



ISSN: 0975-833X

Available online at <http://www.journalcra.com>

**INTERNATIONAL JOURNAL
OF CURRENT RESEARCH**

International Journal of Current Research
Vol. 4, pp. 070-074, May, 2010

REVIEW ARTICLE

Genome sequencing and crop improvement

Shabir H. Wani*, **N. B. Singh****, **H. Nanita Devi****, **Rita Nongthombam**** and **Hitesh K. Saini*****

*Biotechnology Laboratory, Central Institute of Temperate Horticulture, Srinagar, (J&K) 190 007 India.

**Department of Plant Breeding and Genetics, COA, Central Agricultural University, Imphal, Manipur, 795 004

*** Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana 140 004

ARTICLE INFO

Article History:

Received 12th April, 2010

Received in revised form

15th April, 2010

Accepted 17th April, 2010

Published online 2nd May, 2010

ABSTRACT

Most of the crop plants have large and complex genomes. Maize and wheat genomes have a size of 2300 Mb and 16500 Mb respectively. Until recently the sequencing of these complex genomes was considered intractable. But now the genome sequencing technology is undergoing a revolution with the commercialization of second generation technologies capable of sequencing millions of bases in a single run. In the coming years these technologies will further add to the available information regarding the genomes. The second generation sequencing technologies have already been commercialized and the third generations sequencing strategies will be available soon in the near future. Within no time the toughest genomes like that of wheat will be available for utilization in crop improvement. The resulting information about the genes and molecular markers will revolutionize the crop breeding program if this information is applied to our advanced genotyping methodologies.

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INTRODUCTION

The first sequenced genome of a plant, *Arabidopsis thaliana*, was reported a decade ago (Arabidopsis Genome Initiative, 2000). The effort to sequence the rice (*Oryza sativa*) genome and the publication of the data in 2005 took about 8 years and 14 laboratories from nine countries (International Rice Genome Sequencing Project, 2005). In the case of sorghum (*Sorghum bicolor*) with nearly twice the size of the rice genome, its origin dates back to the Plant Animal Genome Meeting in 2005 and it was completed in 2008, with only three production laboratories and a smaller group of analysts than for rice (Paterson et al., 2009). The acceleration and efficiency in genome analysis is thus clearly visible by comparing the time and efforts involved in rice genome sequencing. The recent developments in genome sequencing, through the involvement of second generation sequencing technologies, provide opportunities to develop numerous novel markers, in commercially important crop species, as well as identification of genes of agronomic importance. Identification of all genes within a species permits an understanding of how important agronomic traits are controlled, knowledge of which can be directly translated into crop improvement. Reference genome sequences for several crop species are now becoming available and this information permits both the rapid identification of candidate genes through bioinformatics analysis, and single nucleotide polymorphism (SNP) discovery through comparison of the reference with sequence data from

different cultivars. For crop species such as wheat and barley where no reference sequence is available, gene discovery relies on unassembled genome sequence data and expressed sequence tags (EST). This data can also be applied for SNP and simple sequence repeats (SSR) molecular marker discovery, though without a reference genome sequence, genetic mapping of these markers is required to determine their genomic location. In case of underutilized crops where no sequence data is available, it is now relatively easy to generate sufficient data for computational gene and molecular marker discovery. Molecular genetic markers are based on variation in the genome that can analysed between individuals and across generations. The association of markers with heritable traits is used to associate the genotype of an organism with the expressed phenotype, and the ability to develop millions of novel markers will revolutionize plant genomic research. These markers can be used routinely in crop breeding programs, for rapid crop improvement, for genetic diversity analysis, cultivar identification, phylogenetic analysis, characterization of genetic resources and association with agronomic traits. Markers are used in plant breeding programs to incorporate genetically characterized traits in place of field trials or glass house screens. The inheritance of many agronomic traits is difficult for evaluation in field experiments. The assessment of disease resistance is dependent of the presence of a virulent pathogen, and complex traits such as drought tolerance and yield are influenced by many genetic and environmental factors. In these cases, the use

*Corresponding author: shabirhussainwani@gmail.com

Table1. Genome sequencing and abundance of molecular markers in different crops

Crop	Remarks	Reference
Rice (<i>Oryza sativa</i> L.)	Rice was the first crop genome to be sequenced Genome Size: 415-460Mb, 3533 RFLPs, 133 RAPD, 12922 SSR, 1062 AFLP, 5418373 SNP.	Goff et al., 2002; Yu et al., 2002; Matsumoto et al., 2005, www.gramene.org, www.ncbi.nlm.nih.gov
<i>Arabidopsis</i>	First model plant genome to be sequenced was , <i>Arabidopsis thaliana</i>	<i>Arabidopsis</i> Genome, 2000)
Maize (<i>Zea mays</i>)	Genome size -2300 Mb. 9355 RFLPs, 2243 SSRs, 501 AFLPs, 2018337 SNPs.	(www.gramene.org), www.ncbi.nlm.nih.gov
Wheat (<i>Triticum aestivum</i>)	Genome size 16500Mb, 874 RFLPs, 16 RAPD, 1103 SSR, 731 AFLP, 1051763 SNP	www.gramene.org, www.ncbi.nlm.nih.gov
Sorghum (<i>Sorghum bicolor</i>)	Genome size 750-770, 1082 RFLP, 229 SSR, 5344 AFLP, 209814 SNP	(www.gramene.org), Sasaki and Antonio, 2009
Sugarcane (<i>Saccharum officinarum</i>)	Genome size 10,000 Mb; 57RFLP, 67RAPD, 221 SSR, 614 AFLP, 1632 SNP.	Arruda and Silva 2007, Garcia et al. (2006), Liang et al. (2004), Pan (2006), Aitken et al. (2006),
Oats (<i>Avena sativa</i>)	Genome size : 11300, 507, RFLP, 10RAPD, 3 SSR, 27 AFLP, 485 SNP	www.gramene.org,
Barley (<i>Hordeum vulgare</i>)	Genome size :4900, RFLP 1001, 15 RAPD , 196 SSR, 336 AFLP, 501336 SNP	www.gramene.org,
Banana (<i>Musa spp.</i>)	Genome size 500-600 Mb,47 RFLP, 50 RAPD,12 SSR, 119 AFLP	Venkatachalam et al 2008, Xiao et al 2007
Soybean (<i>Glycine max</i>)	Genome Size: 1115 Mb, 4991 SNP, 874 SSR,	Choi et al 2007, Hyten et al 2008,Schmutzet al., 2010

of molecular markers to select for the underlying genetic determinants of the trait increases the efficiency of crop breeding. Thus genome sequencing is an important tool for gene identification in crop improvement and with recent advances in genome sequencing the whole genome sequencing of commercially important crop plants has become quicker and cost effective

Genome Sequencing Projects

The first plant genome to be sequenced was of *Arabidopsis* (*Arabidopsis* Genome Initiative, 2000). Rice was the first crop genome to be sequenced (Goff et al., 2002; Yu et al., 2002; Matsumoto et al., 2005). Nowadays crop genome sequencing projects are rapidly accelerating, new technology and researchers are adopting second generation sequencing to gain insight into crop genome. To sequence the 430 Mbp genome of *Theobroma cacao*, Roche 454 technology is being used (Scheffler et al., 2009), while Sanger and Roche 454 sequencing is being used to sequence the apple genome (Velasco, 2009; Velasco et al., 2009). A similar approach is being used to sequence the 504 Mbp grape genome (Velasco et al., 2007) where a combination of 6.5x Sanger paired read sequences and 4.2x unpaired Roche 454 reads were assembled into 2093 metacontigs representing an estimated 94.6% of the genome. A combined Illumina Solexa and Roche 454 sequencing approach has been used to characterize the genomes of cotton (Wilkins et al., 2009). Roche 454 sequencing has been used to survey the genome of *Miscanthus* (Swaminathan et al., 2009), while Sanger, Illumina Solexa and Roche 454 sequencing are being used to interrogate the genome of banana

(Hribova et al., 2009). Thus, the advances in DNA sequencing technologies have led to the initiation of genome sequencing of many economically important plants like cucumber (Huang et al., 2009), barley (Wicker et al., 2006, 2008; Stein, 2009) soybean (Schmutz et al., 2010) and tomato (Mueller et al., 2009) Table1. Genome sequencing of large genome crops like wheat is underway and will be available for use in crop improvement in the near future.

Identification of genes of economic importance

The genes responsible for many simply inherited traits have been identified and characterized in detail. In these cases, the biochemical functions of the encoded protein may be studied to know more about the understanding of the mechanisms underlying the trait and whether variation in the gene structure or expression may further improve the trait. Knowledge of the gene underlying a trait enables the transfer of the trait between cultivars and even species using genetic modification. Alternatively, the gene conferring the favourable trait may be incorporated into a cultivar by marker-assisted selection (MAS) breeding. While many of the simple traits have been well characterized at the genome level, there are many other traits which are poorly understood. This is particularly true for complex traits which are controlled by interacting gene networks. Although many aspects of a complex trait, such as yield, may be characterized individually, it is unlikely that the genetic basis underlying all components of yield heritability will be understood in the near future. The identification of all the genes for a crop is only one step towards understanding the inheritance of agronomic traits. The functions of many of the genes identified by genome sequencing remain unknown and the genetic

control of the majority of agronomic traits has yet to be determined. Producing a finished genome sequence for a crop is an important first step and is becoming feasible for an increasing number of crop species (Imelfort and Edwards, 2009).

Genome sequencing and Molecular markers

Molecular techniques for detecting differences in the DNA of individual plants have many applications of value to crop improvement. Several types of molecular markers that have been developed and are being used in plants include restriction fragment-length polymorphisms (RFLPs), amplified fragment-length polymorphism (AFLP), random amplification of polymorphic DNA (RAPD), cleavable amplified polymorphic sequences (CAPS), single strand conformation polymorphisms (SSCP), sequence-tagged sites (STS), simple sequence repeats (SSRs) or microsatellites, and single-nucleotide polymorphisms (SNPs) (Mohan *et al.*, 1997; Rafalski, 2002). Such markers, closely linked to genes of interest, can be used to select indirectly for the desirable allele, which represents the simplest form of marker-assisted selection (MAS), now being exploited to accelerate the backcross breeding and to pyramid several desirable alleles (Singh *et al.*, 2001). The discovery of molecular markers has enabled dissection of quantitative traits into their single genetic components (Tanksley 1993, Pelleschi *et al.*, 2006, Bernier *et al.*, 2007, Kato *et al.*, 2008) and helped in the selection and pyramiding of QTL alleles through MAS (Ribaut *et al.*, 2004; Neeraja *et al.*, 2007; Ribaut and Ragot, 2007). In silico methods of SNP and SSR discovery are now being adopted, providing cheap and efficient methods for marker identification (Barker *et al.*, 2003; Batley *et al.*, 2003; Robinson *et al.*, 2004; Jewell *et al.*, 2006; Duran *et al.*, 2009a,b,c). Large quantities of sequence data are being generated by the latest second generation sequencing technologies and these provide a valuable resource for the mining of molecular markers (Imelfort *et al.*, 2009). The details of abundance of molecular markers in different crop species resulted through advances in plant genome sequencing are given in table 1. The rapid advances in genome sequencing technology will result in whole genome sequencing to be the routine method for estimation of diversity and polymorphism in economically vital crop species.

Conclusion

Improvement in the genome sequencing technology is radically changing biological research and will have a major impact on crop improvement. Now it has become possible to sequence the tough cereal genomes, which was not possible a decade ago. All the genes, gene promoters and ESTs in a genome can now be easily surveyed and identified with relatively low input and high throughput results. Genome wide SNP discovery can be routinely used for sequencing multiple. This new abundance in information related to genomes can be combined with recently developed advanced genotyping methods to provide access to genome wide research, thus combining the genetic variability and trait improvement. The abundance of genes and molecular markers responsible for economically important traits like yield, quality, and tolerance to biotic and abiotic factors will help us to make our breeding programs more robust.

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