Molecular and Cellular Biology Research Interests

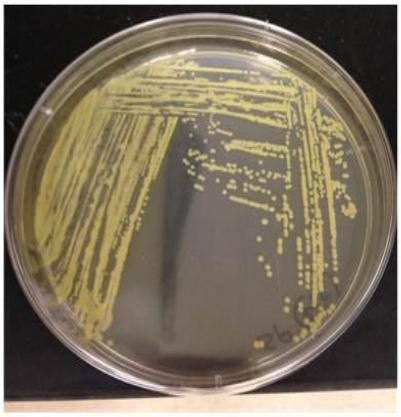
Research Area 1

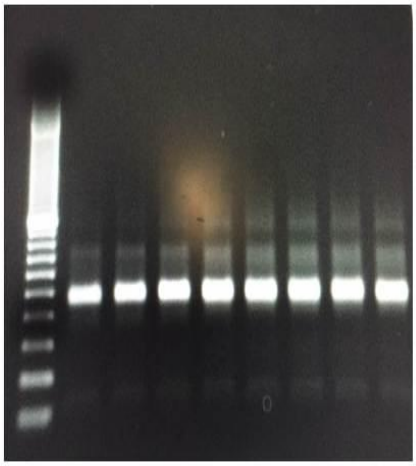
Gene manipulation of peanuts with resistant genes against Aspergillus sps using *Agrobacterium tumefaciens.*

Georgia, Florida and Alabama are known for their peanut cultivation and Georgia alone produces approximately 50% of peanuts in the United States with several hundred million dollars in revenue. In spite of strict control measures, the peanut industry continues to suffer economic loss due to aflatoxin contamination from *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxins are carcinogenic to humans and other animals, and accumulation of aflatoxins in peanut kernels can influence marketing of peanut and peanut products. Breeding for resistant varieties against this mold, has not been very promising, as evidenced by serious economic losses in the peanut market due to aflatoxin outbreaks year after year. Traditional breeding is a costly, laborious and a time consuming process as opposed to developing resistant peanut cultivars which maintain a low level of aflatoxin contamination. The goal of my research is to transform peanuts with resistant genes such as chitinase and β-1, 3-glucanase against *A. flavus*, the most common aflatoxin producing Aspergillus sps. in peanuts and other food and agricultural products.

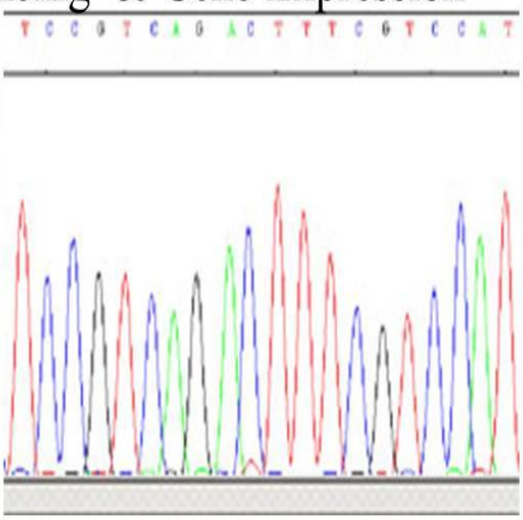
Research also includes expression of these pathogenesis-related (PR) proteins such as chitinase and β-1, 3-glucanase and aflatoxin at cellular and subcellular levels in susceptible, tolerant, and transgenic peanut lines by immunocytochemical approaches. The intellectual merit of this research is to produce basic information about a little studied area, the characterization of PR proteins and disease resistance in transgenic peanuts. Basic molecular and biochemical studies in tolerant/transgenic peanut varieties will provide information on tolerance/resistance and on prevention of aflatoxin accumulation

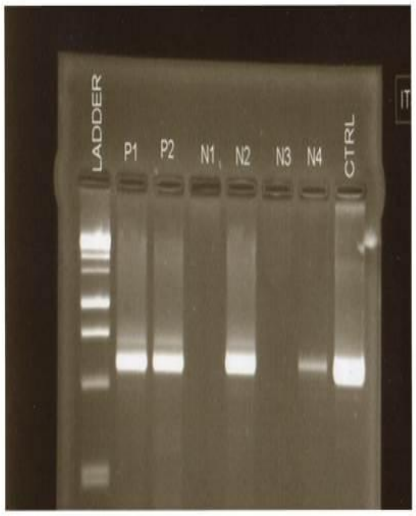
in peanuts. The in Situ localization of induced molecules in the presence of foreign genes from the cyto and immunocytochemical approaches will form the basis for future research in the development of biological control alternatives for *A. flavus* in peanuts. Broader impacts include the research's potential to make progress in developing an effective approach to eliminating aflatoxin accumulation in peanuts. Moreover, developing new peanut transgenic cultivars with resistance using genetic engineering will have great potential in breeding resistance to *A. flavus* infection, and the problem of aflatoxin management could be solved if peanut cultivars are resistant against *A. flavus* colonization. Reducing a lower level of aflatoxin or preventing the production of carcinogenic toxin is certainly beneficial to peanut consumers.





Sequencing & Gene Expression





Agrobacterium Transformation to develop Transgenic Peanuts using Chitinase and Glucanase genes in Georgia peanut

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Abstract

Infection of peanuts by *Aspergillus* significantly threatens the economic viability of not only the US peanut industry but also that of third world countries. During the harvest, storage and transportation *A. flavus* and *A. parasiticus* produce aflatoxins (B1 & B2, B1) which are among the most carcinogenic naturally occurring compounds known to pose significant health risks to human and animals .The goal of this project was to successfully transform peanuts with Glucanase gene against *A. flavus*, the most common aflatoxin producing the *Aspergillus* species found in peanuts. The peanuts from Tifguard and GAO6G ware soaked in distilled water overnight. After 24 hrs the outer seed was removed and the cotyledon and embryo were separated. The embryo were then treated with 1% Bavistin and HgCl . Using the aseptic technique the embryos were incubated in MS media containing 2,4-D and exposed for 20 days in dark treatment to get the callus formation. After 20 days the callus was transferred to modified MS basal media with IAA and BA for shoot and root formation. The embryo and the explants were transformed with *Agrobacterium* culture, EH105 pCAM 638, containing the transgenic glucanase gene. Our study resulted in good callus formation from the embryo after 20 days of incubation. Following callus formation, the explants showed extensive growth of root and shoot after 15 days. Performing a GUS assay confirmed successful gene insert into the transgenic peanut plant.

Introduction

Georgia produces more than 50% of the nation's peanut supply, around 1.7 tons. Benefiting 50,000 Georgian workers, the peanut industry supplies more than 20% of Georgia's agricultural income, making peanuts the state's official state crop . Infection of peanuts seed by *A. flavus* and *A . Parasiticus* is a serious problem that can result in the outbreak of Aflatoxin B1, the most toxic and dangerous carcinogen known to humans and animals. This toxin contains harmful properties that can hasten the spread of diseases by suppressing the immune system's in humans. Having the ability to spread before the harvest, storage or even exportation, contamination can result in $25 million dollars of financial loss. Traditional breeding of peanuts has not resulted in a resistant cultivar again *Aspergillus* species, as evidenced by serious economic losses in the peanut market due to aflatoxin outbreak every year. Genetic engineering has now become a new approach to developing transgenic peanuts over conventional methods (Tyagi and Mohanty 2000). The pathogenesis- related (PR) protein gene induced by the plant's defense response in one of the important and widely used genes to generate fungal resistant transgenic plant. Having the capability to directly attack the cell wall of bacteria and fungus, PR proteins can destroy bacterial and fungal infections to keep plants healthy.

Materials and Methods

Materials: MS Basal Media, Bavistin, Kanamycin,2,4-D, Benzyl Adenine(BA for root formation) , Indole Acetic Acid (IAA for shoot formation), Mercuric chloride.

Development of Callus, Shoot and Root Initiation: Peanut seeds, variety, Tifguard and GA06G, were soaked overnight in distilled water. The outer coat was removed to separate cotyledons and the embryos. Embryos were treated with 1% Bavistin for 30 mins and washed with distilled water. Further, the embryos were treated with 0.1% HgCl, for 20-30 sec, rinsed with distilled water 3-4 times and blotted dried. They were then placed in MS basal media with 15mg/L 2,4-D and incubated in dark at 25 °C . After 20 days of incubation, calluses were transferred to modified MS medium which contain IAA, shoot and BA, root initiation media. The calluses were exposed to 16 hrs photoperiod at 25 °C in growth chamber until root and shoot were formed.

Preparation of culture for Co-cultivation: A single *Agrobacterium* (Chitinase gene) colony containing (EH 105pCAM 638) from Nutrient Agar plate, was inoculated onto 20mL YEP broth containing 50mg/L Kanamycin in sterile flask. The flask was kept in orbital shaker, overnight at 28 °C. The culture, with an OD of 0.6-0.8, at 600 nm was centrifuged at 6,000 rpm for 10 min at 4 °C . The supernatant was discarded and the bacterial pellet was resuspended with half MS media. The explants with shoot and root formation was then immersed in *Agrobacterium* suspension for 10, 20, 30, 40, 60 sec, respectively and dried with sterile blotting paper. After two days on hormone free media, the explants were placed on modified MS media, incubated as described above, for the development of transgenic peanuts. A GUS Histochemical assay was performed to confirm the successful insertion of chitinase gene in the transgenic plant.

Fig. 3 a, b & d: Potted Peanut Plants

Fig. 3 c: Formation of Peanut embryos

Results

Fig. 1 a, b & c: Formation of Callus from Peanut Embryos on Murshige and Skoog (MS) Medium.

Fig. 2:
a. Callus formation
b & c: Shoot and Root formation

Conclusion

Fig. 4 a & b: Fully Developed Transgenic Peanut Plants

References

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Acknowledgments

The authors would like to thank the Department of Biology and Physics & Mentor Protégé, KSU for financial support and Dr. R Gowda, UAS, Bangalore University, India for his expertise.