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Biodiversity Measures in Agriculture Using DNA

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1. Introduction

How to measure biodiversity? One of the possibilities is to use DNA. The mention of DNA can hint evolution, but this question is much more complicated and beyond the proposition of a biodiversity measure. The reasons stated for use DNA instead of other possible molecules could be that it is stable and responsible for the transmission of traits to future generations. But another reason is simple that it is suitable for measures. First, due to universality among all living things. Second, because it is a big molecule, constituted by variations of just four bases, making it easy to make a great number of sequence comparisons and used it on biodiversity measures. When individuals to be compared are similar, a greater number of comparisons have to be made to obtain a quantification of the differences among them. Conservation biology is not concerned only with the extinction of species, but also with diversity within species or subspecies, where accurate measures of diversity may be required. A third reason is that a good amount of methodologies is already developed to study its nature.
DNA markers when used to study biodiversity are frequently designed for a great number of comparisons of similarities or differences among individuals, groups of individuals, or populations. DNA is the code for protein synthesis, but in most of the studies DNA markers are considered exclusively for comparison, which may not to be linked or recognized to be linked with any trait or adaptability of the individual.
There are lots of kinds of markers, and one important thought to have in mind is that there is not a best one among them, but the choice should consider the species to be studied and the objective of the diversity analysis.

2. From nature to agriculture: why we need diversity?

The different crops and/or livestock, as we know, are indispensable components for Humankind daily routine and were domesticated in the course of modern agriculture development. The initials "attempts" of agriculture, which leaded to plants domestication, were mainly intuitive, the selection of seeds and animals were based in traits that best suited

the demand from each civilization. But as soon as the initials "attempts" of agriculture were taken, guiding early "breeding" purposes, the losses of diversity became more and more apparent. After XIX century, these losses tended to increase in a higher speed, since breeder's effort in developing modern, high yield cultivars guided a substitution of wild relatives of modern crops, landraces and local varieties.

The debate about diversity, germplasm conservation and agriculture is not an original topic within breeders, germplasm bank curators, ecologists and others. Even though, each expertise embraces a different perspective about diversity. It is well known that low genetic diversity, within main crops, are due the intensive selection practiced by breeding programs, which are inclined to use few genitors (Donini et al., 2000). Such custom may leave them more vulnerable to a broad set of biotic and abiotic stresses, if not it leads to a decline in genetic variability, reducing the chance of selecting new allelic combinations (Borba et al., 2009). And since genetic variability is the raw material for selection, it is prudent to maintain it at an adequate rate to consent new combinations and therefore the exploitation of "new" desirable traits.

The competent maintenance of diversity within crops and their wild relatives, as well as plants with social-economic potential interest, is strategic not only to breeding programs but to pharmaceutical and biofuels industries, food security among others causes. But despite the immense potential that diversity holds for humankind, its unknown value comprises the major risk factor for its irreparable losses. Among the most prominent causes of diversity losses are the high rates of demographic growth and, as a result, the quick devastation of natural resources (Nass, 2001). Tropical and sub-tropical countries, which hold the greatest proportion of biodiversity (Figure 1, Figure 2), are the ones that undergo higher rates of natural devastation.

Fig. 1. Sampling of common bean (*Phaseolus vulgaris*) diversity. Archives of Embrapa Rice and Beans, Francisco Lins, 1999.

Despite the great proportion of diversity concentrated in a relative small number of countries, no country is self-sufficient in biodiversity. Therefore, the preservation of biodiversity sources, as genetic resources, is the key factor to satisfy nowadays and future needs. Different strategies are available for the maintenance of genetic resources (as a living stock of diversity), the two most "popular" are *ex situ* and *in situ* methods. Both strategies are required and important and the choice for the most suited method must reflect the species characteristics and needs.
The strategies for genetic diversity conservation can be practiced as management strategies and may vary according to the characteristics of the various plant species. Even though different species demand different conservation strategies, there are some few ordinary steps that, if followed, may guide a successful conservation of diversity. Prospection, evaluation and characterization, interchange, regeneration among others are essential for the adequate maintenance of diversity represented by genetic resources.
Along with the available tools for the management of diversity there are molecular markers, or "observing" DNA fragments which can be associated to genetic heritable traits.

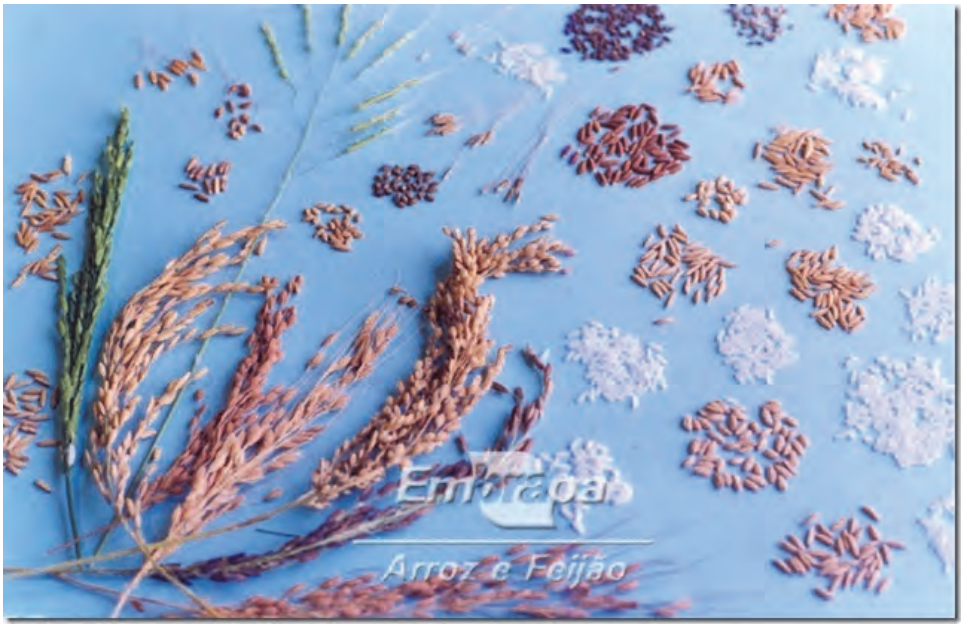

Fig. 2. Sampling of cultivated rice (*Oryza sativa*) diversity. Archives of Embrapa Rice and Beans, Francisco Lins, 1999.

3. Microrganisms and pests associated to plants

Plants shoots and roots are constantly exposed to pests and microorganisms. In soil the various microorganisms frequently starve, and are nurtured and attracted by root exudates. Microorganisms may be symbionts, in an intimate association where important new morphological structures are created, at least at the cellular level; or promote plant growth by, for example, producing beneficial substances or being antagonistic to pathogens (Araújo et al., 2001; Silveira & Freitas, 2007; Torres et al., 2009). Microorganisms may be pathogens, and plants have to defend themselves. Defense can be constitutive or be triggered only by

the contact with the microorganism. When defense reactions are elicited by the presence of pathogens, a system of recognition is necessary, usually performed by cell membrane proteins. Those recognition proteins are specific to pathogens species or races. Constitutive mechanisms can be much more general.

Various research efforts are directed to study important pest or pathogens diversity. It has been frequently performed with markers not related to any function or pathogenicity (Krause-Sakate et al., 2001; Ribeiro et al., 2003). The genetic structure of a pest population is probably related to geographical distances and physical barriers, and may be dependent of alternative hosts off the insect (Cunha et al., 2010). More recently, efforts have been made to identify genes related to pathogenicy and study of diversity is conducted directly with them. This seem to be the ideal measure, when the diversity study is conducted with agronomic purposes, for example, to evaluate the distribution and variability of the pathogen and its virulence effectors, as a way to infer if a given resistance gene, or some resistance genes, would be enough to control the disease. Effectors genes that encode proteins secreted in the host plants have been used to study a soil fungi diversity (Chakrabarti et al., 2011).

Plant genes responsible to resistance to pathogens have been also localized or cloned. Molecular mapping is a use of molecular markers slightly different from the study of the genetic diversity per se, because it has the aim to localize a marker physically linked in the chromosome to a gene that is responsible for a given trait. The link implies that gene and marker recombine the least when gametes are formed, causing gene and marker to cosegregate. The ideal plant population to localize a marker linked to a gene is the offspring of plants derived from a cross between paternal lines which differ specially on the trait to be mapped. Some traits are controlled by various loci, each one contributing a small amount to a quantitative trait, and are called quantitative trait loci (QTL). Plant resistance to pathogens is interesting to be mapped because there are a small number of genes, or a single gene, responsible for the trait (St Clair, 2010), which can be considered qualitative. Furthermore, the general protein structure and protein sequences of various plant resistance genes are conserved among plants, particularly those related to pathogen recognition, and new genes can be isolated by similarity (Bakker et al., 2011).

A series of markers linked to disease resistance genes are available in the literature, and they are useful in breeding programs where plants bearing the marker are selected with the aim to select resistant plants. There is special advantage of using this indirect selection, called marker assisted selection (MAS) is due to the difficulty of inoculating the high number of plants to be selected in a breeding program. Some viruses have to be inoculated through insect vectors, impracticable with a considerable amount of plants. Most of breeding programs are based on selection during natural infection, with the conduction of the experiments in conditions that favor the disease spread. But disease spread is not uniform, and plant by plant selection is sometimes required.

The adaptability of plants introduced to different environment can be improved if a selection for the resistance to particular stressed condition is performed by molecular markers. For example, the introduction of Latin American cassava genotypes to Africa has been more successful when a previous selection to the resistance to the Africa Cassava Mosaic Virus was made (Okogbenin et al., 2007).

4. Genetic markers and molecular markers

Along with the available tools for the management of diversity there are molecular markers, which may assist, reliably, the determination and examination of diversity, its conservation

and, satisfactorily, guide its exploitation. Within the applications of molecular markers is the determination of how the genetic structure of a certain population, or an assemblage of germplasm accessions, is organized. The genetic structure may answer questions about how much diversity such germplasm assemblage holds, or how such genotypes must "react" under natural or artificial selection. Besides, the information resultant from molecular markers' analysis and from biometrics tools might result in the identification of novel marker alleles linked to genes involved in the expression of important traits, which can be extensively explored during cultivar development in breeding programs.

The definition of a genetic marker is not new, it was first given when the concern was not to study diversity, but to understand cosegregation of agronomic interesting traits to others characteristics of the genome, which is known by QTL and genetic mapping. Therefore, a genetic marker is defined as a heritable characteristic that can be associated to an interesting trait. When we do not think in mapping, but in diversity, the genetic marker is any genetic characteristic that is variable, or polymorphic, among the individuals to be studied, and heritable.

The genetic marker can be morphological or molecular (biochemical or DNA/RNA based). The presence or size of a spot in a flower is a morphological marker. The main advantage of molecular markers is that they can be obtained in a virtually infinite number. Furthermore it is not influenced by the environment, as parts of the morphological traits. Small organisms, as bacteria, are practically impossible to be studied through naked eye, or sometimes even with a microscope, consequently difficult to characterize morphological differences, therefore molecular markers can help. Others morphological markers can be assumed to have a relevance greater than the deserved. For example, the traditional cotton *Gossypium barbadense*, which used to be cultivated by native South American inhabitants, was classified in different subspecies when the seed from the same boll were adhered to each other, forming the called kidney seeds. This trait is, presumably, controlled by a single locus (Almeida et al., 2009), but for some authors this single trait is not relevant enough to differentiate subspecies, and molecular markers could be used to explain this process.

Population genetics has been markedly based on studies using neutral molecular markers, and the obtained genetic structure provides information individuals in a population are more related among themselves than with individuals of other populations. It is also possible to conduct population genetic studies based on QTLs or markers linked to any characteristic known to have been selected. The comparison can elucidate relative roles of selection and neutral evolution (Edelaar & Bjorklund, 2011; Stinchcombe & Hoekstra, 2008).

Monitoring forest maintenance by satellites has been criticized because it would not be enough to measure the size of the preserved forest area, but real diversity is not perceived. Species identification must be done *in situ* (Fonseca et al., 2008), and molecular markers distributed along the genome and not linked to the special selected traits may provide general diversity measures as the number of alleles per locus, as well as the population structure (Laurentin, 2009).

5. How measures are taken: a brief review on the simplest and most popular tools

For some time, sequencing was laborious and expensive, and differences among DNA molecules were accessed mainly by DNA fragment size. The amplification of DNA in vitro, or PCR, was an essential methodology to develop DNA markers. The separation can be

carried out by electrophoresis: short molecules migrate faster than long ones. Other tools are restriction enzymes, which, in nature, are enzymes synthesized by bacteria to break infecting virus DNA. Some of them make their cuts in special definite DNA sequences. The precision and reproducibility of the sequence recognition were useful on the recognition of specificities – maintenance and differences among DNA of various individuals – and so, on the development of markers.
We here briefly list some of the most used techniques to obtain markers, focusing not in the methodology but the characteristics of facility of obtaining data and ability to detect polymorphism.

5.1 Random amplified DNA reveals polymorphism

Random Amplified Polymorphic DNA (RAPD) is a friendly marker which compares individuals based on suitability for amplifications which depends on DNA complementation to random small DNA sequences. It has been used largely, but is criticized due to the low repeatability or reproducibility.
It is easy to use, and cheap, because it is based only on a PCR amplification followed by agarose gel electrophoresis. The random small DNA sequences (usually from eight to ten bases long) are used as primers of the PCR reaction. They are smaller to the oligonucleotides used in regular PCR, which are specific, therefore having a greater chance to anneal to any genome: since annealing occurs by complementarity of adenine to thiamine and of guanine to cytosine, the chance to a small sequence to find by chance a complementary sequence in a genome is relatively high. The regular PCRs are performed with longer oligonucleotides as primers therefore to the amplification is specific, chosen by the researcher, and the sequence of the oligonucleotides to be used have to be previously known.
After electrophoresis, DNA is stained, and the differences among individuals are observed as presence or absence of bands (Figure 3). Homozygous and heterozygous individuals cannot be distinguished, and the progeny of intercrossed heterozygous individuals segregates in a 3:1 proportion, therefore RAPD is a dominant marker.

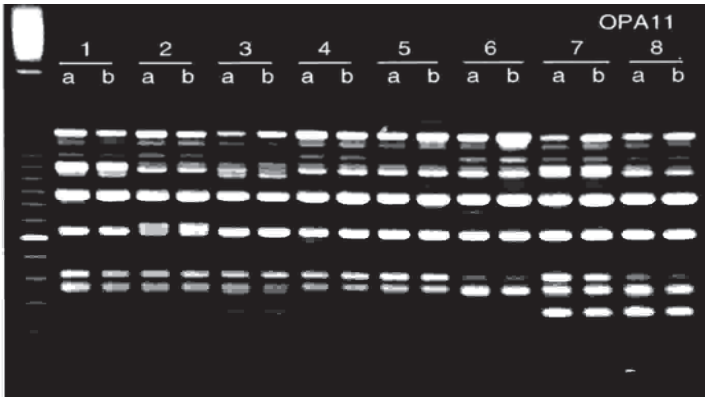

Fig. 3. Eight soybean individuals amplified with the RAPD primer OPA11. Polymorphism is noticed as presence or absence of stained bands, resulting from the number of times this primer annealed in each one of the particular genomes, with production of amplified DNA fragments separated by size in the agarose gel.

The lack of necessity of any previous knowledge of the species DNA was a great advantage of this marker.
Other techniques were developed that did not require any previous information of the species DNA, although with higher reproducibility, as AFLPs (Vos et al., 1995; Zabeau & Vos, 1993) and DART (Akbari et al., 2006; Amorim et al., 2009; Varshney et al.,2010; Wenz et al., 2004).
The RAPD markers have been independently developed by Willians et al. (1990) and Welsh e McClelland (1990).
The use of the marker in plant diversity has been reviewed by Arif et al. (2010).

5.2 Repetitive sequences can be especially polymorphic

Despite the extent of DNA molecule and the differences among individuals, it may be difficult to access variability, especially when studying genetically related individuals. In those cases, sequences with the greatest contrasts are desirable.
Microsatelites or SSR are small repetitive DNAs which are used due to be hypervariable. The repetitive bases are one to six, repeated a few times until around a hundred times, flanked by normal non repetitive sequences. The primers to reveal SSR loci are designed to be complementary to these non repetitive flanking sequences – so the disadvantage of SSR markers is the necessity of knowing the sequences before the primers design. SSR markers are more frequently in no coding regions of the genome, those which will not be transcribed or translated into DNA or proteins (Victoria et al., 2011). SSR are used multialellic, what means that for a single locus more than two forms of the marker, composed by various number of repetitions, may be found (Figure 4). Heteroziguous individuals bearing two alleles can be distinguished from any of the homozygous ones, and observing the alleles of a population derived from the crossing among two heterozygous individuals the proportion 1:2:1 can be noticed. Therefore microsatellites are codominant markers.
Frequently genomes have been sequenced not from the whole DNA of the organisms, but from expressed sequences only. For that, the initially collected material is RNA, instead of DNA. It means that the sequences obtained are all expressed, called expressed sequence tags (EST). These data can be a source to mine SSRs, and when obtained this way the SSRs belong to a expressed sequence.
Following the PCR an electrophoresis is performed to separate fragments of different sizes. Differently from RAPD, SSR of the individuals differ from others just by a few bases, so the separation has to be sharp to lead size identification. For that reason fragments are separated on acrylamide gels, not agarose gels, or capillary electrophoresis in a sequencing equipment.

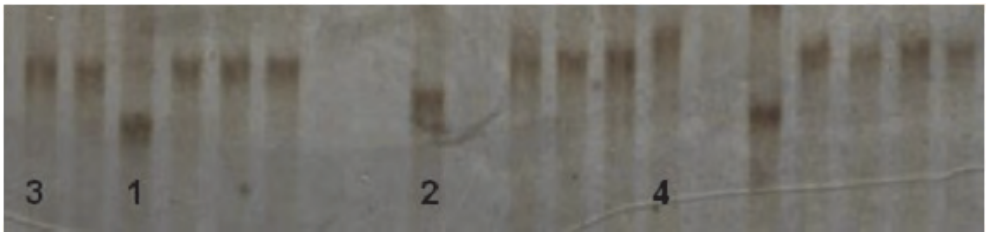

Fig. 4. Fragments of DNA resulting for the amplification with SSR primer pairs separated by size by electrophoresis in an acrylamide gel and stained by silver. For different DNA sizes are shown, corresponding to four different alleles.

5.3 Sequencing and single nucleotide polymorphism

Recently, sequencing became much more reliable, allowing the discovering of differences between individuals of a single nucleotide, or single nucleotide polymorphism (SNP). Differently from the previous cited markers, the definition of single nucleotide polymorphism is not dependent on the methodology used for their detection, which can be various. The most efficient are oligonucleotide arrays (Gupta et al., 2008).
Similarly, small insertion or deletion (INDEL) have been localized and their frequencies measured (Zhidkov et al., 2011).
Those markers are much more abundant and precise, and should turn out to be the most used, at least among the most studied species. They are the new frontier to measure the biodiversity, and have been used to study human pathogens diversity and epidemiology (Baker et al., 2010).
Independently of the marker class, working with DNA fragments may require criteria to demonstrate the reliability of the results. Sharing information by publications and websites may be very useful to verify reproducibility of results.

6. Markers may help to understand evolution

Evolution is rarely accessed experimentally, but by observation and measures taken in natural environment and inference. Hypothesis in this field may look more theoretical than in others.
It is known that natural selection depends on fitness, which may be defined as the ability to produce descendents. Fitness is dependent on the interaction with environment.
The hole of hybridization in evolution has been despised since a various interespecific hybrids present smaller general development and reduced or absent seed production. Molecular markers have lead to show that well established plants are hybrids (Ellstrand, 2003) and may have supplanted their parents (Hegde et al., 2006).
Genetic drift may have importance in evolution, which can be understood by loose of variability, caused for example by death of huge amount of population individuals due to natural phenomena or human actions. It is not unusual that a plant species suffer with an environmental or disturbance by human action causing a marked reduction of the population size. Afterwards, the remaining individuals reproduce so size of the population is recovered, but not with the ancient diversity. This phenomena is called a genetic bottleneck, and molecular markers are able to track them by identifying a population with great number of individuals with genetic diversity smaller then a small population of the same species (Barroso et al., 2010). The smallest diversity reveals disturbance among wild plants.
The importance of genetic drift has been shown experimentally in a for years experiment with *Lolium perenne* (Nestmann et al., 2011).

7. The gains in plant breeding depends of variance

Biodiversity is important not only in nature, but also on agriculture systems. The goals of plant breeding are productive plants, resistant to draught and temperature, pathogens and insects, efficient on nutrients uptake and symbiosis, etc. Novel characteristics or use of plant species can also be a challenge, like production of biofuels (Paterson et al., 2009). The way to achieve this is to find within the species to be bred plants bearing the genes conferring the desired trait or, if not available, within related species which intercrosses with it.
The number of traits that can be introduced by genes of non related plant species by plant genetic transformation is restricted mainly by the number of genes necessary for the

characteristic to be expressed. Only those controlled by a small number of genes can be introduce by genetic transformation, and usually a single gene is introduced. Difficulties on knowing useful genes, which may not have been already isolated and characterized, may also exist.

Productiveness is economically believed to be major challenge to agriculture in face of the human population growth. Plant breeding has a major hole on increase agricultural production by the development of seeds – and for that the selection have to be performed among the plants that already are productive and adapted to cultivation. The continuous procedure causes loss of general biological diversity (Bai & Lindhout, 2007) and genetic diversity, which can be noticed by a loss in allele richness.

The gains achieved by plant breeding may decrease in years of selection due to the loss of genetic richness and allele segregation within the breeding population (Campbell et al., 2010). How genetic variability could be enhanced or preserved? The introduction of the crop relatives not so adapted to the cultivation system is referred as pre breeding, which are crossed to well adapted genotypes. The low productiveness of the offspring compared to the adapted parent and the years of crossing and the years of crossings and selection necessary to recover the initial production level discourages its use. Molecular markers can help hear not to maintain diversity, but otherwise to recover the adapted parent traits, with the use of recurrent selection. The marker assisted selection when used to select to the productive parental genotype may help to recover production levels in a much lesser number of years. Selecting the crop genotype is the aid molecular markers can play to foster introduction of non adapted genotypes to plant breeding.

Colored cotton fibers exist in nature, but cotton breeders have been selected for white fibers, easier to be industrial stained (Figure 5). The development of color cotton varieties avoids environmental pollution caused by staining (Teixeira et al., 2010).

Because breeding programs are expensive, and a great number of the populations which are conducted may not produce interesting seeds of varieties, models have been developed to use the evaluation by molecular data of candidate parents for prediction of the performance of the population resulting from their crossings (Barroso et al., 2003).

Fig. 5. (Continued)

Fig. 5. *Gossipium mustelinum*, a native cotton species endemic to northeast Brazil semiarid region. While the cultivated cotton (A) retains the fiber and seeds, a trait selected by plant domestication, the seeds of the wild cotton are naturally released from the boll (B) and will be dispersed through streams. Young plants survive due the protection from goat feeding by a common thorn plant *Bromelia lacinosa* (C). Adult plants can be high (D) so animals damage but not destroy them.

8. Conclusion

We are in a period of constant innovations in methodologies to access genetic diversity, in which some methodologies in use can be seen as obsolete when faced to newly developed ones. For a number of well the best studied species, genetic diversity measures data are easily obtained and available. The use of molecular data to monitor genetic diversity lead improved understanding over evolution. The increasing amount of data of crops and their relatives should foster the actual use of genetic resources in plant breeding.

9. References

Akbari, M., Wenzl, P., Caig, V., Carling, J., Xia, L., Yang, S., Uszynski, G., Mohler, V., Lehmensiek, A., Kuchel, H., Hayden, M. J., Howes, N., Sharp, P., Vaughan, P., Rathmell, B., Huttner, E. & Kilian, A. (2006). Diversity Arrays Technology (DArT) for high-throughput profiling of the hexaploid wheat genome. *Theoretical and Applied Genetics*, Vol. 113, No. 8, (October 2006), pp. 1409-1420, ISSN 0040-5752

Almeida , V. C., Hoffmann, L. V., Yokomizo, G. K. I., Costa, J. N., Giband, M. & Barroso, P. A. V. (2009). In situ and genetic characterization of *Gossypium barbadense* populations from the states of Pará and Amapá, Brazil. *Pesquisa Agropecuária Brasileira*, Vol. 44, No. 7, (July 2009), pp. 719-725, ISSN 0100-204X

Amorim, E. P., Vilarinhos, A. D., Cohen, K. O., Amorim, V. B. O., Santos-Serejo, J. A., Silva, S. O., Pestana, K. N., Santos, V. J., Paes, N. S., Monte, D. C. & Reis, R. V. (2009). Genetic diversity of carotenoid-rich bananas evalueted by Diversity Arrays Technology (DArT). *Genetics Molecular Biology*, Vol. 32, No. 1, (n. d.), pp. 96-103, ISSN 1415-4757

Araújo, W. L., Maccheroni Jr., W., Aguilar-Vildoso, Barroso, P. A. V., C. I., Saridakis, H. O. & Azevedo, J. L. (2001). Variability and interactions between endophytic bacteria and fungi isolated from leaf tissues of citrus rootstocks. *Canadian Journal of Microbiology*, Vol. 47, No. 3, (March 2001), pp. 229-236, ISSN 0008-4166

Arif, I.A., Bakir, M.A., Khan, H.A., Al Farhan, A.H., Al Homaidan, A.A., Bahkali, A.H., Al Sadoon, M. & Shobrak, M. (2010). A brief review of molecular techniques to assess plant diversity. *International Journal of Molecular Science*, Vol. 11, No. 5, (May 2010), pp. 2079-2096, ISSN 1422-0067

Bai, Y. & Lindhout, P. (2007). Domestication and Breeding of Tomatoes: What have We Gained and What Can We Gain in the Future? *Annals of Botany*, Vol. 100, No. 5, (August 2007), pp. 1085–1094, ISSN 0305-7364

Baker, S., Hanage, W.P. & Holt, K.E. (2010). Navigating the future of bacterial molecular epidemiology. *Current Opinion in Microbiology*, Vol. 13, No.5, (October 2010), pp. 640-645, ISSN: 1369-5274

Bakker, E., Borm, T., Prins, P., Van der Vossen, E., Uenk, G., Arens, M., Boer, J., Van Eck, H., Muskens, M., Vossen, J.,Van der Linden, G.,Van Ham, R., Klein-Lankhorst, R., Visser, R., Smant, G., Bakker, J. & Goverse, A. (2011). A genome-wide genetic map of NB-LRR disease resistance loci in potato. *Theoretical and Applied Genetics,* (May 2011), ISSN 0040-5752

Barroso, P. A. V., Geraldi, I. O., Vieira, M. L. C., Pulcinelli, C. E., Vencovsky, R. & Dias, C. T. S. (2003). Predicting performance of soybean populations using genetic distances estimated with RAPD markers. *Genetics and Molecular Biology*, Vol. 26, No. 3, (n. d.) pp.343-348, ISSN 1415-4757

Barroso, P. A. V., Hoffmann, L. V., Freitas, R. B., Batista, C. E. A., Alves, M. F., Silva, U. C. & Andrade, F. P. (2010). In situ conservation and genetic diversity of three populations of *Gossypium mustelinum* Miers ex Watt. *Genetic Resources and Crop Evolution*, Vol. 57, No. 3, (August 2009), pp. 343-349, ISSN 0925-9864

Borba, T. C. O., Mendes, C. A., Guimarães, E. P., Brunes, T. O., Fonseca, J. R., Brondani, R. V. & Brondani, C. (2009). Genetic variability of Brazilian rice landraces determined by SSR markers. *Pesquisa Agropecuária Brasileira*, Vol. 44, No. 7, (July 2009), pp. 706-712, ISSN 0100-204X

Campbell, B. T., Saha, S., Percy, R., Frelichowski, J., Jenkins, J. N., Parker, W., Mayee, C. D., Gotmare, V., Dessauw, D., Giband, M., Du, X., Jia, Y., Constable, G., Dillon, S., Abdurakhmonov, I. Y., Abdukarimov, A., Rizaeva, S. M., Adullaev, A., Barroso, P. A. V., Padua, J. G., Hoffmann, L. V. & Podolnaya, L. (2010). Status of the global cotton germplasm resources. *Crop science*, Vol. 50, No.4, (July 2010), pp. 1161-1179, ISSN 0011-183X

Chakrabarti, A., Rep, M., Wang, B., Ashton, A., Dodds, P. & Ellis, J. (2011). Variation in potential effector genes distinguishing Australian and non-Australian isolates of the cotton wilt pathogen *Fusarium oxysporum* f.sp. vasinfectum. *Plant Pathology*, Vol. 60, No. 2, (September 2010), pp. 232–243, ISSN 0032-0862

Cunha, F., Gómez, D.R.S., Silva, J.J., Alexandre, T.M. & Moscardi, F. (2010). Genetic diversity of the sunflower caterpillar (*Chlosyne lacinia saundersii* Doubleday and Hewitson) (Lepidoptera: Nymphalidae) populations determined by molecular RAPD markers.

Anais da Academia Brasileira de Ciências, Vol. 82, No. 4, (n.d.), pp. 1127-1136, ISSN 0001-3765

Donini, P., Law, J.R., Koebner, R.M.D., Reeves, J.C. & Cooke, R.J. (2000). Temporal trends in the diversity of UK wheat. *Theoretical and Applied Genetics*, Vol.100, No.6, (n.d.), pp.912-917, ISSN 0040-5752

Edelaar, P. & Bjorklund, M. (2011). If FST does not measure neutral genetic differentiation,then comparing it with QST is misleading. Or is it? *Molecular Ecology*, Vol. 20, No. 9, (March 2011), pp.1805-1812, ISSN: 0962-1083

Ellstrand, N.C. (2003). Current knowledge of gene flow in plants: implications for transgene flow. *Philosophical Transactions of the Royal Society*, Vol. 358, No. 1434 , (May 2003), pp. 1163–1170, ISSN 1471-2970

Fonseca, R.M., Lopes, R., Barros, W.S., Lopes, M. T . G.& Ferreira, F. M. (2008). Morphologic characterization and genetic diversity of *Capsicum chinense* Jacq. accessions along the upper Rio Negro – Amazonas. *Crop Breeding and Applied Biotechnology*, Vol.8, No.3, (n.d.), pp. 187-194, ISSN 1518-7853

Gupta, P.K., Rustgi, S. & Mir, R.R. (2008). Array-based high-throughput DNA markers for crop improvement. *Heredity*, Vol.101, No. 1, (May 2008), pp.5-18, ISSN 0018-067X

Hegde, S. G., Nason, J. D., Clegg, J. M. & Ellstrand, N. C. (2006). The evolution of California'a wild radish has resulted in the extinc of its progenitors. *Evolution*, Vol. 60, No. 6, (n. d.), pp. 1187–1197, ISSN 0014-3820

Krause-Sakate, R., Mello, R. N., Pavan, M. A., Zambolim, E. M., Carvalho, M. G., Le Gall, O. & Zerbini, F. M. (2001). Molecular characterization of two Brazilian isolates of *Lettuce mosaic virus* with distinct biological properties. *Fitopatologia Brasileira*, Vol.26, No. 2, (n.d.), pp.153-157, ISSN 0100-4158

Laurentin, H. (2009). Data analysis for molecular characterization of plant genetic resources. *Genetic Resources and Crop Evolution*, Vol. 56, No. 2, (January 2009), pp. 277–292, ISSN 0925-9864

Nass, L.L. (2001). Utilização de recursos genéticos vegetais no melhoramento, In: *Recursos genéticos e Melhoramento – Plantas*, Nass, L.L., Valois, A.C.C., Melo, I.S. & Valadares-Inglis, M. C, (Ed.), pp.30-55, Fundação MT, Rondonópolis, Brazil

Nestmann, S., Rajicic, T. S., Dehmer, K.J., Fischer, M., Schumacher, J. & Roscher, C. (2011). Plant species diversity and composition of experimental grasslands affect genetic differentiation of *Lolium perenne* populations. *Molecular Ecology*, Vol.20, No. 10, (n.d.), pp. 2188–2203, ISSN: 0962-1083

Okogbenin, E., Porto, M.C.M., Egesi, C., Mba, C., Espinosa, E., Santos, L.G., Ospina, C., Marín, J., Barrera, E., Gutiérrez, J., Ekanayake, I., Iglesias, C. & Fregene, M.A. (2007). Marker-Assisted Introgression of Resistance to Cassava Mosaic Disease into Latin American Germplasm for the Genetic Improvement of Cassava in África. *Crop Science,* Vol.47, No. 5, (n.d.), pp.1895-1904, ISSN 0011-183X

Paterson, A.H., Bowers, J.E., Bruggmann, R., Dubchak, I., Grimwood, J., Gundlach, H., Haberer, G., Hellsten, U., Mitros, T., Poliakov, A., Schmutz, J., Spannagl, M., Tang, H., Wang, X., Wicker, T., Bharti, A.K., Chapman, J., Feltus, F.A., Gowik, U., Grigoriev, I.V., Lyons, E., Maher, C.A., Martis, M., Narechania, A., Otillar, R.P., Penning, B.W., Salamov, A.A., Wang, Y., Zhang, L., Carpita, N.C., Freeling, M.,

Gingle, A.R., Hash, C.T., Keller, B., Klein, P., Kresovich, S., McCann, M.C., Ming, R., Peterson, D.G., Mehboob-ur-Rahman, Ware, D., Westhoff, P., Mayer, K.F.X., Messing, J. & Rokhsar, D.S. (2009). The *Sorghum bicolor* genome and the diversification of grasses. *Nature*, Vol.457, No. 7229, (January 2009), pp. 551-556, ISSN 0028-0836

Ribeiro, S. G., Ambrozevícius, L. P., Ávila, A. C., Bezerra, I. C., Calegario, R. F., Fernandes, J. J. , Lima, M. F., de Mello, R. N., Rocha, H. & Zerbini, F. M. (2003). Distribution and genetic diversity of tomato-infecting begomoviruses in Brazil. *Archives of Virology*, Vol.148, No. 2, (n.d.), pp.281-295, ISSN 0304-8608

Silveira, A. P. D & Freitas, S.S. (Eds.). (2007). *Microbiota do Solo e Qualidade Ambiental,* Instituto Agronômico Campinas, ISBN 978-85-85564-14-8, São Paulo, Brazil

St.Clair, D.A. (2010). Quantitative disease resistance and quantitative resistance Loci in breeding. *Annual Review of Phytopathology,* Vol. 48, (May 2010), pp. 247-268, ISSN 0066-4286

Stinchcombe, J.R. & Hoekstra, H.E. (2008). Combining population genomics and quantitative genetics: finding the genes underlying ecologically important traits. *Heredity*, Vol.100, No. 2, (February 2007), pp. 158-170, ISSN 0018-067X

Teixeira, E. M., Corrêa, A. N., Manzoli, A., Leite, F. L., Oliveira, C. R. & Mattoso, L. R. C. (2010). Cellulose nanofibers from white and naturally colored cotton fibers. *Cellulose*, Vol. 17, No. 3, (February 2010), pp. 595-606, ISSN 0969-0239

Torres, A. R., Cursino, L., Muro-Abad, J. I., Gomes, E. A., Araújo, E. F., Hungria, M. & Cassini, S. T. A. (2009). Genetic diversity of indigenous common bean (*Phaseolus vulgaris* L.) rhizobia from the state of Minas Gerais, Brazil. *Brazilian Journal of Microbiology*, Vol.40, No. 4, (n.d.), pp- 852-856, ISSN 1517-8382

Varshney, R. K., Glaszmann, J. C., Leung, H. & Ribaut, J. M. (2010). More genomic resources for less-studied crops. *Trends in Biotechnology*, Vol. 28, No. 9, (n.d.), pp. 452-460, ISSN 0167-7799

Victoria, F. C., Maia, L. C. & Oliveira, A. C. (2011). In silico comparative analysis of SSR markers in plants. *BMC Plant Biology,* Vol 11, No.1, (n.d.), pp. 11-15, ISSN 1471-2229

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van de Lee, T., Hornes, M., Friters, A., Pot, J., Paleman, J., Kuiper, M. & Zabeau, M. (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research,* Vol. 23, No. 21, (November 1995), pp. 4407–4414, ISSN 0305-1048

Welsh, J. & McClelland, M. (1990). Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Research*, Vol.18, No.24, (n.d.), pp.7213-7218, ISSN 0305-1048

Wenzl, P., Carling, J., Kudrna, D., Jaccoud, D., Huttner, E., Kleinhofs, A. & Kilian, A. (2004). Diversity Arrays Technology (DArT) for whole genome-profiling of barley. *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 26, No. 101, (June 2010), pp. 9915-9920, ISSN 0027-8424

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. & Tingey, S.V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research*, Vol.18, No.22, (n.d.), pp.6531-6535, ISSN 0305-1048

Zabeau, M & Vos, P. (1993). Selective restriction fragment amplification: a general method for DNA fingerprinting. *European Patent Office*, publication 0 534 858 A1, bulletin 93/13.

Zhidkov, I., Cohen, R., Geifman, N., Mishmar, D. & Rubin, E. (2011). CHILD: a new tool for detecting low-abundance insertions and deletions in standard sequence traces. *Nucleic Acids Research*, Vol 39, No.7, (January 2011), pp.1-8. ISSN 0305-1048