

Recent advances in biotic & abiotic stress management



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Biotic stress:

Imperative causal agents are:

- **Fungi** (*P. infestans*, *Alternaria solani*, *Rhizoctonia solani*, *Verticillium dahliae*).
- **Viruses** (PVY, PLRV, PVX, PVM, PVA, PVS).
- **Bacteria** (*Ralstonia solanacearum*, *Erwinia species*, *Streptomyces scabies*, *Pseudomonas solanacearum*) and
- **Nematodes** (*Globodera pallida* and *Globodera rostochiensis*)

These biotic components causes severe losses to potato crop & remains as major impediment in potato production worldwide.

Late Blight

"infectious plant destroyer"

Late blight: caused by *Phytophthora infestans*

Source of resistance:

- Diverse late blight resistance genes are found in wild and cultivated relatives of potato e.g. *S. bulbocastanum*, *S. demissum* and *S. stoloniferum*.
- Other wild species possessing resistance are *S. tuberosum* ssp. *andigena*, *S. pinnatisectum*, *S. polyadenium*, *S. verrucosum*, *S. chacoense*, *S. berthaulti*, *S. vernei*, *S. polytrichon*, *S. simplicifolium* and *S. microdontum*.

An array of 'R' genes have been reported and mapped from wild species against late blight.

Late blight:

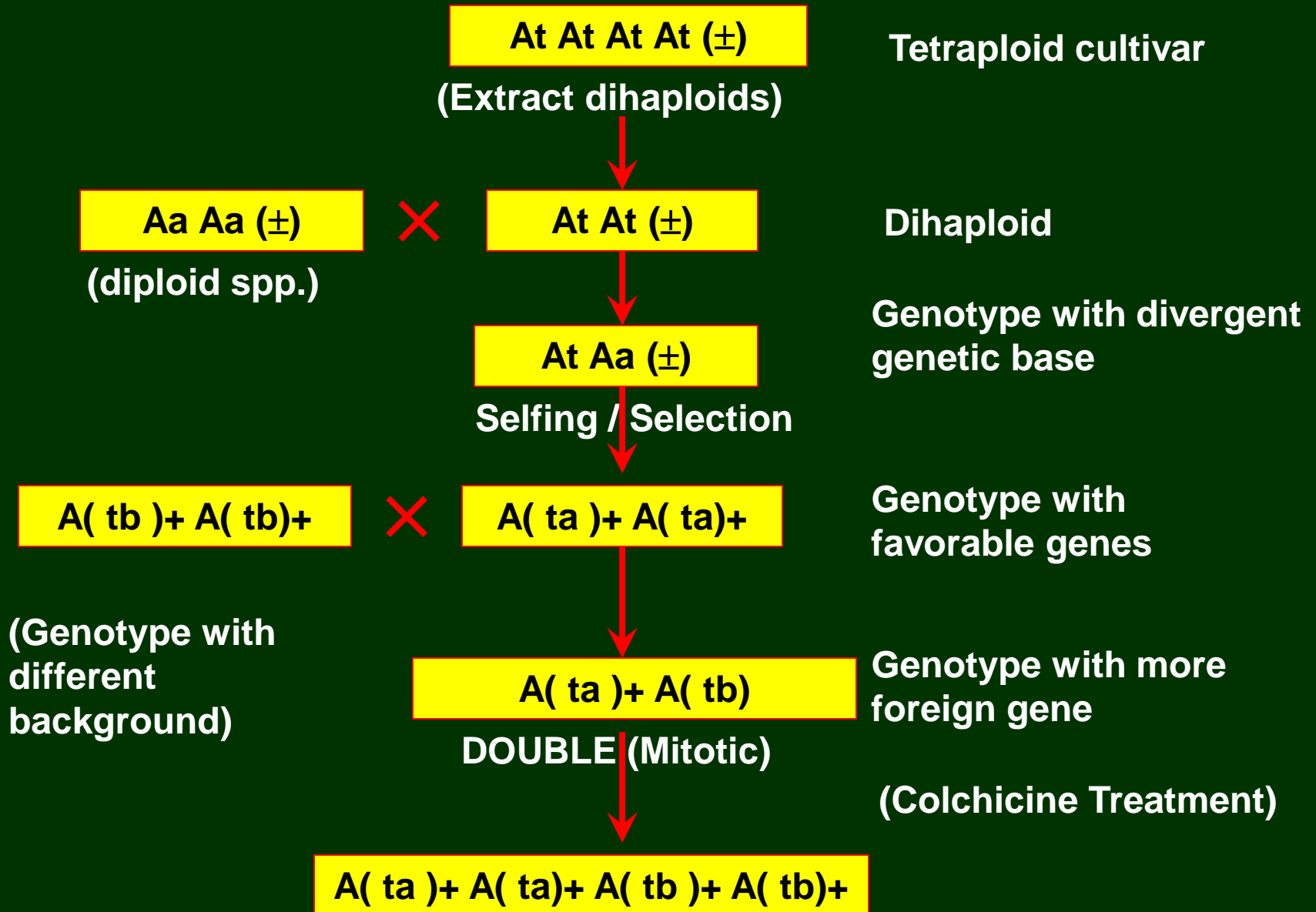
Mechanism of resistance: Resistance can be grouped into two types:

(i) Vertical resistance

- also called race-specific resistance or major gene resistance, qualitative or discontinuous resistance and
- is oligogenic and expressed in the form of hypersensitive response to all the races of *P. infestans* that lack the corresponding virulence to the resistance genes i.e. the *R*-genes specific resistance is governed by major *R*-genes.

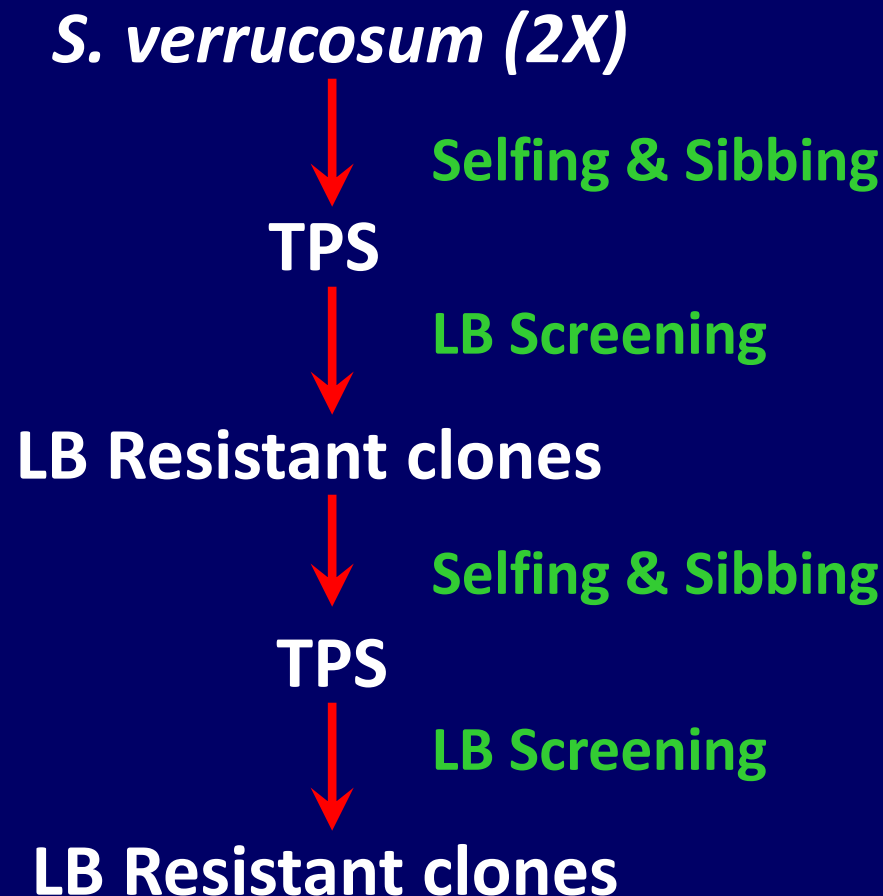
(ii) Horizontal resistance

- also called race non-specific or minor gene resistance, field resistance, polygenic resistance, quantitative or partial resistance.
- in contrast, to vertical resistance. it is non-specific, quantitative, multigenic, durable and field resistance.

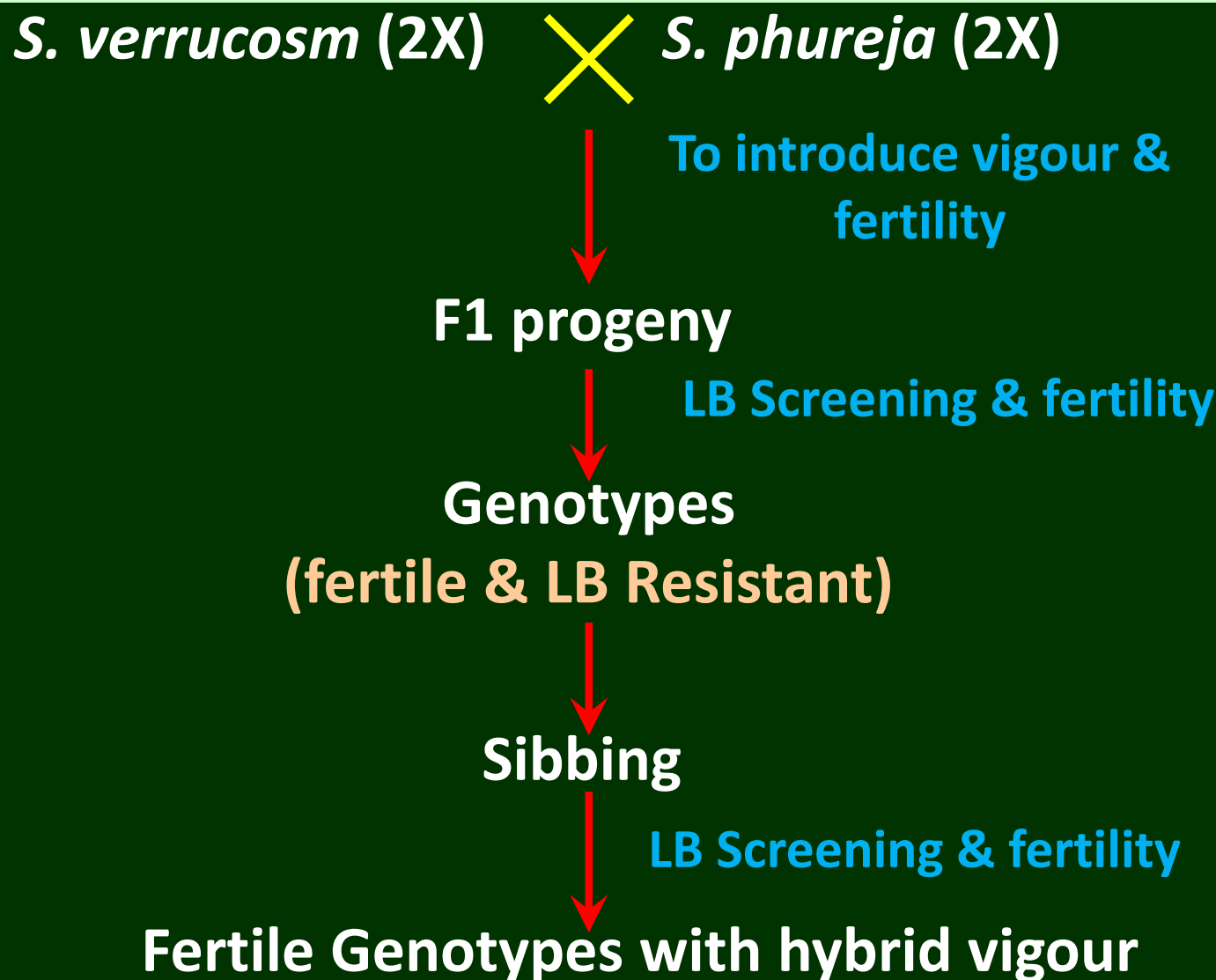


Transfer of field Resistance from *S. verrucosum*

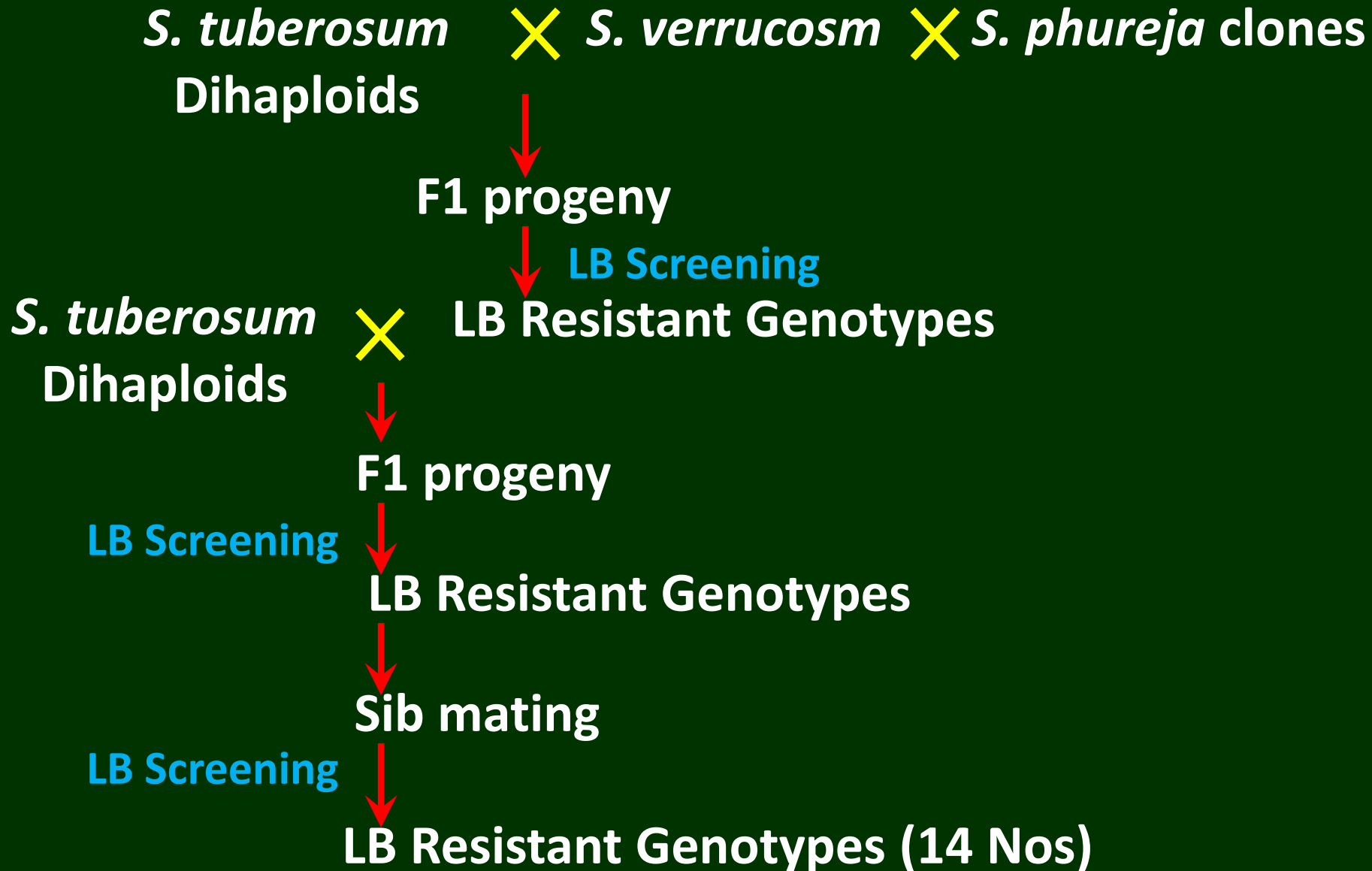
(A) Conversance of LB resistance genes in *S. verrucosum*



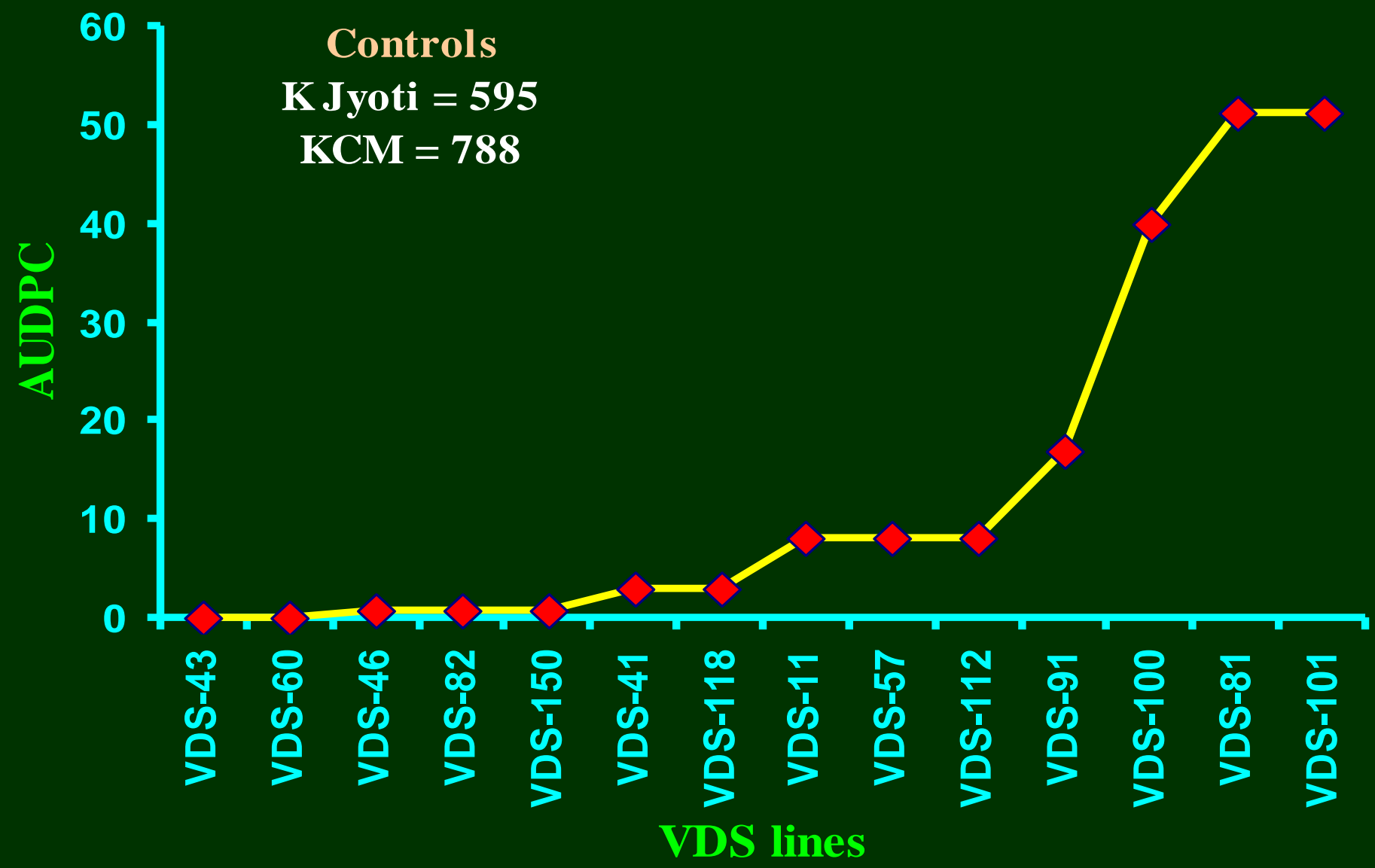
(B) Introduction of hybrid vigour and fertility in *S. verrucosum*



(C) Introduction of *S. tuberosum* genes in *S. verrucosum* derived clones



Field evaluation of VDS lines against potato late blight



(D) Development of Meiotic tetraploids from *S. verrucosum* derived clones

S. tuberosum Cultivars
(KCM, CD) 4X



VDS
(2X)



VMTs developed

BREEDING FOR LATE BLIGHT RESISTANCE :

Conventional breeding:

- The use of new resistance sources to develop and deploy resistant cultivars has been a continuous endeavour across the potato growing nations of the world through:
- Several resistance genes (*Rpi-genes*) from wild species have been introduced in to cultivated potato successfully.
- *Solanum demissum*, having race specific *R1-R11* resistance genes was introgressed in to cultivated potato is one of the earliest potato breeding efforts in **central Mexico** (Reddick, 1934; Black, 1970).

Conventional breeding:

Regardless of the failure of initial Late Blight resistance breeding in developed countries, developing countries have been successful in breeding moderate, to high, resistant potato varieties through traditional approaches like the

- **‘Sarpo varieties’** in Hungary,
- **Cooperation 88 a** potato cultivar grown in Yunnan Province of China resulted from the cross between late blight resistant clone from India I-1085, and bulk pollen of LB resistant population (*Solanum andigena*) from CIP Peru,
- Further **C88** late blight resistant breeding lines are also known in Asian countries (Li, *et al.* 2011), **‘Amarilis’** in Southern America (Salazar, Winters *et al.* 2009), first released by Peru’s National Institute of Agricultural Research (INIA) in 1993.

Potato varieties carrying field resistance to late blight

Kufri Giriraj (Hills)

Kufri Chipsona-1, 2, 3, 4 (Processing)

Kufri Shailja (Hills)

Kufri Himalini (Hills)

Kufri Girdhari (Hills)

Kufri Himsona (Processing for hills)



Late blight:

Non-Conventional/Biotechnological breeding:

- Till date, over **21 functional late blight 'R' genes** have been mapped & cloned to the potato genome through linkage to specific DNA markers.
- Most of the cloned genes belong to the **CC-NBS-LRR class**, and all derived from wild *Solanum* species.
- Besides *S. demissum* other wild species includes:
 - ***S. bulbocastanum*** (Song *et al.*, 2003; van der Vossen *et al.*, 2003, 2005),
 - ***S. stoloniferum* and *S. papita***, (Vleeshouwers *et al.*, 2008),
 - ***S. venturii* and *S. mochiquense*** (Pel *et al.*, 2009; Foster *et al.*, 2009).



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Late blight:



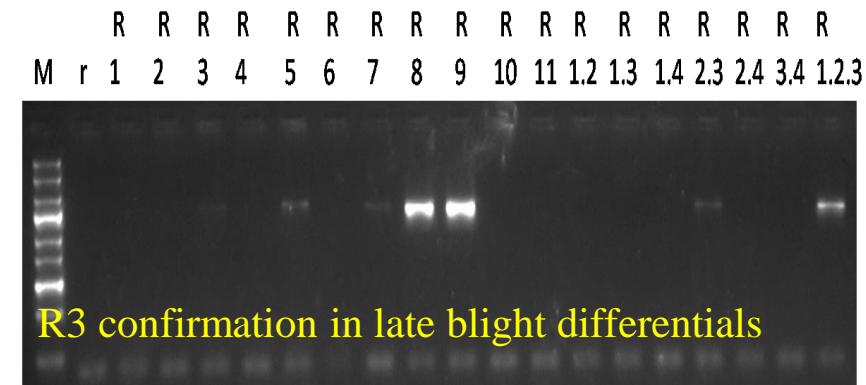
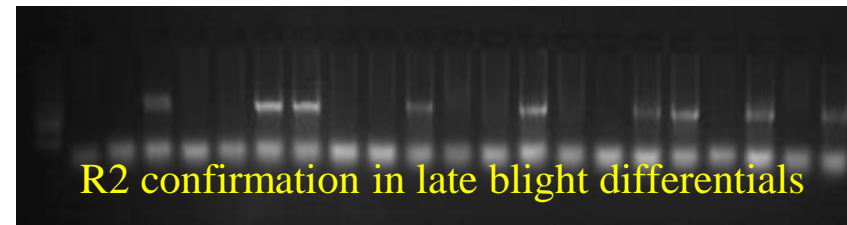
Non-Conventional/Biotechnological breeding:

Development of transgenic/cisgenic potato varieties may be a significant way to breach the worldwide public criticism on use of GM-crops.

‘**Fortuna**’ is a GM potato variety, to be released shortly having two resistance genes, being *Rpi-blb1*, and *Rpi-blb2*, which is highly resistance than conventional bred varieties Toluca and Bionica possessing single resistance gene *Rpi-blb2*.

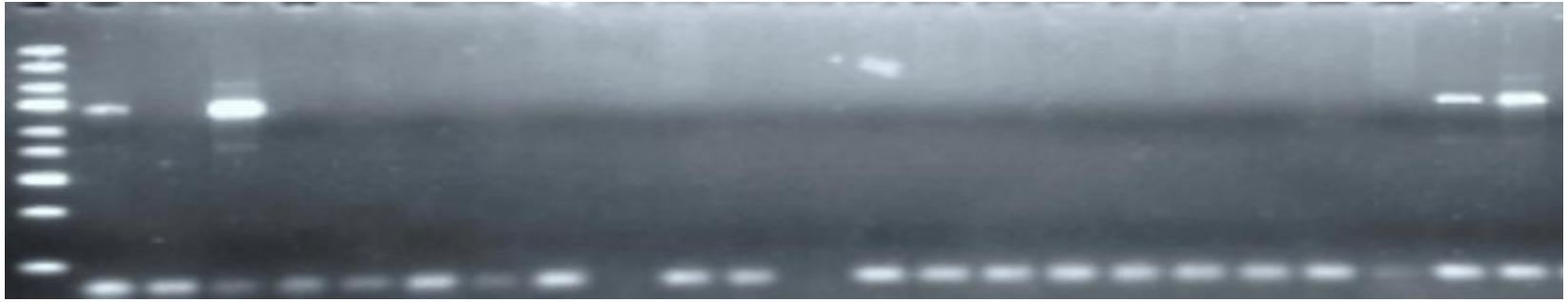
In India, Promising genotypes having RB gene mediated late blight resistance have been developed both by genetic transformation and by crossing with **RB transgenic Katahdin lines** expressing the RB gene and conferring field resistance to late blight for hills and plains

Validation of R-Genes markers in late blight differentials

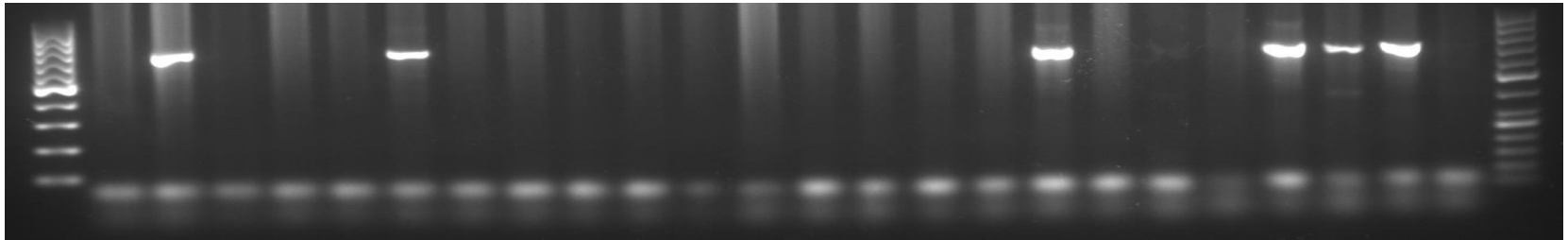


Gene	Marker	Primer sequence
R1	Cos A F Cos A R	F-CTCATTCAAATCAGTTTTGATC R-GAATGTTGAATCTTTTTGTGAAGG
	R1AS F R1AS R	F-CAC TCG TGA CAT ATC CTC ACT A R- CAA CCC TGG CAT GCC ACG
R2-Rpi-abpt	R2 F1 R2 R3	F-GCTCCTGATACGATCCATG R- ACGGCTTCTTGAATGAA
R3 a	R3 1380 F R3 1380 R	F-GCTTCCGACATGTATTGATCTCCC R- GGCAGCCACTTCAGCTTCTTACAG

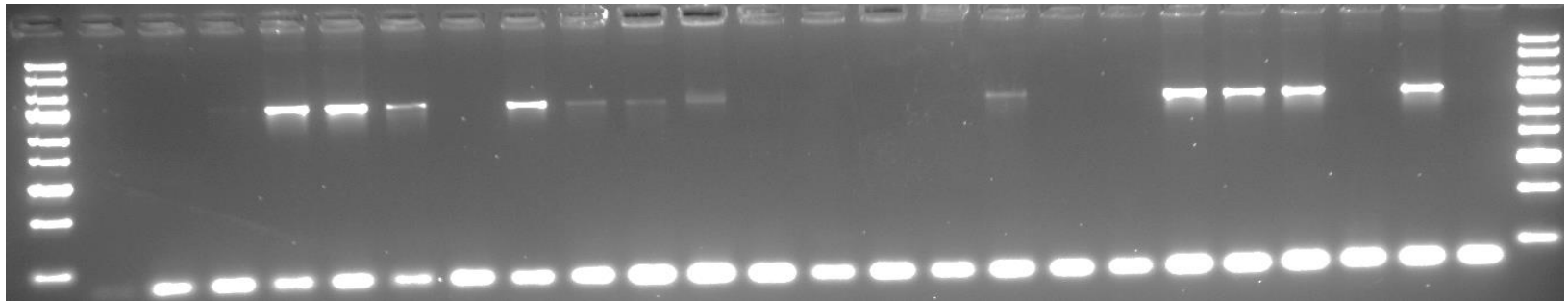
Identification of potato genotypes having different R-genes



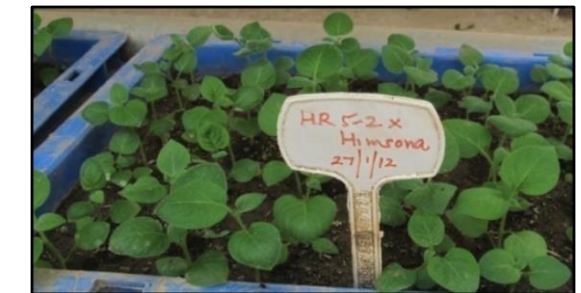
Screening for presence of R1 gene (23 genotypes)



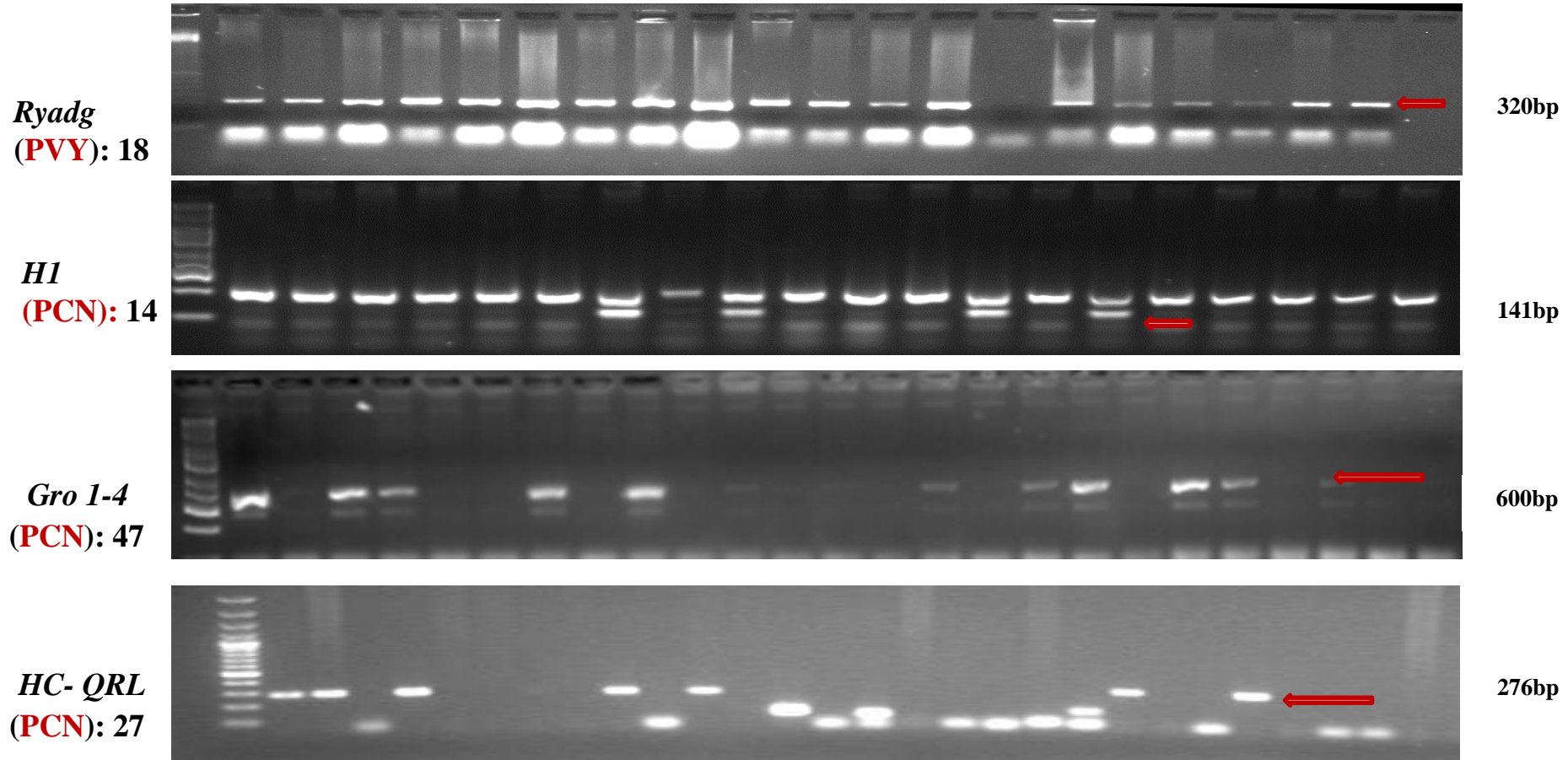
Screening for presence of R2 gene (22 genotypes)



Screening for presence of R3a gene (61 genotypes)



Stacking multiple resistance (LB, PVY & PCN) genes using MAS



Validation of markers for PVY and PCN

LB: R1, R2, R3a

PVY: Ryadg, Rysto

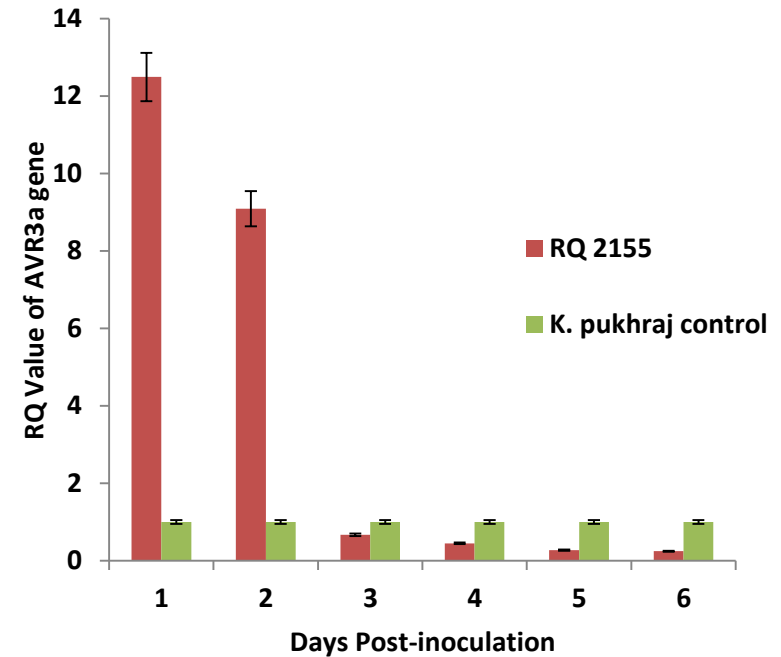
PCN: H1 (and), Gro 1-4 (spg), HC-QRL (ver), Gpa5 & 6 (spg):2

Silencing of RXLR effector gene (*Avr3a*) of *P. infestans* using RNAi approach

i) SiRNA



K. Pukhraj SI2AS1 2155 & K. Pukhraj control

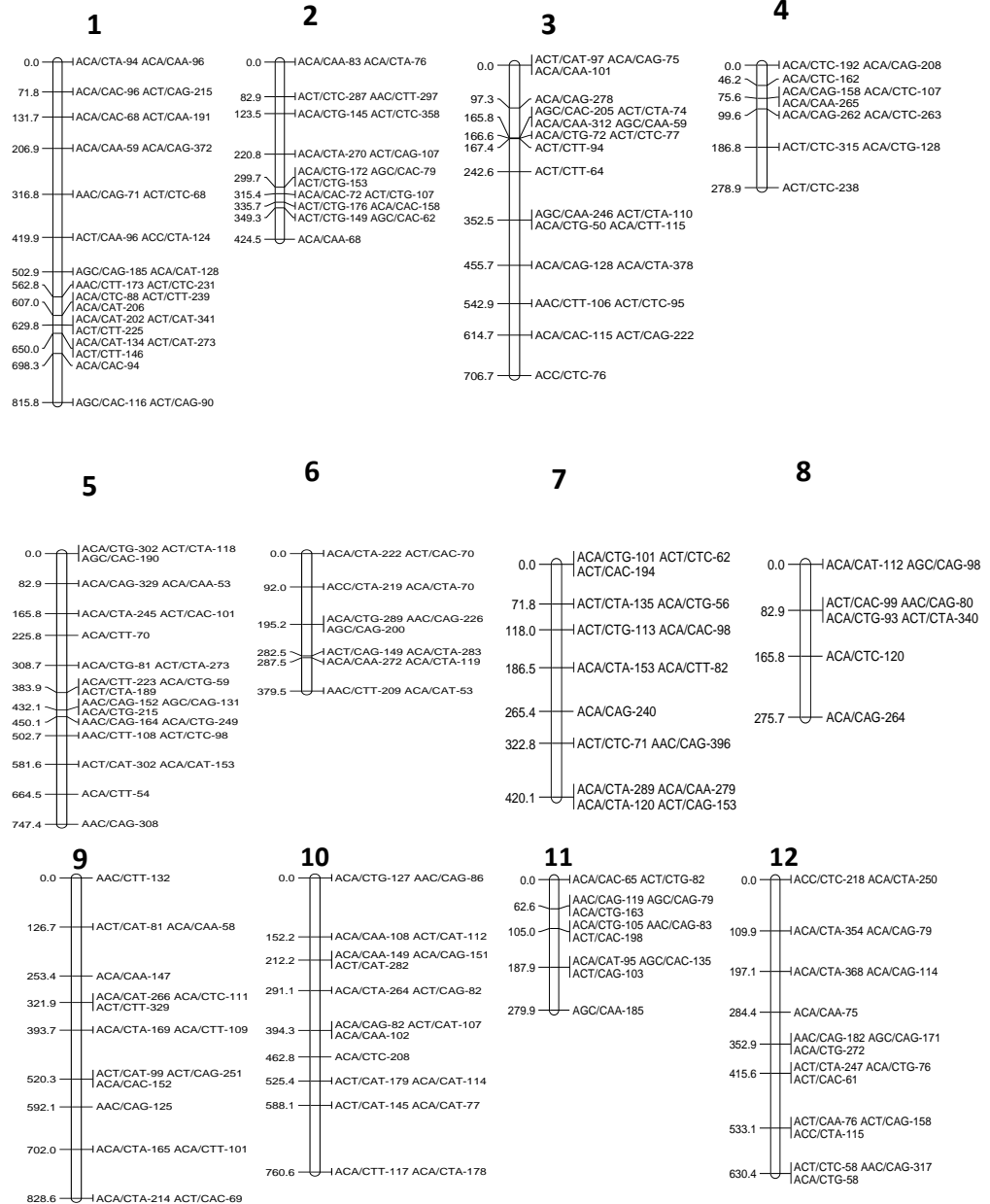


Relative Expression of *Avr3a* gene in transgenic K. Pukhraj SI2AS1 2155

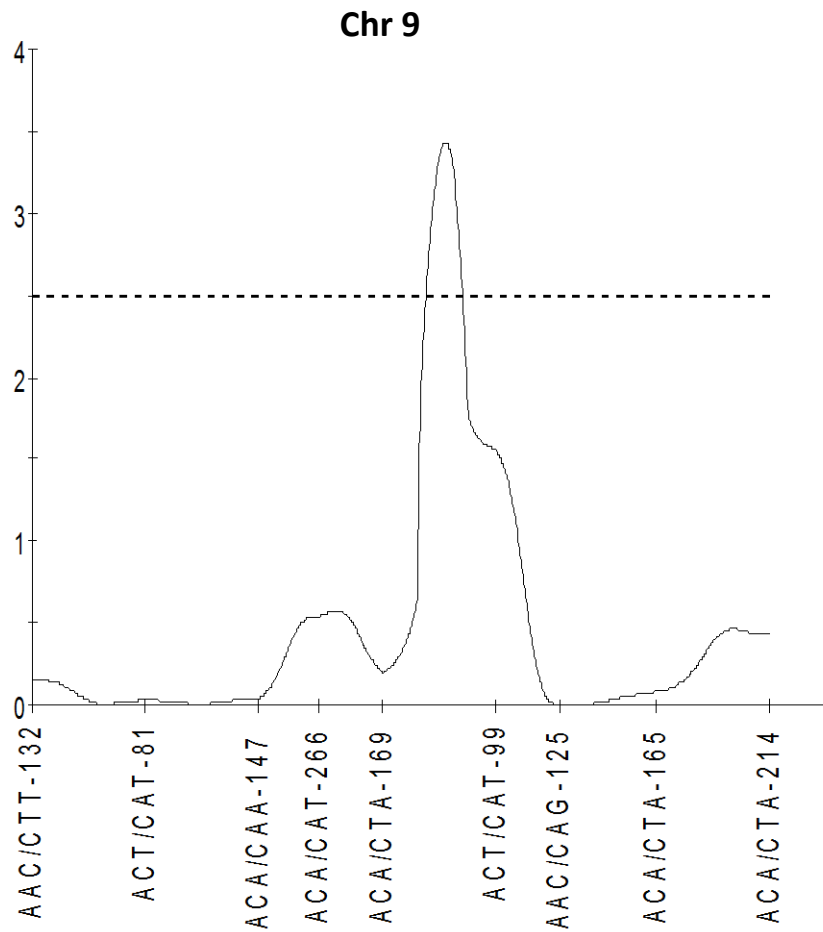
Five lines from SiRNA transgenics and three lines from amiRNA transgenics have been selected.

Real Time PCR analysis of *Avr3a* gene in transformed Potato line (K. Pukhraj SI2AS1 2155) and non - transformed control plants confirming the silencing of the *Avr3a* gene upon *P. infestans* challenge inoculation.

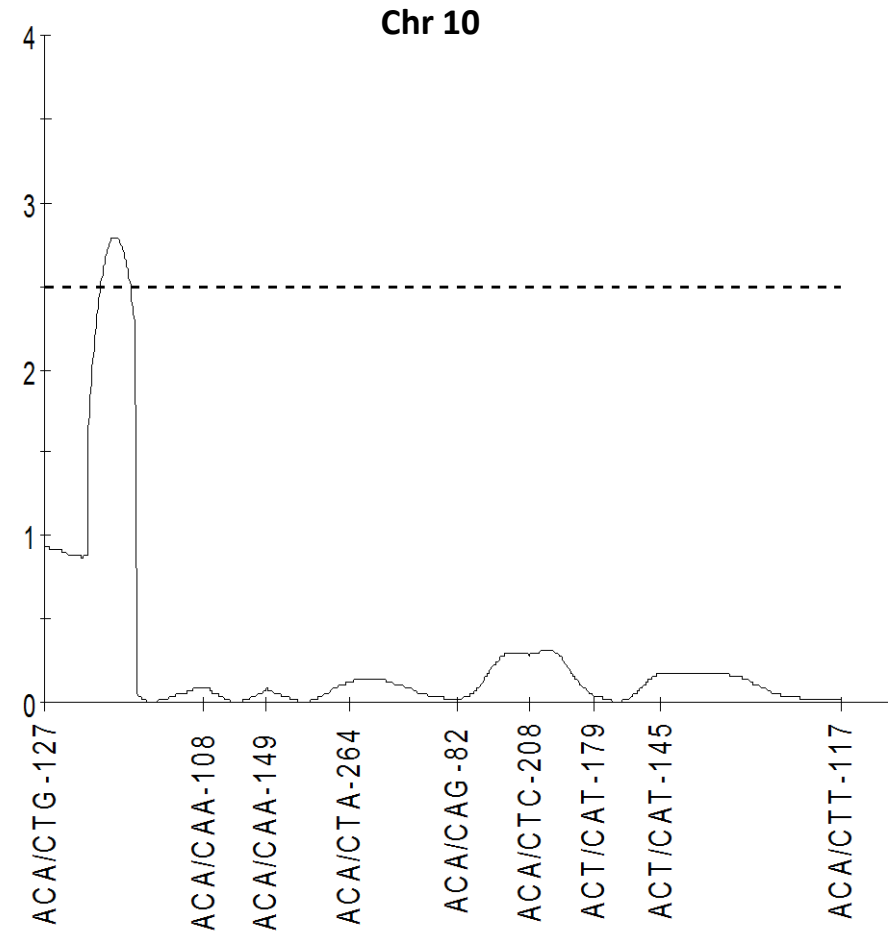
- A mapping population (126) was developed using *S. spegazinii* X *S. chacoense*
- Molecular linkage maps of *S. chacoense* and *S. spegazinii* were prepared containing 208 and 247 markers, respectively.
- Two QTLs have been identified on chromosomes 9 & 10.



QTL identification



LOD 3.41



LOD 2.80

<i>Solanum</i> spp.	Chromosome	R gene (Mapped and some cloned)
<i>S. avilesi</i>	11	<i>Rpi-avl1</i>
<i>S. berthaultii</i>	10	<i>Rpi-ber1</i> and <i>Rpi-ber2</i>
<i>S. brachistotrichum</i>	4	<i>Rpi-bst1</i>
<i>S. bulbocastanum</i>	8 (<i>Rpi-blb1</i> and <i>Rpi-bt1</i>), 6 (<i>Rpi-blb2</i>), 4 (<i>Rpi-blb3</i> and <i>Rpi-abpt</i>)	<i>RB/Rpi-blb1</i> , <i>Rpi-blb2</i> , <i>Rpi-blb3</i> , <i>Rpi-abpt</i> and <i>Rpi-bt1</i>
<i>S. capsicibaccatum</i>	11	<i>Rpi-cap1</i>
<i>S. circaeifolium</i>	11	<i>Rpi-qum1</i>
<i>S. demissum</i>	5 (<i>R1</i>), 4 (<i>R2</i> and <i>Rpi-dmsf1</i>), 11 (<i>R3-R11</i> except <i>R8</i>), 9 (<i>R8</i>)	<i>R1</i> , <i>R2</i> , <i>R3</i> (<i>R3</i> & <i>R3b</i>), <i>R4</i> , <i>R5</i> , <i>R6</i> , <i>R7</i> , <i>R8</i> , <i>R9</i> , <i>R10</i> , <i>R11</i> and <i>Rpi-dmsf1</i>
<i>S. dulcamara</i>	9	<i>Rpi-dlc1</i>
<i>S. edinense</i>	4	<i>Rpi-edn1.1</i>
<i>S. hjertingii</i>	4	<i>Rpi-hjt1.1</i> , <i>Rpi-hjt1.2</i> and <i>Rpi-hjt1.3</i>
<i>S. microdontum</i>	4	<i>Rpi-mcd1</i>
<i>S. mochiquense</i>	9	<i>Rpi-mcq1</i>
<i>S. papita</i>	8	<i>Rpi-pta1</i> and <i>Rpi-pta2</i>
<i>S. phureja</i>	9	<i>Rpi-phu1</i>
<i>S. pinnatisectum</i>	7	<i>Rpi-pnt1</i>
<i>S. polytrichon</i>	8	<i>Rpi-plt1</i>
<i>S. schenckii</i>	4	<i>Rpi-snk1.1</i> and <i>Rpi-snk1.2</i>
<i>S. stoloniferum</i>	8 (<i>Rpi-sto1</i>) and 11 (<i>Rpi-sto2</i>)	<i>Rpi-sto1</i> and <i>Rpi-sto2</i>
<i>S. venturii</i>	9	<i>Rpi-vnt1.1</i> , <i>Rpi-vnt1.2</i> and <i>Rpi-vnt1.3</i>
<i>S. verrucosum</i>	6	<i>Rpi-ver1</i>
Unknown spp.	4	<i>R2-like</i>

RB gene –A broad spectrum resistance gene against late blight from *S. bulbocastabum*

- ❑ Gene RB from *Solanum bulbocastanum* confers broad spectrum resistance against potato late blight pathogen *P. infestans*
- ❑ Wild potato species *S. bulbocastanum* is highly resistant to all known races of *P. infestans*
- ❑ Potato germplasm derived from *S. bulbocastanum* has shown durable and effective resistance in the field against late blight
- ❑ In Wisconsin, IUSA, major resistance gene RB was cloned by using map based approach in combination with a long range PCR strategy
- ❑ In India, introgression of this gene into major potato cultivars has been done

Development of LB resistant varieties using RB gene in India

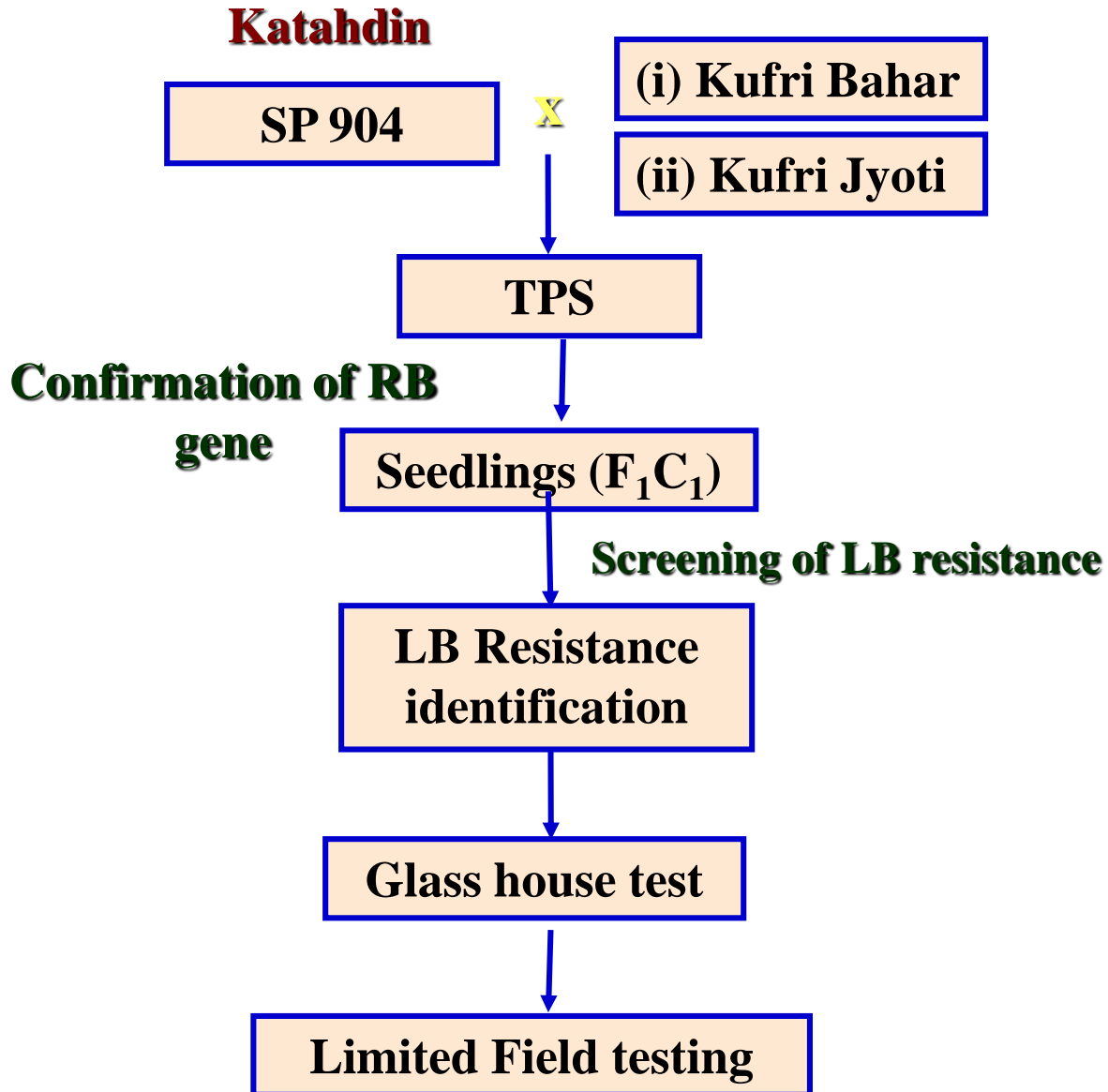
**RB transgenic
Katahdin**

**RB gene
construct**

**Breeding
Approach**

**Transgenic
Approach**

BREEDING APPROACH



Potato viruses

- **Viral diseases are an important constraint for potato crop because of their systemic distribution in the host and are mainly responsible for the degeneration of seed stocks.**
- **While more than 40 viruses naturally affect the potato around the world.**
- **But at least twelve viral diseases are known to infect potato crop in India and elsewhere which includes:**
- **Viruses PVX, PVY, PVS, PVA, PVM and PLRV are most important.**

And other viruses are:

PMTV, TRV, GBNV, ToLCNDV-potato and one viroid i.e. PSTVd.

Potato viruses:

Sources of resistance:

New resistance genes are still under investigation but no doubt, many others as yet to explore. Few sources of virus resistance genes are :

Virus	Resistant source species
PLRV	<i>S.etuberosum</i> , <i>S. tuberosum gp.andigenum</i> , <i>S.chacoense</i> , <i>S.brevidens</i>
PVY & PVA	<i>S. stoloniferum</i> , <i>S. tuberosum gp.andigenum</i> , <i>S. demissum</i> , <i>S. hougasii</i> , <i>S. chacoense</i> , <i>S.phureja</i> , <i>series etuberosa</i>
PVX	<i>S.tuberosum gp. andigenum</i> , <i>S. acaule</i> , <i>S.chacoense</i> , <i>S. tuberosum</i> , <i>S.sparsipilum</i> , <i>S. phureja</i> , <i>S. sucrense</i>
PVS	<i>S. tuberosum gp.andigenum</i>
PVM	<i>S.gourlayi</i> , <i>S. megistacrolobum</i>

Potato viruses

Types of resistance

Virus resistance can be achieved through various genetically controlled reactions which include:

- **Resistance to infection** also known as mature plant resistance, and is polygenic in nature
- **Mature plant resistance,**
- **Resistance to virus accumulation/movement/multiplication, virus vector resistance,**
- **Hypersensitivity resistance (HR), & extreme resistance (ER) or immunity.**

Virus	Nature of Resistant
PLRV	Polygenic and can be achieved through simple genetic control of a single dominant
PVY & PVA	Monogenically inherited in cultivated and wild potato species
PVS	Polygenic and recessive; therefore it is a poor genetic source of resistance in a breeding programme
PVM	Inheritance was found to be monogenic dominant.

Potato Viruses

Non conventional/biotechnological breeding:

- Four different known *R* genes: *Ryadg*, *Rysto*, *Ryhou* and *Rychc*, confer ER to PVY and four *N* genes, viz., *Nychc*, *Nydms*, *Nctbr* and *Nyadg* that confer HR. Two new genes *Nytbr* and *Ny-1* were also identified.
- The PCR based CAPS marker **ADG2/BbvI** was the first diagnostic marker for selection of PVY resistant genotype in potato (Sorri et al., 1999).
- The SCAR marker **RYSC3** developed for the detection of *Ryadg* (Kasai et al. 2000) was widely used in breeding for PVY resistance.
- Hosaka et al. (2001) discovered the gene *Rychc* that confers ER to PVY in Japanese cv. Konafubuki

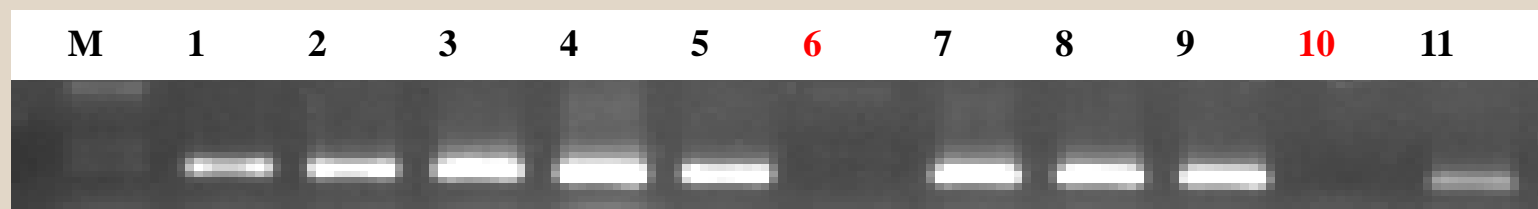
Potato Viruses

Non conventional/biotechnological breeding:

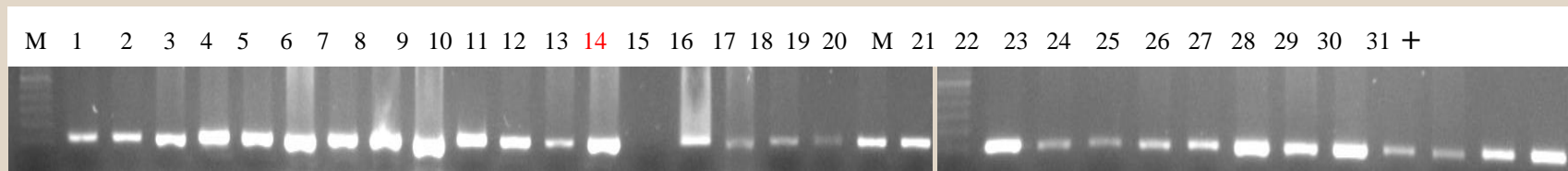
- R genes conferring extreme resistance are against PVX are $R_{x_{adg}}$ (R_{x1}), $R_{x_{tbr}}$, $R_{x_{acl}}$ (R_{x2}), $R_{x_{HB}^{scr}}/R_{x_{CP}^{scr}}$ and N genes are $N_{x_{acl}}$, $N_{x_{chc}}$, $N_{x_{tbr}}$, $N_{x_{tbr}^{spl}}$, $N_{x_{phu}}$.
- A single dominant gene N_s was reported which confers HR in *S. tuberosum gp. andigenum* to PVY and PVA
- The PVM resistance gene R_m originates from *S. megistacrolobum* which provides HR against PVM infection (Dziewonska and Ostrowska 1977).
- The R genes conferring ER to PVA were R_{ysto} , R_{asto} , R_{aadg} and R_{yho} whereas N genes were N_{aadg} , N_{asto} , N_{ychc} , N_{adms} , N_{ydms} , N_{atbr} and $N_{aKE brc}$ confers HR to PVA,

Identification of triplex parental line possessing PVY extreme resistance gene using Marker Assisted Selection

Segregation of SCAR marker among 11 offspring of the cross nulliplex xYY-13/3 C76



Segregation of SCAR marker among 31 offspring of the cross nulliplex xYY-6/3 C11



Potato is afflicted by six bacterial diseases viz.

- **bacterial wilt** or brown rot (*Ralstonia solanacearum* (Smith),
- **soft rot of stem** and tuber (*Erwinia carotovora*, *Bacillus spp.*, *Pseudomonas spp.*),
- **ring rot** (*Clavibacter michiganensis* ssp. *sepedonicus* (Spieck and Koth),
- **common scab** (*Streptomyces spp.*),
- **pink eye** (*Pseudomonas spp*) and
- **leaf spot** (*Xanthomonas vesicatoria*).

Ring rot and pink eye do not occur, while the leaf spot is a minor disease in India

Bacterial wilt or brown rot (*Ralstonia solanacearum*)

- Bacterial wilt, caused by *Ralstonia solanacearum* (Yabuuchi et al. 1995) is the second most important potato disease after late blight locally and globally (Kaguongo et al. 2008).

- Traditionally, *R. solanacearum* has been divided into five races (related to the ability to wilt members of the family *Solanaceae*)
 - r1,
 - r2 banana,
 - r3 potato & tomato in temperate
 - r4 ginger
 - r5 mulberry
- Based on this classification, potatoes are affected by two races of *R. solanacearum*,
 - r1, frequent at warmer areas & lower elevations in the tropics
 - r3, more common in higher elevations or latitudes

(Martin & French 1985)

(Buddenhagen et al. 1962; He et al. 1983; Pegg & Moffett 1971),



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Bacteria



Resistance: inheritance and expression:

Resistance to bacterial wilt is of polygenic and quantitative in nature involving genes.

Certain genes, other than those governing resistance show pleiotropic effect conferring resistance in certain set of environmental conditions.

Bacterial wilt resistance in potato is strain or temperature dependent and the so called resistant cultivars are tolerant as they harbor a large population of the pathogen in the system.



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Bacteria



BREEDING FOR BACTERIAL WILT

Conventional Breeding:

Cultivars such as **Cruza 148** and **Molinera** were found to have some degree of tolerance to bacterial wilt but still transmit latent infection to their progeny tubers (French 1994).

Resistance in ***S phureja*** is strain as well as temperature specific and breaks down in warm climate.

Varieties **Molinera**, **Caxamarca** and **Serrena**, reported to be resistant to bacterial wilt in S America becomes susceptible under Indian condition.

Potato Cyst Nematodes (PCN)

- **Two types of PCN have been reported worldwide:**
 - I. *Globodera pallida* (white cyst nematode)**
 - II. *Globodera rostochiensis* (golden cyst nematode)**
- *Both are quarantine pests causing serious yield losses to the potato crop worldwide including India*

Symptoms:

Potato plants heavily infested with PCN have stunted growth, yellowing of the foliage occurs, wilting and eventually die prematurely while plants with evenly distributed infestation levels, there is gradual reduction in yield over the years

Pathotypes:

- Depending on the classification scheme used,
 - *G. rostochiensis* has five pathotypes and four races,
 - whereas *G. pallida* has three pathotypes and seven races.
- In India, the differential host reactions of PCN populations from Nilgiris and Kodaikanal hills revealed that the pathotypes **Ro₁** of *G. rostochiensis* and **Pa₂** of *G. pallida* were the most prevalent forms accounting for 75% of the total populations.
- Other pathotypes Pa₁ accounted for 15% followed by Ro₂ at 7%. The least prevalent pathotypes Pa₃ and Ro₅ accounted for only 3% but were able to multiply distinctly on differential hosts indicating their virulent capacity (Prasad 1996).

Potato Cyst Nematodes (PCN)

Source of resistance:

The wild species mostly exploited in PCN resistance breeding are *S. tuberosum ssp. andigena*, *S. vernei* and *S. spagazzini*, *S. gourlayi*, *S. sparsipilum*, *S. chacoense*, *S. phureja*, *S. demissum*, *S. gourlayi*, *S. microdontum*, *S. sucrense*, *S. tarijense*, *S. acaule*, *S. fendleri*, *S. multidissectum*, *S. oplocense*.

A number of PCN resistance genes have been mapped in different potato chromosomes conferring specific and partial resistance.

Major Genes imparting specific resistance to *G.rostochiensis* are *H1*, *GroVI*, *Gro1* while *Gpa2*, *GpaV* and *GpaXI* genes against *G.pallida*.



Potato Cyst Nematodes (PCN)



BREEDING FOR PCN

Conventional Breeding:

Toxopeus and Huijsman (1953) reported PCN resistance in *S. tuberosum ssp. Andigena*, through a single dominant gene **H1** conferring effective resistance against pathotypes Ro 1 and Ro 4 of *G. rostochiensis*. This major gene was subsequently incorporated in breeding programme throughout Europe and in the USA.

Maris Piper in 1963 and **Saturna** in 1964, **Pentl** and **Javel** in 1968 were first resistant cultivars released possessing this gene.

The Scottish cultivar **Spey** with three copies of *H1* and cv. **Picasso** with two copies of *H1* were bred by intercrossing cultivars possessing *H1* gene. (Mackay, 2005).



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Potato Cyst Nematodes (PCN)



Non conventional/Biotechnological Breeding:

So far 19 genes/major and minor QTLs have been placed on potato chromosome map, conferring resistance against PCN.

The first PCN resistance gene mapped using RFLP markers was the oligogenic dominant *Gro1* gene against *G. rostochiensis* located on chromosome VII.

H1 is a dominant gene that confers durable resistance to pathotype Ro1 of *G. rostochiensis*.

Abiotic stress:

Imperative Factor includes:

- Heat/high temperature
- Drought
- Frost

Potato yield is optimal under growing conditions with adequate light, water and cool temperatures. Heat and drought stress greatly influence potato yield. Total and marketable yield substantially decrease after short periods of severe stress. There are, however, variations in the degree to which cultivars are affected by these stresses.



Heat/high temperature



- The potato has long been considered a crop for cool and temperate climates.
- Higher temperatures inhibit yield by overall reduction of plant development due to heat stress or by reduced partitioning of assimilates to tubers.
- Tuberization is reduced at night temperatures above 20°C with complete inhibition of tuberization above 25°C.
- Exposure of potato plants to heat stress alters the hormonal balance in the plants.

Heat/high temperature

Effects of high temperature:

- Strongly reduce the harvest index in potato.
- Stolon initiation/development is significantly delayed
- Branching and growth of stolons is stimulated but tuberization is delayed.
- Delay, impede or even inhibit tuber initiation. Potato tuber formation is badly affected due to high night temperatures of above 20°C

Heat/high temperature

Breeding Strategy:

While breeding for heat tolerant variety, the factors that are considered important and should be taken into account includes

- Plant ability to tuberize at night temp. of 22^o C and above,
- Low shoot/root ratio at high temp. & early maturity of crop.
- CPRI has developed K. Surya which has potential to tuberize up to 22^oC temperature and few more are in advanced stages of trial for release as a variety.
- The known heat tolerant genotypes used in the breeding programme at CPRI, Shimla are LT-1, LT-2, LT-5, LT-7, LT-8, LT-9, DTO-28, DTO-33 (received from CIP) Katahdin, Desiree & K. Lauvkar.

Frost Stress

- **Temperatures below -2°C in the field can produce partial or complete loss of the crop.**
- **In temperate zones, frosts can occur during spring when the crop is establishing itself, or during autumn when the crop is maturing.**
- **Higher crop losses occur in tropical highlands and subtropical plains where frosts can occur any time during the crop growth period.**
- **In India more than 80% of the potatoes are grown during winter in plains and the crop is prone to frosts during the months of December and January.**

BREEDING FOR FROST STRESS

Genetic variability exists in the genus *Solanum* with respect to frost injury. *S. acaule* has the ability to withstand extracellular ice formation up to -5°C , which gives this species frost tolerance.

This species can be used in the breeding programmes to transfer frost tolerance to *S. tuberosum*.

In North India, frost occurs in most years during December and January in the plains of Punjab and Eastern UP. Cultivars **Kufri Sheetman** and **Kufri Dewa** released by the Central Potato Research Institute possess resistance to frost.

*High degree of frost resistance was observed in other 28 hybrids from crosses involving *S. acaule*.*



THANKS