Somatic hybridization between potato (*S.tuberosum*) and *S.phureja* to transfer bacterial wilt (*Ralstonia solanacearum*) resistance traits

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Introduction

Bacterial wilt

Potato

↓

266 pests and diseases

(23 viruses, 38 fungus, 2 mikoplasm, 1 viroid,
6 bacteria, 68 nematodes, 128 nematodes)

↓

Most Important disease:
Bacterial wilt (*Ralstonia solanacearum*)

↓

Chemicals control are not effective

↓

Breeding for resistance to bacterial wilt

↓

Resistance traits from wild species:
(S.chacoense, S.sparsipillum, S. multidissectum, S phureja)

↓

Sexual incompatibilities

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Somatic hybridization/Protoplast fusion

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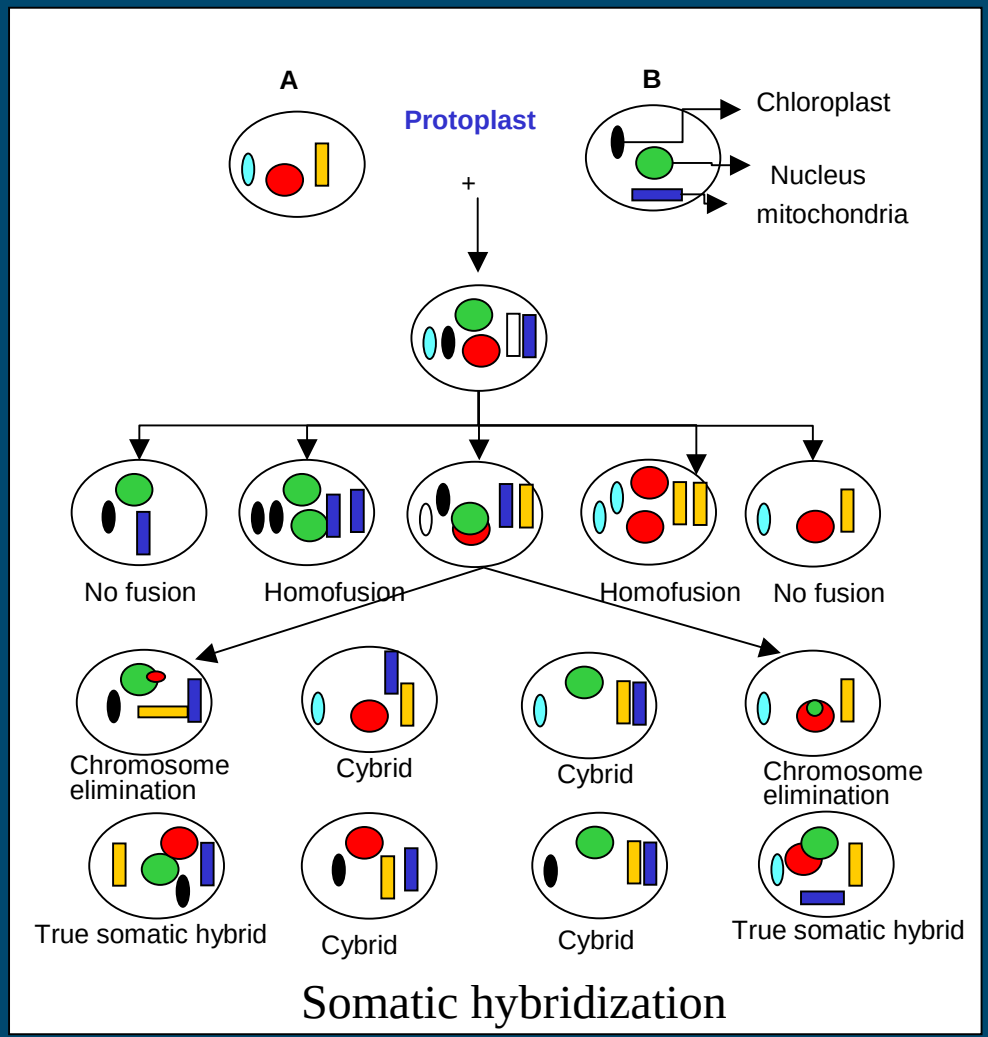
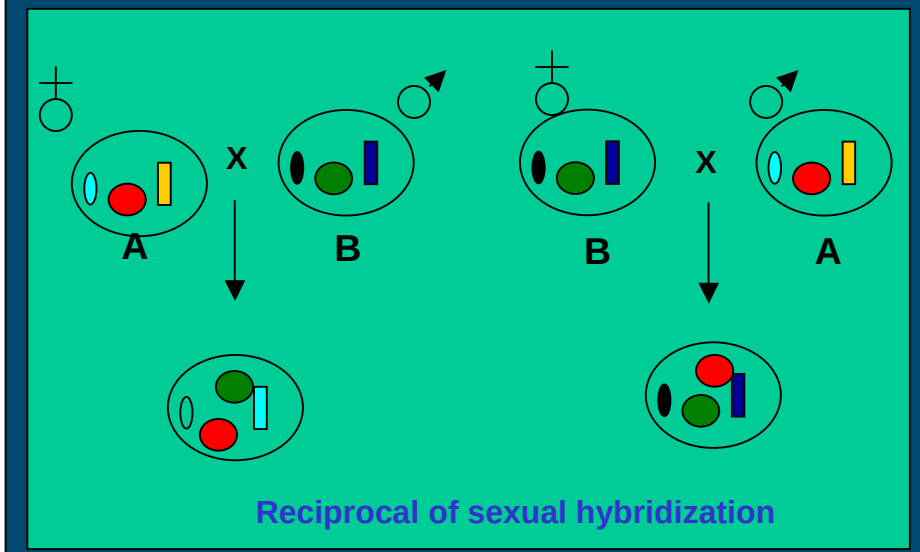
Somatic and Sexual Hybridizations

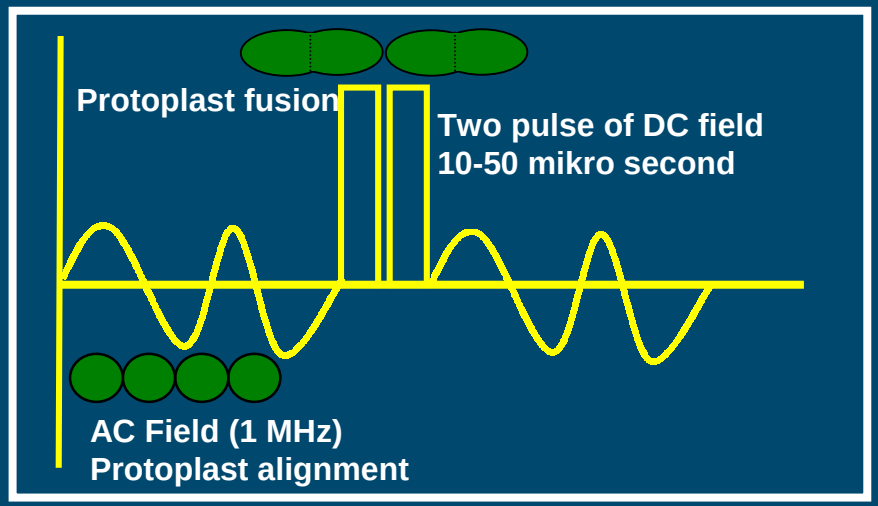


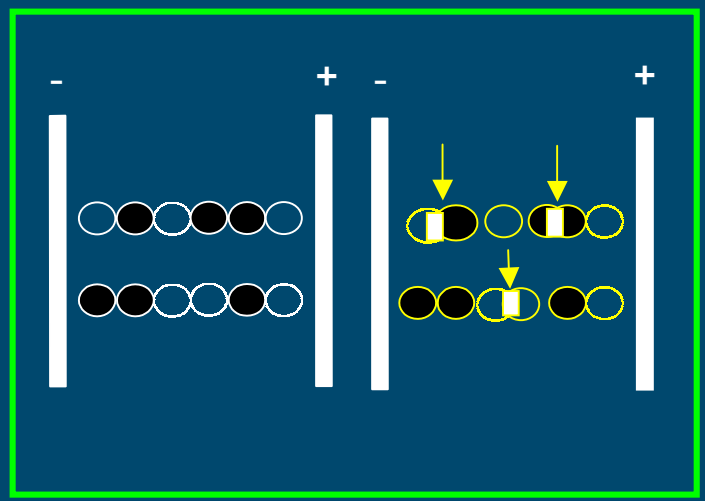
Somatic hybridization



Reciprocal of sexual hybridization

Electro fusion





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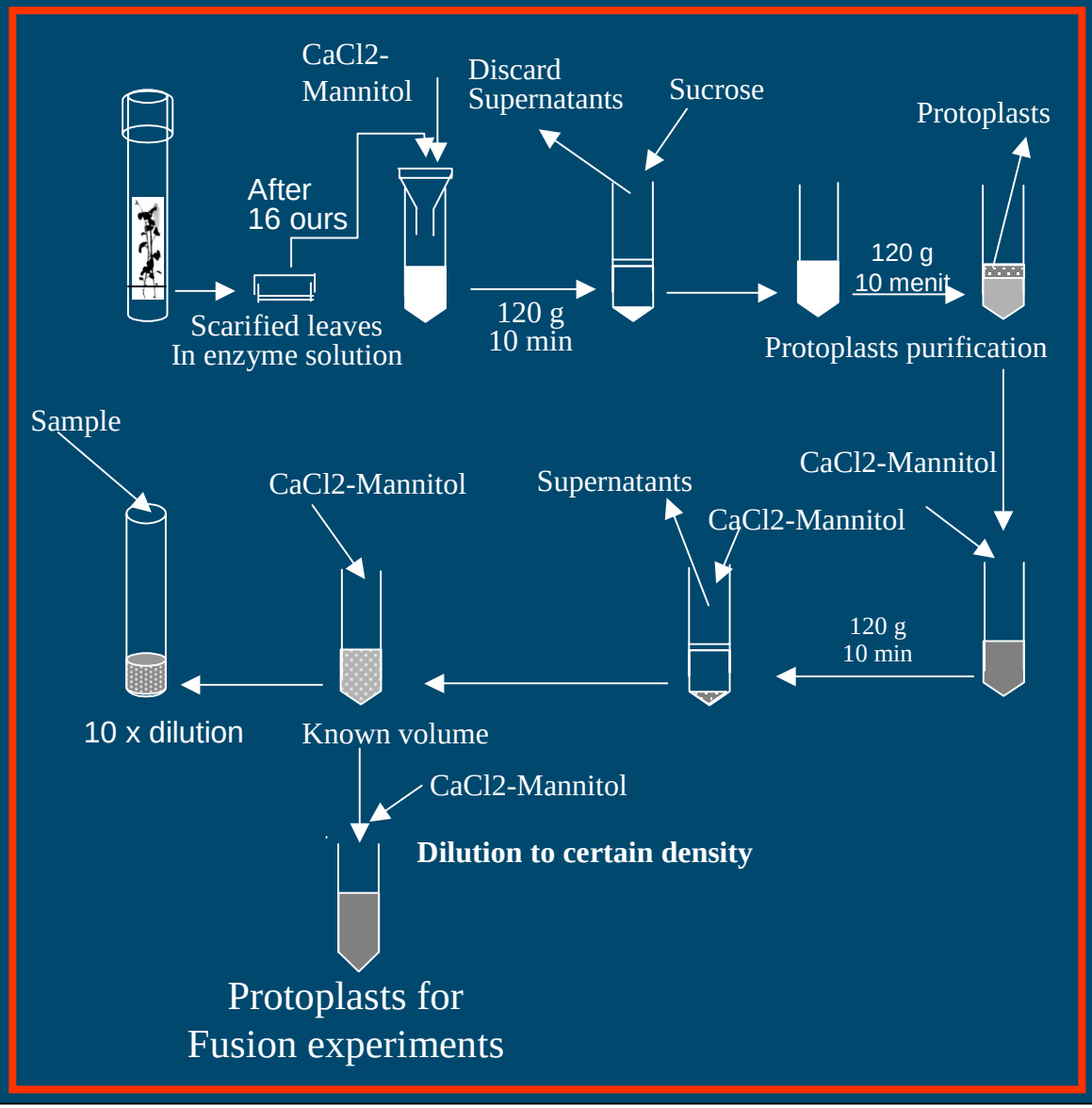
Protoplasts isolation



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Culture and Regeneration after Protoplasts fusion

Electro fusion

S.tuberosum cv BF15 (2n=2x=24) + *S.phureja* (2n=2x=24)

↓

Plating protoplasts

VKM medium+0.2 mg/l 2,4-D+0.5 mg/l zeatin+1.0 mg/l NAA

↓ *After 2 weeks*

Dilution of cultures

10x on the VKM medium+0.1 mg/l 2,4-D dan 2 mg/l BAP

↓ *After 2 weeks*

Microcalli enlargement

Solid MS medium+0.5 mg/l NAA dan 0.5 mg/l zeatin

↓ *3-4 mm diameter*

Regeneration medium

MS medium+2.0 mg/l zeatin+0.1 mg/l IAA

↓

Shoot multiplication

Hormone-free MS medium

Identification and Characterization of somatic hybrids

Identification

Regenerants
(Fusion products)
↓
Isoenzim analysis
(Esterases and peroxidases)
↓
RAPD
↓
Chromosome counting
↓
Somatic hybrids

Characterization

Somatic hybrids

- **In vitro test for bacterial wilt resistance**
- **In vitro microtuber production**
- **Field performance**

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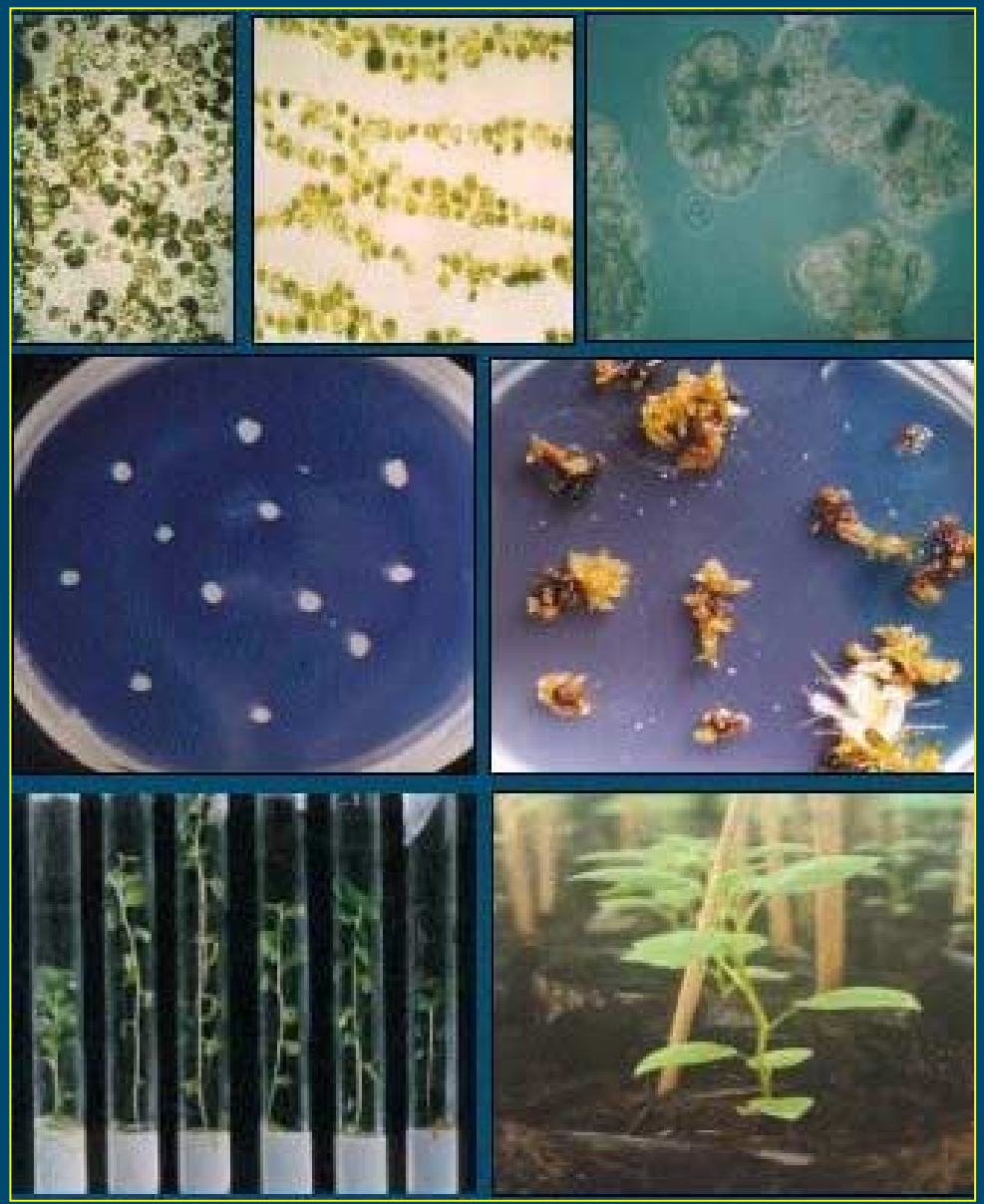

322 selected calli

↓

55 regenerants (Shoots (17%)

↓

10 Shoots are somatic hybrids
(confirmed by Isoenzyme and RAPD)

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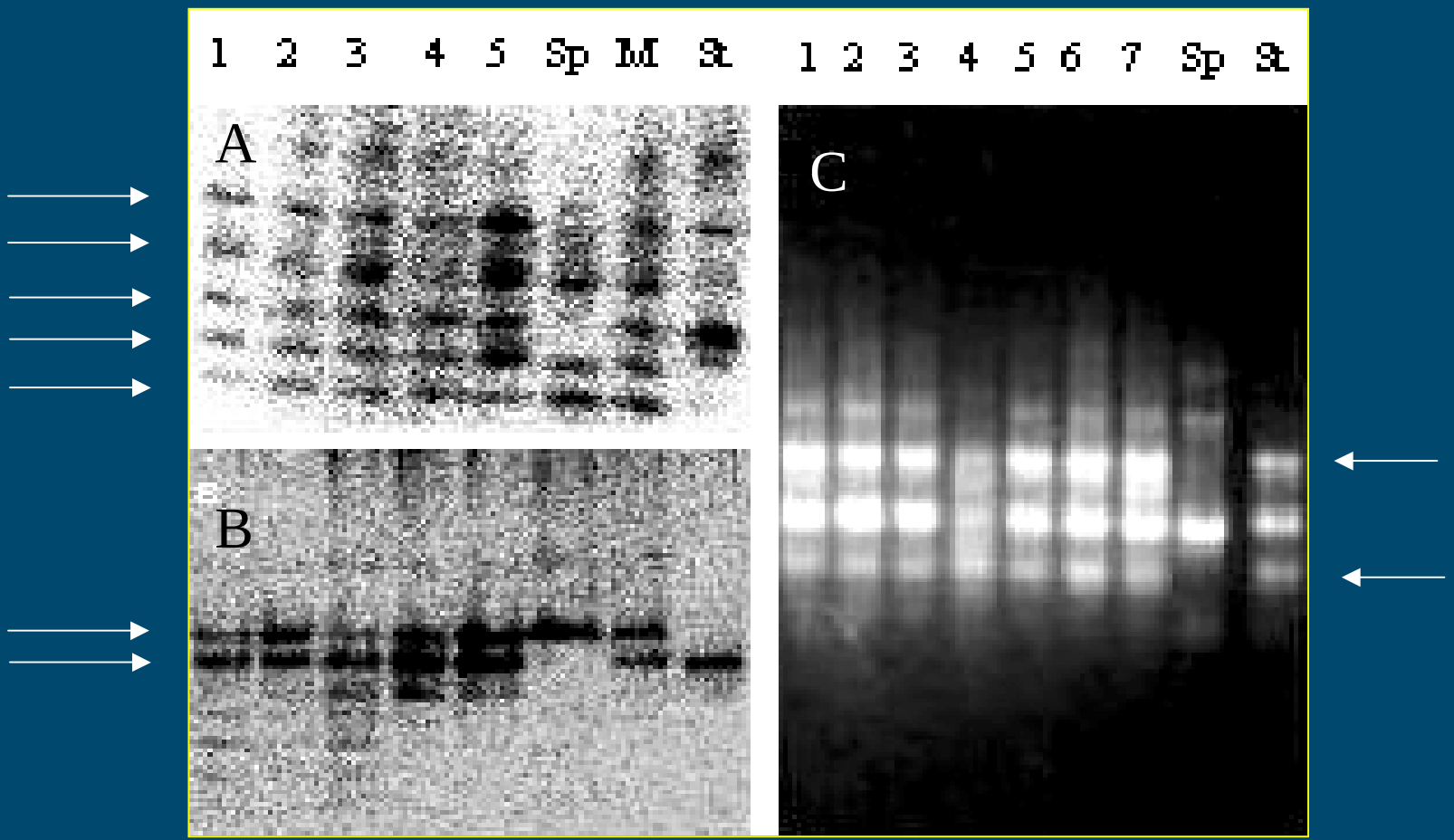
Isoenzymes and RAPD analysis



A: Esterases
B: Peroxidases
C: RAPD with OPA 18 primer (5'-GCTATCTGAC-3')

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Chromosome number of somatic hybrids

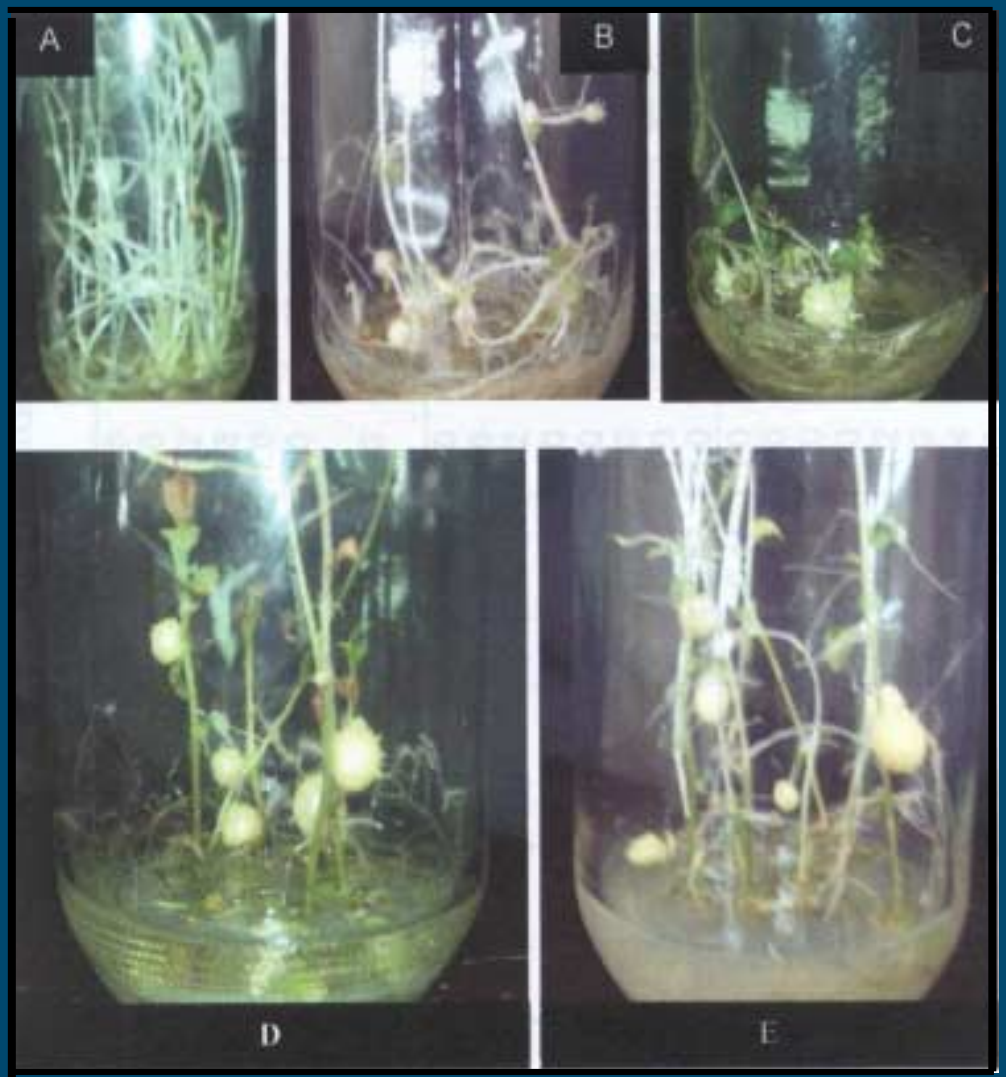
Diploid
2n=2x=24

Tetraploid
2n=4x=48

Oktoploid
2n=6x=72

Clones of somatic hybrids	Number of chromosomes
BP1	mixoploid
BP3	48
BP4	48
BP6	48
BP8	mixoploid
BP9	67
BP13	70
BP14	48
BP15	48
BP16	47

In vitro microtuber induction of somatic hybrids



6 weeks old in vitro plantlets
In hormone-free
of liquid MS medium

↓

Added with liquid MS medium
+ 400 mg/l CCC+ 5 mg/l BAP
+ 90 g/l sucrose

↓

Stored in the dark at 18-21 C
for 8 weeks

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Production of *in vitro* micro tuber of tetraploid somatic hybrids and their parental line

Genotype	Number of tuber per plant	Average fresh weight per tuber (mg)	% dry weight
BP3	2.1b	125b	12.1±1.7
BP4	3.2c	149c	15.2±0.9
BP6	1.4a	112a	13.4±2.1
BP9	1.1a	97a	10.2±1.2
BP15	2.2b	131b	12.7±0.7
BP16	1.6a	119b	14.2±2.2
S.phureja	3.1c	87a	15.7±3.7
BF15	2.4bc	92a	14.2±1.6
Granola	2.2b	137b	11.7±1.3

Test for resistance to bacterial wilt in vitro

Bacterial suspension (10^9 cfu/ml)

↓

Inoculation to in vitro nodal cutting

↓

Transfer to hormone-free semi solid MS medium

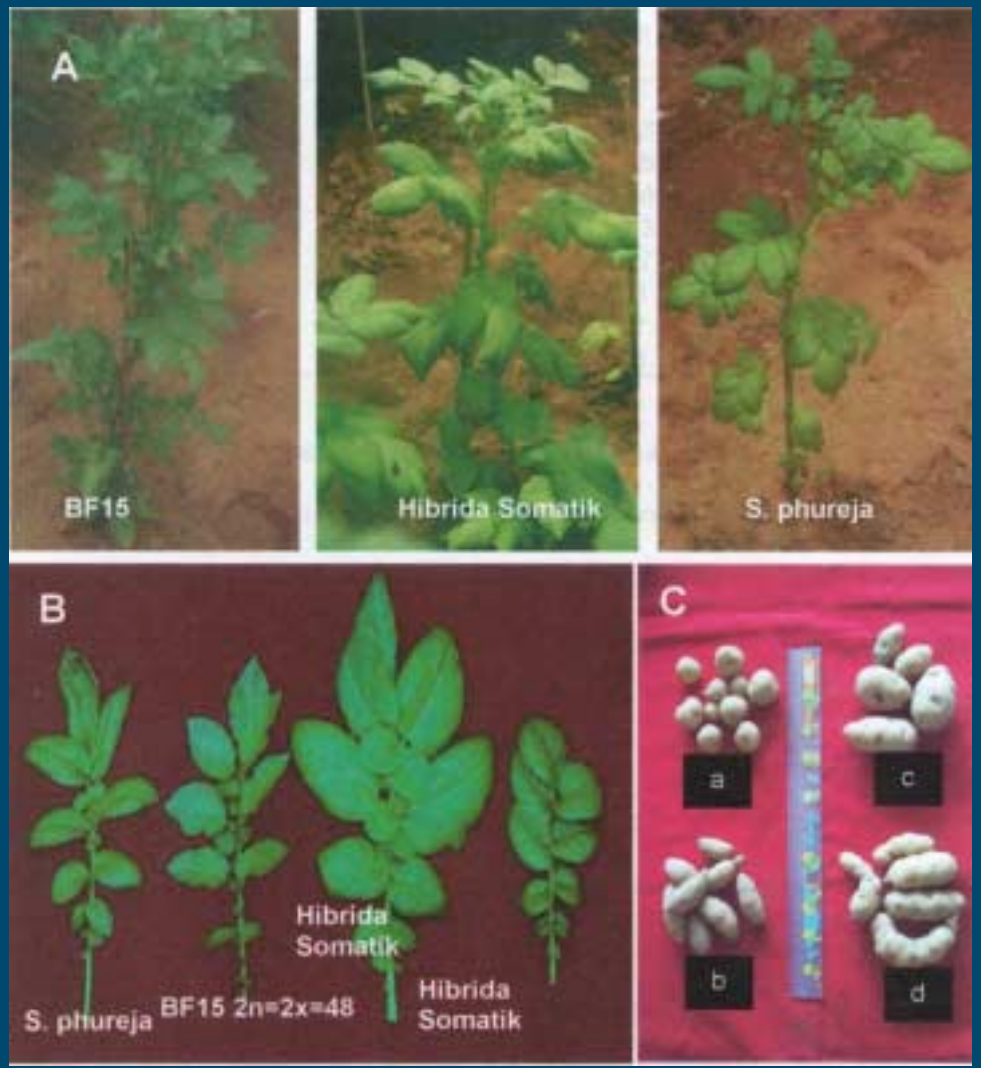
↓

Observed for incubation period and diseases incidence

The rate of resistance of somatic hybrids

Clones	Incubation periods (days after inoculation)	Diseases incidence (%)	Degree of resistance
R. solanacearum **race**1 strain			
BP3	18	19.2	Resistant
BP4	16	16.6	Resistant
BP6	6	42.1	Susceptible
BP9	14	17.6	Resistant
BP15	8	57.3	Susceptible
BP16	8	66.4	Susceptible
S.phureja	17	15.8	Resistant
BF15	5	100.0	Susceptible
Granola	5	87.5	Susceptible
R. solanacearum **race 3 strain**			
BP3	14	37.6	Moderate Resistant
BP4	13	17.7	Resistant
BP6	17	16.3	Resistant
BP9	10	23.7	Moderate Resistant
BP15	10	27.3	Moderate Resistant
BP16	6	10.3	Resistant
S.phureja	15	17.5	Resistant
BF15	5	100	Susceptible
Granola	7	75.5	Susceptible

Performance of somatic hybrids in the field



In vitro Plantlets

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Micro cutting

↓

Acclimatization

↓

Mini cutting

↓

Rooting

↓

Transfer to green house

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Performance among somatic hybrids in the greenhouse

Performance of tetraploid somatic hybrids and their parental line in the field

Genotype	Height of plant (cm)	Number of tuber per plant	Fresh weight of tuber per plant (g)	% Dry matter
BP3	56.7a	2.2a	33.7a	15.4±1.9
BP4	72.8c	8.2cd	211.7	17.8±1.5
BP6	77.4cd	3.9a	67.2b	13.7±2.2
BP9	43.7a	5.1b	94.7bc	12.2±1.3
BP15	60.7b	4.2ab	75.9bc	14.2±0.9
BP16	82.3d	6.4bc	117.3c	15.1±2.7
S.phureja	73.4c	9.6d	96.4bc	17.1±1.9
BF15	61.7b	5.7b	121.7c	16.2±1.8
Granola	68.9b	7.4c	177.4d	14.3±1.4

Conclusion

1. Number of regenerants produced from 322 selected microcalli was 55 or 17.0 percent, and 18.2 percent of them or 10 plants were somatic hybrids

2. All somatic hybrids were resistant or moderate resistant to bacterial wilt race 3 and three of them were resistant to race 1

3. All five tetraploid somatic hybrids were able to produce microtuber in comparable yield with cv Granola. This result were also confirmed by the experiment done in the greenhouse.

4. Somatic hybrid BP4 was promising clone, therefore further field experiment is needed.

THANK YOU

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