *Cell Research*, 22, 1666–1680
- [25] Redona ED, Mackill DJ. 1998. Quantitative trait locus analysis for rice panicle and grain characteristics. *Theor Appl Genet* 96:957–963
- [26] Shomura A, Izawa T, Ebana K, Ebitani T, Kanegae H, Konishi S, Yan, M. 2008. Deletion in a gene associated with grain size increased yields during rice domestication. *Nat Genet* 40: 1023–1028.
- [27] Song X J, Huang W, Shi M, Zhu M Z, Lin H X. 2007. A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. *Nature Genetics*, 39, 623–630
- [28] Sun H, Qian Q, Wu K, Luo J, Wang S, Zhang C, Ma Y, Liu Q, Huang X, Yuan Q, Han R, Zhao M, Dong G, Guo L, Zhu X, Gou Z, Wang W, Wu Y, Lin H, Fu X. 2014. Heterotrimeric G proteins regulate nitrogen-use efficiency in rice. *Nat Genet* 46:652–656
- [29] Sun XY, Wu K, Zhao Y, Kong FM, Han GZ, Jiang HM, Huang XJ, Li RJ, Wang HG, Li SS. 2009. QTL analysis of kernel shape and weight using recombinant inbred lines in wheat. *Euphytica* 165:615–624
- [30] Suwannaporn P, Linnemann A. 2008. Rice-eating quality among consumer in different rice grain preference countries. *J of Sensory Std* 23:1–13

- [31] Taguchi-Shiobara F, Kawagoe Y, Kato H, Onodera H, Tagiri A, Hara N, Miyao A, Hirochika H, Yano M, Toki S. 2011. A loss-of-function mutation of rice DENSE PANICLE 1 causes semi-dwarfness and slightly increased number of spikelets. *Breed Sci* 61:17–25
- [32] Takano-Kai N, Doi K, Yoshimura A. 2011. GS3 participates in stigma exertion as well as seed length in rice. *Breed Sci* 61:244–250
- [33] Takano-Kai N, Jiang H, Kubo T, Sweeney M, Matsumoto T, Kanamori H, Padhukasahasram B, Bustamante C, Yoshimura A, Dio K, McCouch S. 2009. Evolutionary history of GS3, a gene conferring grain length in rice. *Genetics* 182:1323–1334
- [34] Tan YF, Xing YZ, Li JX, Yu SB, Xu CG, Zhang Q. 2000. Genetic bases of appearance of quality of rice grains in Shanyou 63, an elite rice hybrid. *Theor Appl Genet* 101:823–829
- [35] Tanabe S, Kurinami S, Ashikari M, Kitano H, Iwasaki Y. 2007. Mapping of small and round seed 3 gene in rice. *Rice Genet News* 23:56–58
- [36] Terashima K, Saito Y, Sakai N, Watanabe T, Ogata T, Akita S. 2001. Effects of high air temperature in summer of 1999 on ripening and grain quality of rice. *Jpn J Crop Sci* 70:449–458
- [37] Tokunaga T, Miyahara K, Tabata K, Esaka M. 2005. Generation and properties of ascorbic acid-overproducing transgenic tobacco cells expressing sense RNA for L-galactono-1,4-lactone dehydrogenase. *Planta* 220; 854–863
- [38] Unnevehr L, Duff B, Juliano BO. 1992. Consumer Demand for Rice Grain Quality: Terminal Report of IDRC Projects, National Grain Quality (Asia), and International Grain Quality Economics (Asia): Int. Rice Res. Inst.
- [39] Wan X, Weng J, Zhai H, Wang J, Lei C, Liu X, Guo T, Jiang L, Su N, Wan J. 2008. Quantitative trait loci (QTL) analysis for rice grain width and fine mapping of an identified QTL allele *gw-5* in a recombination hotspot region on chromosome 5. *Genetics* 179, 2239e2252.
- [40] Wan XY, Wan JM, Jiang L, Wang JK, Zhai HQ, Weng JF, Wang HL, Lei CL, Wang JL, Zhang X, Cheng ZJ, Guo XP. 2006. QTL analysis for rice grain length and fine mapping of an identified QTL with stable and major effects. *Theor. Appl. Genet.* 112, 1258e1270.
- [41] Wan XY, Wan JM, Weng JF, Jiang L, Bi JC, Wang CM, Zhai HQ. 2005. Stability of QTLs for rice grain dimension and endosperm chalkiness characteristics across eight environments. *Theor Appl Genet* 110:1334–1346
- [42] Wang C, Chen S, Yu S. 2011. Functional markers developed from multiple loci in GS3 for fine marker-assisted selection of grain length in rice. *Theor Appl Genet* 122:905–913
- [43] Wang E, Wang J, Zhu X, Hao W, Wang L, Li Q, Zhang L, Lu B, Lin H, Ma H, Zhang G, He Z. 2008. Control of rice grain-filling and yield by a gene with a potential signature of domestication. *Nat Genet* 40:1370–1374
- [44] Wang E, Xu X, Zhang L, Zhang H, Lin L, Wang Q, Li Q, Ge S, Wang W, He Z. 2010. Duplication and independent selection of cell wall invertase genes *GIF1* and *OsCIN1* during rice evolution and domestication. *BMC Evol Biol* 10:108
- [45] Wang S, Wu K, Yuan Q, Liu X, Liu Z, Lin X, Zeng R, Zhu H, Dong G, Qian Q, Zhang G, Fu X. 2012. Control of grain size, shape and quality by *OsSPL16* in rice. *Nat Genet* 44: 950-954.
- [46] Wang S, Wu K, Yuan Q, Liu X, Liu Z, Lin X, Zeng R, Zhu H, Dong G, Zhang G, Fu X. 2012a. Control of grain size, shape and quality by *OsSPL16* in rice. *Nat Genet* 44:950–954
- [47] Wang Y, Li H, Zhang L, Lü W, Wang J. 2012b. On the use of mathematically-derived traits in QTL mapping. *Mol Breed* 29:661–673
- [48] Weng J, Gu S, Wan X, Gao H, Guo T, Su N, Lei C, Zhang X, Cheng Z, Guo X, Wang J, Jiang L, Zhai H, Wan J. 2008. Isolation and initial characterization of *GW5*, a major QTL associated with rice grain width and weight. *Cell Res* 18:1199–1209
- [49] Xu JL, Yu SB, Luo LJ, Zhong DB, Mei HW, Li ZK. 2004. Molecular dissection of the primary sink size and its related traits in rice. *Plant Breed* 123:43–50
- [50] Yan S, Zou G, Li S, Wang H, Liu H, Zhai G, Guo P, Song H, Yan C, Tao Y. 2011. Seed size is determined by the combinations of the genes controlling different seed characteristics in rice. *Theor Appl Genet* 123:1173–1181
- [51] Yi X, Zhang Z, Zeng S, Tian C, Peng J, Li M, Lu Y, Meng Q, Gu M, Yan C. 2011. Introgression of *qPE9-1* allele, conferring the panicle erectness, leads to the decrease of grain yield per plant in japonica rice (*Oryza sativa* L.). *J Genet Genomics* 38:217–223
- [52] Yoon DB, Kang KH, Kim HJ, Ju HG, Kwon SJ, Suh JP, Jeong OY, Ahn SN. 2006. Mapping quantitative trait loci for yield components and morphological traits in an advanced backcross population between *Oryza grandiglumis* and the *O. sativa japonica* cultivar *Hwaseongbyeo*. *Theor. Appl. Genet.* 112, 1052e1062.
- [53] Yoshioka Y, Iwata H, Tabata M, Ninomiya S, Ohsawa S. 2007. Chalkiness in rice: potential for evaluation with image analysis. *Crop Sci.* 47; 2113–2120.
- [54] Yu S, Li J, Xu C, Tan Y, Gao Y, Li X, Zhang Q, Maroof M. 1997. Importance of epistasis as the genetic basis of heterosis in an elite rice hybrid. *Proc Natl Acad Sci* 94:9226–9231
- [55] Zhang X, Wang J, Huang J, Lan H, Wang C, Yin C, Wu Y, Tang H, Qian Q, Li J, Zhang H. 2012. Rare allele of *OsPPKL1* associated with grain length causes extra-

large grain and a significant yield increase in rice.
Proceedings of the National Academy of Sciences of the
United States of America, 109, 21534–21539.