

Genetic Engineering for development of salt tolerant pigeon pea plants

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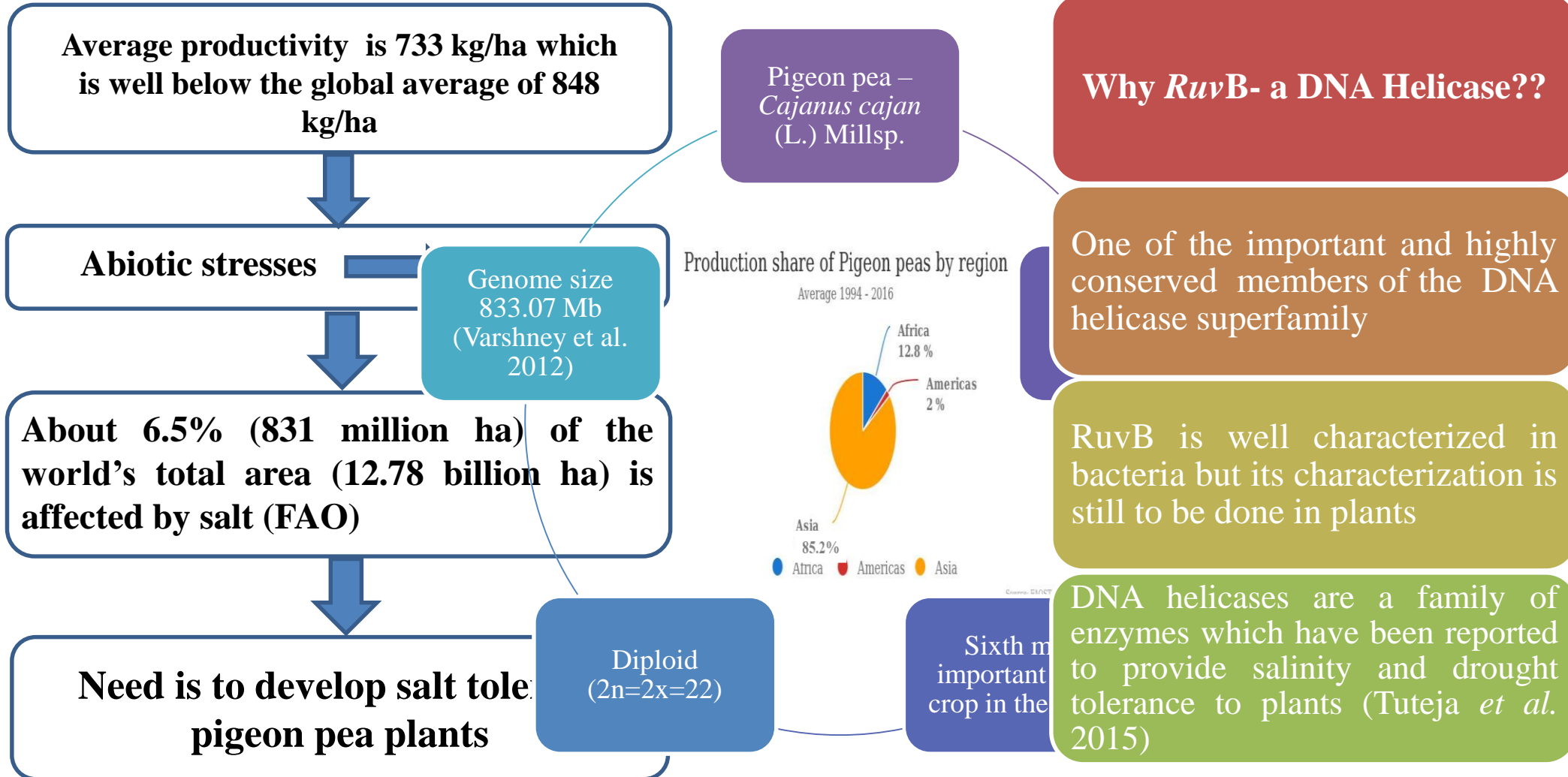
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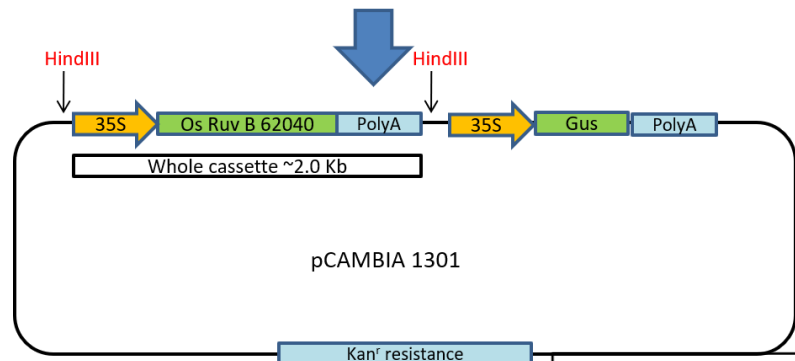


Introduction



Materials and Methods

Agrobacterium tumefaciens LBA 4404



Pushpa Kharb, **Rakshita Singh** and Parveen Batra. 2018. An efficient method for transformation in pigeon pea. (Patent Application No. 201811012099)

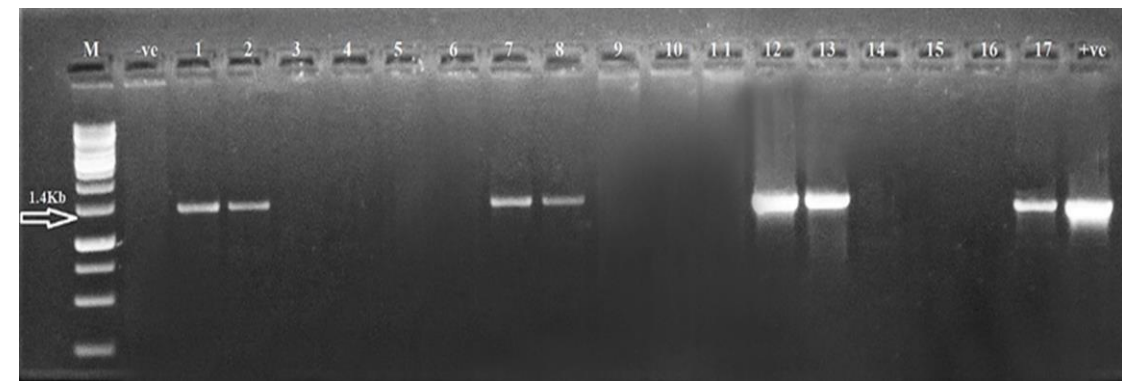
Molecular characterization of T₀ transgenic plants using PCR, Southern Hybridisation and Real-time PCR



Putative T₀ transgenic pigeon pea (cv. Manak) plant

Physio-biochemical analysis of 6 selected transgenic lines in T₁ generation

Molecular characterization of transgenic pigeon pea plants



Agarose gel showing PCR amplification of *OsRuvB* gene using gene specific primers (Lanes: M: 1 kb DNA ladder (Fermentas); -ve: negative control; 1-17: T₀ plant samples; +ve: positive control)

| No. of plants screened | No. of PCR positive plants | Transformation efficiency |
|------------------------|----------------------------|---------------------------|
| 70 | 25 | 35.7% |

Real-time PCR for detection of copy number in pigeon pea lines

| Line no. | L-10 | L-17 | L-32 | L-37 | L-66 | L-107 |
|----------|------|------|------|------|------|-------|
| Copy no. | 1 | 1 | 1 | 1 | 1 | 2 |

Segregation analyses of the transgene in T₁ generation of transgenic pigeon pea based on PCR analysis

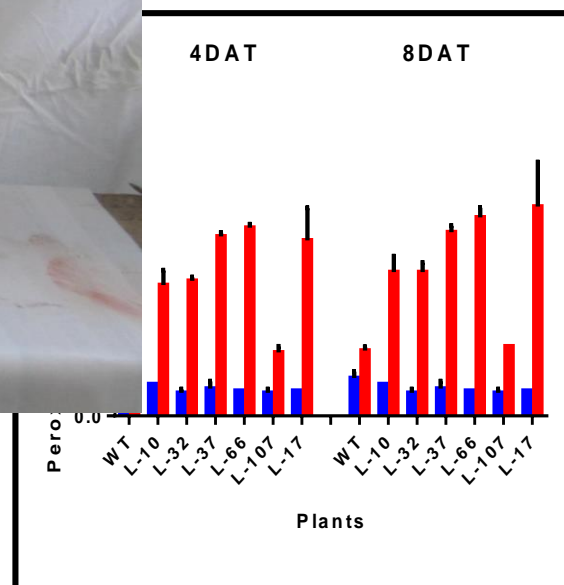
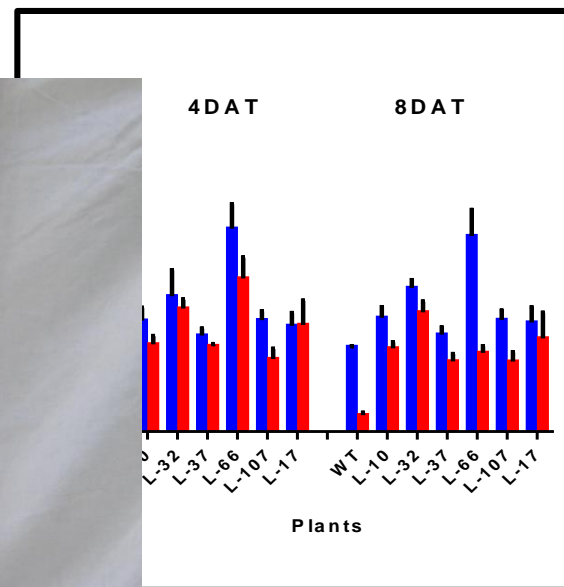
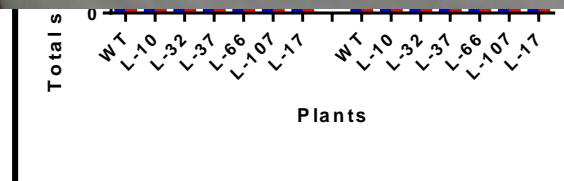
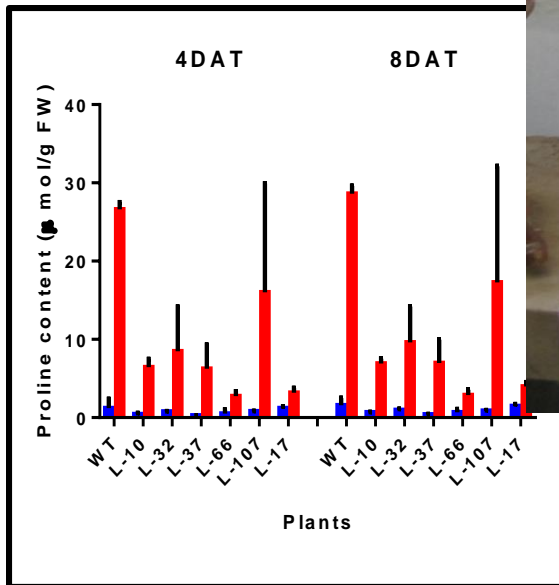
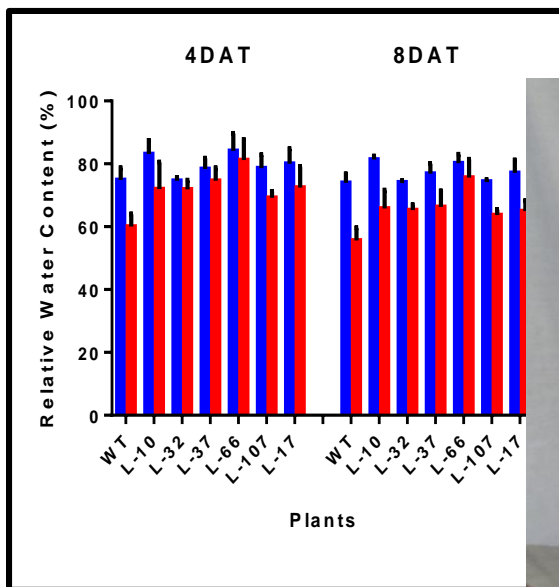
| S. No. | Line | No. of T ₁ plants screened | OsRuv B ⁺ | OsRuv B ⁻ | Observed Ratio | c ² -value | p-value |
|--------|-------|---------------------------------------|----------------------|----------------------|----------------|-----------------------|---------|
| 1 | L-10 | 43 | 31 | 12 | 2.6:1 | 0.21 | 0.64 |
| 2 | L-17 | 42 | 32 | 10 | 3.2:1 | 0.12 | 0.72 |
| 3 | L-32 | 47 | 34 | 13 | 2.6:1 | 0.10 | 0.75 |
| 4 | L-37 | 50 | 36 | 14 | 2.6:1 | 0.09 | 0.76 |
| 5 | L-66 | 45 | 33 | 12 | 2.8:1 | 0.11 | 0.74 |
| 6 | L-107 | 36 | 33 | 3 | 11:1 | 0.52 | 0.47 |



Southern blot analysis of transgenic plants

(Lanes: -ve: DNA from Wild type plant; +ve: DNA from pCAMBIA1301-*OsRuvB*; 1-6: DNA from transgenic lines where 1: L-66, 2: L-37, 3: L-32, 4: L-17, 5: L-107)

Physio-biochemical analysis of wild type and T₁ generation transgenic pigeon pea plants



Control Salt stress



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