Review article

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Critical considerations for molecular breeding in rice

BCY Collard[1,2]*, C Raghavan and MR Islam[1]

[1]International Rice Research Institute (IRRI), Philippines
[2]Sugar Research Australia (SRA), Queensland, Australia
**Corresponding author e-mail: bcycollard@gmail.com*

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ABSTRACT

The last 20 years has seen a tremendous growth in molecular genetics research and breeding rice. While there have been some outstanding successful examples of rice molecular breeding, there have also been many failures. Careful analysis of successes and failures indicates several critical factors including parameters of the quantitative trait loci (QTL) mapping experiment and subsequent testing which is referred to as QTL and marker validation. In rice, validation research is seldom reported and more activities are needed, as has been done in other cereals. In order to leverage the wealth of publicly-available genomics resources in rice for molecular breeding in the future, breeders will need: carefully planned and well-executed QTL mapping experiments, QTL and marker validation activities, efficient genotyping systems and processes, cheaper genotyping systems and more breeder-friendly analytical tools. It is imperative that future activities focus on bridging the disconnection between breeders and molecular geneticists and achieving improved co-ordination and collaboration. Arguably, the highest priority to achieve success and synergy regarding varietal/germplasm development in the short-term and the future is the need to integrate breeding with molecular genetics research.

Key words: *Rice breeding, marker-assisted selection (MAS), molecular breeding, quantitative trait loci (QTL) mapping, association mapping, marker validation, genotyping, integration*

Rice breeders have made a large contribution to rice production and food security by developing new improved varieties (Peng and Khush 2003). The majority of inbred varieties have been developed using conventional breeding methods, such as pedigree or bulk breeding methods (Khush and Virk 2005; Collard *et al*. 2013). Rice breeding programs involve a large amount of work including hybridization, screening segregating populations (*i.e.*, by eliminating unwanted lines and promoting desirable ones) and conducting field trials across several locations and years. In general, tens to hundreds of crosses are made and each breeding population usually consists of 1000 to 3000 individual plants. Therefore, rice breeders work with large plant populations (*i.e.*, often tens of thousands of plants or more), which must be screened to eliminate undesirable plants and retain and promote lines with desirable genetic combinations.Behind the scenes are considerable logistical activities involving seed processing, organization and archiving. Rice breeders also manage large amounts of data (*e.g.*, using spreadsheets or in-house or commercial data management systems), and perform considerable data analysis using a range of statistical methods. These activities are critical activities that are critical to develop new varieties.

DNA markers or molecular markers may be used as tools by breeders to increase the accuracy or efficiency of selection (Dwivedi *et al.* 2007; Xu and Crouch 2008). Using DNA markers can translate into benefits of efficiency for plant breeding programs. These markers have been developed over decades on research in molecular biology and genetics. They have numerous applications including DNA fingerprinting, genetic diversity analysis, parental characterization of known genes and marker assisted selection

(MAS)(Collard and Mackill 2008). DNA markers have been a powerful tool for plant breeders to select for major genes or quantitative trait loci (QTLs) with large effects for critical or important traits, and thus have enormous potential benefits to breeders. The development of new genotyping methods and systems offers even more potential for modern rice breeders (Thomson 2014).

Some successful examples of rice molecular breeding (*e.g.,* Sub1 variety development) have been regarded as case studies for crop species since varieties were developed, released, disseminated and adopted on a large scale (Singh *et al.* 2013). Furthermore, several large-scale rice molecular breeding projects have been undertaken and successfully completed (Singh *et al.* 2016). However, despite some success and a large investment of resources in this applied research area, there are many examples where the use of markers has not led to the development of new germplasm in public rice breeding program (Young 1999; Dwivedi *et al.* 2007; Collard and Mackill 2008). These findings are usually anecdotal and reports of unsuccessful molecular breeding activities are usually unpublished.In this review, we explore some critical factors required for QTL mapping and successful MAS in rice. Importantly, some of these issues are not just technical ones but are related to how research programs are designed and managed, and due to the culture of science research.

Background and context

As discussed above, rice breeders have been very successful in selecting for a wide range of traits using only conventional methods. Therefore, it is pertinent to ask the question "when should a rice breeder use MAS?" As pointed out by Collard and Mackill (2008), breeders do not need to use markers for every trait, even if perfect markers are available. An example of this is flowering time (or heading date). Many genes/ QTLs have been extensively characterized for this trait and many actual genes in the flowering pathway have been cloned. However, this trait is very easy to select for in the field using visual assessment within a typical rice breeding program, so in practice, markers are not needed specifically for this trait by breeders. So therefore, the answer to the above question is: when markers provide an advantage of conventional phenotyping or breeding. In other words, when they save time, labour, resources or money. The full advantage of MAS can be realized when markers are used to do something that cannot be done using conventional methods such as marker-assisted backcrossing (MABC) or marker-assisted pyramiding (Collard and Mackill 2008). The advantages of using markers compared to conventional phenotypic screening may be determined by doing a cost-benefit analysis, although this has rarely been reported by rice molecular geneticists or breeders.

A brief overview of QTL mapping

Marker-trait associations are identified by QTL mapping experiments which are usually performed by molecular geneticists. In brief for classical QTL mapping experiments, a segregating population (*e.g.*, F2, recombinant inbred lines) is used as the experimental population for phenotypic evaluation of traits of interest (McCouch and Doerge 1995; Yano and Sasaki 1997; Collard *et al.* 2005; Semagn *et al.* 2010). DNA is extracted from each individual plant and then genotyped so that the DNA marker information is used to generate a linkage map. This map is then used to locate genomic regions associated with the trait using statistical methods such as 'interval mapping'. The positions of QTLs controlling the traits are then mapped relative the markers used in map construction. The importance of a QTL is usually indicated by a logarithm of odds (LOD) score and the R^2 value which indicates the effect of the QTL. The higher the LOD score, the more likely the presence of a QTL in the region; the larger the R2 value, the greater the effect of the QTL in terms of its contribution to the trait phenotype. In rice, because full genome sequences are available, markers can be correlated to the physical position of the rice genome sequence.

An alternative approach is called association mapping or genome wide association study (GWAS) in which a panel or set of accessions or breeding lines is used (Zhu *et al.* 2008; Korte and Farlow 2013). This method is often used when the objective is to identify QTLs from a gene bank sample of accession (referred to as a 'panel'), however breeding material may also be used (Begum *et al.* 2015). Instead of interval mapping methods, association-based methods based on linkage disequilibrium (LD) are used to detect associations

between marker and trait (Flint-Garcia *et al*. 2003). An important step is to determine the population structure or relatedness in order to minimize the detection of false positive QTLs. The main advantages of this method is the convenience of using a readily-available set of germplasm (*i.e.*, a segregating population does not need to be developed) and the resolution is higher because a large number of markers are used. However, this latter point necessitates the need for high-throughput marker systems (usually single nucleotide polymorphism or SNPs) to be used (Rafalski 2002).

Critical factors in QTL mapping

It should be emphasized that a QTL map is just a unique output of the experiment that was performed. There are many critical factors that determine the accuracy and relevance of the experiment. Sources of error could involve trait or phenotypic data, marker or genotypic data, or analytical errors may occur. Based on review of the literature, the main factors that determine the accuracy of QTL mapping are indicated in Table 1.

Based on practical experience, the two most critical factors are: (1) accuracy of the phenotypic data; and (2) population size of the mapping populations. The importance of accurate trait data has long been reported (Young 1999; Myles *et al*. 2009). Often, ordinal scales are used for trait characterization because they are commonly used by rice breeders (see for example IRRI standard evaluation system; (IRRI 2014). Furthermore, scoring scales should not be based on subjective ratings scales (Poland and Nelson 2010). In order to properly characterize quantitative traits, reliable phenotyping methods are required to maximize the detection of genetic variation (Cobb *et al*. 2013), but this is not usually the role of molecular geneticists.

Population size ultimately determines the precision of the QTL experiment in terms of identifying the number, location and effects of QTLs (Raghavan and Collard 2012). Estimation of QTL effects may be greatly over-estimated or even underestimate due to small population sizes.Heritability (*e.g.*, broad-sense) should be calculated in order to understand the proportion of genetic variance that has been explained by the QTLs.

Relevance to breeders and external validity

Like any biological experiment, the rational and objectives should be clearly defined and ultimately associated with field conditions. First and foremost, the trait(s) should be a high priority for rice breeders, and defined by the breeders. Phenotyping methods should be highly correlated with field testing. Markers may be highly reliable for a specific assay (*e.g.*, greenhouse-

Table 1. Important factors in accuracy of QTL mapping

Factor	Comments	Some relevant references
Population size	Typical population sizes used are 100-250 individuals but more are required to detect QTLs with small effects and improve precision. For association mapping, panels of >300 are typical.	Raghavan and Collard (2012) Wang *et al.* (2012)
Accuracy of phenotypic data	QTLs can only be reliably mapped with accurate data. Garbage in, garbage out.	Cobb *et al.* (2013) Poland and Nelson (2010)
Marker order	Topic rarely discussed in rice due to ordering based on physical map. However, caution should be noted because there are differences between *indica* and *japonica*.	Collard *et al.*(2009) Chen *et al.* 1997
Number and coverage of markers	Even chromosome coverage is ideal but can be difficult in some elite mapping populations in some chromosomal regions.	
Reliability of marker systems used	More relevant to older marker systems such as random amplified polymorphic DNA (RAPDs). Markers often need to be converted or new ones developed going from "maps to MAS"	Parsons *et al.* (1997)
QTL mapping methods used	There are inherent limitations of the methods. There are also unreported differences between software programs methods.	Li *et al.* (2007)
False positive QTL results	Possible in any experiment, but less likely in larger population sizes. Population structure needs to be accounted for in GWAS.	Raghavan and Collard (2012) Wang *et al.* (2012)

based disease or abiotic stress tolerance test) but there would be little value using these markers if this assay was not an effective predictor of field resistance or tolerance. In practice, information regarding the trait under investigation is lacking and can undermine the effectiveness of subsequent marker-based selection.

Of equal importance are critical factors regarding the validity of the experiment for actual breeding in field conditions. QTL experiments often do not consider genotype by environment (G x E) interactions. Breeders are well aware that G x E interactions that may complicate traits phenotyping. Variation in phenotypic data exists within and between greenhouse and field trials. For field trials, there are usually large variance components for locations and years within multi-environment trials (Atlin *et al.* 2011).

The relevance of parents used to develop the mapping populations should be considered for breeders. Often mapping populations are derived from parents that show a large contrast for the trait being investigated. While this has some advantages for QTL mapping, the extremes being may not provide an accurate picture of the value of the QTL alleles being identified when they are introgressed into elite genetic backgrounds. In other words, breeders develop elite material that often have "good" alleles for many traits, although they may not be the "best alleles". For example, a cross could be made between a very resistant (VR) and a very susceptible (VS) parent. Large effect QTLs may be detected from this study, however the effect of allelic substitution may be less than expected because moderate effect QTL alleles may already be present at this locus in elite breeding material. Finally, the effect of genetic background must be considered. Genes and QTLs may behave differently in different recipient parents due to interactions or epistasis (Hayes *et al.* 2008; Islam *et al.* 2011).

Some molecular geneticists claim in publications that MAS can proceed directly after QTL mapping research. However in our experience, this is certainly not the case. Due to all of the potential pitfalls described above, it has been previously advised that performing QTL and marker validation activities are critical prior to MAS in breeding programs (Collard and Mackill 2008; Collard *et al.* 2008). Breeders must know several details about the markers tools they are using (*i.e.*, reliability in terms of selection accuracy for trait, effectiveness in different genetic backgrounds) and this information should be provided by molecular geneticists. Surprisingly this is not often reported in rice, although there are more examples of these research activities in wheat and barley (Eagles *et al.* 2001; Hayes *et al.* 2008). The need to perform QTL and marker validation activities is one of the main take-home messages from this paper.

What limits the use of MAS in actual rice breeding programs?

There are literally thousands of QTLs that have been previously published and characterized, which implies that there are thousands of markers available for breeders used in selection. Interestingly, surveying or discussions with current rice breeders indicates that this is not the case (*i.e.*, today rice breeders only have a very limited number of validated markers ready to use). For arguments sake, let's assume that all QTLs that have been published are real and accurate. The question then posed is "why have there been relatively few published examples of rice varieties that have been developed using successful MAS?" Although breeders do not usually prioritize publishing their activities, we believe that there are several factors (discussed below) that explain this observation.

The cost of marker genotyping is surely one of the biggest obstacles to wider implementation of molecular markers. The majority of public sector rice breeding programs have extremely limited funds for genotyping and there is simply no scope to implement routine MAS. In practice, options are extremely limited, complicated and risky to substitute a component of the conventional breeding program for a new technology, because there are a suit of essential processes that breeders must do to develop a new variety. Although there are many promising SNP marker platforms, there are still considerable initial challenges to establishing routine marker genotyping systems, via in-house labs or outsourcing (Thomson 2014).

Access to routine marker labs is another problem. Some public sector breeding programs in some Asian countries lack the funds to establish a molecular breeding labs which require a significant initial investment and sufficient human expertise (*i.e.*,

dedicated staff with specialized training). While several central breeding institutes have access to a biotechnology lab, regional satellites rarely have a well-defined pathway for routine marker screening. Although out-sourcing options provided by commercial genotyping service laboratories are becoming more common and accessible, in practice there are still several important bottlenecks such as the logistics of sample collection and processing, and high throughput DNA extraction.

In contrast, some of the larger private breeding companies have highly-efficient and effective molecular breeding support teams and pipelines that are fully integrated with breeding objectives (Eathington *et al.* 2007). Consequently these molecular breeding support systems generate large amounts of molecular data which is directly used in molecular breeding. Unfortunately the public sector and many smaller rice breeding companies in Asia do not have the initial resources or organizational structure to establish equivalent molecular breeding support teams.

A question that we have often pondered is "why is the research carried out by molecular geneticists so disconnected with what breeders need?" Collard and Mackill (2008) described two critical gaps that limit the implementation of MAS in breeding: 'knowledge gap' and 'application gaps'. The knowledge gap was described as the two-way lack of understanding of essential concepts required in molecular and conventional breeding. Many breeders may not understand topics in molecular breeding in sufficient detail and molecular geneticists often do not understand breeding processes well enough to identify the most strategic stage to apply markers. This also prevents integration between breeding and molecular geneticists including sharing of germplasm such as mapping populations or sharing of information or trait data.

The 'application gap' refers to the different motivations of breeders and molecular geneticists. Breeders are usually focused on developing new varieties whereas molecular geneticists are focused more on gene/QTL discovery and ultimately publications. This has been attributed to the culture of scientific research community (Van Sanford *et al.* 2001; Collard and Mackill 2008). Molecular geneticists may also be more interested in trying to go to the gene level or investigate gene expression. While these are valid endeavours from a basic science perspective, they may detract from goals of molecular-assisted germplasm development. This situation could change if management of molecular geneticists in the public sector could place more emphasis on applied outputs and outcomes as in done in private breeding companies. Furthermore, the location of breeding and molecular genetics research is often separate, and may occur at different institutions (*i.e.*, regional breeding station and university or central government headquarters) which can undermine effective integration.

Funding for molecular genetics and/or breeding research can also be sub-optimal. Some funding agencies can fund molecular breeding projects that are imbalanced towards molecular genetics research and fail to provide adequate resources for applying results in breeding programs. Breeders may be attached to projects as a 'token' gesture, rather than by the genuine need to integrate. The opposite situation may also be true where large scale breeding programs do not provide enough 'wet lab' resources for QTL mapping experiments, highlighting the need for careful management of project activities and allocation of resources. The bridging of all of the above-mentioned gaps in the public sector would ensure efficient use of resources and that outputs lead to positive outcomes for rice breeding programs.

Future prospects and conclusion

Recently, there have been encouraging developments in options to outsource genotyping (Thomson 2014) and in new molecular support tools, although the lack of open-source, publicly available tools is still an obvious gap (Varshney *et al.* 2015).There have been some encouraging developments in new molecular breeding schemes in rice such as 'genomic selection' (Heffner *et al.* 2010; Nakaya and Isobe 2012; Desta and Ortiz 2014; Heslot *et al.* 2015). This method is based on using very large numbers of markers (usually SNPs) to predict trait phenotypes based on the detailed phenotypic and genotypic characterization of a 'training set'. This method generally uses very large numbers of markers (*e.g.*, 1000 to >10,000 markers) located all over the genome rather than markers which are specifically associated with genes or QTLs. Recent research has indicated that including known genes or QTLs from QTL mapping experiments can increase the accuracy

of prediction (Spindel *et al.* 2016). However cost per sample, rather than cost per marker data point and open-source breeder-friendly software tools are still huge obstacles to overcome for this scheme to be routinely applied. Considerable further empirical testing is still needed in rice.

In conclusion, QTL mapping experiments are the first step in identifying loci controlling traits using conventional interval mapping or association mapping-based methods. There are many subsequent steps required before the ultimate use of associated markers in actual rice molecular breeding. Currently, most rice breeders do not have a large arsenal of marker kits to select for their high priority traits. Molecular geneticists and breeders must work closely together on common objectives and research needs to be integrated. Highly efficient and cheap genotyping systems are desperately needed for routine MAS to serve rice breeders, because it is likely that MAS will focus on major genes or large-effect QTLs in the short to medium term.

In order to leverage the wealth of publicly-available genomics resources in rice for molecular breeding in the future, breeders will need: carefully planned and well-executed QTL mapping experiments, QTL and marker validation activities, efficient genotyping systems and processes, cheaper genotyping systems and more breeder-friendly analytical tools. Integration between breeders and molecular geneticists will be critical to develop new germplasm using markers. With the continual and rapid developments in rice genomics (The 3 2014; Matsumoto *et al.* 2016), it cannot be emphasized enough that breeders and molecular geneticists will need to work more closely than before in order to effectively integrate activities. Improved co-ordination and collaboration regarding germplasm development between breeders and molecular geneticists and some fresh thinking will also be important to achieve synergy.

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The opinions expressed in this article are those of the authors and do not reflect the view of IRRI.

REFERENCES

Atlin GN, Kleinknecht K, Singh GP and Piepho HP 2011. Managing Genotype x Environment Interaction in Plant Breeding Programs: A Selection Theory Approach. Journal of the Indian Society of Agricultural Statistics 65: 237-247

Begum H, Spindel JE, Lalusin A, Borromeo T, Gregorio G, Hernandez J, Virk P, Collard B and McCouch SR 2015. Genome-wide association mapping for yield and other agronomic traits in an elite breeding population of tropical rice (*Oryza sativa*). PLoS ONE10, 3

Chen X, Temnykh S, Xu Y, Cho YG and McCouch SR 1997. Development of a microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.). Theoretical and Applied Genetics 95: 553-567 doi:10.1007/s001220050596

Cobb JN, DeClerck G, Greenberg A, Clark R and McCouch S 2013. Next-generation phenotyping: Requirements and strategies for enhancing our understanding of genotype-phenotype relationships and its relevance to crop improvement. Theoretical and Applied Genetics 126: 867-887 doi:10.1007/s00122-013-2066-0

Collard BCY, Ismail AM and Hardy B 2013. 'EIRLSBN: Twenty years of achievements in rice breeding.' (BC. Collard, AM Ismail, and B Hardy, Eds.). International Rice Research Institute: Los Banos, Philippines

Collard BCY, Jahufer MZZ, Brouwer JB and Pang ECK 2005. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. Euphytica 142: 169-196 doi:10.1007/s10681-005-1681-5

Collard BCY, Mace ES, McPhail M, Wenzl P, Cakir M, Fox G, Poulsen D and Jordan D 2009. How accurate are the marker orders in crop linkage maps generated from large marker datasets? Crop and Pasture Science 60: 362-372

Collard BCY and Mackill DJ 2008. Marker-assisted selection: An approach for precision plant breeding in the twenty-first century. Philosophical Transactions of the Royal Society B: Biological Sciences 363: 557-572 doi:10.1098/rstb.2007.2170

Collard BCY, Vera Cruz CM, McNally KL, Virk PS and Mackill DJ 2008. Rice molecular breeding laboratories in the genomics era: Current status and future considerations. International Journal of Plant Genomics 2008 doi:10.1155/2008/524847

Desta ZA and Ortiz R 2014. Genomic selection: Genome-wide prediction in plant improvement. Trends in Plant Science 19: 592-601 doi:10.1016/j.tplants.2014.05.006

Dwivedi SL, Crouch JH, Mackill DJ, Xu Y, Blair MW, Ragot M, Upadhyaya HD and Ortiz R 2007. The Molecularization of Public Sector Crop Breeding: Progress, Problems, and Prospects. Advances in Agronomy 95: 163-318 doi:10.1016/S0065-2113(07)95003-8

Eagles HA, Bariana HS, Ogbonnaya FC, Rebetzke GJ, Hollamby GJ, Henry RJ, Henschke PH, Carter M, Eagles HA, Bariana HS, Ogbonnaya FC, Rebetzke GJ, Hollamby GJ, Henry RJ, Henschke PH and Carter M 2001. Implementation of markers in Australian wheat breeding. Australian Journal of Agricultural Research 52: 1349 doi:10.1071/AR01067

Eathington SR, Crosbie TM, Edwards MD, Reiter RS and Bull JK 2007. Molecular markers in a commercial breeding program. Crop Science 47: S154-S163

Flint-Garcia SA, Thornsberry JM and Buckler ESI 2003. Structure of linkage disequilibrium in plants. Annual Review of Plant Biology 54: 357-374. doi:10.1146/annurev.arplant.54.031902.134907

Hayes PM, Marquez-Cedillo L, Mundt CC, Richardson K and Vales MI (2008) Perspectives on Finding and using Quantitative Disease Resistance Genes in Barley. 'Plant Breed. Arnel R. Hallauer Int. Symp.' pp. 182-200 (Blackwell Publishing: Ames, Iowa, USA) doi:10.1002/9780470752708.ch13

Heffner EL, Lorenz AJ, Jannink JL and Sorrells ME 2010. Plant breeding with Genomic selection: Gain per unit time and cost. Crop Science 50: 1681-1690 doi:10.2135/cropsci2009.11.0662.

Heslot N, Jannink J-L and Sorrells ME 2015. Perspectives for Genomic Selection Applications and Research in Plants. Crop Science 55: 1-12 doi:10.2135/cropsci2014.03.0249

IRRI (2014) 'Standard evaluation system for rice.' International Rice Research Institute, Manila, Philippines

Islam MR, Gregorio GB, Salam MA, Collard BCY, Tumimbangraiz E, Adorada DL, Mendoza RD, Singh RK and Hassan L 2011. Validation of a major QTL for salinity tolerance on chromosome 1 of rice in three different breeding populations. Agrochimica 55: 355-366

Khush GS and Virk PS 2005. 'IR varieties and their impacts.'Los Baños (Philippines): International Rice Research Institute pp.163

Korte A and Farlow A 2013. The advantages and limitations of trait analysis with GWAS: a review. Plant Methods 9 doi:10.1186/1746-4811-9-29

Li H, Ye G and Wang J 2007. A modified algorithm for the improvement of composite interval mapping. Genetics175: 361-74 doi:10.1534/genetics 106.066811

Matsumoto T, Wu J, Itoh T, Numa H, Antonio B and Sasaki T 2016. The Nipponbare genome and the next-generation of rice genomics research in Japan. Rice 9: 33 doi:10.1186/s12284-016-0107-4

McCouch SR and Doerge RW 1995. QTL mapping in rice. Trends in genetics?11: 482-7 http://www.ncbi.nlm.nih.gov/pubmed/8533164

Myles S, Peiffer J, Brown PJ, Ersoz ES, Zhang Z, Costich DE and Buckler ES 2009. Association mapping: critical considerations shift from genotyping to experimental design. The Plant Cell 21: 2194-2202 doi:10.1105/tpc.109.068437

Nakaya A and Isobe SN 2012. Will genomic selection be a practical method for plant breeding? Annals of Botany 110: 1303-1316 doi:10.1093/aob/mcs109.

Parsons BJ, Newbury HJ, Jackson MT, and Ford-Lloyd BV 1997. Contrasting genetic diversity relationships are revealed in rice (*Oryza sativa* L.) using different marker types. Molecular Breeding 3: 115-125 doi:10.1023/A:1009635721319

Peng S and Khush GS 2003. Four Decades of Breeding for Varietal Improvement of Irrigated Lowland Rice in the International Rice Research Institute. Plant Prod. Sci. 6: 157-164 doi:10.1626/pps.6.157

Poland JA and Nelson RJ 2010. In the Eye of the Beholder: The Effect of Rater Variability and Different Rating Scales on QTL Mapping. Phytopathology 101: 290-298

Rafalski A 2002. Applications of single nucleotide polymorphisms in crop genetics. Current Opinion in Plant Biology 5: 94-100 http://www.ncbi.nlm.nih.gov/pubmed/11856602

Raghavan C and Collard B 2012. Effect of small mapping population sizes on reliability of quantitative trait locus (QTL) mapping. African Journal Of Biotechnology 4: 10661-10674 doi:10.5897/AJB11.2032

Van Sanford D, Anderson J, Campbell K, Costa J, Cregan P, Griffey C, Hayes P and Ward R 2001. Discovery and deployment of molecular markers linked to fusarium head blight resistance: An integrated system for wheat and barley. Crop Science 41: 638-644 doi:10.2135/cropsci2001.413638x

Semagn K, Bjornstad A and Xu Y 2010. The genetic dissection of quantitative traits in crops. Electronic Journal of Biotechnology 13: 1-45 doi:10.2225/vol13-issue5-fulltext-21

Singh US, Dar MH, Singh S, Zaidi NW, Bari MA, Mackill DJ, Collard BCY, Singh VN, Singh JP, Reddy JN, Singh RK and Ismail AM (2013) Field performance, dissemination, impact and tracking of submergence tolerant (Sub1) rice varieties in South Asia. SABRAO Journal of Breeding and Genetics 45: 112-131

Singh R, Singh Y, Xalaxo S, Verulkar S, Yadav N, Singh S, Singh N, Prasad KSN, Kondayya K, Rao PVR, Rani MG, Anuradha T, Suraynarayana Y, Sharma PC, Krishnamurthy SL, Sharma SK, Dwivedi JL, Singh AK, Singh PK, Nilanjay, Singh NK, Kumar R, Chetia SK, Ahmad T, Rai M, Perraju P, Pande A, Singh DN, Mandal NP, Reddy JN, Singh ON, Katara JL, Marandi B, Swain P, Sarkar RK, Singh DP, Mohapatra T, Padmawathi G, Ram T, Kathiresan RM, Paramsivam K, Nadarajan S, Thirumeni S, Nagarajan M, Singh AK, Vikram P, Kumar A, Septiningshih E, Singh US, Ismail AM, Mackill D and Singh NK 2016. From QTL to variety-harnessing the benefits of QTLs for drought, flood and salt tolerance in mega rice varieties of India through a multi-institutional network. Plant Science 242: 278-287 doi:10.1016/j.plantsci.2015.08.008

Spindel JE, Begum H, Akdemir D, Collard B, Redoña E, Jannink J-L and McCouch S 2016. Genome-wide prediction models that incorporate de novo GWAS are a powerful new tool for tropical rice improvement. Heredity116: 395-408 doi:10.1038/hdy.2015.113

Spindel J, Begum H, Akdemir D, Virk P, Collard B, Redoña E, Atlin G, Jannink J-L and McCouch S-R 2015. Correction: Genomic Selection and Association Mapping in Rice (*Oryza sativa*): Effect of Trait Genetic Architecture, Training Population Composition, Marker Number and Statistical Model on Accuracy of Rice Genomic Selection in Elite, Tropical Rice Breeding. PLoS Genetics11 doi:10.1371/journal.pgen.1005350

The 3 000 rice genomes project 2014. The 3,000 rice genomes project. GigaScience 3. doi:10.1186/2047-217X-3-7

Thomson MJ 2014. High-Throughput SNP Genotyping to Accelerate Crop Improvement. Plant Breeding and Biotechnology 2: 195-212

Varshney RK, Singh VK, Hickey JM, Xun X, Marshall DF, Wang J, Edwards D and Ribaut JM 2015. Analytical and decision support tools for genomics-assisted breeding. Trends in Plant Science 1360-1385(15)00277-0., 1-10. doi:10.1016/j.tplants.2015.10.018

Wang H, Smith KP, Combs E, Blake T, Horsley RD and Muehlbauer GJ 2012. Effect of population size and unbalanced data sets on QTL detection using genome-wide association mapping in barley breeding germplasm. Theoretical and Applied Genetics 124: 111-124. doi:10.1007/s00122-011-1691-8

Xu Y and Crouch JH 2008. Marker-Assisted Selection in Plant Breeding?: From Publications to Practice. Crop Science 48: 391-407. doi:10.2135/cropsci2007.04.0191

Yano M and Sasaki T 1997. Genetic and molecular dissection of quantitative traits in rice. Plant Molecular Biology 35: 145-153. doi:10.1023/A:1005764209331

Young ND 1999. A cautiously optimistic vision for marker-assisted breeding. Molecular Breeding 5: 505-510. doi:10.1023/A:1009684409326

Zhu C, Gore M, Buckler ES and Yu J 2008. Status and prospects of association mapping in plants. The Plant Genome Journal 1: 5-20 doi:10.3835/plantgenome2008.02.0089