RPF - III

PROFORMA FOR SUBMISSION OF ANNUAL PROGRESS REPORT OF RESEARCH PROJECTS

Part - I: GENERAL INFORMATION

800 Project Code :
8001 Institute Project Code No. : Biotech XI (813)
8002 ICAR Project Code No. :

801 Name of the Institute and Division :
8011 Name and address of Institute : Indian Institute of Spices Research
8012 Name of Division/Section : Cardamom Research Centre
8013 Location of Project : Appangala

802 Project Title : Identification of molecular markers linked to Katte resistance genes in small Cardamom (Elettaria cardamomum (L.) Maton)

803 Priority Area :
8031 Research Approach:

Applied Research	Basic Research	Process/Technology development	Transfer of Technology
01*	02*	03	04

804 Specific area : Cardamom genetic resources
805 Duration of Project :
8051 Date of start : 2009
8052 Date of completion : 2011

806 Total cost /Expenditure Incurred : 14.20 lakhs
(Give reasons for variation, if any from original estimated cost)
Since the transfer of Dr.Usha Rani, the original principal investigator of the project 10 lakhs difference is there with original proposal i.e 24 lakhs.

807 Executive Summary

DNA was isolated from parental lines viz GG, a susceptible parent as well as from NKE 12, a resistant parent. Parental polymorphism was studied using ISSR markers. The ISSR profiling revealed that four (834a, 866, 812, 815) out of seven primers worked and

Two primers (866, 815) have shown polymorphism, one (866) for Susceptible parent-GG and other (815) for Resistant parent-NKE12. The F2 mapping populations were maintained in the insect proof glass house for phenotypic screening. Twenty plants which belong to the population were inoculated with viruliferous aphids following standard inoculation procedures. All the plants were found to be susceptible to katte disease.

Key words: CdMV, Markers, *Katte* resistance

PART - II: INVESTIGATOR PROFILE

(Please identify clearly changes, if any in project personnel)

810 Principal Investigator

8101	Name	: Dr. R. Senthil kumar
8102	Designation	: Sr. Scientist (Horticulture)
8103	Division/Section	: Crop Improvement and Biotechnology
8104	Location	: Appangala
8105	Institute Address	: Indian Institute of Spices Research, Cardamom Research Centre, Appangala, Madikeri-571 201, Karnataka.

811 Co-Investigator

8111	Name	: Dr. K. Nirmal Babu
8112	Designation	:Project Coordinator
8113	Division/Section	: Division of Crop Improvement & Biotechnology
8114	Location	: IISR, Kozhikode
8115	Institute Address	: Indian Institute of Spice Research, Kozhikode

812 Co-investigator:

8121	Name	: Dr. D. Prasath
8122	Designation	: Senior Scientist
8123	Division/Section	: Division of Crop Improvement & Biotechnology
8124	Location	: IISR, Kozhikode
8125	Institute Address	: Indian Institute of Spice Research, Kozhikode

813 Co-investigator:

8131	Name	: Dr. R. Praveena
8132	Designation	: Scientist
8133	Division/Section	: Division of Crop Protection
8134	Location	: Cardamom Research Centre, Appangala
8135	Institute Address	: Indian Institute of Spice Research, Kozhikode

PART - III: TECHNICAL DETAILS

820 Introduction and Objectives:

8201 Project objectives :

a) To develop mapping populations for Katte disease resistance
b) To tag genes responsible for Katte disease resistance in cardamom
c) To construct molecular map of Katte disease resistance genes
d) To carry out marker aided selection and Katte resistance breeding in cardamom against katte disease.

8202 Background information and importance of the Project :

National status: Indian spices and spice product are main foreign exchange earners and holds premier position in the global spice market. At present, India produces around 2.75 million tons of different spices valued at approximately 4.2 billion US $. Among spices, Indian cardamom has been preferred choice worldwide because of its superior quality with characteristic flavour and aroma. However in the last two decades, the production of cardamom was greatly affected by a mosaic disease, which is caused by *Cardamom mosaic virus* (CdMV). This has resulted in a steep reduction in the overall yield as well as of the export of the spice (Varmudi, 2000).

CdMV-induced disease is manifested by the characteristic mosaic symptoms on the leaves and pseudostem, followed by stunted growth and reduction in the yield by 70–100%, in 1–3 years. Plants become virtually unproductive after the third year of the disease. The viral nature of the mosaic disease in cardamom was first reported by Uppal *et al.* (1945), followed by a number of studies on the mode of transmission by the aphid *Pentalonia nigronervosa*(Varma and Capoor, 1958; Rajan, 1981; Naidu and Venugopal, 1986). Recently, Jacob and Usha (2001) analyzed the nucleic acid sequence of coat protein and 3' untranslated region of isolate of katte virus originating from Sakleshpur in Karnataka and concluded that katte virus belong to genus Macluravirus of the family potyviridae. The purification and solubility properties of bacterially expressed wild type and mutant forms of the CP of CdMV have been reported (Jacob and Usha, 2001). Jebsingh *et al.* (2008) reported expression, solubilization and purification of Nuclear Inclusion b (NIb) protein of CdMV for structural characterization.

Keeping these in view, this research project was proposed in order to address the viral disease problem in small cardamom. This project will help in mapping and tagging of *katte* resistance genes using molecular markers. Various researchers have attempted molecular characterization of some elite genotypes of cardamom for genetic diversity studies (Johnson *et al.*, 2006; Kizhakkayil *et al.* 2006). The tightly linked markers can be further used in MAS for *katte* disease resistance breeding in cardamom.

Molecular characterization of some elite genotypes of cardamom has been attempted to find out the genetic relationship between them. However no work has been carried on identification of marker linked to *katte* resistance. Three non-anchored ISSR primers were successfully used in amplifying inter-microsatellite regions of small cardamom, large cardamom, and different species of Vanilla and *Piper* for assessing the genetic diversity (Johnson *et al.*, 2006). Kizhakkayil *et al.* (2006) characterized exported cardamoms from India, Sri Lanka and Guatemala based on physical, biochemical parameters and molecular techniques and concluded molecular profiling using RAPD/ISSR primers did not reveal much polymorphism among them. Eight operon primers out of

100 were found to be polymorphic in characterization studies of fourteen elite cardamom genotypes (Radhakrishnan and Mohanan, 2005).

821 Project Technical Profile

8211 Technical Programme
(Indicate briefly plan of procedure, techniques, instruments and special materials, organisms, special environments etc.)

1. Searching and designing of new primers
2. Standardization of PCR conditions for SSR and ISSR
3. Parental polymorphism study using available molecular marker techniques
4. Development of F2 mapping population
5. Phenotyping of F2 mapping population for Katte resistance
6. Bulked segregant analysis and genotyping of F2 mapping population
7. Linkage analysis and construction of map for Katte resistance

Total man months involvement of component project workers

Investigator	Man months	Total man months
1. R. Senthil kumar	3+3	06
2. T.R.Usha Rani	3	03
3. K.Nirmal Babu	3	03
4. D.Prasath	3	03
5. R.Praveena	3+3	06
		21

822 Final Report on the Project

Detailed report containing all relevant data with a summary of results (not exceeding 2-5 pages)

8221 Achievements in terms of targets fixed for each activity

Standardization of PCR conditions for SSR and ISSR primers and Parental polymorphism study using available ISSR primers:
DNA was isolated from parental lines (GG and NKE 12) using CTAB method.

DNA isolation from the parental lines (GG and NKE 12)

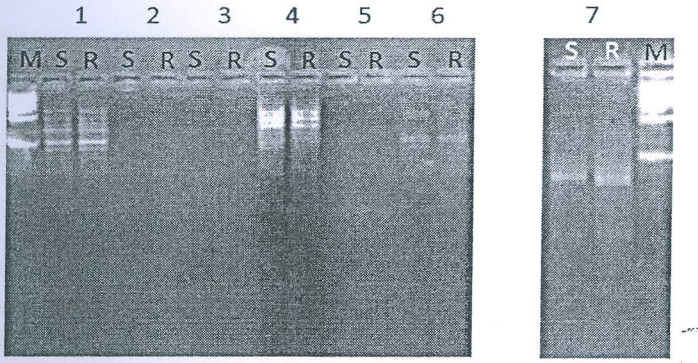
Standardization of PCR conditions with 7 sets of ISSR primers (834a, 841a, 854a, 866, 867, 812, 815) and Agarose gel Electrophoresis of PCR product.

PCR reaction Mixture: Primer 2µl, PCR buffer 3 µl, dNTP's mix 0.4 µl, $MgCl_2$ 1.5 µl Taq 0.3 µl, Water 6.8 µl, DNA 1 µl

PCR Condition:

94°C -5min
94°C -1min
50°C -1min
72°C -1min 30 sec
72°C -7min

ISSR profiling revealed that four (834a, 866, 812, 815) out of seven primers worked and Two primers (866, 815) have shown polymorphism, one (866) for Susceptible parent-GG and other (815) for Resistant parent-NKE12



M=Marker,1=834a,2=841a,3=857a,4=866,5=867,6=812,7=815

ISSR profiling of the parents with seven ISSR primers for GG, a susceptible parent as well as for NKE 12, a resistant parent

The F2 mapping populations were maintained in the insect proof glass house for phenotypic screening. Twenty plants which belong to the population were inoculated with viruliferous aphids following standard inoculation procedures. All the plants were found to be susceptible to katte disease.The characteristic symptoms of katte disease were manifested on the inoculated plants fifteen days after inoculation.The symptoms included, slender chlorotic flecks on the youngest leaf which later developed into pale green discontinuous stripes. (Plate 1)The results indicated that, all the plants belonging to the mapping population were

susceptible to katte disease.

8222 Questions - Answered:

What is the practical application of this study?

Molecular tagging of Katte resistance genes will help in resistance breeding in cardamom.

8223 Process/Product/Technology/Developed:

The lead obtained in the project will be utilized in the breeding cardamom for high yield and disease resistance project.

8224 Practical Utility
(not more than 150 words)

Systematic efforts have been made periodically at Indian Institute of Spices Research, Cardamom Research Centre, Appangala since inception of this project (2009) for molecular tagging of katte resistance genes, based on so for obtained leads in the project will be utilized in the ongoing breeding cardamom for high yield and disease resistance project.

8225 Constraints, if any:
Since the transfer of Dr.Usha Rani, the original principal investigator of the project some of the objective will be continued in the ongoing breeding project.

823 Publications and Materials Development:
(One copy each to be supplied with this proforma)

8231 Research papers: Nil