International Journal of Scientific World, 2 (1) (2014) 16-20
©Science Publishing Corporation
www.sciencepubco.com/index.php/IJSW
doi: 10.14419/ijsw.v2i1.1785
Review Paper

Molecular marker assisted selection as approach to increase the selection efficiency of drought tolerant genotypes

M.R. Shiri *, K. Akhavan

Agricultural and Natural Resources Research Center of Ardebil Province, Moghan, Iran
**Corresponding author E-mail: mohammadrezashiri52@gmail.com*

Abstract

Identifying the complete-linked molecular markers with target gene and mapping its chromosome locus is an important goal in plant breeding for gene cloning and marker-aided selection. Due to complexity of the interactions, in most of the agronomic traits, especially the interaction between the grain yield and the environmental factors, classic methods do not function appropriately in improving agronomic traits at present. If the selection is made based on genotype by DNA markers, the efficiency of selection will increase considerably. In a genetic evaluation program, the combination between the data from the linkage between marker position and quantitative traits loci (QTL) as well as the phenotypic data can be used to increase the accuracy of the assessments and thereby the accuracy of selection. The selection in which inherited values are used along with the marker data in selection of superior genotypes in a breeding program is called Marker-assisted-selection (MAS).

Keywords: *MAS, QTL, Water Stress, Maize.*

1. Introduction

Conventional plant breeding is dependent on appropriate environmental conditions in which to identify and select desirable plants. Typically, breeders improve crops by crossing plants with desired traits, such as high yield or drought tolerance, and selecting the best offspring over multiple generations of testing. A new variety could take 8 to 10 years to develop. Breeders are very interested in new technologies to speed up this process or make it more efficient. Since the mid-1990's, the term 'marker-assisted selection' has entered the plant breeders and genetics science [28]. The MAS term largely refers to all the form of the selections based on genetic data.

There are so many drawbacks in the classic selection method in which phenotypic measures are used as follows [18]:

a) Environmental factors reduce the accuracy of the selection for important commercial traits.
b) It is difficult to measure several traits.
c) In classical breeding programs, measurements are performed in a broad sense. It is both costly and time consuming.

Given the above-mentioned drawbacks, selection using genetic markers could increase both efficiency and accuracy of the selection compared with selection based on phenotypic data [32].

If it is not possible to analyze both the traits and the pedigree in a broad sense in breeding programs, genetic markers of the major genes can be used provided that they have significant economic impacts [32].

An appropriate method is the one in which molecular markers data is combined with statistical methods. This increases the accuracy, reduces the generation gap, and ultimately increases the response to selection. The advantage of marker-assisted selection on a trait compared to selection based on the phenotype depends on the heritability. Marker-assisted selection is advantageous for following cases [18]:

a) When the heritability of the trait is low
b) When the traits that are difficult and costly to measure
c) When there is no information on the parents of the present population

The disadvantage of marker-assisted selection only lies in the recombination probability, which reduces the usefulness of this method.
The direct information obtained from the level of genes is effective in breeding programs using these three general following methods [32]:

a) The Markers can reduce the generation gap and allow the selection to take place in the early stages of growth.
b) The accuracy of selection increases by providing more information to estimate.
c) The marker increases the severity of selection and provides the possibility to select the major candidates among many candidates for selection.

Any feature of the living organisms whose inheritance can easily be checked and tracked is called a marker.
Genetic markers are the biological features that are determined by allelic forms of genes or genetic loci and can be transmitted from one generation to another, and thus they can be used as experimental probes or tags to keep track of an individual, a tissue, a cell, a nucleus, a chromosome or a gene [32].
The marker should have two following features, so that it can be useful and applicable:

a) The marker should differ the individuals from each other, i.e. it should be polymorphic.
b) The marker should be passed from one generation to another generation unchanged.

In addition to the characteristics mentioned above, a favorable marker should have the following characteristics for genetic and breeding studies [32]:

a) It should be co-dominant.
b) It should not be dependent on both plant tissue and developmental stage of the plant.
c) It should be neutral in terms of phenotypic expression; however, if possible, it should be linked with the gene (or the genes), which control the expression of the desired trait.
d) It should be repeatable.
e) It should be easy to measure and simple to use.
f) It should also be easy to interpret the results obtained from marker analysis.
g) It should not be dependent on application of hazardous materials such as radioactive materials.

Genetic markers fall into one of the three broad classes: those based on visually assessable traits (morphological and agronomic traits), those based on gene product (biochemical markers), and those relying on a DNA assay (molecular markers) [15].

2. The use of biomarkers in quantitative traits selection

Although the idea for marker-assisted selection dates back to 1923, when Sax in 1923 first reported association of a simply inherited genetic marker with a quantitative trait in plants when he observed segregation of seed size (polygenic, quantitatively inherited trait) associated with segregation for a seed coat colour(monogenic trait) marker in beans (Phaseolus vulgaris L)and drew the conclusion that the single gene controlling seed color must be linked to one or more of the polygene controlling seed size[14]. This concept was further elaborated by Thoday (1961), who suggested that if the segregation of simply inherited monogenes could be used to detect linked QTLs, then it should eventually be possible to map and characterize all QTLs involved in complex traits [13]. At first, traits showing quantitative variation were studied by statistical analysis of appropriate experimental populations based on the means, variances and covariances of relatives, with no actual knowledge of the number and location of the genes that underlie them [25]. Working with morphological markers, the main practical limitation of his work was the fact that only few suitable markers were available.
By the early 1980s, allozyme markers were being employed as a tool for the discrimination of genotypes, replacing the previously used morphological markers. Allozyme markers are based on protein polymorphisms; they are allelic forms of enzymes and can be separated on electrophoretic gels and detected by staining the gels. Advantages of this method are the low costs, technical simplicity and the co-dominant nature of the marker. Co-dominance means that alleles of both parents can be detected in the F1, thus homozygous and heterozygous genotypes can be distinguished. However, the limited number of suitable allozyme loci in the genome and the requirement of fresh tissue of the right developmental stage are clear disadvantages [16], [31]. Realization of this potential has been limited by the lack of markers.
With the advent of DNA-based genetic markers in the late 1970s, for the first time, the situation changed and researchers could begin to identify large numbers of markers dispersed throughout the genetic material of any species of interest and use the markers to detect associations with traits of interest, thus allowing MAS finally to become a reality. In fact, new interest in QTL mapping in crops was generated when studies on fruit traits of tomato [1] and the morphological and agronomic characters of maize [4] successfully demonstrated that some molecular markers explained a substantial proportion of the phenotypic variance of quantitative traits. With DNA markers, more polymorphisms can be revealed and breeders could identify large numbers of markers dispersed throughout the genome of any species of interest, using the markers to detect associations with traits of interest, independent of their stage specific expression. Finally, the idea of MAS became a reality [10].

Various types of molecular markers have been described in the literature, which are as follows: allele specific associated primers (ASAP), allele specific oligo (ASO), allele specific polymerase chain reaction (AS-PCR), amplified fragment length polymorphism (AFLP), anchored microsatellite primed PCR (AMP-PCR), anchored simple sequence repeats (ASSR), arbitrarily primed polymerase chain reaction (AP-PCR), cleaved amplified polymorphic sequence (CAPS), degenerate oligo nucleotide primed PCR (DOP-PCR), diversity arrays technology (DART), DNA amplification fingerprinting (DAF), expressed sequence tags (EST), inter-simple sequence repeat (ISSR), inverse PCR (IPCR), inverse sequence-tagged repeats (ISTR), microsatellite primed PCR (MP-PCR), multiplexed allele-specific diagnostic assay (MASDA), random amplified microsatellite polymorphisms (RAMP), random amplified microsatellites (RAM), random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), selective amplification of microsatellite polymorphic loci (SAMPL), sequence characterized amplified regions (SCAR), sequence specific amplification polymorphisms (S-SAP), sequence tagged microsatellite site (STMS), sequence tagged site (STS), short tandem repeats (STR), simple sequence length polymorphism (SSLP), simple sequence repeats (SSR), single nucleotide polymorphism (SNP), single primer amplification reactions (SPAR), single stranded conformational polymorphism (SSCP), site-selected insertion PCR (SSI), strand displacement amplification (SDA), and variable number tandem repeat (VNTR). Although some of these marker types are very similar (e.g., ASAP, ASO and AS-PCR), some synonymous (e.g., ISSR, RAMP, RAM, SPAR, AMP-PCR, MP-PCR, and ASSR), and some identical (e.g., SSLP, STMS, STR and SSR), there are still a wide range of techniques for researchers to choose upon [14].

Restriction fragment length polymorphisms (RFLPs) are reliable and yield co-dominant data, but are also time-consuming and expensive, requiring relatively large amount of highly purified DNA and they do not lend themselves to automation [28]. Random amplified polymorphic DNA (RAPD) markers are unreliable with poor replication success among laboratories [3], [9]. Sequence characterised amplified regions (SCAR) markers are more reliable than RAPD markers, but are often developed from RAPD markers [9], which might limit their utility. Simple sequence repeats (SSR) markers, however, combine reliability and genomic abundance with high levels of polymorphism and co-dominance [5]. Recently, there is increasing use of single nucleotide polymorphism markers (SNPs) in maize [26]. The main drawback of SSRs is the initial identification of primer sites to amplify SSR loci, a procedure which is time- and resource demanding. In the present case, a large number of SSR markers are already available.

There are five main considerations for the use of DNA markers in MAS as follows:

Reliability: Markers should be tightly linked to target loci, preferably less than 5 CM genetic distance. The use of flanking markers will greatly increase the reliability of the markers to predict phenotype.

DNA quantity and quality: Some marker techniques require large amounts and high quality of DNA, which may sometimes be difficult to obtain in practice, and this adds to the cost of the procedures.

Technical procedure: The level of simplicity and the time required for the technique are critical considerations. High-throughput simple and quick methods are highly desirable.

Level of polymorphism: Ideally, the marker should be highly polymorphic in breeding material.

Cost: The marker assay must be cost-effective in order for MAS to be feasible.

The most widely used markers in major cereals are called simple sequence repeats (SSRs) or microsatellites [28], [29]. They are highly reliable (i.e. reproducible), co-dominant in target locus reliability for selection. The only disadvantages of SSRs are that they typically require polyacrylamide gel electrophoresis and generally give information only about a single locus per assay, although multiplexing of several markers is possible. These problems have been overcome in many cases by selecting SSR markers that have large enough size differences for detection in Agarose gels, as well as multiplexing several markers in a single reaction. SSR markers also require a substantial investment of time and money to develop, and adequate numbers for high-density mapping are not available in some orphan crop species. STS, SCAR or SNP markers that are derived from specific DNA sequences of markers (e.g. RFLPs) that are linked to a gene or quantitative trait locus are also extremely useful for MAS [2], [27], [34].

3. Steps for MAS

Generally the first step is to map the gene or quantitative trait locus (QTL) of interest first by using different techniques and then use this information for marker assisted selection.

The two general goals of QTL mapping in plants are to (a) increase our biological knowledge of the inheritance and genetic architecture of quantitative traits, both within a species and across related species, and (b) identify markers that can be used as indirect selection tools in breeding [15].

Based on our experiences, we suggest the following steps be taken to enjoy a successful marker-assisted selection:

1) Find markers that are less than 5 cM away from the desired gene. Zheng et al. (1995) empirically showed that the selection process will be 99.75 percent accurate if markers are found that are less than 5 cM away from the desired gene.
2) Transform non- specific markers to specific or STS markers. The linkage of the main genes in crop plants with, or their distance from, markers (especially the RFLP and RAPD markers) has been established. Although these markers can be used in marker-assisted selection, their transformation to STS markers will greatly increase the

efficiency of the selection process because, if this transformation takes place, utilization of the speed, accuracy, and efficiency of PCR will lead to a more successful and faster selection that needs less DNA.

3) Produce and detect specific amplicon polymorphism so that PCR can be used in marker-assisted selection. In plants QTL mapping is generally achieved using bi-parental cross populations; a cross between two parents which have a contrasting phenotype for the trait of interest are developed. Commonly used populations are recombinant inbred lines (RILs), doubled haploids (DH), back cross and F2. Linkage between the phenotype and markers which have already been mapped is tested in these populations in order to determine the position of the QTL. Such techniques are based on linkage and are therefore referred to as "linkage mapping".
4) Carry out marker-assisted selection. A successful marker-assisted selection requires identification of an important gene. Those genes must be more emphasized that are more difficult, or impossible, to study by using classic and field studies. Moreover, markers should not be used in selection for traits that are easily recognized in the field. Marker-assisted selection should be utilized as a complement to classic methods. We should not forget that the technology of using molecular markers is still relatively expensive and complex and specialists are needed to utilize it.

In contrast to two-step QTL mapping and MAS, a single-step method for breeding typical plant populations has been developed. In such an approach, in the first few breeding cycles, markers linked to the trait of interest are identified by QTL mapping and later the same information in used in the same population. In this approach, pedigree structures are created from families that are created by crossing number of parents (in three-way or four way crosses). Both phenotyping and genotyping is done using molecular markers mapped the possible location of QTL of interest. This will identify markers and their favorable alleles.

4. Use of marker-aided selection for drought tolerant genotypes selection

Drought tolerance liking other environmental stresses in higher plants is a complex genetic and physiologic trait. Most plant processes which are critical in drought tolerance have little inheritance and show a continual variation and are also under the influence of environmental conditions. Previous genetic studies revealed that both additive and dominance gene effects in inheritance are included in almost all traits related to drought [22], [23].

In maize, about 148 QTLs for grain yield have been detected. However, fewer QTLs were identified under water-stressed conditions (about 20 QTLs) [26].

In maize, most research efforts have been directed toward the development of microsatellite marker systems for genetic mapping and germplasm analysis [8], [33]. The study on mapping or tagging presents information about the number of genes controlling the trait and the place of these genes in linkage map.

Dubey et al. (2009) in an effort to identify SSR markers of drought tolerance in 24 tropical maize lines with different responses to drought stress came to the conclusion that dupssr12, umc1042, bnlg1866, umc1056 ,dup13, umc1069, umc1962, bnlg1028 and c1344 markers were among those drought related markers in the susceptible and drought tolerant genotypes under investigation[17].

Despite numerous reports of QTLs in maize [26], including QTLs for adaptation to water-limited conditions, reviewed by Tuberosa et al.(2002) and Sawkins et al. (2006), little has been published on the implementation of MAS based on these QTLs in breeding programmes[22], [30]. Successful MAS applications have been reported for introgression breeding in maize, including introgressions of transgenes [20] and conversions involving simple [19] or complex traits [24]. Several marker-assisted recurrent selection (MARS) strategies have also been proposed and evaluated. When aimed at population improvement, MARS involves selecting individuals based on their marker genotypes and intermating them at random to produce the next generation [6].

In a wider context, QTL might be used to identify genes that are important in drought tolerance and it is noteworthy, given the complexity of drought tolerance, that so few QTL are identified within any given genome. This may be an indication that traits are determined by a limited number of sites and/or that genes associated with physiological traits are clustered on chromosomes. However, the fact that a QTL represents many, perhaps hundreds, of genes remains a problem to finding key loci within a QTL. The easiest way forward may be through the identification of candidate genes [12].

5. Conclusion

Molecular genetic polymorphisms can be used to achieve substantial increases in the efficiency of artificial selection. There is no doubt that the use of molecular markers maize breeding programmes has increased significantly over the last few years. However, there is more challenge for MAS that will be in the integration of this diverse and disparate information and interpretation in a specific biological context to convert it into knowledge. Modern maize breeding for complex traits, a combination of molecular and phenotypic selection, should become routine in breeding programmes, but success will largely be dependent on the accuracy of plant phenotyping and the capacity to determine the Genotype environment interaction; two major components that affect the prediction of the allelic value on the plant phenotype in new populations.

References

[1] A. H. Paterson, E.S. Lander, J.D. Hewitt, S. Peterson, S. E. Lincoln, S. D. Tanksley, *Resolution of quantitative traits into Mendelian factors using a complete linkage map of restriction fragment length polymorphisms*, Nature, 335, 1988, 721-726.

[2] A.C. Sanchez, D.S. Brar, N. Huang, Z. Li, G.S. Khush, *Sequence tagged site marker-assisted selection for three bacterial blight resistance genes in rice*. Crop Science, 40, 2000, 792–797. DOI:10.2135/cropsci2000.403792x

[3] C. Hallden, M. Hansen, N.O. Nilsson, *Competition as a source of errors in RAPD analysis*, Theoretical and Applied Genetics, 93, 1996, 1185-1192. DOI: 10.1007/BF00223449

[4] C.W. Stuber, S.E. Lincoln, D.W. Wolff, T. Helentjaris, E.S. Lander, *Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers*, Genetics, 132, 1992,823-839.

[5] E.C.L. Chin, M.L. Senior, , H. Shu, J.S.C. Smith, *Maize simple repetitive DNA sequences: abundance and allele variation*, Genome, 39, 1996, 866-873.

[6] F. Hospital, L. Moreau, F. Lacoudre, A. Charcosset, A. Gallais, *More on the efficiency of marker-assisted selectio*n, Theoretical and Applied Genetics, 95, 1997,1181-1189. DOI:10.1007/s001220050679

[7] G. Taramino, S. Tingey, *Simple sequence repeats for germplasm analysis and mapping in maize*, Genome, 39, 1996, 277–287. DOI:10.1139/g96-038

[8] I. Paran, RW. Michelmore, *Development of reliable PCR based markers linked to powdery mildew resistance genes in lettuce*. Theor. Appl. Genet., 85, 1993, 985-993. DOI:10.1007/BF00215038

[9] J. Ruane, A. Sonnino, *Marker-Assisted Selection as a Tool for Genetic Improvement of Crops, Livestock, Forestry and Fish in Developing Countries:* an Overview of the Issues. In: Guimaraes, E. P., Ruane, J., Scherf, B. D., Sonnino, A. and Dargie, J. D. (Eds.): Marker-assisted Selection - Current Status and Future Perspectives in Crops, Livestock, Forestry and Fish, Food and Agriculture Organization of the United Nations (FAO), 2007, 3-13.

[10] J.M. Ribaut, M. Banziger, T. Setter, G. Edmeades, D. Hoisington, *Genetic dissection of drought tolerance in maize: a case study.* In: Nguyen H, Blum A, eds. Physiology and biotechnology integration for plant breeding. New York: Marcel Dekker Inc., 2004, 571–611.

[11] J.M. Ribaut, M. Ragot, *Marker-assisted selection to improve drought adaptation in maize: the backcross approach, perspectives, limitations, and alternatives*, Journal of Experimental Botany, 58, 2007, 351–360.

[12] J.M. Thoday, *Location of polygenes*. Nature, 191,196, 368-370.

[13] K. Sax, *The association of size differences with seed-coat pattern and pigmentation in Phaseolus vulgaris*. Genetics, 8, 1923, 552-560.

[14] K. Semagn, Å. Bjørnstad, M.N. Ndjiondjop, *An overview of molecular marker methods for plants*, African Journal of Biotechnology, 5,2006, , 2540-2568.

[15] K. Weising, H. Nybom, K. Wolff, W. Meyer, *DNA fingerprinting in plants and fungi*, CRC Press, Boca Raton Ann Arbor London Tokyo, 1995, 321 pp.

[16] L. Dubey, B.M. Prasanna, B. Ramesh, *Analysis of drought tolerant and susceptible maize genotypes using SSR markers tagging candidate genes and consensus QTLs for drought tolerance*, The Indian Journal of Genetics and Plant Breeding, 69,2009,344–351.

[17] M. Farsi, A. Bagheri, *Principles of plant biotechnology*, Mashhad University Pub., Mashhad, Iran, 2011, 554p.

[18] M. Morris, K. Dreher, J.M. Ribaut, M. Khairallah 2003. *Money matters (II): costs of maize inbred line conversion schemes at CIMMYT using conventional and marker-assisted selection*. Molecular Breeding 11, 235–247. DOI:10.1023/A:1022872604743

[19] M. Ragot, M. Biasiolli, M.F. Delbut, A. Dell'Orco, L. Malgarini, P. Thevenin, J. Vernoy, J. Vivant, R. Zimmermann, G. Gay. *Marker-assisted backcrossing: a practical example.* In: A. Berville, M. Tersac, eds. Les Colloques, No72, Techniques reutilizations des marqueurs mole´ culaires, Paris,INRA, 1995, 45–56.

[20] M. Sawkins, J. Meyer, J.M. Ribaut, *Drought adaptation in cereal crops drought adaptation in maize*. In: Ribaut J.M, ed. Drought tolerance in cereals. Binghamtown, NY: The Haworth Press Inc., 2006, 356–387.

[21] M. Shiri, R. Choukan, R.T. Aliyev, *Drought tolerance evaluation of maize hybrids using biplot method*. Trends Applied Sciences Research, 5, 2010a, 129-137. DOI:10.3923/tasr.2010.129.137

[22] M. Shiri, R.T. Aliyev, R. Choukan, *Water stress effects on combining ability and gene action of yield and genetic properties of drought tolerance indices in maize*, Research Journal of Environmental Science, 4, 2010b, 75-84. DOI:10.3923/rjes.2010.75.84

[23] M. Willcox, M. Khairallah, D. Bergvinson, *Selection for resitance to southwaestern corn borer using marker-assisted and conventional backcrossing*, Crop Science, 42, 2002, 1516–1528. DOI:10.2135/cropsci2002.1516

[24] M.J. Kearsey, and A.G.L. Farquhar, *QTL analysis in plants; where are we now*?, Heredity, 80, 1998,137–142. DOI:10.1046/j.1365-2540.1998.00500.x

[25] *Maize Genetics and Genomics Database* (2010). http://www.maizegdb.org.

[26] P. J. Sharp, *Validation of molecular markers for wheat breeding*, Aust. J. Agric. Res., 52, 2001, 1357–1366. DOI:10.1071/AR01052

[27] P. K.Gupta, R.K.Varshney, P.C. Sharma, B. Ramesh, *Molecular markers and their applications in wheat breeding*, Plant Breeding, 118, 1999, 369-390. DOI: 10.1046/j.1439-0523.1999.00401.x

[28] P.K. Gupta, R.K. Varshney, *The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat*, Euphytica, 113, 2000,163-185. DOI:10.1023/A:1003910819967

[29] R. Tuberosa, S. Salvi, M.C. Sanguineti, P. Landi, M. MacCaferri, S.Conti, *Mapping QTLs regulating morpho-physiological traits and yield: case studies, shortcomings and perspectives in drought-stressed maize*, Annals of Botany 89, 2002, 941–963. DOI: 10.1093/aob/mcf134

[30] S. D. Tanksley, *Mapping Polygenes*, Annual Review of Genetics, 27, 1993, 205-233. DOI: 10.1146/annurev.ge.27.120193.001225

[31] S.A. Mohammadi, *Statistical methods in genetics*, Proceedings of 6th Statistics International Conference, Tarbiat Modares University, Tehran, Iran, 26-28 August, 2002, p.371-394.

[32] T.L. Phelps, A.E. Hall, B. Buckner, *Microsatellites repeat variation within the y1 gene of maize and teosinte*, J. Hered., 87,1996, 396–399.

[33] X. Shan, T.K. Blake, L.E. Talbert, *Conversion of AFLP markers to sequence-specific PCR markers in barley and wheat*, Theor. Appl. Genet., 98, 1999, 1072-1078. DOI: 10.1007/s001220051169.