



**IFAR 2008 Professional Development Program Completion Report (800 words)**

**Instructions:**

Please submit the completion report by email, using this form, through the sponsoring CGIAR Center to [ifar@ifar4dev.org](mailto:ifar@ifar4dev.org) within two months after the completion of the fellowship.

*Please check if Thalwitz Scholarship*

**No**

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**Name of Applicant**

**G. Kalyani**

**Sponsoring CGIAR Center**

**ICRISAT**

**Work program goals achieved (maximum length: 200 words)**

**Project Title:** Development and evaluation of transgenic groundnut plants for resistance against foliar fungal diseases (Late leaf spot and rust)

Late leaf spot (LLS) disease caused by *Phaeoisariopsis personata* (Berk. & Curt.) Van Arx, and rust caused by *Puccinia arachidis* Speg. are economically important fungal diseases of groundnut (*Arachis hypogaea* L.) causing 50-80% yield losses in the semi-arid tropic regions (Waliyar, 1991). The annual economic losses caused by LLS and rust in groundnut account for losses of over US\$599 m and US\$467 m, respectively (FAO, 2004). The primary focus of this project was to develop transgenic groundnut for resistance to foliar fungal diseases (LLS and rust) through constitutive expression of the rice chitinase gene (*RChit*). Genetic transformation of the *RChit* gene in groundnut varieties was done using *Agrobacterium tumefaciens*-mediated gene transfer. Overall, 75 transgenic events, including 16 events of var. JL 24, 10 events of ICGV 89104 and 4 events of ICGV 86031 were produced using pCAMBIA 1302:RChit construct having *hpt* as the selectable marker and 45 events of var. JL 24 were produced using pCAMBIA 2300:RChit construct without selectable marker. All the transgenic plants produced were morphologically normal. PCR (Polymerase chain reaction) analysis proved the presence of *RChit* and *hpt* genes in the putative transformants. The transcript and accumulation of chitinase in transgenic groundnut was confirmed by RT-PCR. Copy number and integration patterns of the transgene were investigated using Southern blot analysis, proving the presence of single copies in most of the transformants. Mendelian (3:1) inheritance pattern was observed for both *hpt* and *RChit* genes in T<sub>1</sub> and T<sub>2</sub> generations indicating stable and successful inheritance of the transgenes. Preliminary assessment for in vitro seed colonization with *A. flavus* showed that all the transgenic events had 0-40% infection as compared to 70% infection in the untransformed controls. In fungal bioassay using detached leaf technique, 15 transgenic events showed 50-90% disease reduction in terms of infection frequency for both LLS and rust as compare to non-transgenic control plants in both T<sub>1</sub> & T<sub>2</sub> generations. Transgenic events showed higher chitinase activity ranging from 0.15 to 2.01  $\mu\text{mol GlcNac min}^{-1} \text{g}^{-1}$  of protein as compare to non-transgenic control plants (0.1  $\mu\text{mol GlcNac min}^{-1} \text{g}^{-1}$  of protein).

**Plans for follow-up (maximum length: 200 words)**

The promising transgenic events identified in this study will undergo further testing under contained greenhouse and field conditions to further confirm the resistance to LLS and rust. As fungal cell wall is composed of chitin and  $\beta$ -1,3-glucan, disease resistance would be improved further by pyramiding *RChit* gene along with glucanase gene. Submission for publication research relating to production of marker-free transgenic plants and development and evaluation of transgenic groundnut for resistance to foliar fungal diseases (LLS and rust) are underway. The transgenic events with stable resistance to LLS and rust would be made available to breeders for use in breeding programs.

**Report budget utilization including whether budget has spent as planned (maximum length: 100 words)**

The fund was spent according to the approved budget.

The fund (US\$ 11,000 total) was spent as follows:

1. Applicant = 1,800
2. Research Consumables = 6,675
3. Travel = 2,000
5. ICRISAT Overhead = 525

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Total Expenditure = US\$ 11,000  
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Research consumables cover part of tissue culture, molecular characterization, containment greenhouse for growing transgenic plants, and carry out fungal bioassays.

**Assessment of fellowship experience and general comments (maximum length: 300 words)**

I am very much delighted with the acceptance and funding of my proposal. This grant significantly helped me to continue my ongoing studies on “Development of transgenic groundnut for resistance to foliar fungal diseases”. This was very important contribution to my both professional and personal lives. It improved my knowledge and skill. I would like to express my gratitude to Dr. S.N Nigam (Principal scientist, Groundnut Breeding, ICRISAT, Patancheru), Dr. K.K Sharma (Principal scientist, Genetic Transformation Laboratory, ICRISAT, Patancheru) and Dr. Farid Waliyar (Director, West & Central Africa, ICRISAT, Niamey) for their valuable comments, co-direction and for great support in completing this research. I would like to thank IFAR organization for this generous support and providing opportunity to conduct this research.