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A Review on the Study of Genetic Diversity in *Allium* Species

KEYWORDS: Genetic diversity, RAPD, ISSR, molecular marker, Allium species

Packia Lekshmi N C J
Department of biotechnology, Sathyabama university, Chennai

Viveka S
Department of Biotechnology, Udaya School of Engineering, Vellamodi

Jeeva S
Department of Microbiology, Udaya College of Arts and Science, Vellamodi.

Raja Brindha J
Department of Microbiology, Udaya College of Arts and Science, Vellamodi.

ABSTRACT *Onion and garlic are best known for their pungent aromas, but these potent veggies have powerful effects on health and also there is urgent need to identify superior populations, quickly characterize and select elite candidates and breed new varieties for achieving current as well as future food and global health security needs. Hence this review article is focused on the analysis of the genetic diversity of Allium cepa (onion) and Allium sativum (garlic) using molecular markers.*

Introduction

Plant diversity is very essential for the balance of quality atmosphere, fragile ecosystems, current/future global health and good security. They provide valuable traits needed for adapting plants to changing climatic conditions, biotic and abiotic stresses and outbreak of pandemic diseases. Plants cater many human needs such as food, fuel, fibre, oil, herbs, spices, medicine and wood for furniture, paper pulp and industrial crops and as forage and fodder for domesticated animals. Despite ~ 27000 plant species around the world, studies have estimated that only 30 crops provide 95% of human food energy needs with just four of them, viz., rice, wheat, maize and potatoes, provide more than 60%. With even tremendous medical advances, only 20% of the world's plant species have ever been tested for their medical potential (Nageswara Rao and Soneji 2010).

Given the significance of a relatively small number of crops for global food-health security, it is critically important to conserve the diversity within these plants. The challenge of feeding a world population which is growing steadily lies on a handful of domesticated crop plants and will require an astonishing increase in food production. Studies project that, by 2050, world population will increase from the current level of about 6 billion to more than 8 billion people (Hoisington et al 1999). It is estimated that world will need to produce as much food during the next 50 years as was produced since the beginning of agriculture 10,000 years ago (Hoisington et al 1999). Thus, there is urgent need to identify superior populations, quickly characterize and select elite candidate(s) and breed new varieties for achieving current as well as future food and global health security needs (Singh et al 2008).

In addition to plant genetic engineering and tissue cultures techniques, classical breeding is a well-recognized tool for increasing yield, conferring resistance and improving agricultural traits. Both ways involve the presence of simple and precise molecular markers for speeding up breeding programmes and genome analysis. Traditional methods, based on morphological, karyotypic analysis of metaphase chromosomes and isozyme analysis have been used to determine genetic variations in somaclones and to identify parental hybrids and cultivars (Brown et al 1993). The major disadvantages of such analysis are time consuming-blurred by environmental analysis only minor portion of the genome is represented and sometimes it is impossible to analyse high number and small size chromosomes (Rani et al 1995; Wang et al 1994).

Evolution of genus has been accompanied by ecological diversification. The majority of species grow in open, sunny, rather dry sites in arid and moderately humid climates. However, *Allium* sp. have adapted to many other ecological niches. Different types of forests, European subalpine pastures and moist subalpine and alpine grasslands of the Himalayan and Central Asian high mountains all contain *Allium* species, and gravelly places along river banks do as well. Even saline and alkaline environments are tolerated by some taxa (Cox 2011).

Classical approaches for the identification of *Allium* cultivars are based on morphological traits. The assessment of these traits is difficult and their evaluation can be subjective considering that most of these cultivars are related.

Many cultivars have been selected and used in daily life as food; however, their genetic background was unknown. Genetic diversity has been studied in plant species using a variety of morphological, chemical and molecular descriptors.

Clonal lineages within *Allium* species show a remarkably high degree of phenotypic diversity. New genotypes have not been obtained through hybridization, but through the selection of spontaneous mutation expressing traits of horticultural interest. More than 200 named garlic clones are commercially available in US. The US National Plant Germplasm System (NPGS) maintains 193 main accessions at the Western Regional Plant Introduction Station (WRPIS) in Pullman, Wash. The diversity of these clones is described by a set of phenotypic and morphological descriptors known to be phenotypic and morphological de-

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scriptors known to be plastic (Al-Zahim et al 1997; Ipek et al 2003).

The agricultural traits of garlic germplasms have normally shown wide variations in characteristics such as bulb weight, coat layer, leaf length, growth habit, and stress resistance (Fan et al 1997; Lu et al 2001; Volk and Stern 2009). Assessment of germplasm resources is necessary for their effective use (Kamentsky 2007). Evaluation of genetic diversity using molecular markers is a cornerstone for understanding genome structure, the characterization and maintenance of genetic variation in plant germplasm, identifying genes underlying important traits and devising optimal breeding strategies for crop improvement (Hayden et al 2010). Applying markers and recognition of polymorphic nucleotide sequences dispersed throughout the genome have provided new possibility for evaluating diversity and determining of inter and intra-species genetic relationship (Gostimsky et al 2005). Several molecular markers are available for the investigation of genetic diversity. SSR (Becker and Heun 1994), RAPD (Williams et al 1990), AFLP (Vos et al 1995), and ISSR (Zietkiewciz et al 1994) were the most important of them.

The Randomly Amplified Polymorphic DNA (RAPD) technique (Welsh and McClelland 1990; Williams et al 1990) based on PCR has been used for invitro variability identification and on genetic diversity studies in roses, peach, tea, cymbidium (ObaraOkeya and Kako 1998; Zhu et al 2006; Choi et al 2006) and annanas. The RAPD technique has also been used to determine the purity of hybrid seed in tomato.

Molecular markers and molecular sequences contain useful information about evolutionary history (Clegg 1993; Haymer 1994). Recently the use of RAPD has become popular. The arbitrarily primed PCR amplifies anonymous fragments of DNA from any genome (Williams et al 1990; Welsh and McClelland 1990). The size distribution of amplified fragments varies among species. However, closely related species have similar fragment distribution, while distantly related ones are more divergent (Wilkie et al 1993). Thus, RAPD bands (fragments) distribution contains considerable phylogenetic information (Campos et al 1994; Charmet et al 1997).

The development of RAPD approach (Williams et al 1990) has allowed simple, easy and less time consuming genome analysis at the DNA level compared with RFLP (Restriction Fragment Length Polymorphism). Numerous investigators have successfully employed RAPD to find molecular markers that could be used for genetic analysis of micropropagated and regenerated plants (Taylor et al 1995); taxonomic studies and classification (Castiglione et al 1993; MaaB and Klaas 1995); cultivar identification (Corniquel and Mercier 1994); genotypic screening (Chunwongse et al 1993) and breeding programmes (Chen et al 1995). Some success has been reported in correlating isozymes alleles with morphological traits (Pooler and Simon 1993; Siqueira et al 1985), whereas MaaB and Klaas (1995) applied both isozyme and RAPD analysis to the study of the origin of garlic with respect to the geographical distribution and genetic variation of unclassified germplasm.

The ISSR, as a relatively new class of molecular markers, is based on inter tandem repeats of short DNA sequences. These inter repeats are highly polymorphic in their sizes even among closely related genotypes due to the lack of evolutionary functional constraints in these non-functioning regions (Rizkalla et al 2012).

Inter Simple Sequence Repeat markers (ISSR) are one among the molecular markers considered as more discerning than RAPD (Qian et al 2001). Identification of closely related cultivars (Fang and Roose 1997) and characterization of genebank accessions (Charters and Wilkinson 2000), mapping of plant genome (Cheghamirza et al 2004) have been done by ISSR. Genetic diversity are estimated based on diallelic (1= band present, 0 = band absent) characteristics in ISSR technique (Xue-Jun et al 2005). ISSR is a rapid and inexpensive method to amplify and evaluate genetic variations among different plant cultivars and is used extensively.

Major limitations of these methods were low reproducibility of RAPD, high cost of AFLP and need to know the flanking sequences to design specific primers for SSR markers. The ISSR markers overcome most of these limitations (Reddy et al 2002) where, they have advantage of relatively low cost, high polymorphism and good reproducibility (Gonzalez et al 2002).

There are numerous reasons to study garlic. For one, garlic is the most important culinary seasoning throughout the world, and its production has become restricted to a small number of countries, which are in competition with each other. Furthermore, several garlic-importing countries have attempted to protect their domestic garlic production by setting up tariffs and other nontariff barriers. For example, many countries have strict import regulations that represent barriers against purchasing garlic from select foreign origins. This situation have been exploited by unscrupulous importers, who dodge such barriers by shipping agricultural products from a trade-restricted country to a second trade-unrestricted country, whereas, other countries have import subsidies. Therefore, characterization of the geographic origin of specific crops permits both the protection of domestic production and the inhibition of illegal trade. Determining the geographic origin of crops through the use of mineral profiling strategies represents an empowering instrument, as evidenced by the large number of studies published about the technique (Camargo et al 2010).

Unlike cultivated garlic (*A.sativum* L.), which has been the focus of several studies targeting morphological, agronomic and molecular characteristics (Baghalian et al 2005; Maab and Klaas 1995), studies on rosy garlic are rare. Also, these studies are mainly dedicated to its morphological (Guetat et al 2009a; Jendoubi et al 2001), chemical (Najjaa et al 2007) and karyological characteristics (Ferchichi 1997; Guetat et al 2009b). Since 2001, agronomic and morphological characterisation of Tunisian *A.roseum* material has been carried out in southern Tunisia, which is where the reference collection of this species was planted (near the Arid Land Institute (IRA) in Medicine, Tunisia).

At the time of the above research, genetic diversity in *A.roseum* at the molecular level had not been investigated. Guetat et al (2010) therefore undertook the study to investigate the genetic diversity of this species. The resulting information will contribute to the pool of background genetic information regarding rosy garlic, which may then facilitate the selection of a suitable conservation program, because the *Allium* chloroplast genome is maternally inherited (Corriveau and Coleman 1988). Guetat et al (2010) studied the genetic diversity using chloroplast DNA (cpDNA) to avoid the possibility of hybridisation between the

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different populations cultivated at the Arid Land Institute.

Conclusion

The molecular evidence given in this review article showed high interspecific diversity and intraspecific divergence among the *Allium* species. This may be due to the distinct features in different cultivation sites resulting in random genetic drift.

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