DEVELOPMENT OF MOLECULAR MARKER FOR RAPID IDENTIFICATION OF *Fusarium* WILT INFECTING SAFFLOWER

*SUBMITTED TO*

SCIENTIST IN-CHARGE

PROJECT REGISTRY CELL

DEPARTMENT OF BIOTECHNOLOGY

BLOCK 2, 7th FLOOR

C.G.O. COMPLEX, LODI ROAD

NEW DELHI – 110 003

*BY*

Dr. S.S. SHENDE

ASSOCIATE PROFESSOR

MARATHWADA AGRICULTURAL UNIVERSITY

COLLEGE OF AGRICULTURAL BIOTECHNOLOGY, LATUR
PROFORMA FOR SUBMISSION OF PROJECT PROPOSALS ON RESEARCH AND DEVELOPMENT, PROGRAMME SUPPORT

PART I: GENERAL INFORMATION

1. Name of the Institute/University/Organisation submitting the Project Proposal:
    Marathwada Agricultural University,
    College of Agricultural Biotechnology, Latur.

2. State: Maharashtra

3. Status of the Institute: State University

4. Name and designation of the Executive Authority of the Institute/University forwarding the application:
   Director of Research,
   Marathwada Agricultural University,
   Parbhani (Maharashtra)

5. Project Title: Development of molecular marker for rapid identification of *Fusarium* wilt infecting safflower

6. Category of the Project (Please tick): R&D/ Programme Support

7. Specific Area: 1.11: Molecular Diagnostic

8. Duration: 3 years

9. Total Cost (Rs.) 31,42,000/-

10. Is the project Single Institutional or Multiple-Institutional (S/M) ? : Multiple Institutional

11. If the project is multi-institutional, please furnish the following: Yes
    Name of Project Coordinator: Dr. S.S. Shende
    Affiliation: Marathwada Agricultural University, Parbhani
Address: College of Agricultural Biotechnology, Nanded Road, Latur -413 512 (M.S)

12. Scope of application indicating anticipated product and processes
   i. Genetic variability of the *Fusarium* species infecting safflower will be find out. This will be helpful in *Fusarium* wilt resistant breeding programme.
   ii. The marker developed will be helpful for rapid identification of *Fusarium* species present in soil.
   iii. So, soil analysis before sowing by developed marker will reduce time for identification and mitigation measures to control the disease before planting.

13. Project Summary

   --Please see Annexure-I—

PART II: PARTICULARS OF INVESTIGATORS

Principal Investigator:

14. Name: Dr. S.S. Shende

   Date of Birth: 22 May 1977 Sex (M/F): F

   Designation: Associate Professor

   Department: Plant Biotechnology

   Institute/University: Marathwada Agricultural University,

   College of Agricultural Biotechnology, Latur

   Address: College of Agricultural Biotechnology, Nanded Road, Latur (M.S) PIN: 413 512

   Telephone: 07588611023 Fax: 02382-251366 E-mail: sarikasshende@gmail.com

   Number of research projects being handled at present: Nil
Co-Investigator:

15. Name: Dr. M.K. Rai

Date of Birth: 28/08/1955       Sex(M/F): M

Designation: Professor & Head

Department: Biotechnology

Institute/University: Sant Gadge Baba Amravati University, Amravati

Address: Department of Biotechnology,
Sant Gadge Baba Amravati University,
Amravati (M.S)       PIN: 444 602

Telephone: 9422857196   Fax 91-721-2660949, 2662135

E-mail: mkrai123@rediffmail.com

Number of Research projects being handled at present: 10
PART III: TECHNICAL DETAILS OF PROJECT

16. Introduction

16.1 Origin of the proposal

Safflower (*Carthamus tinctorius* L.), an oilseed crop is a member of the family Asteraceae. The crop has tremendous potential to be grown under varied conditions and to be exploited for various purposes. Safflower, a multipurpose crop has been grown for centuries in India for the orange red dye, carthamin extracted from its brilliantly colored flowers, leaf and stem as vegetables and for its quality oil (linoleic acid, 67-83% and oleic acid, 74-80%) (Oyen *et al*., 2007). Thus, each part of safflower has a value attached to it.

India is ranked number one in global safflower production and acreage. India accounts for 3,00,000 ha of area under safflower cultivation globally. However, area under safflower cultivation has declined by 64% since 1991 and is continuously declining over the last 10 years. Production has also declined by 41% during the same period. In India key safflower producing states are Maharashtra and Karnataka contributing 55% and 31% respectively. Maharashtra, Karnataka along with Gujarat and Andhra Pradesh accounts for 94% of Indian area under safflower cultivation and about 99% of country's safflower production (Anonymous, 2011).

Many bacterial, fungal and viral pathogens infect the safflower crop causing diseases. Among all these *Fusarium* wilt is observed as one of the most common and destructive disease. *Fusarium*, a mitosporic *hypocreales*, is a diverse genus consisting of an array of species responsible for damping-off, root rots and vascular wilts on a multitude of economically important plant species (Nelson *et al*., 1981). Being soil borne in nature, the fungus survives in the soil as chlamydospores in diseased plant debris without losing viability (Chakraborty, 1980). Wilt of safflower is mainly caused by *Fusarium oxysporum* f. sp. *carthami*. But, other species of *Fusarium* are also reported causing wilt (Shende *et al*., 2011). It invades the roots mainly through wounds and proceeds into and throughout the vascular system, leading to functional collapse, systemic wilting and often death of the infected plant. The disease becomes severe when the plant reaches about 15 cm in height. The variation in temperature and soil moisture exerts an influence on the fluctuation
of the disease expression and severity. Infected plants may either totally wilt and
 die, or persist in a weakened state, producing a reduced number of inferior fruits
 (Jarvis and Shoemaker, 1978).
 Laboratory species designation based solely on microscopic and morphologic traits
 are time consuming and can be prone to misinterpretations. In addition, micro and
 macroscopic characteristics may be influenced by media, age of culture and
 identification pattern. In contrast, the use of PCR and sequence analysis is capable
 of identification of isolates to the genus and species level in less than 24 hours.
 rDNA gene internal transcribed spacer (ITS) as a genetic marker, has been widely
 used in classification and identification of microbial strains, genetic diversity and
 phylogenetic relationships, pathogen diagnostics, phylogenetic studies on different
 aspects of research. (Vilatersana et al., 2000).
 Physiological and pathogenic specialization in an organism makes the task of
disease management more difficult. A newly emerged race/strain of a pathogen may
 affect the production of cultivars. It is therefore very important to analyze the
 diversity amongst isolates of *Fusarium* species from different geographical areas
 and to develop marker for rapid identification.

16.2 (a) **Rationale of the study supported by cited literature**

rDNA genes provide a means analyzing phylogenetic relationships over a wide
range of taxonomic levels and designed primers for amplification of various
segments of the nuclear and mitochondrial rDNA genes of fungi (White et al.,
1990). ITS PCR primers for assessing fungal diversity in environmental samples
(Anderson et al., 2003). Two taxon selective primers for quick identification of the
*Fusarium* genus are developed, ITS-Fu-f and ITS-Fu-r by comparing the aligned
sequences of ITS for a range of *Fusarium* species (Abd-Elsalam et al., 2003). Both
ITS-1 and ITS-2 regions are needed for accurate identification of fungi, *Aspergillus*
at the species level (Henry et al., 2000). *Fusarium oxysporum* isolates of weeds
striga species are characterized on the basis of partial DNA sequence of the ITS
region of nuclear ribosomal RNA genes (Elzein et al., 2008).

(b) **Hypothesis**—The developed marker will be used as a diagnostic tool for rapid
identification of *Fusarium* species infecting safflower and other crops.
(c) **Key questions**-What is need of marker based identification, can molecular marker be developed? Will it be possible to identify *Fusarium* species infecting other crops.

### 16.5 Current status of research and development in the subject (both international and national status)

Very meager information is available on *Fusarium* wilt in safflower.

**International status:** *Fusarium oxysporum* f. sp. *carthami* was reported as a causal organism for the wilt disease of safflower (*Carthamus tinctorius* Linn.) for the first time by Klisiewicz and Houston (1962) and collected 12 isolates from different areas of Sacramento valley (USA). Since, then the incidence has been reported from California (Zimmer *et al*., 1963) and Yolo (Stout, 1963).

White *et al.* (1990) designed primers for amplification of various segments of the nuclear and mitochondrial rDNA genes of fungi. In addition to the standard ITS-1+ITS-4 primers used by most laboratories, several taxon specific primers have been developed that allow selective amplification of fungal sequences (Gardes and Bruns, 1993). Abd-Elsalam *et al.* (2003) developed two taxon selective primers for quick identification of the *Fusarium* genus. These primers, ITS-Fu-f and ITS-Fu-r were designed by comparing the aligned sequences of internal transcribed spacer regions (ITS) of a range of *Fusarium* species. The primers showed good specificity for the genus *Fusarium*, and amplified approx. 389 bp product. Elzein *et al.* (2008) characterized and sequenced *Fusarium oxysporum* isolates of weeds striga species on the basis of partial DNA sequence of the ITS region in order to more clear distinction than other morphologically similar pathogenic *Fusarium oxysporum* strains. Castillo *et al.* (2010) isolated and identified the wilting agent present in cotton lines by PCR using the universal primers ITS-1 and ITS-4, and the specific primers FOV1, FOV2 for future race identification. Chehri *et al.* (2011) isolated and identified disease causing *Fusarium* species from infected cucurbit plants, to ascertain their pathogenecity and phylogenetic relationships based on ITS RFLP analysis. This was the first report on identification and pathogenecity of major plant pathogenic *Fusarium* species causing root and stem rot on cucurbits in Iran.
National status: Nirmal et al. (1989) reported the disease from Marathwada region of Maharashtra with an incidence of 25 percent. Deokar et al. (1998) recorded the incidence of safflower wilt disease up to 40 percent in Solapur, Osmanabad, Latur, Parbhani and Buldhana districts of Maharashtra. Prasad et al. (2007) studied molecular variability among 45 isolates belonging to 8 sections of the genus *Fusarium* by using PCR based RAPD, ISSR and ITS-RFLP analysis and suggested need of ITS sequencing.

16.6 The relevance and expected outcome of the proposed study

The *Fusarium* species causing wilt in safflower will be isolated and identified. The genetic variability of *Fusarium* species will be determined. The marker developed from the conserved ITS sequences of the *Fusarium* species will be used for identification of *Fusarium* wilt infection in safflower as well as in other crops.

16.7 Preliminary work done so far

*Fusarium* species causing safflower wilt has been isolated and identified from various regions of Marathwada. The 550bp rDNA region has been amplified by ITS-1 and ITS-4 universal primer pair. The ITS region is cloned and sequenced for analysis of genetic variability. Two species of *Fusarium* viz., *F. equiseti* and *F. moniliforme* are reported for the first time from Marathwada region, proving its pathogenecity on safflower.

17. Specific objectives (should be written in bulleted form, a short paragraph indicating the methods to be followed for achieving the objective and verifiable indicators of progress should follow each specific objective)

i. Isolation and identification of *Fusarium* species:

The *Fusarium* species will be isolated from wilted safflower plants. Purification of fungal cultures will be done by hyphal tip method (Dohroo and Sharma, 1992). The isolates of the *Fusarium* species will be identified based on characters and spores morphology (Booth, 1971). Pink coloration of fungal species on PDA medium and formation of microconidia, macroconidia and intercalary spores on microscopic observations confirm *Fusarium* species.

ii. Amplification, Cloning and sequencing of ITS fragment:
DNA amplification of *Fusarium* species will be done using ITS-1 and ITS-4 primer (Prasad et al., 2007). The amplified fragment will be cloned in vector and transformed in host. The recombinant plasmid will be recovered from transformed host and sequenced. The PCR amplification of 550bp fragment by ITS-1 and ITS-4 primers confirms ITS region. Cloning in vector followed by transformation in host and appearance of white colonies (blue white screening) confirms transformation and presence of ITS gene in vector and host.

**iii. Sequence analysis by bioinformatics tool to study genetic variability:**

Data generated by sequencing ITS-1 and ITS-4 region will be analysed by MEGA4 or PHYLIP software to study the genetic variability among *Fusarium* species.

**iv. Development of molecular marker for identification of *Fusarium* species:**

The marker will be developed by comparing the conserved region of *Fusarium* species with other pathogens of infecting safflower. The amplification of ITS region by newly developed marker in *Fusarium* species and not in other pathogens of the safflower confirms the validation of marker.

18. **Work Plan:** should not exceed 3-4 pages (the section can be divided according to the specific aims and under each specific aim, the following should be stated clearly as sub headings)

18.1 Work plan (methodology/experimental design to accomplish the stated aim)

**i. Collection of diseased samples**

Wilt affected safflower root samples will be collected from different regions of Maharashtra, and preserved at 4°C in refrigerator for further studies.

**ii. Isolation and identification of the pathogen**

Isolations will be made from safflower plants, naturally infected and showing typical wilt symptoms collected from farmer’s field during survey. All the diseased roots and stem of infected plants wash in tap water then disinfected with 0.1% mercuric chloride for one minute, washed thoroughly with sterile distilled water for 2-3 times to remove the traces of mercuric chloride, blot dried and aseptically transferred on PDA medium containing streptomycin (0.12gm/lit), and
the plates will be incubated at 25±2°C for 5-6 days. Fungus growth in plate will examined and then sub-cultured on PDA slants.

iii. Identification and maintenance of *Fusarium* isolates
All the isolates of the pathogen will be purified by hyphal tip method (Dohroo and Sharma, 1992) and identified based on characters and spores morphology (Booth, 1971). All the isolates were maintained on PDA medium at 4°C. Pathogenicity of *Fusarium* isolates will tested by proving Koch’s postulates using safflower variety Nira and applying soil inoculation method (Nene *et al*., 1981).

iv. Genetic variability
Internal Transcribed Spacers (ITS) sequence analysis will used to detect the variations among the isolates of *Fusarium* species. Standardized protocols will be used for the isolation of DNA (Cenis *et al*., 1992) and ITS analysis (Prasad *et al*., 2007).

v. Cloning and sequencing of rDNA ITS region
The amplified rDNA fragment of *Fusarium* species will be cloned in vector and transformed into competent *E. coli* cells by heat shock treatment. Plasmid will isolated from transformed cells by alkaline lysis method (Sambrook and Rusell, 2001).

vi. Sequencing of clones
The plasmid containing insert will be sequenced. The sequenced data thus obtained will be used for phylogenetic analysis by MEGA4 software.

vii. Phylogenetic tree
The phylogenetic trees will be constructed in order to study evolutionary relationship between gene sequences using molecular evolutionary genomic analyzer software MEGA 4. Neighbor-joining algorithm will used for the phylogenetic analyses and Kimura 2-parameter distance model will employed for phylogeny of all nucleotide positions.

viii. Pairwise distance and homology calculation
Pairwise (P) distance between and among the sequences of the selected strains and those identified in the present study for phylogenetic analysis will be calculated by using P distance and pairwise deletion method in MEGA 4.0 software. The corresponding percent homologies will be calculated using P-distance converter.

**ix. Marker development**

The ITS sequence data of Fusarium species thus obtained will be compared with the ITS sequences of other safflower pathogens *viz.*, *Alternaria*, *Phytophthora*, *Pithium*, *Cercospora*, *Puccinia*, *Erysiphe*. The sequence which is present in *Fusarium* and not in other pathogens and which can exclusively amplify more than 150 bp sequence of *Fusarium* with standard primer parameters will be selected.

The developed marker will be validated by its ability to amplify ITS region of *Fusarium* and not other pathogens. The marker will also be tested to amplify ITS region of *Fusarium* infecting other crops.

**18.2 Connectivity of the participating institutions and investigators**

(in case of multi-institutional projects only)

Dr. M.K. Rai, Sant Gadge Baba Amravati University, Amravati: For identification and maintenance of fungal samples.

**18.3 Alternate strategies**: Nil

**19. Timelines**: (Please provide quantifiable outputs)

<table>
<thead>
<tr>
<th>Period of study</th>
<th>Achievable targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 Months</td>
<td>Collection of wilted safflower samples from various safflower growing regions of Maharashtra</td>
</tr>
<tr>
<td>12 Months</td>
<td>Isolation, identification and maintenance of <em>Fusarium</em> species</td>
</tr>
<tr>
<td>18 Months</td>
<td>DNA isolation and PCR amplification of ITS region of <em>Fusarium</em> species</td>
</tr>
<tr>
<td>24 Months</td>
<td>Cloning and transformation of amplified ITS region. Confirmation of clones</td>
</tr>
<tr>
<td>30 Months</td>
<td>Sequencing of cloned ITS region and sequence analysis for analysis of genetic diversity</td>
</tr>
<tr>
<td>36 Months</td>
<td>Development and validation of marker</td>
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### 20. Name and address of 5 experts in the field

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Name</th>
<th>Designation</th>
<th>Address</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dr. R.D. Prasad</td>
<td>Principal Scientist</td>
<td>Directorate of Oil Seed Research, Rajendra Nagar, Hyderabad</td>
</tr>
<tr>
<td>2</td>
<td>Dr. P.K. Chakraborty</td>
<td>Principal Scientist</td>
<td>Central Institute for Cotton Research, Shankar Nagar, Nagpur</td>
</tr>
<tr>
<td>3</td>
<td>Dr. S.P. Mehtre</td>
<td>Safflower Breeder</td>
<td>AICRP on Safflower, Marathwada Krishi Vidyapeeth, Parbhani</td>
</tr>
<tr>
<td>4</td>
<td>Dr. Kumaravadivel</td>
<td>Professor</td>
<td>Centre for Plant Molecular Biology, TNAU, (T.N)</td>
</tr>
<tr>
<td>5</td>
<td>Dr. Y. K. Bansal</td>
<td>Professor</td>
<td>Dept. of Biosciences, R.D. University, Jabalpur (M.P)</td>
</tr>
</tbody>
</table>

### PART IV: BUDGET PARTICULARS

**Budget (In Rupees)**

**A. Non-Recurring (e.g. equipments, accessories, etc.)**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Item</th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
<th>Total</th>
</tr>
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<tbody>
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<td>Microscope with photographic</td>
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<td>--</td>
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<td>1,50,000</td>
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<td></td>
<td>arrangement</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2.</td>
<td>BOD incubator</td>
<td>80,000</td>
<td>--</td>
<td>--</td>
<td>80,000</td>
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<tr>
<td>3.</td>
<td>Electrophoresis assembly</td>
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<td>4.</td>
<td>Laminar Air Flow</td>
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<td>70,000</td>
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<td>5.</td>
<td>Refrigerator</td>
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<td>6.</td>
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<td>7.</td>
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<td>8.</td>
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**Sub-Total (A)**

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<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
<th>Total</th>
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<tbody>
<tr>
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<td>8,50,000</td>
<td>1,50,000</td>
<td>12,30,000</td>
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</table>
**Justification:** Laminar air flow is required for aseptic culture of wilted specimen. Camera is required for photography of experimental results. Microscope with camera is required for morphological characterization and identification of fungal cultures. BOD incubator is needed for proper growth of fungal culture. Thermocycler is required for ITS amplification of *Fusarium* species. Gel documentation system is necessary to observe the result DNA isolation and ITS amplification. Cooling centrifuge is required for extraction of DNA.

B. Recurring (For College of Agril. Biotechnology, Latur)

### B.1 Manpower

<table>
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<tr>
<th>S. No.</th>
<th>Position No.</th>
<th>Consolidated Emolument</th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
<th>Total</th>
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</thead>
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<td>JRF-01</td>
<td>12,000/-</td>
<td>1,44,000/-</td>
<td>1,44,000/-</td>
<td>1,68,000/-</td>
<td>4,56,000/-</td>
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<tr>
<td>2.</td>
<td>Technical Assistant</td>
<td>5,000/-</td>
<td>60,000/-</td>
<td>60,000/-</td>
<td>60,000/-</td>
<td>1,80,000/-</td>
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<tr>
<td></td>
<td>Sub-Total (B.1)</td>
<td></td>
<td>17,000/-</td>
<td>2,04,000/-</td>
<td>2,04,000/-</td>
<td>6,36,000/-</td>
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### B.2 Consumables

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<th>S. No.</th>
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<th>Quantity</th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
<th>Total</th>
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</thead>
<tbody>
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<td>1.</td>
<td>Glasswares</td>
<td></td>
<td>50,000/-</td>
<td>30,000/-</td>
<td>20,000/-</td>
<td>1,00,000/-</td>
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<tr>
<td>2.</td>
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<td>50,000/-</td>
<td>1,00,000/-</td>
<td>1,00,000/-</td>
<td>2,50,000/-</td>
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<tr>
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<td><strong>Sub-Total (B.2)</strong> =3,50,000/-</td>
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### Other items

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<td>70,000/-</td>
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<td><strong>B.4 Contingency</strong></td>
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<tr>
<td><strong>B.5 Overhead</strong> (If applicable)</td>
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**Sub-total of B (B.1+B.2+B.3+B.4+B.5) Rs. 12,01,000/-**

**Grand Total (A + B) Rs. 24,31,000/-**

B. Recurring (For SGBAU, Amravati)
B.1 Manpower

<table>
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<td>4,56,000/-</td>
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**Sub-Total (B.1)**

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<td></td>
<td></td>
<td>12,000/-</td>
<td>1,44,000/-</td>
<td>1,44,000/-</td>
<td>1,68,000/-</td>
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B.2 Consumables

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<td>30,000/-</td>
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**Sub-Total (B.2) = 1,90,000/-**

<table>
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<th>Total</th>
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</thead>
<tbody>
<tr>
<td>B.3 Travel</td>
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<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>B.4 Contingency</td>
<td>--</td>
<td>25,000/-</td>
<td>20,000/-</td>
<td>20,000/-</td>
<td>65,000/-</td>
</tr>
<tr>
<td>B.5 Overhead (If applicable)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

**Sub-total of B (B.1+B.2+B.3+B.4+B.5) = Rs. 7,11,000/-**

**Grand Total (College of Agril. Biotech. Latur+ SGBAU, Amravati) = 24,31,000+7,11,000=31,42,000**

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**PART V : EXISTING FACILITIES**
Resources and additional information

1. Laboratory:
   a. Manpower-NIL
   b. Equipments
      1. Autoclave
      2. Centrifuge
      3. Shaker
      4. Mini electrophoresis assembly
      5. Refrigerator
      6. Laminar for plant tissue culture work
      7. Spectrophotometer
      8. Biosafety cabinet
      9. GC-MS
     10. HPLC
     11. Fermentor
     12. Weighing balance
     13. pH meter
     14. Gel documentation system

2. Other resources such as clinical material, animal house facility, glass house.
   Experimental garden, pilot plant facility etc.
      1. Green house
      2. Poly house
PART VI: DECLARATION/CERTIFICATION

It is certified that

a) the research work proposed in the scheme/project does not in any way duplicate the work already done or being carried out elsewhere on the subject.

b) the same project proposal has not been submitted to any other agency for financial support.

c) the emoluments for the manpower proposed are those admissible to persons of corresponding status employed in the institute/university or as per the Ministry of Science & Technology guidelines (Annexure-III)

d) necessary provision for the scheme/project will be made in the Institute/University/State budget in anticipation of the sanction of the scheme/project.

e) if the project involves the utilisation of genetically engineered organisms, we agree to submit an application through our Institutional Biosafety Committee. We also declare that while conducting experiments, the Biosafety Guidelines of the Department of Biotechnology would be followed in toto.

f) if the project involves field trials/experiments/exchange of specimens, etc. we will ensure that ethical clearances would be taken from concerned ethical Committees/Competent authorities and the same would be conveyed to the Department of Biotechnology before implementing the project.

g) it is agreed that any research outcome or intellectual property right(s) on the invention(s) arising out of the project shall be taken in accordance with the instructions issued with the approval of the Ministry of Finance, Department of Expenditure, as contained in Annexure-V.

h) we agree to accept the terms and conditions as enclosed in Annexure-IV. The same is signed and enclosed.

i) the institute/university agrees that the equipment, other basic facilities and such other administrative facilities as per terms and conditions of the grant will be extended to investigator(s) throughout the duration of the project.

j) the Institute assumes to undertake the financial and other management responsibilities of the project.

Signature of Project Coordinator:  
(applicable only for multi-institutional projects)  
Date: 11/11/2011

Signature of Principal Investigator:  
Date: 11/11/2011

Signature of Executive Authority of Institute/University with seal:  
Date: 23/11/2011

Signature of Co-Investigator:  
Date: 11/11/2011
PART VII: PROFORMA FOR BIOGRAPHICAL SKETCH OF INVESTIGATORS

Name: Dr. S. S. Shende  
Designation: Associate Professor  
Department/Institute/University: Department of Plant Biotechnology, College of Agricultural Biotechnology, Latur  
Date of Birth: 22/05/1977  
Sex (M/F): F  
SC/ST: SC  

Education (Post-Graduation onwards & Professional Career)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Institution Place</th>
<th>Degree Awarded</th>
<th>Year</th>
<th>Field of Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Amravati University, Amravati (M.S.)</td>
<td>M.Sc</td>
<td>2001</td>
<td>Biotechnology</td>
</tr>
<tr>
<td>2</td>
<td>Amravati University, Amravati (M.S.)</td>
<td>Ph.D</td>
<td>2005</td>
<td>Biotechnology</td>
</tr>
<tr>
<td>3</td>
<td>ICAR, Delhi</td>
<td>NET</td>
<td>2001</td>
<td>Plant Biotechnology</td>
</tr>
<tr>
<td>4</td>
<td>CSIR-UGC, Delhi</td>
<td>NET</td>
<td>2002</td>
<td>Life Science</td>
</tr>
<tr>
<td>5</td>
<td>VMOU, Kota (Raj.)</td>
<td>PGDESD</td>
<td>2006</td>
<td>Environmental Science</td>
</tr>
</tbody>
</table>

A. Position and Honors

Position and Employment (Starting with the most recent employment)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Institution Place</th>
<th>Position</th>
<th>From (Date)</th>
<th>To (date)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>College of Agricultural Biotechnology, Latur (M.S)</td>
<td>Associate Professor</td>
<td>28/01/2010</td>
<td>Till date</td>
</tr>
<tr>
<td>2</td>
<td>Department of Agril. Biotechnology, Marathwada Agricultural University,</td>
<td>Assistant Professor</td>
<td>31/01/2007</td>
<td>27/01/2010</td>
</tr>
</tbody>
</table>
Parbhani (M.S)

<table>
<thead>
<tr>
<th>3</th>
<th>College of Computer Science and Information Technology, Latur (M.S)</th>
<th>Head and Lecturer</th>
<th>06/11/2006</th>
<th>30/01/2007</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>International College for Girl’s Jaipur (Raj.)</td>
<td>Assistant Professor</td>
<td>02/01/2004</td>
<td>31/10/2006</td>
</tr>
</tbody>
</table>

**Honors/Awards:** Nil

**Professional Experience and Training relevant to the Project:**

Working in the field of molecular markers *viz.*, Random Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeat (ISSR), Simple Sequence Repeat (SSR) and Internal Transcribed Spacer (ITS) for molecular characterization of agricultural crops and microbial genotypes since last five years.

**B. Publications** (Numbers only)

- Books: Nil
- Research Papers, Reports: 31
- General articles: 03
- Patents: Nil
- Others (Please specify): Nil

**Selected peer-reviewed publications (Ten best publications in chronological order)**


List maximum of five recent publications relevant to the proposed area of work:


### C. Research Support

- **Ongoing Research Projects**: Nil
- **Completed Research Projects**: Nil

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**Place**: Latur

**Date**: 11/11/2011

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**PART VII: PROFORMA FOR BIOGRAPHICAL SKETCH OF INVESTIGATORS**

- **Name**: Dr. M.K. Rai
- **Designation**: Professor and Head
- **Department/Institute/University**: Department of Biotechnology,
Sant Gadge Baba Amravati University, Amravati
(M.S)

Date of Birth: **28/08/1955**  Sex (M/F) **M**  SC/ST: **NA**

**Education** (Post-Graduation onwards & Professional Career)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Institution Place</th>
<th>Degree Awarded</th>
<th>Year</th>
<th>Field of Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>R. D. University, Jabalpur (M.P.)</td>
<td>M.Sc</td>
<td>1978</td>
<td>Botany (Sp-Microbiology)</td>
</tr>
<tr>
<td>2</td>
<td>R. D. University, Jabalpur (M.P.)</td>
<td>Ph.D</td>
<td>1982</td>
<td>Mycology</td>
</tr>
</tbody>
</table>

**D. Position and Honors**

**Position and Employment** (Starting with the most recent employment)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Institution Place</th>
<th>Position</th>
<th>From (Date)</th>
<th>To (date)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Department of Biotechnology, SGB Amravati University, Amravati (M.S.)</td>
<td>Professor and Head</td>
<td>2001</td>
<td>Till date</td>
</tr>
<tr>
<td>2</td>
<td>Danielson College, Chindwara (M.P.)</td>
<td>Associate Professor</td>
<td>1996</td>
<td>2000</td>
</tr>
<tr>
<td>3</td>
<td>Danielson College, Chindwara (M.P.)</td>
<td>Assistant Professor</td>
<td>1982</td>
<td>1995</td>
</tr>
</tbody>
</table>

**Honors/Awards**

- Obtained merit scholarship upto HSSC
- National Scholar for 5-years
- Obtained Junior, Senior and PDF, CSIR, New-Delhi
- Father T.A. Mathias award, 1989 by All India Association for Christian Higher Education, New Delhi.
- Satpuda Award in the field of Environment conservation by Y.M.C.A., 1992.
- Fellow of Academy of Environmental Biology
- Awarded SERC visiting fellowship by DST, New Delhi, 1996
- Honorary research associateship for 1-year, Sydney, Australia, 1997
- INSA visiting fellowship, 1998
- Visiting scientist award (from April-June, 1999), Turin, Italy
- Medini Award for book on *Herbal Medicines*, by Govt of India, June 5, 2001
- Associateship for 3-years by TWAS, Trieste, Italy to work on AM fungi in Brazil, 2003
- Visiting Scientist, Department of Bioenergetics, University of Geneva, Switzerland, May-June, 2004
- Hungarian Scholarship by Hungarian Scholarship Board, to visit Department of Plant Protection, Debrecen University, Hungary (October 2005-January 2006).
- Awarded by IDVL for yeoman services in 2005
- Awarded Austrian Scholarship for 2006
- Hungarian Scholarship by Hungarian Scholarship Board, to visit Department of Plant Protection, Debrecen University, Hungary, 2008
- In advisory board of “International Medicinal Mushroom Conference” to be held in Croatia in 2011

**Professional Experience and Training relevant to the Project**

- Central Drug Research Institute, Lucknow  
  Oct. 1-30, 1985
- SERC visiting fellow, Jawaharlal Nehru University, New Delhi  
  Oct. -Dec., 1996
- Department of Botany, Jodhpur,  
  1991 (1-month)
- School of Life Sciences, JNU, New Delhi,  
  1994 (1-month)
- INSA visiting fellow, Jawaharlal Nehru University, New Delhi  
  Jan.-March, 1998
- Visiting scientist, Turin, Italy  
  April-June, 1999
- Associateship from Third World Academy of Sciences -  
  May-July, 2003
- UNESCO for 3- years (First visit)
B. Publications (Numbers only)

Books : 20  Research Papers, Reports : 200  General articles : 100
Patents : 03  Others (Please specify) : Nil

Selected peer-reviewed publications (Ten best publications in chronological order)


List maximum of five recent publications relevant to the proposed area of work


E. Research Support

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Title of Project</th>
<th>Funding Agency</th>
<th>Amount</th>
<th>Date of sanction and Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Screening of different</td>
<td>DST, New Delhi</td>
<td>20,00,000/-</td>
<td>01/04/2009-</td>
</tr>
<tr>
<td>No.</td>
<td>Project Title</td>
<td>Investigator</td>
<td>Budget</td>
<td>Duration</td>
</tr>
<tr>
<td>-----</td>
<td>---------------</td>
<td>--------------</td>
<td>--------</td>
<td>----------</td>
</tr>
<tr>
<td>1</td>
<td><em>Fusarium</em> species for synthesis of silver nanoparticles and their potential for development of novel antimicrobial agents (PI)</td>
<td></td>
<td></td>
<td>31/03/2012</td>
</tr>
<tr>
<td>2</td>
<td>Diversity of Endophytic Fungi in Selected Medicinal Plants of Melghat Forest (PI)</td>
<td>Department of Environment and Forest, New Delhi</td>
<td>17,00,000/-</td>
<td>03/08/2009-02/08/2012</td>
</tr>
<tr>
<td>3</td>
<td>Exploitation of plant pathogens for the mycosynthesis of silver nanoparticles for the development of novel antimicrobial (PI)</td>
<td>Rajiv Gandhi S &amp; T Commission, Govt. of Maharashtra, Mumbai</td>
<td>32,00,000/-</td>
<td>15/06/2009-14/06/2012</td>
</tr>
<tr>
<td>4</td>
<td>Rapid detection of toxigenic species of <em>Fusarium</em> secreting T-2 toxin (PI)</td>
<td>DRDO, New Delhi</td>
<td>15,00,000/-</td>
<td>27/10/2009-26/10/2012</td>
</tr>
</tbody>
</table>
## Completed Research Projects (State only major projects of last 3 years)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Title of Project</th>
<th>Funding Agency</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Search for antimycotic activity in medicinal plants of Chhindwara and suburbs against local strains of dermatophytes, sponsored by DST, New Delhi, 1987 (PI) under young scientist programme</td>
<td>DST, New Delhi</td>
<td>56,000/-</td>
</tr>
<tr>
<td>2</td>
<td>Environmental Conservation Programme in Chhindwara district with special reference to tribal areas, sponsored by Department of Environment, New Delhi, 1991 (PI)</td>
<td>Ministry of Environment and Forest, New Delhi</td>
<td>1,35,000/-</td>
</tr>
<tr>
<td>3</td>
<td>Screening of medicinal plants of family Asteraceae for their antimycotic activity against human pathogenic fungi, UGC, New Delhi, 1996 (PI)</td>
<td>UGC, New Delhi</td>
<td>2,65,800/-</td>
</tr>
<tr>
<td>4</td>
<td>Role of mycorrhizae in tissue-culture raised medicinal plants, DBT, New Delhi, 1998 (CO-PI)</td>
<td>DBT, New Delhi</td>
<td>24,00,000/-</td>
</tr>
<tr>
<td>5</td>
<td>Overproduction of active principles of <em>Withania somnifera</em> (Aswagandha) in root organ culture., 2006 (CO-I)</td>
<td>UGC, New Delhi</td>
<td>11,19,100/-</td>
</tr>
<tr>
<td>6</td>
<td>Process development for production of <em>Artemisinin</em> through root organ culture of <em>Artemisia annua</em> DRDO, LSRB- 103 / FS/2006 (CO-I)</td>
<td>DRDO, New Delhi</td>
<td>9,96,000/-</td>
</tr>
</tbody>
</table>
Project Summary

Safflower (Carthamus tinctorius L.), an oilseed crop is a member of the family Asteraceae. The crop has tremendous potential to be grown under varied conditions and to be exploited for various purposes. Safflower, a multipurpose crop has been grown for centuries in India for the orange red dye, carthamin extracted from its brilliantly colored flowers and for its quality oil.

Many bacterial, fungal and viral pathogens infect the safflower crop causing diseases. Among all these Fusarium wilt is observed as one of the most common and destructive disease. The genus Fusarium poses a multifaceted threat to global crop production and animal/human health. Being soil borne in nature, the fungus survives in the soil as chlamydospires in diseased plant debris without losing viability (Chakraborty, 1980) and invades the tap root of safflower directly or through its root hairs by mechanical means.

Since the beginning of the 1990s, there has been a marked increase in the incidence of diseases caused by Fusarium. The risk of infection has increased through changes in crop production, because these pathogens produce mycotoxins, which may directly limit the use of infected grain in food and feed production. The most important fusaritoxins produced are nivalenol (NIV), deoxynivalenol (DON), triticale and zearalenon (ZEA).

Fusarium is a diverse genus consisting of an array of species responsible for damping-off, root rots and vascular wilts on a multitude of economically important plant species. Fusarium oxysporum f.sp. carthami is known to cause wilt in safflower, but some other species of Fusarium viz., F. equiseti and F. moniliforme which are reported for the first
time from Marathwada region, proving its pathogenicity on safflower are also responsible for safflower wilt and making difficulty in management of disease. Therefore, the present work is proposed to isolate, identify and evaluate the genetic diversity of *Fusarium* species causing wilt in safflower and also to develop a marker for rapid identification of *Fusarium* from soil itself, helping in better management of disease before sowing. The wilted samples will be collected from safflower growing regions of Maharashtra. The samples will be sterilized and cultured on PDA medium for fungal growth. The isolates will be identified by morphological and microscopic characterization. The DNA will be isolated from all *Fusarium* species and subjected to PCR amplification using universal ITS-1 and ITS-4 primer pairs. The amplified product will be cloned in vector and transformed in host for multiplication. The positive clones will be selected and isolate the recombinant plasmid. The cloning conformation will be done of the isolated plasmid and sequenced. The sequenced data thus generated will be analysed for genetic diversity using bioinformatics software like MEGA-4 or PHYLIP. The marker will be developed from conserved region of *Fusarium* species by comparing with the ITS region of other pathogens of safflower. The developed marker will be validate for its ability to amplify ITS region of *Fusarium* species. The developed marker will be used as diagnostic tool for rapid identification of *Fusarium* wilt of safflower as well as other crops.
Literature Cited


deleniation and dysploidy in the genera, Cardencellus, Carthamus and Phomus

Identification of a Randomly Amplified Polymorphic DNA Marker Linked to the
Fom-2 Fusarium wilt resistance gene in Muskmelon MR-1. Molecular Plant
Pathology. 85(10): 1245-1249.

and fungal ribosomal RNA genes for phylogenetics. In: Innis, M. A., Gelfand, D.,
315-324.

Zang, Y. and J. R. Stommel (2001). Development of SCAR and CAPS markers linked to
the Beta gene in tomato. Crop Sci. 41: 1602-1608.