Genetic Engineering of chickpea (*Cicer arietinum* L.) for insect resistance (IR)

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Chickpea (*Cicer arietinum* L.)

- Earliest cultivated legume (~ 7,500 yrs old)
- Protein rich (20-25 %) grain legumes (fam: *Fabaceae*)
- Symbiotic Nitrogen Fixation (SNF)
- Climate Smart Crop
- Important commodity: Producer, consumer and importer
Breeding pursuits to enhance production led to development of improved cultivars

*Genetic bottlenecks* and subsequent *founder effects* during domestication resulted in narrow genetic base

Average nucleotide diversity: Low

Cultivated (desi vs kabuli): 0.00125-0.000915 bp⁻¹
(Cultivated vs Wild): 0.0031 bp⁻¹

Highly self pollinated (Cleistogamy)

Insect resistant (IR) trait *not identified* in gene pool

Incompatible issues with wild relatives

Genome-wide variations in breeding signatures (SNPs, CNVs, InDel & PAVs) between desi and kabuli chickpea

Gram pod borer (*Helicoverpa armigera* Hubner): Devastating insect pest of pulses

- Fortnight after germination
- Voracious during reproductive stage
- Skeletonise leaves leaving only veins
- Circular holes and feed on the developing grains rendering empty pods

**Yield loss: 10-40% annually**

Bt technology provide protection to this predator
Genetic Engineering of chickpea

*In vitro* regeneration system coupled with system of gene transfer

Chickpeas are recalcitrant to genetic transformation and *in vitro* regeneration of plantlets

Extremely low frequency of transformation and recovery of transgenic plants

Responding explant: Cotyledonary node and parts thereof

Exudates from chickpea explants:
**Phenolics** (Gallic acid, chlorogenic acid, caffeic acid, sinapinic acid, coumeric acid, ferulic acid)
**Flavonoids** (Daidzein, genistein, kaempferol, biochanin A, quercetin, rutin),
**Salicylic acid**
Exhibit inhibitory activity against *Agrobacterium tumefaciens* cells

Polyphenol oxidase (PPO): Hypersensitive response: tannins
Tannins lead to intense browning and necrosis and formation of dead cell

**PPO Inhibitors**: L-cysteine, sodium thiosulphate, DTT

Yadav et al 2016
Binary Vector harboring cry1Ac

Agrobacterium strain: LBA 4404; Binary Vector: pBinAR

Eco RI, Bam HI, Eco RI, Bam HI

LB 35S P cry1Ac nos T nosT nptII nos P RB

M1 - Lamda DNA EcoR I, Hind III
M2 - 1 Kb plus ruler
1 - EcoR I HindIII digested
2 - EcoR I digested
3 - HindIII digested
4 - Bgl II digested
5 - uncut
6 - SalI, BamHI digested
7 - BamHI digested
8 - SalI digested
9 - uncut
In vitro regeneration of chickpea

Srivastava et al 2012, Das et al 2017
Generation of large no of putative transformants
Molecular Analysis of transgenic lines

- Leaf tissues are homogenized in Extraction Buffer
  - Strip was placed in homogenate
  - Homogenate ascent and band development
Molecular Characterization of transgenic lines

Transcript based assays

RT-PCR screening in T₃ chickpea progenies

M1   PC   T₃ chickpea lines   NC   R   NTC  M2

RT PCR in transgenic chickpea

M1: 100 bp ladder, PC: Positive control, NC: Negative control (DCP 92-3)
R: RNA as template, NTC: No template Control, M2: 1 Kb ladder
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PCR analysis with gene specific primers

M- 1Kb plus DNA ladder; Lanes 1 to 15: chickpea lines
Molecular Characterization of transgenic lines

Southern Hybridization in T₁ Chickpea

Copy No: Hind III
Cassette Release: Eco RI
Molecular Characterization of transgenic lines

Protein based assays

A. Qualitative ELISA, B. Quantitative ELISA, C. Western blot.
Molecular Characterization of transgenic lines

Protein expression ranging from 3.08-42.72 ng/mg TSP

- Pre-flowering > Post flowering
- Leaf > Pod wall > Immature seed
Insect Bioassay of transgenic lines

A. Detached leaf and pod assay  B. Insect Percent Mortality

A. Detached leaf and pod assay  B. Insect Percent Mortality

Das et al 2017
Insect bioassay of transgenic lines

A. Leaf consumed by larvae  
B. Larval Weight Gain

Das et al 2017
Whole Plant Bioassay of transgenic chickpea

Das et al 2017
Way Forward

- Confined Field Trials
- Select the best event
- Detailed characterization (regulatory requirements)
- Agronomic performances
- Food/feed and environmental safety tests
Biology Document of Chickpea

- Origin of crop: Chickpea (SE Turkey and Syria)
- Crucial Growth Stages: Duration of crops
- Wild species and their distribution in India
- List of Reported sexually compatible species
- Methods of Reproductive Isolation: Flower and pollination
- Chickpea: Highly self-pollinated, low out crossing (upto 1%)
- Isolation distance: 10 m (Chickpea)

Customized Standard Operating Procedures (SOPs)

ICAR Guidelines for working with transgenic crops

Awareness Program on GE and Biosafety
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