Mol Biotechnol (2011) 49:90–95
DOI 10.1007/s12033-011-9382-x

RESEARCH

Fine Mapping QTL for Drought Resistance Traits in Rice (*Oryza sativa* L.) Using Bulk Segregant Analysis

Arvindkumar Shivaji Salunkhe · R. Poornima · K. Silvas Jebakumar Prince · P. Kanagaraj · J. Annie Sheeba · K. Amudha · K. K. Suji · A. Senthil · R. Chandra Babu

Published online: 5 February 2011

Abstract Drought stress is a major limitation to rice (*Oryza sativa* L.) yields and its stability, especially in rainfed conditions. Developing rice cultivars with inherent capacity to withstand drought stress would improve rainfed rice production. Mapping quantitative trait loci (QTLs) linked to drought resistance traits will help to develop rice cultivars suitable for water-limited environments through molecular marker-assisted selection (MAS) strategy. However, QTL mapping is usually carried out by genotyping large number of progenies, which is labour-intensive, time-consuming and cost-ineffective. Bulk segregant analysis (BSA) serves as an affordable strategy for mapping large effect QTLs by genotyping only the extreme phenotypes instead of the entire mapping population. We have previously mapped a QTL linked to leaf rolling and leaf drying in recombinant inbred (RI) lines derived from two locally adapted *indica* rice ecotypes viz., IR20/Nootripathu using BSA. Fine mapping the QTL will facilitate its application in MAS. BSA was done by bulking DNA of 10 drought-resistant and 12 drought-sensitive RI lines. Out of 343 rice microsatellites markers genotyped, RM8085 co-segregated among the RI lines constituting the respective bulks. RM8085 was mapped in the middle of the QTL region on chromosome 1 previously identified in these RI lines thus reducing the QTL interval from 7.9 to 3.8 cM. Further, the study showed that the region, RM212–RM302–RM8085–RM3825 on chromosome 1, harbours large effect QTLs for drought-resistance traits across several genetic backgrounds in rice. Thus, the QTL may be useful for drought resistance improvement in rice through MAS and map-based cloning.

Keywords Bulk segregant analysis · Fine mapping · Microsatellites · QTLs · MAS · Drought resistance · Rice · *Oryza sativa*

Introduction

Rice, (*Oryza sativa* L.) is a principal food for majority of the population in Asia, Africa and South America [1]. It is grown on 153 million hectares (Mha) globally [2], about half of which is in rainfed ecosystems where drought stress is a serious limitation. In Asia alone, about 34 Mha of rainfed lowland rice and 8 Mha of upland rice [3] are subjected to frequent drought stress. The global rice yield loss due to drought is estimated at 18 million tonnes annually [4]. Improving rice yields in these marginal environments is critical to meet the increasing demand for food by the growing population.

Genetic improvement of rice for adaptation to drought based on yield per se under stress, though feasible [5], is slow due to unpredictability of drought events, in terms of timing and severity, across years and locations [6]. Alternatively, incorporation of secondary traits contributing in drought resistance as selection indices in breeding will hasten development of drought-resistant cultivars. Several putative traits conferring drought resistance in rice have been proposed [1, 7, 8], and selection of these traits in breeding will increase rice yields in water-limited environments as demonstrated in wheat [9]. Leaf rolling is the first visual symptom of drought reaction and occurs due to

A. S. Salunkhe · R. Poornima · K. S. J. Prince · P. Kanagaraj · J. A. Sheeba · K. Amudha · K. K. Suji · A. Senthil · R. C. Babu (✉)
Department of Plant Molecular Biology and Biotechnology, Centre for Plant Molecular Biology, Tamil Nadu Agricultural University, Coimbatore 641003, India
e-mail: chandrarc2000@yahoo.com

wilting (turgor loss) and inability of leaves to sustain the transpiration demand [10, 11]. Visual leaf rolling is an efficient method for detecting drought resistance in rice, especially in vegetative stage [12, 13]. Leaf rolling is the most important criteria found useful in assessing levels of drought resistance in large scale screening of rice [14]. Visual drought scoring by an experienced researcher based solely on leaf rolling and leaf drying is quite effective in discriminating drought avoidance in rice [15]. Leaf rolling and drying are negatively correlated with leaf-relative water content [16]. Delayed leaf rolling and drying are positively related to drought resistance under field conditions [17]. Leaf drying score can be used to determine drought resistance at all stages of rice growth and has moderate to high heritability under stress [18].

However, incorporation of secondary traits such as leaf rolling and leaf drying as selection criteria is hampered due to complexity of the drought environment, different mechanisms of drought resistance adapted by rice and the interaction between the two as well as genetic complexity of the traits [19]. Molecular markers allow breeders to track the genetic loci linked to such complex traits and help in their indirect selection without the need for difficult phenotypic measurements, thus saving in time and resources [1]. Molecular markers are also not environmentally regulated and can be detected at all stages of plant growth [20].

Identifying molecular markers through QTL mapping is, however, not affordable by many researchers. In the conventional QTL mapping, each progeny of mapping population (normally in multiples of hundred) should be genotyped with numerous molecular markers which is time consuming and costly [21]. Several strategies have been proposed to identify molecular markers near a gene/QTL of interest with reduced number of plants to be genotyped. The two main strategies are selective genotyping [21, 22] and bulk segregant analysis (BSA) [23]. Selective genotyping is relatively a low-cost approach to detect QTL with large effects by genotyping individuals from the two tails of the phenotypic distribution [21, 22]. BSA further saves cost by genotyping pooled DNA from groups of individuals with similar phenotypes and is a rapid technique as it can detect large effect QTL alleles in a large sample of progenies relatively at low cost and in very quick time [21, 24]. Possibility of using DNA pooling strategies for identifying markers involving various mapping populations has been discussed earlier [25]. Though the potential of BSA has been demonstrated to tag genes controlling qualitative traits, the method could now be extended for analysis of complex traits such as drought resistance. Markers linked to drought-resistance traits have been identified using BSA in barley [26], wheat [27] and maize [28]. Markers linked to yield [29] and yield under drought stress in rice [24] have also been identified using BSA. We have recently mapped three minor QTLs for leaf drying by genotyping a subset of 250 RI lines derived from two locally adapted rice lines [30]. BSA of the extreme phenotypes detected a common QTL on chromosome 1 for leaf rolling and leaf drying in these RI lines. The QTL interval is large, 7.9 cM [31]. Fine mapping the QTL will facilitate its application in MAS for drought resistance [32]. QTLs for leaf rolling are good targets for MAS for drought avoidance in rice [19]. Thus, this study was conducted with the objective to fine map the QTL and also to identify other major effect QTLs linked to leaf rolling and leaf drying under drought stress in these rice lines using BSA.

Materials and Methods

Plant Materials

A mapping population consisting of a total of 397 F_7 recombinant inbred (RI) lines was developed from a cross between IR20 and Nootripathu by single seed descent. These two rice lines are well adapted to rainfed rice production environment in south India. IR20 is a semi-dwarf *indica* ecotype with high yield potential and good grain quality. However, it is drought sensitive and has shallow and thin roots [33]. Nootripathu, a landrace from southern State of Tamil Nadu, India is drought resistant and possess long and thick roots. From the total of 397 F_8 RI lines, a subset of 330 RI lines was selected randomly for drought phenotyping, and further a subset of 250 out of these 330 RI lines was used for genotyping and QTL mapping of drought resistance and plant production traits in target production environment [30]. BSA of pooled DNA of 11 drought-resistant and 12 drought-susceptible RI lines, selected based on leaf rolling and leaf drying scores under drought stress from the field experiment [30], identified three markers, RM212, RM302 and RM3825 on chromosome 1 to be linked to leaf rolling and leaf drying under stress in these rice lines [31]. The region spanned an interval of 7.9 cM. These RI lines, excluding RI line # 37 [31] were further used in this study to fine map the above QTL region and also to detect additional large effect QTLs, if any, for drought resistance through BSA.

DNA Extraction and Bulking

Leaf samples of the selected RI lines and their parents were collected from field-grown seedlings, and freeze dried. DNA was extracted from the leaves using cetyl trimethyl ammonium borate buffer [34]. The quantity and quality of DNA was assessed in 0.8% agarose gel, and concentration was adjusted to 25 ng/μl by comparing DNA standards.

Equal quantity (50 µl each) of diluted DNA from 10 drought-tolerant and 12 drought-susceptible RI lines were pooled separately into resistant and susceptible bulks, respectively.

Bulk Segregant Analysis

A subset of 343 rice microsatellite (RM) markers (Agile Life Science Tech Ltd, India) were selected covering the entire genome based on their map location with approximately one marker for every 5 cM [35]. The genomic DNA of the two parental lines was initially screened using these 343 primers. The polymorphic primers were then used to screen the resistant and susceptible bulks. The primers showing polymorphism between the bulks were tested for co-segregation by genotyping individual RI lines constituting the respective bulks.

Polymerase chain reactions (PCR) were performed in a volume of 15 µl in a PTC-100, MJ thermo cycler (MJ Research Inc, USA). The reaction mixture contained each primer at 1 µM, 100 µM deoxy nucleotide, 1× *Taq* buffer, 0.1 U *Taq* polymerase, and template DNA at 50 ng. After 5 min at 94 °C, the PCR involved 35 cycles of amplification, each cycle comprising 1 min at 94 °C, 1 min at either 55 or 57 or 60 °C (depending on the primer), 1 min at 72 °C and with a final extension step of 5 min at 72 °C. The completely co-segregated polymorphic primer amplicons were separated on 3% MetaPhor agarose gels [36].

Results and Discussion

Bulk Segregant Analysis

Out of the 343 microsatellite markers, 96 primers were polymorphic between the parents. Out of these 96, seven primers viz., RM85, RM163, RM540, RM1337, RM3215, RM5925 and RM8085 were polymorphic between the bulks and were tested for co-segregation among the individual RI lines constituting the bulks. Among these polymorphic loci, RM8085 alone showed complete co-segregation among individual RI lines constituting the respective bulks with clear bands (Plate 1). Similar method of using only those polymorphic markers which showed clearly visible differences in band intensity between the high and low tails of phenotype in BSA has been reported in rice [24]. The other six markers showed partial co-segregation among the individual RI lines and were not followed further. Similar results were reported earlier [24, 37, 38]. The drought-resistant RI lines had majority of alleles as that of the resistant parent, Nootripathu, and the susceptible RI lines had most alleles similar to IR20, the susceptible parent. RM8085 is mapped on chromosome 1 at 139.9 cM [35] and is linked to leaf rolling and leaf drying under drought stress in this study (Fig. 1). Earlier, three microsatellite markers, RM212, RM302 and RM3825 linked to leaf rolling and leaf drying under drought stress in these RI lines were identified using BSA at this genomic region [31]. RM212–RM302–RM3825 covered a length of 7.9 cM and RM8085, identified in this study through BSA, reduced the QTL interval to 3.8 cM, which is approximately 73 genes in rice [39]. Thus, BSA is helped to fine map and reduce the confidence interval of the QTL. The QTL region RM212 on chromosome 1 was also linked to plant height and biomass under stress in these RI lines [30].

Comparative Mapping

Comparative mapping indicated co-location of many QTLs for various drought-resistance traits in several rice lines at this genomic region (Fig. 1). The integrated linkage map [35] was used as a bridge to compare maps across different populations. RM6703 (139.1 cM; 34.5 Mb), which is above RM8085 (139.9 cM; 34.8 Mb) has been linked to

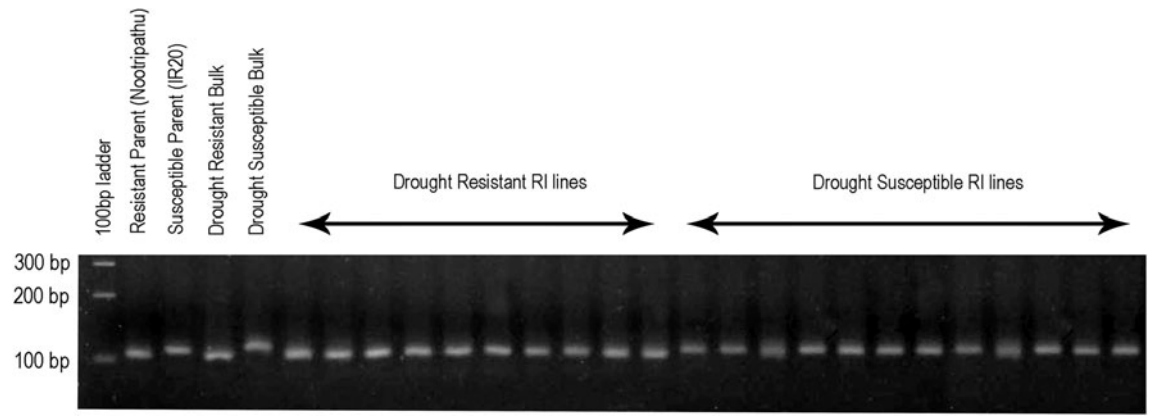

Plate 1 Alleles showing co-segregation of the SSR primer RM8085 among the parents, bulks and RI lines

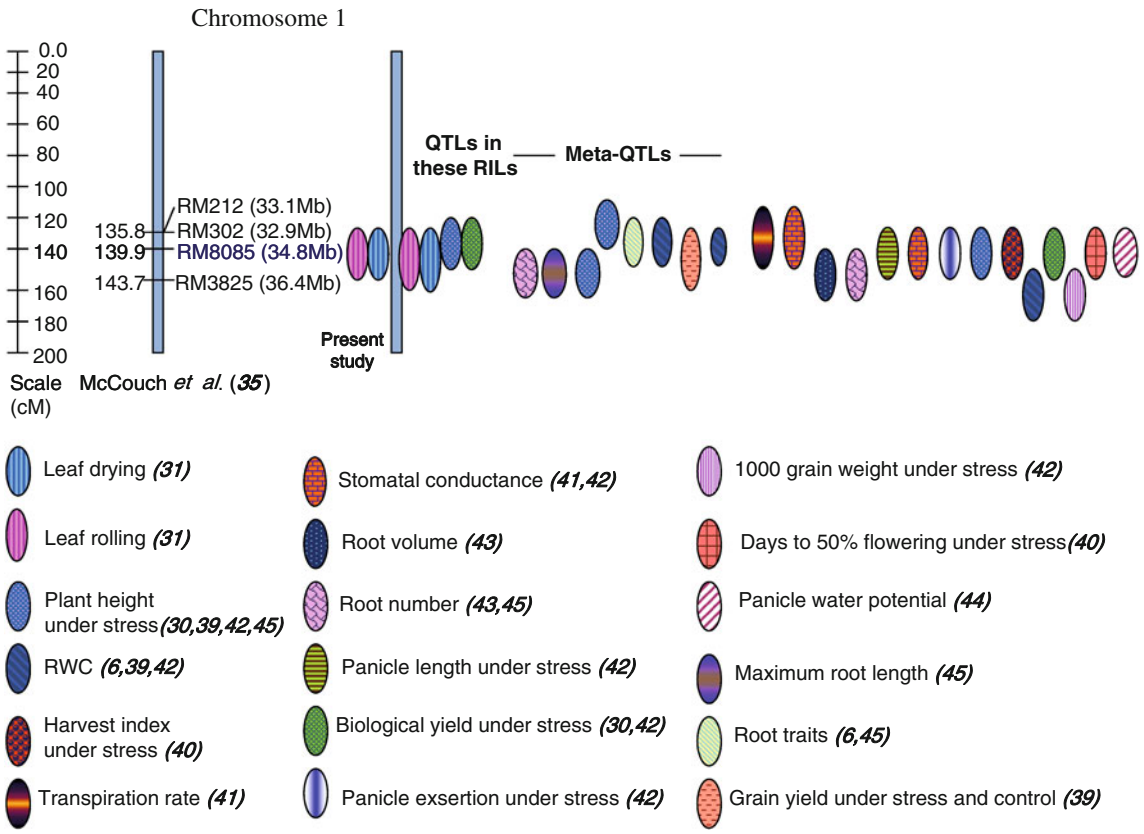

Fig. 1 Co-location of QTLs linked to drought resistance traits mapped on chromosome 1 in several rice lines

harvest index and days to 50% flowering under drought stress in IR64/Apo $F_{2:3}$ lines [40]. RM8085 was mapped close to the region, OSR27 (126.5 cM; 30.7 Mb)–RM212 (135.8 cM; 33.1 Mb) linked to stomatal conductance and transpiration rate under drought stress in Teqing/Lemont rice introgression lines [41]. The marker, RM302 (135.8 cM; 33.9 Mb) was linked to plant height, panicle exsertion, panicle length, biomass and stomatal conductance and RM315 (143.7 cM; 36.7 Mb) was linked to relative water content and 1000 grain weight under drought stress in IR64/Norungan and IR50/Norungan rice RI lines [42]. The 9.6 cM genomic region between RM1003 (136.8 cM; 33.4 Mb), RM472 (146.4 cM; 37.8 Mb) and RM1198 (146.4 cM; 37.6 Mb), which flanked RM8085 identified in this study, was associated with root volume and root number in IRAT109/Yuefu rice RI lines [43]. QTLs for spikelet density between RM302 and RM476B (142.2 cM), and panicle water potential between RM315 and RM472 under drought stress were mapped in Zhenshan97B/IRAT109 rice lines [44].

Meta QTL analysis is highly valuable in identification of positional candidate genes underlying QTL [39, 45]. Meta-analysis of QTLs from 15 mapping populations identified this genomic region on chromosome 1 as a candidate for MAB for drought resistance in rice, wherein several QTLs for plant type, primary, secondary and integrated traits co-located [6]. Meta-analysis identified true QTLs for root traits at this locus (35–40 Mb) on chromosome 1 [45]. *Sd1* gene controlling the plant height was very close to these true QTLs with the narrowest confidence interval. However, meta-analysis of 1650 QTLs from Bala/Azucena mapping population confirmed root traits meta-QTL at this locus and also not likely to be associated with *Sd1* gene [39]. This gives scope for selectively combining plant type and drought resistance traits in rice.

The general approaches to identify candidate genes underlying the QTLs were reviewed [46, 47]. The strategies followed in rice include expressed sequence tags [48, 49], development of near isogenic lines (NILs) [50], map-based cloning [51], microRNAs [52], functional study of gene in a relevant developmental pathway [53–55], gene expression studies [56] and mutant studies with t-DNA insertion approach [57]. A total of 587 genes (including 207 hypothetical genes, 69 known function genes, 173 putative function genes, 11 similar to function genes, 65 expressed genes and 62 transposon-related genes) underlying this 32–36 Mb region of chromosome 1 in rice physical map [58] could be mined using 'RiceGeneThresher' [59]. This region, RM212–RM302–RM8085–RM3825 is reported to have a gene density of 5933 base

pair/gene and average gene length of 2695 base pairs. It is evident that RM212–RM302–RM8085–RM3825 region on chromosome 1 is consistently linked to several drought-resistance traits across genetic backgrounds and will be useful in MAS and map-based cloning of genes for drought resistance in rice. Several NILs carrying the desired QTL introgressions from Azucena failed to have improved root characteristics [60] as inntrogressed QTL intervals were large (e.g. 41.8 cM) and had small proportion of phenotypic effect (6–18%). These results indicate that only fine-mapped alleles with large confirmed effects on performance under stress and consistent across different genetic backgrounds and environments are appropriate targets for MAS [4]. Thus, BSA can identify major QTLs linked to complex traits such as drought resistance at considerable ease and savings in genotyping effort and cost, allowing resources to be focussed on precise localization of QTLs with large effects. Moreover, BSA facilitates fine mapping of candidate QTLs with large intervals and being prone to recombination during crossing.

References

1. Nguyen, H. T., Babu, R. C., & Blum, A. (1997). Breeding for drought resistance in rice: Physiology and molecular genetics considerations. *Crop Science, 37*, 1426–1434.
2. FAO (Food and Agricultural Organization). (2007). http//www.fao.org.
3. Huke, R. E., & Huke, E. H. (1997). *Rice area by type of culture: South, Southeast and East Asia.* Los Banos: IRRI.
4. Bernier, J., Kumar, A., Serraj, R., Spaner, D., & Atlin, G. N. (2008). Breeding upland rice for drought resistance. *Journal of the Science of Food and Agriculture, 88*, 927–939.
5. Venuprasad, R., Lafitte, H. R., & Atlin, G. N. (2007). Response to direct selection for grain yield under drought stress in rice. *Crop Science, 47*, 285–293.
6. Kamoshita, A., Babu, R. C., Boopathi, N. M., & Fukai, S. (2008). Phenotypic and genotypic analysis of drought-resistance traits for development of rice cultivars adapted to rainfed environments. *Field Crops Research, 109*, 1–23.
7. Fukai, S., & Cooper, M. (1995). Development of drought-resistant cultivars using physio-morphological traits in rice. *Field Crops Research, 40*, 67–86.
8. Lafitte, R., Blum, A., & Atlin, G. (2003). Using secondary traits to help identify drought-tolerant genotypes. In *Breeding rice for drought-prone environments*. Los Banos: IRRI.
9. Richards, R. A., Rebetzke, G. J., Watt, M., Condon, A. G., Spielmeyer, W., & Dolferus, R. (2010). Breeding for improved water productivity in temperate cereals: Phenotyping, quantitative trait loci, markers and the selection environment. *Functional Plant Biology, 37*, 85–97.
10. Blum, A. (1988). *Plant breeding for Stress environments*. Boca Raton, FL: CRC Inc.
11. Fischer, K. S., & Fukai, S. (2003). How rice responds to drought. In K. S. Fischer, R. Lafitte, S. Fukai, G. Atlin, & B. Hardy (Eds.), *Breeding rice for drought prone environments* (pp. 32–36). Los Banos: IRRI.
12. De Datta, S. K., Malabuyot, J. A., & Aragon, E. L. (1988). A field screening technique for evaluating rice germplasm for drought tolerance during the vegetative stage. *Field Crops Research, 19*, 123–134.
13. Courtois, B., McLaren, G., Sinha, P. K., Prasad, K., Yadav, R., & Shen, L. (2000). Mapping QTL associated with drought avoidance in upland rice. *Molecular Breeding, 6*, 55–66.
14. Chang, T. T., Loresto, G., & Tagum, P. O. (1974). Screening rice germplasm for drought resistance. *SABRAO Journal, 6*(1), 9–16.
15. O'Toole, J. C., & Moya, T. B. (1978). Genotypic variation in maintenance of leaf water potential. *Crop Science, 18*, 873–876.
16. Price, A. H., Cairns, J. E., Horton, P., Jones, H. G., & Griffiths, H. (2002). Linking drought-resistance mechanisms to drought avoidance in upland rice using a QTL approach: Progress and new opportunities to integrate stomatal and mesophyll responses. *Journal of Experimental Botany, 53*, 989–1004.
17. Singh, B. N., & Mackill, D. J. (1991). Genetics of leaf rolling under drought stress. In *Rice genetics II* (pp. 159–166). Manila: IRRI.
18. Lafitte, R. (2003). Managing water for controlled drought in breeding plots. In K. S. Fischer, R. Lafitte, S. Fukai, G. Atlin, & B. Hardy (Eds.) *Breeding rice for drought-prone environments* (pp. 23–26). Los Banos: IRRI.
19. Khowaja, F. S., & Price, A. H. (2008). QTL mapping rolling, stomatal conductance and dimension traits of excised leaves in the Bala × Azucena recombinant inbred population of rice. *Field Crops Research, 106*, 248–257.
20. Mohan, M., Nair, S., Bhagwat, A., Krishna, T. G., Masahiro, Y., Bhatia, C. R., et al. (1997). Genome mapping, molecular markers and marker-assisted selection in crop plants. *Molecular Breeding, 3*, 87–103.
21. Sun, Y., Wang, J., Crouch, J. H., & Xu, Y. (2010). Efficiency of selective genotyping for genetic analysis of complex traits and potential applications in crop improvement. *Molecular Breeding, 26*, 493–511.
22. Navabi, A., Mather, D. E., Bernier, J., Spaner, D. M., & Atlin, G. N. (2009). QTL detection with bidirectional and unidirectional selective genotyping: Marker-based and trait-based analyses. *Theoretical and Applied Genetics, 118*, 347–358.
23. Michelmore, R. W., Paranand, I., & Kessele, R. V. (1991). Identification of markers linked to disease resistance genes by bulk segregant analysis: A rapid method to detect markers in specific genome using segregant population. *Proceedings of the National Academy of Science, 88*, 9828–9832.
24. Venuprasad, R., Dalid, C. O., Del Valle, M., Zhao, D., Espiritu, M., Sta Cruz, M. T., et al. (2009). Identification and characterization of large-effect quantitative trait loci for grain yield under lowland drought stress in rice using bulk-segregant analysis. *Theoretical and Applied Genetics, 120*, 177–190.
25. Wang, G. L., & Paterson, A. H. (1994). Assessment of DNA pooling strategies for mapping of QTLs. *Theoretical and Applied Genetics, 88*, 355–361.
26. Altinkut, A., Kazan, K., & Gozukirmizi, N. (2003). AFLP markers linked to water-stress-tolerant bulks in barley. *Genetics and Molecular Biology, 26*, 77–82.
27. Altinkut, A., & Gozukirmizi, N. (2003). Search for microsatellites associated with water stress tolerance in wheat through bulked segregant analysis. *Molecular Biotechnology, 23*, 97–106.
28. Quarrie, S. A., Lazic-Jancic, V., Kovacevic, D., Steed, A., & Pekic, S. (1999). Bulked segregant analysis with molecular markers and its use for improving drought resistance in maize. *Journal of Experimental Botany, 50*, 1299–1306.
29. Shashidar, H. E., Vinod, M. S., Naveen, S., Sharma, G. V., & Krishnamurthy, K. (2005). Markers linked to grain yield using bulk segregant analysis approach in rice (*Oryza sativa* L.). *Rice Genetics Newsletter, 22*, 69–71.

30. Gomez, S. M., Boopathi, N. M., Kumar, S. S., Ramasubramanian, T., Chengsong, Z., Jeyaprakash, P., et al. (2010). Molecular mapping and location of QTLs for drought-resistance traits in *indica* rice lines adapted to target environments. *Acta Physiologiae Plantarum, 32*, 355–364.
31. Kanagaraj, P., Prince, K. S. J., Sheeba, J. A., Biji, K. R., Paul, S. B., Senthil, A., et al. (2010). Microsatellite markers linked to drought resistance in rice (*Oryza sativa* L.). *Current Science, 98*(6), 836–839.
32. Bernier, J., Kumar, A., Ramaiah, V., Spaner, D., & Atlin, G. (2007). A large-effect QTL for grain yield under reproductive-stage drought stress in upland rice. *Crop Science, 47*, 505–516.
33. Babu, R. C., Shashidhar, H. E., Lilley, J. M., Thanh, N. D., Ray, J. D., Sadasivam, S., et al. (2001). Variation in root penetration ability, osmotic adjustment and dehydration tolerance among accessions of rice adapted to rainfed lowland and upland ecosystems. *Plant Breeding, 120*, 233–238.
34. Gawel, N. J., & Jarret, R. L. (1991). A modified CTAB DNA extraction procedure for *musa* and *ipomoea* plant. *Molecular Biology Reports, 9*, 262–266.
35. McCouch, S. R., Teytelman, L., Xu, Y., Lobos, K. B., Clare, K., Walton, M., et al. (2002). Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). *DNA Research, 9*, 199–207.
36. Sambrook, J., & Russell, D. W. (2006). Detection of DNA in agarose gels. *Cold Spring Harbor Protocols*. doi:10.1101/pdb.prot4022.
37. Kumari, S., Sheba, J. M., Marappan, M., Ponnusamy, S., Seetharaman, S., Pothi, N., et al. (2010). Screening of IR50 × Rathu Heenati F7 RILs and identification of SSR Markers linked to brown planthopper (*Nilaparvata lugens* Stal) resistance in rice (*Oryza sativa* L.). *Molecular Biotechnology, 46*, 63–71.
38. Steele, K. A., Edwards, G., Zhu, J., & Witcombe, J. R. (2004). Maker-evaluated selection in rice: Shifts in allele frequency among bulks selected in contrasting agricultural environments identify genomic regions of importance to rice adaptation and breeding. *Theoretical and Applied Genetics, 109*, 1247–1260.
39. Khowaja, F. S., Norton, G. J., Courtois, B., & Price, A. H. (2009). Improved resolution in the position of drought-related QTLs in a single mapping population of rice by meta-analysis. *BMC Genomics, 10*, 276–290.
40. Venuprasad, R., Bool, M. E., Dalid, C. O., Bernier, J., Kumar, A., & Atlin, G. N. (2009). Genetic loci responding to two cycles of divergent selection for grain yield under drought stress in a rice breeding population. *Euphytica, 167*, 261–269.
41. Zhao, X. Q., Xu, J. L., Zhao, M., Lafitte, R., Zhu, L. H., Fu, B. Y., et al. (2008). QTLs affecting morph-physiological traits related to drought tolerance detected in overlapping introgression lines of rice (*Oryza sativa* L.). *Plant Science, 174*, 618–625.
42. Subashri, M., Robin, S., Vinod, K. K., Rajeswari, S., Mohanasundaram, K., & Raveendran, T. S. (2009). Trait identification and QTL validation for reproductive stage drought resistance in rice using selective genotyping of near flowering RILs. *Euphytica, 166*, 291–305.
43. Qu, Y., Ping, M., Hongliang, Z., Chen, Y., Gao, Y., Tian, Y., et al. (2008). Mapping QTLs of root morphological traits at different growth stages in rice. *Genetica, 133*, 187–200.
44. Liu, G., Mei, H., Liu, H., Yu, X., Zou, G., & Luo, L. (2010). Sensitivities of rice grain yield and other panicle characters to late-stage drought stress revealed by phenotypic correlation and QTL analysis. *Molecular Breeding, 25*, 603–613.
45. Courtois, R., Ahmadi, N., Khowaja, F., Price, A. H., Rami, J. H., Frouin, J., et al. (2009). Rice root architecture: Meta analysis from a drought QTL database. *Rice, 2*, 115–128.
46. Hubner, N., Wallace, C. A., & Zimdahl, H. (2005). Integrated transcriptional profiling and linkage analysis for identification of genes underlying disease. *Nature Genetics, 37*, 243–253.
47. Zhu, M., & Zhao, S. (2007). Candidate Gene Identification Approach: Progress and Challenges. *International Journal of Biological Sciences, 3*(7), 420–427.
48. Zheng, B. S., Yang, L., Zhang, W. P., Mao, C. Z., & Wu, Y. R. (2003). Mapping QTLs and candidate genes for rice root traits under different water-supply conditions and comparative analysis across three populations. *Theoretical and Applied Genetics, 107*, 1505–1515.
49. Zheng, B. S., Mao, C. Z., Zhang, W. P., & Wu, Y. R. (2006). QTLs and candidate genes for rice root growth under flooding and upland conditions. *Acta Genetica Sinica, 33*(2), 33141–33151.
50. Xiong, L. (2009). *Drought frontiers in rice—Crop improvement for increased rainfed production*. Singapore: World Scientific Publishing Co. Pte. Ltd.
51. Yano, M., Kojima, S., Takahashi, Y., Lin, H., & Sasaki, T. (2001). Genetic control of flowering time in rice, a short day plant. *Plant Physiology, 127*, 1425–1429.
52. Jian, X., Zhang, L., Li, G., Zhang, L., Wang, X., Cao, X., et al. (2010). Identification of novel stress-regulated microRNAs from *Oryza sativa*. *Genomics, 95*, 47–55.
53. Malamy, J. E. (2005). Intrinsic and environmental response pathways that regulate root system architecture. *Plant Cell and Environment, 28*, 67–77.
54. Nibau, C., Gibbs, D. J., & Coates, J. C. (2008). Branching out in new directions: The control of root architecture by lateral root formation. *New Phytologist, 179*(3), 595–614.
55. Rebouillat, J., Dievart, A., Verdeil, J. L., Escoute, J., Giese, E., & Breitler, J. C. (2008). Molecular genetics of rice root development. *Rice, 2*(1), 15–34.
56. Norton, G. J., Aitkenhead, M. J., Khowaja, F. S., Whalley, W. R., & Price, A. H. (2008). A bioinformatic and transcriptomic approach to identifying positional candidate genes without fine mapping; an example using rice root-growth QTLs. *Genomics, 92*, 344–352.
57. Larmande, P., Gay, C., Lorieux, M., Perin, C., Bouniol, M., & Droc, G. (2008). Oryza Tag Line, a database for the phenotypic characterization of the genoplante rice insertion line library. *Nucleic Acids Research, 36*, 1022–1027.
58. Ouyang, S., Zhu, W., Hamilton, J., Lin, H., Campbell, M., Childs, K., et al. (2007). The TIGR Genome Annotation Resource: Improvements and new features. *Nucleic Acids Research, 35*, 883–887.
59. Thongjuea, S., Ruanjaichon, V., Bruskiewich, R., & Vanavichit, A. (2009). Rice Gene Thresher: A web-based application for mining genes underlying QTL in rice genome. *Nucleic Acids Research, 638*, 1–5.
60. Shen, L., Courtois, B., McNally, K. L., Robin, S., & Li, Z. (2001). Evaluation of near-isogenic lines of rice introgressed with QTLs for root depth through marker-aided selection. *Theoretical and Applied Genetics, 103*, 75–83.