Introgression of cassava mosaic disease resistance in to elite clones of Cassava (Manihot esculenta Crantz) in India

Sheela, M.N., Abhilash, P.V.- and Makesh Kumar
ICAR-Central Tuber Crops Research Institute
Thiruvananthapuram, India
CASSAVA BREEDING IN INDIA

EXPERIMENTAL BREEDING
- METHODOLOGY STANDARDISATION
- GERPLASM EVALUATION
- CLONAL SELECTION
- HYBRID PRODUCTION

1963-1990

6 Varieties

DIRECTED BREEDING
- HYBRID PRODUCTION BASED ON GCA/SCA
- TRIPLOID PRODUCTION
- INTERSPECIFIC HYBRIDS
- TRUE SEED CULTIVATION

1990-2010

6 Varieties

MARKER ASSISTED BREEDING

2010 onwards

4 Varieties

World Congress on Root and tuber Crops, Jan 18-22, 2016, Nanning, China
Production of Tripoids: Induction of tetraploids

Procedure: *In vivo & In vitro*
- Induction of tetraploidy by colchicine treatment (0.5% for 12 hr.)
- Isolation of apparent tetraploids based on leaf size, shape and thickness
- Confirmation of the ploidy status by cytological screening (2n=72)
- Screening of 4x plants for flowering and pollen fertility
- Isolation of fertile clones for use as male parents
Comparison of diploids, triploids & tetraploids

![Bar chart showing Tuber no (Mean) and Tuber Yield (kg/plant) for 2n, 3n, and 4n.]

- Tuber no (Mean): 2n = 5, 3n = 8.8, 4n = 7.4
- Tuber Yield (kg/plant): 2n = 2.5, 3n = 4.8, 4n = 2.7

World Congress on Root and tuber Crops, Jan 18-22,2016, Nanning, China
High starch triploid varieties Sree Apoorva and Sree Athulya
Released centrally in 2013

Sree Apoorva

Yield: 40-45 t ha$^{-1}$
Extractable starch: 30%

Sree Athulya

World Congress on Root and tuber Crops, Jan 18-22, 2016, Nanning, China
Sree Pavithra : K efficient Cassava variety

• High yield (35-45 t ha^{-1}),
• Excellent cooking quality,
• Low cyanogenic glucoside (25.8 ppm)
• High K efficiency (243.65 kg tuber/kg K absorbed)
• Suitable for cultivation in Kerala soils, which are inherently low to marginal in soil exchangeable K.
Sree Swarna

Sree Swarna is a cassava variety having

• High yield (40 t ha\(^{-1}\))
• Early bulking (7 months)
• Good culinary quality
• Yellow flesh colour &
• Tolerance to CMD
Breeding for CMD resistance

- Occurs in more severe form in Kerala and Tamil Nadu
- Emerging as a problem in other states
- It causes yield loss ranging from 25 – 80%

Factors:
- Indiscriminate use of infected planting material
- Non adoption of rouging / clean cultivation practices

Types of cassava mosaic viruses
- ICMV
- SLCMV
Source of CMD Resistance

• Interspecific breeding
• Inbreeding to release variability
• MNGa-1 and its breeding lines
• CR lines and their hybrids (CIAT)
BREEDING FOR CASSAVA MOSAIC DISEASE TOLERANCE FOR RESISTANCE THROUGH INTERSPECIFIC BREEDING PROGRAMME

MANIHOT SPECIES
- M.caerulescens
- M.tristis
- M.peruviana
- M.flabellifolia
- M.glaziovii
- M.pseudoglaziovii
- M.epruinosa
- M.dichotoma
- M. anomala

CMD RESISTANT BACKCROSS PROGENY (BC₄)

Wedge grafting

World Congress on Root and tuber Crops, Jan 18-22,2016, Nanning, China
Inbreeding

- Inbreeding in cassava was not universally deleterious, but was beneficial for the segregation and release of several desirable recessive alleles.

- **Cassava mosaic disease** inbreds were developed from heterozygous susceptible parents.

- Identification of cassava clones with rare recessive traits especially biochemical traits led to the development of value added hybrids.
CASSAVA BREEDING SCHEME : CTCRI

**Problems**

- Non flowering in elite non/less branching clones
- Lack of synchronisation in flowering
- Less seed set in wide crosses and selfs

<table>
<thead>
<tr>
<th>Year</th>
<th>Activity</th>
<th>No. of genotypes</th>
<th>Plants/genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Crosses among elite clones</td>
<td>Up to 6,000</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Evaluation of F₁ seedlings</td>
<td>3,000</td>
<td>1</td>
</tr>
<tr>
<td>III</td>
<td>Clonal evaluation trial (Row trail)</td>
<td>500</td>
<td>6-12</td>
</tr>
<tr>
<td>IV</td>
<td>Preliminary yield trial (PYT/IET)</td>
<td>200</td>
<td>20-80</td>
</tr>
<tr>
<td>V</td>
<td>Advanced yield trial (AYT)</td>
<td>50</td>
<td>100-500</td>
</tr>
<tr>
<td></td>
<td>Regional trials/MLTs</td>
<td>5-20</td>
<td>500-5000</td>
</tr>
</tbody>
</table>

Problems:
- Non flowering in elite non/less branching clones
- Lack of synchronisation in flowering
- Less seed set in wide crosses and selfs
Screening: Field, Grafting, PCR
Promising hybrids were screened for the presence of virus using multiplex PCR with SLCMV and ICMV specific primers.

<table>
<thead>
<tr>
<th>Upper Lane</th>
<th>Lower Lane</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Ladder 100 bp plus (fermentas)</td>
<td>1- Ladder 100 bp plus (fermentas)</td>
</tr>
<tr>
<td>2- CR 42-3</td>
<td>2- M4</td>
</tr>
<tr>
<td>3- 9S 165</td>
<td>3- 4-2</td>
</tr>
<tr>
<td>4- 9S 127</td>
<td>4- SREE REKHA</td>
</tr>
<tr>
<td>5- 9S 107</td>
<td>5- SREE VIJAYA</td>
</tr>
<tr>
<td>6- 9S 132</td>
<td>6- SREE PADMANABHA</td>
</tr>
<tr>
<td>7- 9S 272</td>
<td>7- SREE JAYA</td>
</tr>
<tr>
<td>8- CR 54 A-41</td>
<td>8- MVD-1</td>
</tr>
<tr>
<td>9- CR 24-4</td>
<td>9- CO-1 (ICMV infection)</td>
</tr>
<tr>
<td>10- CR 54 A-19</td>
<td>10- CO-2 (faint band)</td>
</tr>
<tr>
<td>11-CR 21-10</td>
<td>11- CO-3</td>
</tr>
<tr>
<td>12- CI 273</td>
<td>12- CO-4</td>
</tr>
<tr>
<td>13- POSITIVE CONTROL</td>
<td>13- SREE SAHYA</td>
</tr>
<tr>
<td>14- NEGATIVE CONTROL</td>
<td>14- SREE HARSHA</td>
</tr>
<tr>
<td>15- BLANK WELL</td>
<td>15- 5-3</td>
</tr>
<tr>
<td>16- BLANK WELL</td>
<td>16- NEGATIVE CONTROL</td>
</tr>
</tbody>
</table>

World Congress on Root and tuber Crops, Jan 18-22,2016, Nanning, China
Promising CMD resistant CIAT clones selected

CR 43-11

CR 43-7

CR 54A-19

World Congress on Root and tuber Crops, Jan 18-22, 2016, Nanning, China
Transfer of CMD resistance to elite Indian clones

- Hybrids with multiple resistance (MNga-1 & CR lines) were developed.

- Promising CMD resistant hybrids selected for onfarm trial includes 9S273, 9S127, 9S113, 9S117, 9S163, 9S278, 8S501-1.

- Promising inbred lines which combine higher yield, CMD resistance, good cooking quality, high dry matter & low cyanogen content were evolved.
<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Entries</th>
<th>Yield t ha⁻¹</th>
<th>Dry matter(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9S127</td>
<td>27.48</td>
<td>41.4</td>
</tr>
<tr>
<td>2</td>
<td>CR24-4</td>
<td>52.84*</td>
<td>39.9</td>
</tr>
<tr>
<td>3</td>
<td>CR54A3</td>
<td>48.91*</td>
<td>35.0</td>
</tr>
<tr>
<td>4</td>
<td>CR43-7</td>
<td>54.14*</td>
<td>33.9</td>
</tr>
<tr>
<td>5</td>
<td>CR21-10</td>
<td>43.16</td>
<td>32.4</td>
</tr>
<tr>
<td>6</td>
<td>CR43-2</td>
<td>51.47</td>
<td>43.2</td>
</tr>
<tr>
<td>7</td>
<td>CR20A2</td>
<td>27.44</td>
<td>37.3</td>
</tr>
<tr>
<td>8</td>
<td>CR52A41</td>
<td>46.47*</td>
<td>32.6</td>
</tr>
<tr>
<td>9</td>
<td>CI-273</td>
<td>48.09*</td>
<td>32.5</td>
</tr>
<tr>
<td>10</td>
<td>CR43-11</td>
<td>61.75*</td>
<td>31.8</td>
</tr>
<tr>
<td>11</td>
<td>Sree Padmanabha</td>
<td>24.29</td>
<td>32.2</td>
</tr>
<tr>
<td>12</td>
<td>Sree Athulya</td>
<td>47.35*</td>
<td>40.5</td>
</tr>
<tr>
<td></td>
<td>CD(0.05)</td>
<td>15.10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CV(%)</td>
<td>20.08</td>
<td></td>
</tr>
</tbody>
</table>
Evaluation of CMD resistant hybrids

- **No. of Tubers/plant**
  - CO-1
  - Sree Prakash
  - 9S127
  - CR43-7

- **Wt.of Tuber/plant(kg)**
  - CO-1
  - Sree Prakash
  - 9S127
  - CR43-7

World Congress on Root and tuber Crops, Jan 18-22,2016, Nanning, China
## MLT on Cassava mosaic resistant varieties

<table>
<thead>
<tr>
<th></th>
<th>Loc1</th>
<th>Loc2</th>
<th>Loc3</th>
<th>Loc4</th>
<th>Loc5</th>
<th>Loc6</th>
<th>Mean</th>
<th>CMD score</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCMS-1</td>
<td>39.5</td>
<td>42.0</td>
<td>37.8</td>
<td>54.3</td>
<td>44.4</td>
<td>55.6</td>
<td>45.59*</td>
<td>0</td>
</tr>
<tr>
<td>TCMS-2</td>
<td>45.7</td>
<td>49.4</td>
<td>32.1</td>
<td>49.4</td>
<td>43.2</td>
<td>44.4</td>
<td>44.03*</td>
<td>0</td>
</tr>
<tr>
<td>CR43-7</td>
<td>45.7</td>
<td>37.4</td>
<td>30.9</td>
<td>32.1</td>
<td>42.0</td>
<td>46.9</td>
<td>39.15</td>
<td>0</td>
</tr>
<tr>
<td>CR43-2</td>
<td>37.0</td>
<td>39.5</td>
<td>50.1</td>
<td>55.6</td>
<td>37.0</td>
<td>49.4</td>
<td>44.77*</td>
<td>0</td>
</tr>
<tr>
<td>H-226</td>
<td>32.1</td>
<td>30.9</td>
<td>24.7</td>
<td>34.6</td>
<td>43.2</td>
<td>37.0</td>
<td>33.74</td>
<td>3</td>
</tr>
<tr>
<td>Sree Padmanabha</td>
<td>37.0</td>
<td>34.6</td>
<td>37.0</td>
<td>39.5</td>
<td>34.6</td>
<td>43.2</td>
<td>37.65</td>
<td>0</td>
</tr>
<tr>
<td>Local</td>
<td>37.0</td>
<td>30.9</td>
<td>34.6</td>
<td>37.0</td>
<td>29.6</td>
<td>28.4</td>
<td>32.92</td>
<td>4</td>
</tr>
<tr>
<td>CD(0.05)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.81</td>
</tr>
<tr>
<td>CV(%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14.55</td>
</tr>
</tbody>
</table>

Yield (t ha⁻¹)
PROMISING CMD RESISTANT HYBRIDS DEVELOPED
Introgression of transgenes into popular varieties

Problems anticipated

• Flowering behaviour of transgenics?
• Majority of popular varieties are non branching, non flowering
Introgression of transgenes in promising cassava varieties

FLOWERING BEHAVIOUR OF PARENTS

Flowing (up to four peaks) is associated with branching.

Most flower buds formed at early growth are abortive.

There is variation in flowering time (from 4 months to > 8 months) and in number of flowers between varieties.

There is differential genotype-environment interaction on flowering among varieties, as well as a strong environmental effect on number of flowering peaks within the same variety.
Crossing of CMD resistant transgenic cassava with high starch accessions

Transgenic clones received from ETH were micropropagated & hardened under containment facility

Induction of flowering in erratic flowering types was undertaken through pruning & grafting on profusely floweing clones

Flowering was induced in non branching varieties viz. Sree Vijaya at 3 months after pruning
CMD Resistant Breeding programme

Transgenic cassava developed through Transformation

Conventional breeding-CMD resistant triploids & early bulking clones

Gene pyramiding through MAS

Clones with transgene introgressed through breeding

CMD/White fly resistant variety
I hereby acknowledge ISCB for sponsoring my trip for attending World Congress on Root and Tuber Crops, Nanning, 18-22, Jan 2016