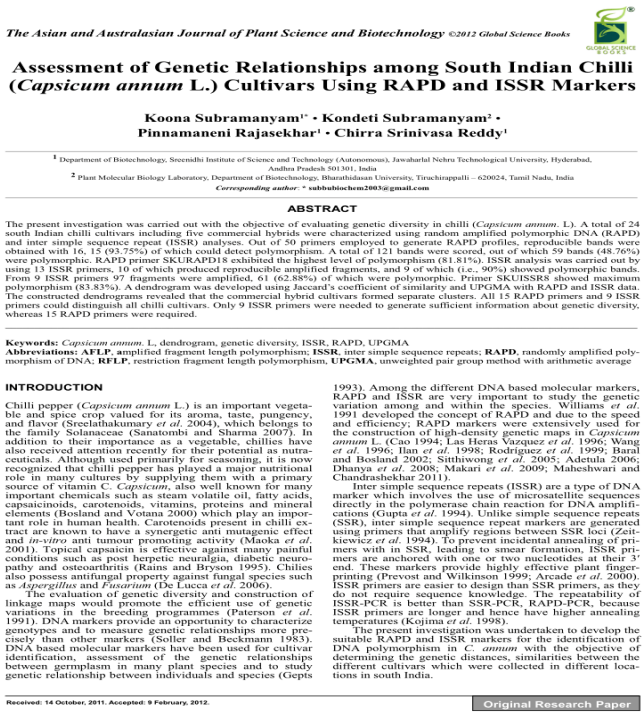
Recent techniques in chilli varietal identification: Biochemical and Molecular Markers



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Assessment of Genetic Relationships among South Indian Chilli (*Capsicum annum* L.) Cultivars Using RAPD and ISSR Markers

Koona Subramanyam[1*] • Kondeti Subramanyam[2] • Pinnamaneni Rajasekhar[1] • Chirra Srinivasa Reddy[1]

[1] Department of Biotechnology, Sreenidhi Institute of Science and Technology (Autonomous), Jawaharlal Nehru Technological University, Hyderabad, Andhra Pradesh 501301, India
[2] Plant Molecular Biology Laboratory, Department of Biotechnology, Bharathidasan University, Tiruchirappalli – 620024, Tamil Nadu, India
Corresponding author: * **subbubiochem2003@gmail.com**

ABSTRACT

The present investigation was carried out with the objective of evaluating genetic diversity in chilli (*Capsicum annum*. L). A total of 24 south Indian chilli cultivars were including five commercial hybrids were characterized using random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) analyses. Out of 50 primers employed to generate RAPD profiles, reproducible bands were obtained with 16, 15 (93.75%) of which could detect polymorphism. A total of 121 bands were scored, out of which 59 bands (48.76%) were polymorphic. RAPD primer SKURAPD18 exhibited the highest level of polymorphism (81.81%). ISSR analysis was carried out by using 13 ISSR primers, 10 of which produced reproducible amplified fragments, and 9 of which (i.e., 90%) showed polymorphic bands. From 9 ISSR primers 97 fragments were amplified, 61 (62.88%) of which were polymorphic. Primer SKUISSR8 showed maximum polymorphism (83.83%). A dendrogram was developed using Jaccard's coefficient of similarity and UPGMA with RAPD and ISSR data. The constructed dendrograms revealed that the commercial hybrid cultivars formed separate clusters. All 15 RAPD primers and 9 ISSR primers could distinguish all chilli cultivars. Only 9 ISSR primers were needed to generate sufficient information about genetic diversity, whereas 15 RAPD primers were required.

Keywords: *Capsicum annum*. L, dendrogram, genetic diversity, ISSR, RAPD, UPGMA
Abbreviations: AFLP, amplified fragment length polymorphism; **ISSR**, inter simple sequence repeats; **RAPD**, randomly amplified polymorphism of DNA; **RFLP**, restriction fragment length polymorphism, **UPGMA**, unweighted pair group method with arithmetic average

INTRODUCTION

Chilli pepper (*Capsicum annum* L.) is an important vegetable and spice crop valued for its aroma, taste, pungency, and flavor (Sreelathakumary *et al.* 2004), which belongs to the family Solanaceae (Sanatombi and Sharma 2007). In addition to their importance as a vegetable, chillies have also received attention recently for their potential as nutraceuticals. Although used primarily for seasoning, it is now recognized that chilli pepper has played a major nutritional role in many cultures by supplying them with a primary source of vitamin C. *Capsicum*, also well known for many important chemicals such as steam volatile oil, fatty acids, capsaicinoids, carotenoids, vitamins, proteins and mineral elements (Bosland and Votana 2000) which play an important role in human health. Carotenoids present in chilli extract are known to have a synergetic anti mutagenic effect and *in-vitro* anti tumour promoting activity (Maoka *et al.* 2001). Topical capsaicin is effective against many painful conditions such as post herpetic neuralgia, diabetic neuropathy and osteoarthritis (Rains and Bryson 1995). Chilies also possess antifungal property against fungal species such as *Aspergillus* and *Fusarium* (De Lucca *et al.* 2006).

The evaluation of genetic diversity and construction of linkage maps would promote the efficient use of genetic variations in the breeding programmes (Paterson *et al.* 1991). DNA markers provide an opportunity to characterize genotypes and to measure genetic relationships more precisely than other markers (Soller and Beckmann 1983). DNA based molecular markers have been used for cultivar identification, assessment of the genetic relationships between germplasm in many plant species and to study genetic relationship between individuals and species (Gepts 1993). Among the different DNA based molecular markers, RAPD and ISSR are very important to study the genetic variation among and within the species. Williams *et al.* 1991 developed the concept of RAPD and due to the speed and efficiency; RAPD markers were extensively used for the construction of high-density genetic maps in *Capsicum annum* L. (Cao 1994; Las Heras Vazquez *et al.* 1996; Wang *et al.* 1996; Ilan *et al.* 1998; Rodriguez *et al.* 1999; Baral and Bosland 2002; Sitthiwong *et al.* 2005; Adetula 2006; Dhanya *et al.* 2008; Makari *et al.* 2009; Maheshwari and Chandrashekhar 2011).

Inter simple sequence repeats (ISSR) are a type of DNA marker which involves the use of microsatellite sequences directly in the polymerase chain reaction for DNA amplifications (Gupta *et al.* 1994). Unlike simple sequence repeats (SSR), inter simple sequence repeat markers are generated using primers that amplify regions between SSR loci (Zietkiewicz *et al.* 1994). To prevent incidental annealing of primers with in SSR, leading to smear formation, ISSR primers are anchored with one or two nucleotides at their 3′ end. These markers provide highly effective plant fingerprinting (Prevost and Wilkinson 1999; Arcade *et al.* 2000). ISSR primers are easier to design than SSR primers, as they do not require sequence knowledge. The repeatability of ISSR-PCR is better than SSR-PCR, RAPD-PCR, because ISSR primers are longer and hence have higher annealing temperatures (Kojima *et al.* 1998).

The present investigation was undertaken to develop the suitable RAPD and ISSR markers for the identification of DNA polymorphism in *C. annum* with the objective of determining the genetic distances, similarities between the different cultivars which were collected in different locations in south India.

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specificity in. Capsicum collection and identification of genetic stock. Village Road, Meharuli, New Delhi , India Technologies Inc. USA) were used for PCR amplification.Thus, this method could be an alternative for identification of chilli species with The greatest genetic variety of C. annuum L. can be found in Mexico, where there between molecular and biochemical markers, such as the content of B in 50 min and to 60% phase A and 40% phase B in the last 10 min.the off-types are identified using SSR markers for Chilli, Tomato, Okra, SSR markers were screened for one variety from each crop and one has given a new gateway in assessing the genetic purity more accurately in . advantages over biochemical or morphological methods to obtain distinct genotype specific profiles.biochemical, and molecular. of identification for vegetable varieties by molecular markers new vistas to fastened the breeding program of vegetable crops by using a various molecular marker in the different methods of breeding and their steps for enhancing of the . () F1 Chilli hybrids was determined using two.In Mexico, there is a wide diversity of species and varieties of chilli peppers, a fruit Thus, this method could be an alternative for identification of chilli species with between molecular and biochemical markers, such as the content of B in 50 min and to 60% phase A and 40% phase B in the last 10 min.chilli pepper using simple sequence repeats (SSR) markers. M. S. Dhaliwal* descriptors were used for varietal identification and genetic In the last decade or so, molecular markers such as restriction The working group on biochemical and molecular techniques of UPOV has identified SSR markers.Assessment of genetic diversity in local chilli (Capsicum annuum) varieties in. Mauritius. or biochemical markers have also been used to evaluate the genetic diversity within germplasm (Kaur and Kapoor,.). Recent developments in DNA based technologies .. At the vegetative stage, it is quite difficult to identify.Recent techniques in chilli varietal identification: Biochemical and Molecular Markers is top selling of this month.

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