Biosafety Data Generation from the Putatively Transformed Peanut (Arachis hypogaea L.) Using afp Gene

Tanjina Akhter Banu, Iffat Ara Rahman, Tahmina Islam, R.H. Sarker, M. Imdadul Hoque

Plant Breeding and Biotechnology Lab
Department of Botany
University of Dhaka
Peanut (*Arachis hypogaea* L.) is one of the most important species among legumes. It is an annual, self pollinated, economically important oil and protein rich crop.

It is one of the major legumes securing third largest source of edible oil. Seeds contain 40-60% oil, 20-40% protein and 10-20% carbohydrate.

It has the ability to fix nitrogen from atmosphere and thus improve soil fertility.

Peanut oil is used for cooking and also in pharmaceutical industries, cattle feed etc.

It is grown in marginal lands mainly in “Char” areas of Noakhali, Faridpur, Kishoreganj, Dinajpur, Patuakhali, Barisal, Dhaka and Pabna.

Figure 1: A typical groundnut plant
Yield Constraints

**Biotic stresses**
- Major constraints of peanut production in our country as well as in many countries of the world are the fungal foliar diseases.
- Various bacterial and viral diseases, insect, pests also causes damage to this crop at varying degrees
- Aflatoxin contamination are also an important biotic factors.

**Abiotic stresses**
- The abiotic stress includes multiple stresses such as drought, salinity, water logging, high temperature, low pH, and low temperatures and so on.

### The common fungal diseases of peanut

<table>
<thead>
<tr>
<th>Disease</th>
<th>Causal agent</th>
<th>Yield loss/damage*</th>
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</thead>
<tbody>
<tr>
<td>Early &amp; late leaf spot</td>
<td>Cercospora arachidicola &amp; Phaeoisariopsis personata Berk &amp; Curt</td>
<td>30-40%</td>
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<tr>
<td>Leaf rust</td>
<td>Puccinia arachidis</td>
<td>~50%</td>
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<tr>
<td>Stem rot</td>
<td>Sclerotium rolfsi</td>
<td>&gt;27%</td>
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*Source: Production technology of oilcrops, Oil seed research centre, BARI*
Objectives

➢ Development of genotype independent *Agrobacterium*-mediated genetic transformation system for local peanut varieties using selectable and screenable marker genes.

➢ Introduction of desired fungal disease resistance gene construct into a high yielding peanut variety.

➢ Molecular analysis of putative transgenic plants using appropriate molecular techniques.

➢ Generation of Biosafety data from the putatively transformed peanut plants.
Transformation and Regeneration of Putative Transgenic Plants

Regeneration ——— Transformation ——— Generation of Biosafety data

Explants were transferred to different regeneration medium

Initiation of regeneration (22-28 days)

Shoot bud elongation

Root induction

Transplanted to pot containing soil

Incubation (explants were incubated in the bacterial suspension for 15 mins)

Co-cultivation (3 days)

Transferred to regeneration medium without selection pressure

Regeneration under selection pressure

Maintenance of Biosafety guidelines during lab condition

Transplantation of putative transgenic by following biosafety guidelines

Generation of putative transgenic plants under double layered insect proof net house condition

Destruction of died leaves and other parts

Generation of molecular data
**In vitro regeneration**

Fig. 2. Different stages of shoot regeneration from decapitated embryo attached cotyledon (DEAC) explants of Dhaka-1 variety (a). DEAC explants of Dhaka-1 (b). Arrow indicates greening of decapitated embryo attached cotyledon explants on SIM1 (c). Induction of adventitious shoot buds from explants after 15 days of culture on SIM2 (d). Multiple shoot regeneration on SEM (e). Shoot elongation after 15 days on SEM. (f). Developed shoots on rooting medium.

Fig. 3. Different stages of shoot regeneration from de-embryonated cotyledon (DEC) explants of Dhaka-1 variety (a). DEC explants of Dhaka-1 (b). Greening of DEC explants on SIM1 (c). Induction of adventitious shoot buds from explants after 15 days of culture on SIM2 (d). Multiple shoot regeneration on SEM (e). Shoot elongation after 15 days on SEM (f). Developed shoot on root induction medium.
Agrobacterium mediated genetic transformation

Transformation with pBI121-GUS

Transformation with pCAMBIA2300 polyA AFP
Molecular analysis of $T_1$ transgenic lines
Development and flowering of the T₁ transformants of Dhaka-1 variety. (a) T₁ seeds of Dhaka-1 variety. (b) Germination of T₁ seeds on cotton bed. (c) Positive healthy plantlet of Dhaka-1 variety, (d) Putative transgenic of plantlets were maintained under contained condition. (e) Plantlet with flower and (f) Peg formation from T₁ plants.
Maintenance of the $T_1$ transgenic plants of Dhaka-1 variety under net house condition. (a) $T_1$ plants of Dhaka-1 variety. (b) Peg formation and rescue of upright pegs of transgenic of plantlets (c) Double net house for maintaining transgenic plants.
Maintenance of $T_1$ plants following Biosafety Guidelines

- Proper labeling of Transgenic Plant
- Collection of seeds with adequate safety measures
- Preservation of Transgenic seeds
- Destruction of waste materials by autoclaving
Acknowledgments