Validation of SSR Markers Linked to the Bean Common Mosaic Virus (BCMV) Resistance in Cowpea (*Vigna unguiculata* L.) Genotypes

N. Manjunatha
Indian Grassland and Fodder Research Institute, India

K. T. Rangaswamy
University of Agricultural Sciences, India

N. Nagaraju
University of Agricultural Sciences, India

H. A. Prameela
University of Agricultural Sciences, India

P. Rudraswamy
University of Agricultural Sciences, India

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Manjunatha N.[1]*, Rangaswamy K. T.[2], Nagaraju N.[2], Prameela H. A.[2], Rudraswamy P.[3]
[1]IGFRI, Jhansi, India
[2]Department of Plant Pathology, University of Agricultural Sciences, Bangalore, India
[3]AICRP on Arid legumes, University of Agricultural Sciences, Bangalore, India
*corresponding author e-mail : manju.ars@rediffmail.com

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Introduction

Cowpea, *Vigna unguiculata* (L.) is one of the important grain and fodder legume crops in the world, especially in tropics and subtropics. Which can adapted different climatic conditions such as high temperature and drought. It is not only important as a grain and fodder but also improve fertility of poor soils by fixing atmospheric nitrogen. An estimated cultivated area of cowpea is 12.5mha in worldwide (FAOSTAT, 2013). The grain and fodder productivity of cowpea are greatly affected by a number of biotic factors such as plant pathogens and insect pests. Among plant pathogens, viruses are considered to be a major constraint (Gioi *et al*., 2010). More than 40 viruses are reported from cowpea growing areas of the world.

Among viruses, Bean common mosaic virus (BCMV) is very serious problem on cowpea in southern part of India especially cowpea growing areas of Karnataka. The BCMV is a member of potyvirus group and it can infect other legume crop family. Virus is seed borne and easily spread by aphids. Its chemical and cultural control neither economical nor ecofriendly. The host plant resistance has been proved the best strategy for its management. Hence it is necessary to identify stable resistance sources for BCMV disease in cowpea. Therefore large scale screening is needed to identify the durable resistance among diverse genotypes of cowpea. The basic requirements for any crop improvement programme are genetic variation for the trait of interest.

Assessment of the extent of genetic variability is fundamental for breeding. Earlier morphological traits were used for assessing thegenetic diversities. However morphological characterization alone does not reliably portray the genetic diversity and relationships among the genotypes due to environmental interactions and largely unknown genetic control (Kumar *et al*., 2014). On other hand DNA based molecular markers such as RAPD, RFLP and AFLP assess the genetic diversity in more precise way. Among the molecular markers simple sequence repeat (SSR) or microsatellites are more being used widely. SSR or micro satellites are co-dominant markers that are frequently used to study the genetic diversity in different legume crops. The variation in the number of tandem repeats results in different PCR product lengths. SSRs have become the tool for wide range of application in genotype identification, diversity analysis, QTL analysis, Marker assisted breeding and identification of molecular markers linked to agronomically important genes (Gioi *et al*., 2010; Kumar *et al*., 2014). Therefore study is undertaken to validate SSR markers which are linked to BCMV resistance in cowpea genotypes.

Materials and Methods

Plant material: The present investigation was conducted using cowpea germplasm lines obtained from AICRP on Arid legumes, UAS, GKVK, Bangalore. Ninety genotypes were used for screening against BCMV disease under field and glass house conditions using 0-5 disease scoring scale (Diwakar and Mali, 1976). After repeated screening of cowpea genotypes 4 clearly resistant (Immune), 14 resistance, 10 moderately resistance, 10 moderately susceptible 42 susceptible and 10 highly susceptible lines were obtained. Validation of SSR markers *viz*., M15, M80, M135 and Y96 linked to BCMV resistance was carried out in randomly selected 25 genotypes comprise of susceptible (C-152) and resistant genotype (V-5).

Marker analysis: Genomic DNA was isolated from the leaf samples of 3 to 4 week old seedlings of randomly selected cowpea genotypes using CTAB extraction method (Sambrook *et al*., 2001). The amplification of template DNA was performed in PCR reaction. Total volume of PCR reaction mixture was made to 25µl, which contains PCR master mix 12.5µl, forward primer 2µl, reverse primer 2µl and template 4µl. PCR conditions for the SSR marker analysis included, an initial denaturation step of 5 minutes at 94^0 C and following 35 cycles of 94^0 C for 1 min (denaturing), 55^0 C for 30 sec

(annealing) and 72^0C for 1mins (extension), with the final step of extension was carried out at 72 for 10 minutes. The amplified products were separated on 4% agarose gel by electrophoresis. Scoring was done manually in terms of positions of the bands relative to the ladder sequentially from the smallest to the largest-sized bands and were transformed to binary matrices and data obtained was analyzed by NTSYSpc (version 2.02).

Results and Discussion

To identify polymorphism between the BCMV susceptible and resistant genotypes randomly 25 cowpea genotypes which showed susceptible and resistant disease reaction were selected for the validation of BCMV resistance linked markers. All markers were amplified to cowpea genotypes clearly. The UPGMA (Unweighted Paired Group Method Using Arithmetic Averages) cluster analysis was performed using amplification data of SSR markers which could be validated and all the 25 genotypes were broadly grouped into two major cluster. Two major cluster again divided in two sub clusters (Sc) at 35% similarity. The 20 genotypes of Sc-I separated as three groups with 65% to 75% similarity, whereas, 5 genotypes of Sc-II formed two different groups with 60% similarity. The clustering of resistant and susceptible genotypes couldn't find clearly among the genotypes. However, genotypes viz., V-5 and IC 8996 clustered in one group showing resistant reaction (Fig. 1). The same pattern of differentiation between resistant and susceptible genotypes was observed in three dimensional principal coordinate analysis (Fig. 2).The reason for improper clustering among genotypes is low level of SSR polymorphism in cowpea than other crops (Gioi *et al*., 2010). The pattern of clustering of resistant and susceptible genotypes against plant diseases using SSR markers analyzed by different researchers in different crops such as in peanut against foliar diseases (Gajjar *et al*., 2014), against yellow mosaic virus in soybean (Kumar *et al*., 2014), yellow mosaic virus resistance in cowpea (Gioi *et al*., 2012). The resistance obtained in this study would serve as source for development of resistance variety against BCMV. This showed that SSR marker can be used to estimate the genetic basis of BCMV resistance in cowpea.

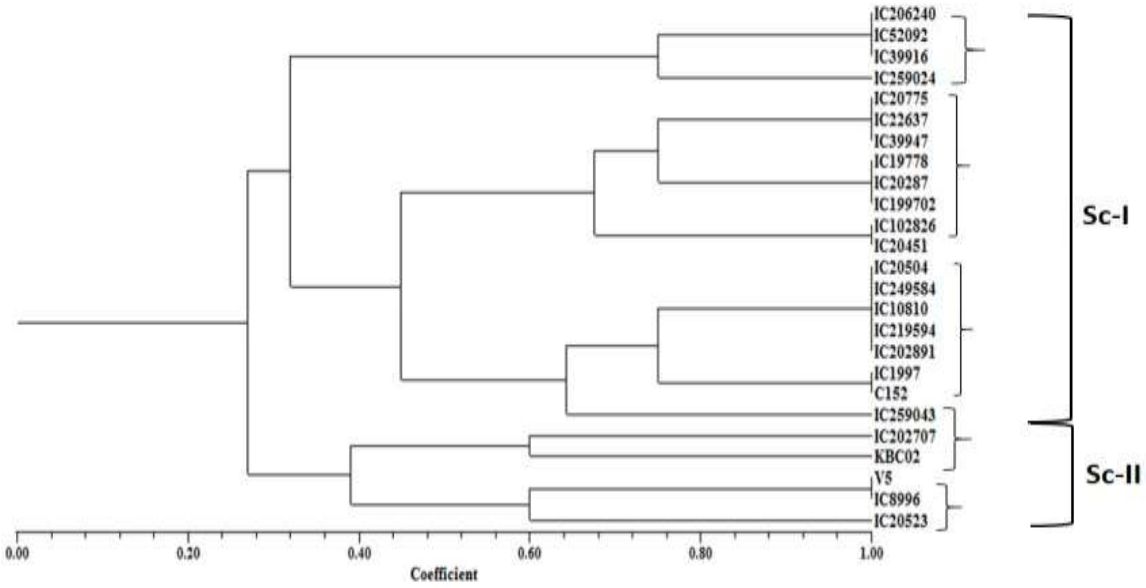

Fig 1. Dendrogram generated using unweighted pair of group method with arithmetic average analysis showing relationship between resistant and susceptible genotypes of cowpea using four SSR markers

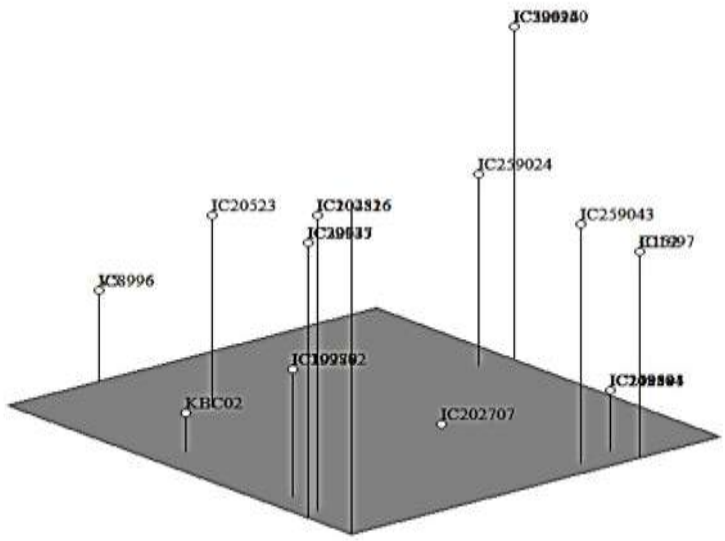

Fig 2. Genetic similarity of 25 cowpea genotypes revealed by three dimensional viw of dendrogram

Conclusion

For disease resistance molecular markers have been validated in different varieties of crop. In present study SSR or microsatellite markers linked to BCMV resistance in cowpea were validated. The information provided by the markers would be very useful in breeding programme to select cowpea genotypes resistance to BCMV disease. These markers may also useful in marker assisted selection for breeding of cowpea resistance to BCMV.

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