Bacterial Wilt and Virus Diagnostics and Control in Potatoes

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Presentation Outline

INTRODUCTION

PATHOGEN DETECTION

DISEASES MANAGEMENT

DATA BIRO PUSAT STATISTIK, 2019

Provinsi	Produksi Tanaman Sayuran Kentang (Ton) 2019
ACEH	26529
SUMATERA UTARA	118778
SUMATERA BARAT	50730
RIAU	0
JAMBI	111812
SUMATERA SELATAN	672
BENGKULU	4093
LAMPUNG	297
KEP. BANGKA BELITUNG	0
KEP. RIAU	0
DKI JAKARTA	0
JAWA BARAT	245418
JAWA TENGAH	294015
DI YOGYAKARTA	0
JAWA TIMUR	320209
BANTEN	13
BALI	208
NUSA TENGGARA BARAT	1503
NUSA TENGGARA TIMUR	530
KALIMANTAN BARAT	0
KALIMANTAN TENGAH	0
KALIMANTAN SELATAN	0
KALIMANTAN TIMUR	0
KALIMANTAN UTARA	0
SULAWESI UTARA	87543
SULAWESI TENGAH	1294
SULAWESI SELATAN	50629
SULAWESI TENGGARA	5
GORONTALO	0
SULAWESI BARAT	289
MALUKU	1
MALUKU UTARA	0
PAPUA BARAT	18
PAPUA	71

TOTAL POTATO PRODUCTION IN INDONESIA YEAR 2019 IS 1.314.657 TON

Fig. 10.2 Symptoms of potato brown rot with bacteria oozing from cut vascular tissues (**a**) and eyes (**b**) (UK Crown Copyright—Courtesy of Fera Science Ltd.), and wilted plant in the field (**c**) (Courtesy of International Potato Center)

Fig. 10.4 Foliar symptoms of *Dickeya dianthicola* on potato. Initial symptoms are either a lack of emergence or leaf curling (**a**). The base of the stem turns dark brown or black and this necrosis can extend several centimeters from the soil line (**b**). The pith inside symptomatic stems is often decayed and the xylem are necrotic for several centimeters above the external stem necrosis and the pith decay (**c**). Disease symptoms may only develop on one stem of a multi-stem plant (**d**) (Courtesy of Amy O. Charkowski, Colorado State University)

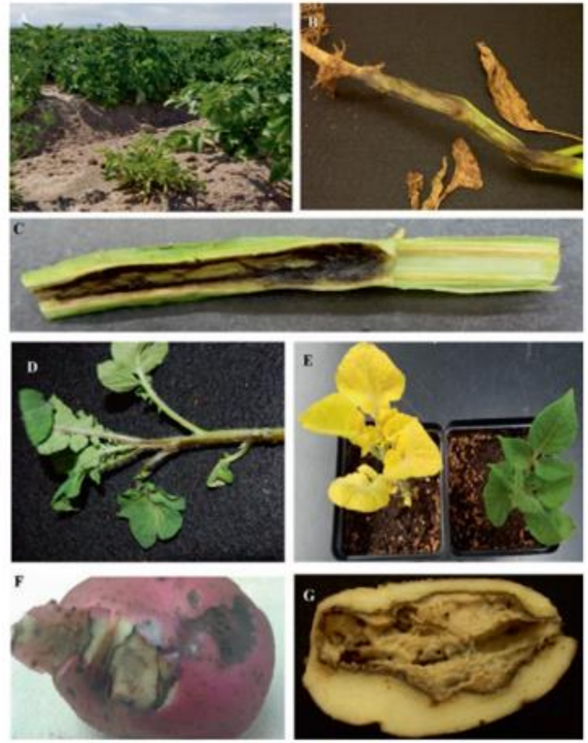
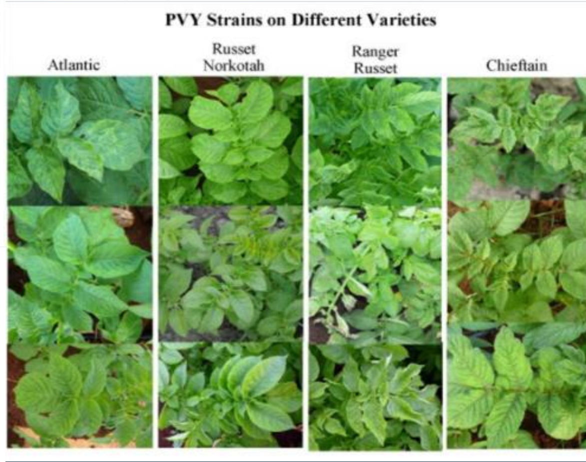


Fig. 10.3 Bacterial blackleg and tuber soft rot symptoms caused by *Pectobacterium* on potato. (**a**) Plants with blackleg are shorter and have curled leaves (A), the stem is blackened on the outside (**b**), the pith inside is decayed and the xylem are brown (**c**). Brown or black decay may spread into leaves (**d**) or leaves may turn bright yellow (**e**). Tubers may have swollen lenticels and sunken lesions (**f**). The soft rot bacteria may enter the tuber through the stolen and decay the center of the tuber (**g**) (Courtesy of Amy O. Charkowski, Colorado State University)



Identification of the pest, understanding its biology and seasonal population trends, damaging life stages and their habitats, nature of the damage and its economic significance, the vulnerability of each life stage for one or more control options, host preference and alternate hosts, predictability of pest occurrence based on the environment, cropping trends, farming practices, and other influencing factors, and **all the related information is critical for identifying an effective control strategy**

Dara, S.K., 2019

Veg-Impact Team conducted two visits in 2018 to seed production sites in Indonesia and made an inventory of bacterial and virus problems.

Samples were taken on FTA-cards and analysed by EWINDO in Purwakarta and Wageningen Research in Wageningen. The results of both labs were compared

Bacteria tests: PCR-tests were carried out to detect Dickeya spp., *Pectobacterium brasiliense* (Pb), *Pectobacterium parmentieri* (Pp), *Pectobacterium atrosepticum* (Pa) and *Ralstonia solanacearum* (Rs).

For *Ralstonia* spp., EWINDO used primers published by Opina et al., 1997. WUR used primers published by Sedighan et al., 2020. Both sets of primers will detect both, *R. solanacearum* and *R. pseudosolanacearum*.

For *Pectobacterium,* EWINDO used EXPCCF/EXPCCR primers which detect *Pectobacterium carotovorum*. WUR used primers able to detect the more aggressive *Pectobacterium* spp. *P. parmentieri*, *P. brasiliense*, *P. atrosepticum* and *Dickeya* spp.

				Symptoms	*R. solanacearum*		*P. carotovorum*	*P. brasiliense*	*P. parmentieri*	
Sample	Date	Location	Cultivar	observed	Ewindo	WUR	Ewindo	WUR	WUR	Conclusion
1	15-sep	Ampelgading	Granola	bacterial wilt	Rs	Rs	-	-	-	Rs
2	15-sep	Ampelgading	Granola	bacterial wilt	Rs	RS	-	Pb	-	Pb + Rs
3	15-sep	Ampelgading	Granola	bacterial wilt	Rs	Rs	-	Pb	-	Pb + Rs
8	17-sep	Kaligedang	Granola	Blackleg	-	Rs	-	Pb	Pp	Pb + Pp + Rs
12	17-sep	Kaligedang	Granola	Bacteria	-	Rs	-	Pb	pp	Pb + Pp + Rs

				Symptoms	*R. solanacearum*		*P. carotovorum*	*P. brasiliense*	*P. parmentieri*	
Sample	Date	Location	Cultivar	observed	Ewindo	WUR	Ewindo	WUR	WUR	Conclusion
5	27-11-2018	Pangalengan	unknown	bacteria	-	-	-	Pb	-	Pb
9	5-12-2018	Ampelgading	Atlantic	bacteria	Rs	Rs	-	Pb	-	Pb + Rs
10	5-12-2018	Ampelgading	Atlantic	bacteria	-	-	-	Pb	-	Pb
11	5-12-2018	Ampelgading	Atlantic	bacteria	-	-	-	Pb	-	Pb
12	5-12-2018	Ampelgading	Atlantic	bacteria	-	-	-	Pb	-	Pb
13	5-12-2018	Ampelgading	Atlantic	bacteria	-	-	-	Pb	-	Pb
14	5-12-2018	Ampelgading	Granola	bacteria	-	-	-	Pb	-	Pb
15	5-12-2018	Ampelgading	Granola	bacteria	-	-	-	Pb	-	Pb
26	6-12-2028	Ampelgading	Granola	bacteria	-	-	-	Pb	-	Pb
27	6-12-2028	Ampelgading	Granola	bacteria	-	-	-	Pb	-	Pb
28	6-12-2028	Ampelgading	Granola	bacteria	-	-	-	Pb	-	Pb

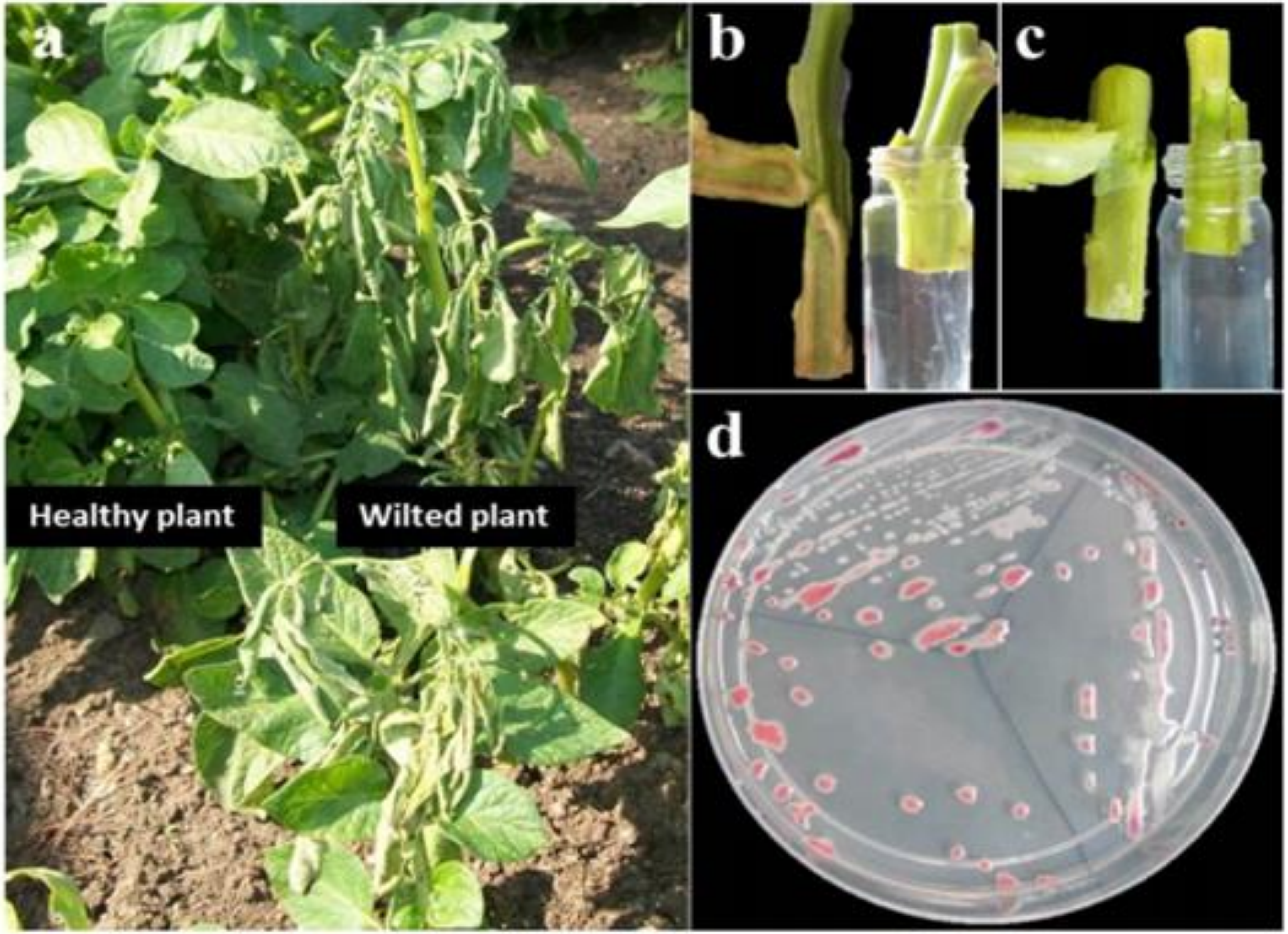
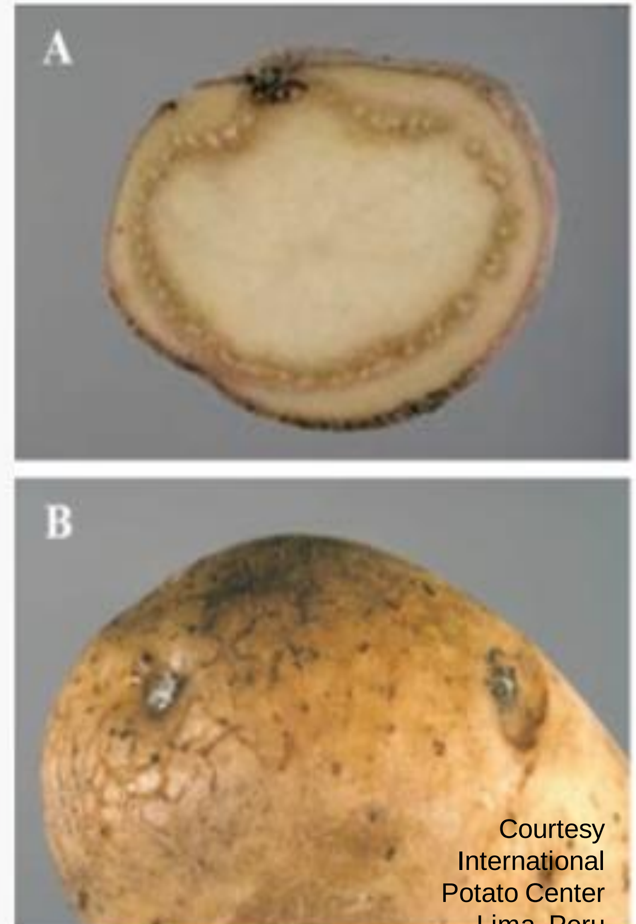


Figure 1. Symptoms of bacterial wilt of potato caused by *R. solanacearum* (a); brown discoloration of vascular tissues in stem and bacterial streaming in clear water from stem of infected plant (b) in comparison with healthy plant stem (c) and typical *R. solanacearum* colonies on TZC agar medium (d).

Domain: Bacteria
Phylum: Proteobacteria
Class: Betaproteobacteria
Order: Burkholderiales
Family: Ralstoniaceae
Genus: Ralstonia
Species: *Ralstonia solanacearum*



Courtesy International Potato Center Lima, Peru

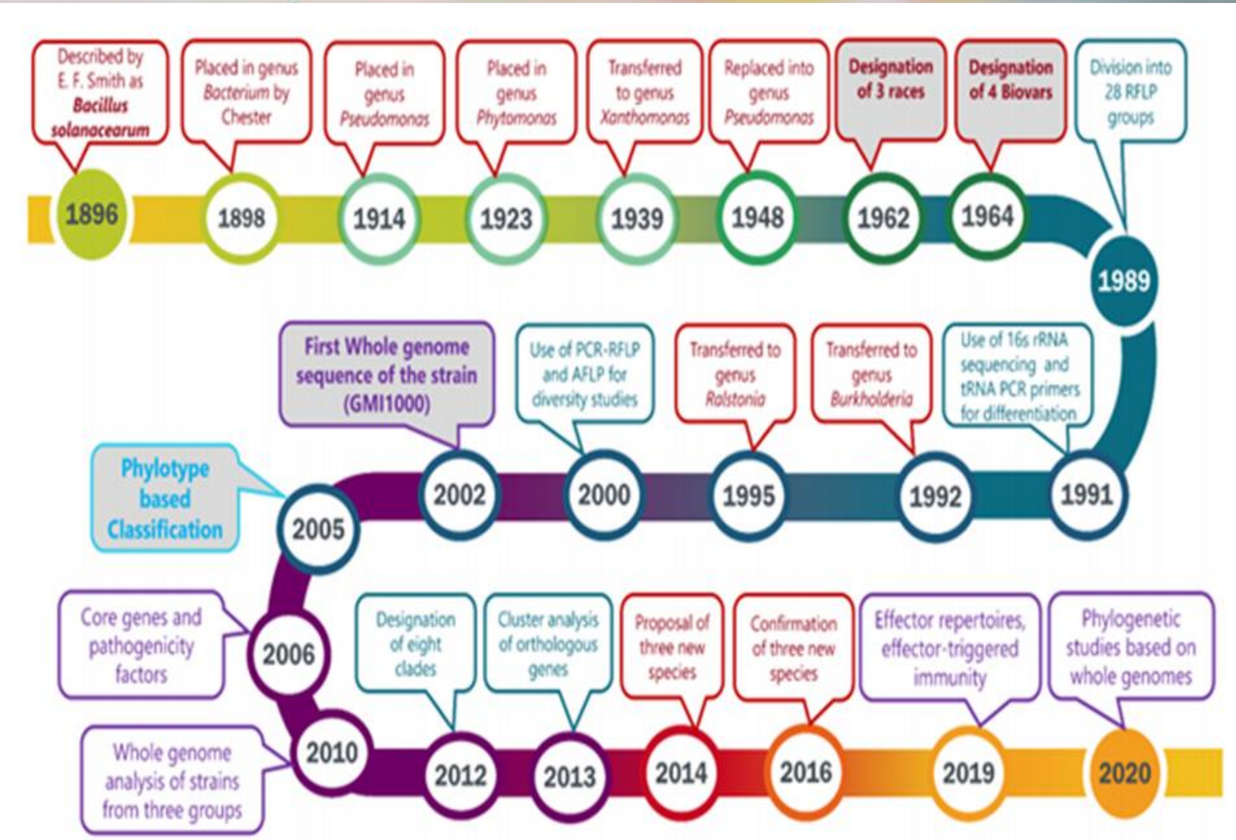
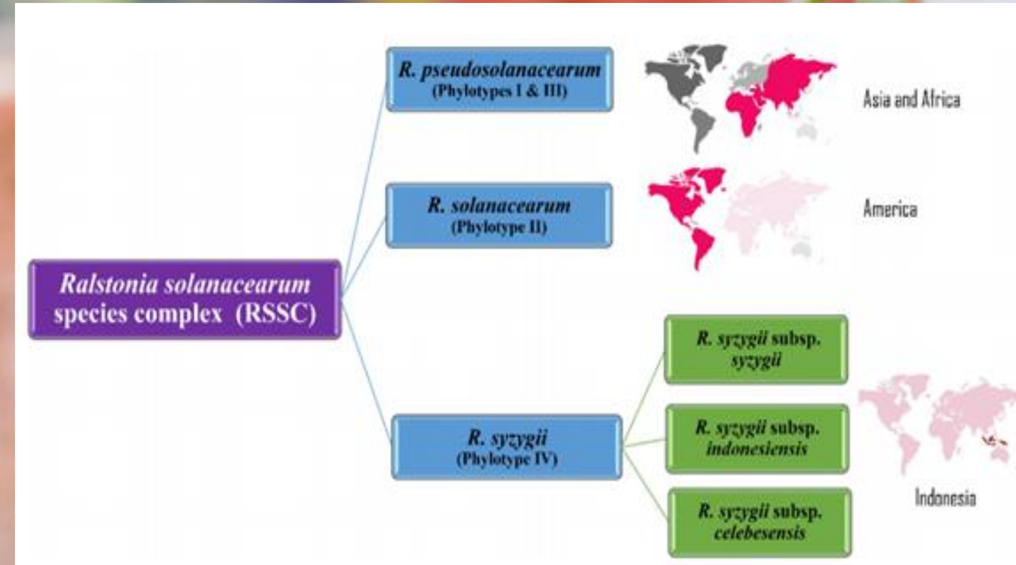


Figure 1. Timeline highlighting the major events based on taxonomic and phylogenetic studies of the bacterial wilt pathogen now in the *Ralstonia solanacearum* species complex (RSSC). Events in red boxes with red fonts indicate major changes in taxonomy and nomenclature. Blue boxes with blue fonts indicate milestones in the DNA-based analyses of RSSC. Purple boxes with purple fonts represent genomic advancements in understanding the diversity of multiple strains of RSSC. Bold labels with grey backgrounds indicate landmark taxonomic and genomic events. Landmark publications associated with each date are provided in the text associated with the date.

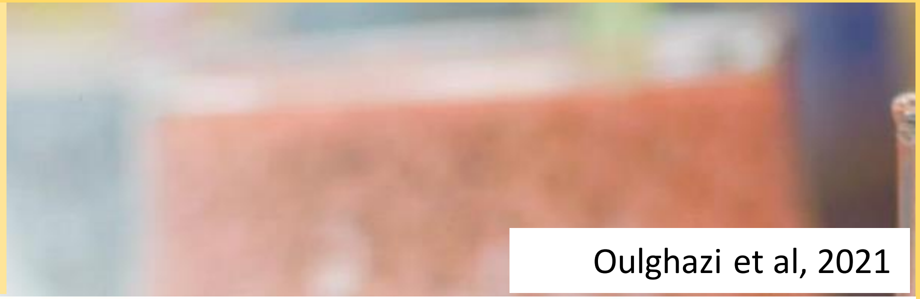
Paudel et al., 2020



Species	Phylotypes	Sequevar
Ralstonia solanacearum	Phylotype IIA	6, 7, 24, 35, 36, 37, 38, 39, 40, 41, 50, 52, 53
	Phylotype IIB	1, 3, 4, 25, 26, 27, 28, 51, 54, 55, 56 57
Ralstonia pseudosolanacearum	Phylotype I	12, 13, 14, 15, 16, 17, 18, 31, 34, 44, 45, 46, 47, 48, 54, 55, 56, 57
	Phylotype III	19, 20, 21, 22, 23, 29, 42, 43, 49, 58, 59, 60
Ralstonia syzygii	Phylotype IV	8, 9, 10, 11

P. brasiliense was first isolated from blackleg-diseased potato plants in Brazil 2004 Since then, it has been found in association with many plants causing symptoms in many parts of the world.

Domain: Bacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Enterobacteriales
Family: Enterobacteriaceae
Genus: Pectobacterium
Spesies: *Pectobacterium brasiliense*



Oulghazi et al, 2021

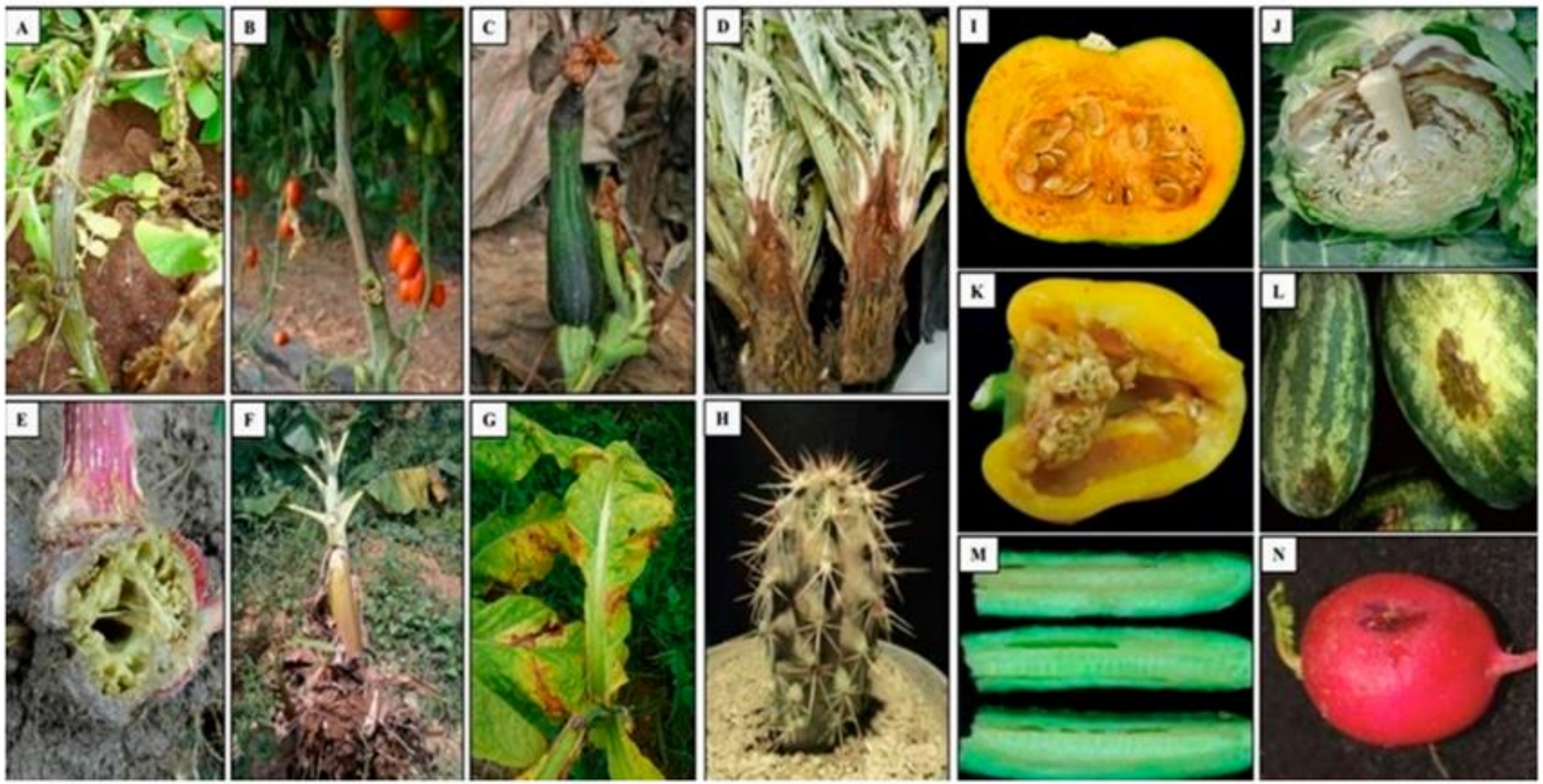


Figure 3. Symptoms caused by Pbr in several plant species. **(A)** Potato (*S. tuberosum*), **(B)** Tomato (*Solanum lycopersicum*), **(C)** Zucchini (*Cucurbita pepo*), **(D)** Artichoke (*Cynara cardunculus* var. *scolymus*), **(E)** Amaranth (Amaranthus), **(F)** Banana (*Musa* sp.), **(G)** Tobacco (*Nicotiana tabacum*), **(H)** Tetecho (*Neobuxbaumia tetetzo*), **(I)** Squash (*Cucurbita pepo*), **(J)** Cabbage (*Brassica oleracea*), **(K)** Pepper (*Capsicum annuum*), **(L)** Watermelon (*Citrullus lanatus*), **(M)** Cucumber (*Cucumis sativus*), **(N)** Raphanus (*Raphanus sativus*) [6,7,63,64,82,85–87].

There are 11 families in Dicotyledon and 1 family in Monocotyledon reported as host of *P. Brasiliense*

- **Solanaceae**: potato (*S. tuberosum* L.), tomato (*S. lycopersicum*), pepper (*C. annuum*), eggplant (*S. melongena*), and tobacco (*N. tabacum*)
- **Cucurbitaceae**: Cucumber (*Cucumis sativus*), Zucchini (*Cucurbita pepo*) and watermelon (*Citrullus lanatus)*
- **Brassicaceae**: Cabbage (*Brassica oleracea* var. capitata), Chinese cabbage (*Brassica rapa* ssp. pekinensis and chinensis), Raphanus (*Raphanus sativus*)
- **Asteraceae**: Chrysanths (*Chrysanthemum*), Artichoke (*Cynara cardunculus* var. scolymus)
- **Amaranthaceae**: Sugar beet (*Beta vulgaris*)
- **Chenopodiaceae:** Amaranth (*Amaranthus*)
- **Cactaceae**: Tetecho (*Neobuxbaumia tetetzo*)
- **Nepenthaceae**: Nepenthes (*Nepenthes*)
- **Malvaceae**: Bull Mallow (*Malva nicaensis*), *Gossypium sp*,
- **Primulaceae**: *Cyclamen sp*
- **Caricaceae**: *Carica papaya*
- **Musaceae**: Banana (Musa sp.)

P. parmentieri can cause tuber soft rot and blackleg. Affected plants are wilted and stunted with black soft rot extending upwards from the mother tuber. **The symptoms are very similar to those caused by the common soft rot bacterium, common blackleg and *Dickyea***

The university of Maine
Cooperative Extension, Bulletin 2448

Recent comprehensive analysis on *P. wasabiae* genomes resulted in reclassification of all *P. wasabiae* potato-originating isolates into a newly established species *P. parmentieri* (CABI)

Source of inoculum: The bacterium is found in the seed, usually at the stem end of the tubers or in and around the lenticels.

Disease Development: Infected seed can break down in the field reducing stands. Stems which do emerge are often weak with wilted foliage. These plants then spread the disease to healthy plants nearby.
***P. parmentieri* develops most rapidly during warm, wet summers and often kills the entire plant.**

Disease Spread: *P.parmentieri* can spread in the field below-ground, when water moves in soil saturated by rain or irrigation. Infected tubers contaminate harvesting and handling equipment. The infested equipment can transfer bacteria to tubers dug from healthy fields. The infected asymptomatic tubers may show no soft rot and may not decay in storage because of the low temperatures. However, the bacteria are likely to cause and spread disease if such tubers are planted.

Following the principles of IPM for Bacterial Wilt Diseases:

Prevention

- Crop rotation with non-Solanaceous crops;
- Avoid planting in infected fields;
- Use disease-free seed or resistant varieties;
- Sterilize seedling media;
- Check and maintain soil pH (6.2-6.5);
- Add organic matter in the soil;
- Raise beds to improve drainage;
- Avoid furrow irrigation;
- Wash hands after handling infected plants;
- Disinfect used tools;

Monitoring

- Observe initial symptoms: sudden wilting of shoot tips with no yellowing;
- Do the bacterial ooze test to confirm that it is bacterial wilt
- Print on FTA card and send to Ewindo lab for identification

Direct measures

- Uproot all infected plants and destroy (burn);
- Solarization or bio-fumigation (with Brassica residues);
- Fungicides or bactericides are NOT effective to control bacterial wilt.

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Virus tests: Elisa tests were used to detect PVY, PVA, PVX, PVS, PVM and PLRV. In addition, an RT-PCR-test + NAD5 check was carried out to detect PLRV.

PVY: WUR used a serological test (ELISA) whereas Ewindo used a generic PCR for potyviruses Poty MJ-1/Poty MJ-2 (Marie-Jane et al., 2000)

					Virus	
Sample	**Date**	**Location**	**Cultivar**	**Observed symptoms**	Ewindo	WUR
5	15-sep	Ampelgading	Granola	Virus	none detected	none detected
6	15-sep	Ampelgading	Granola	Virus	none detected	PVY
7	15-sep	Ampelgading	Granola	Virus	none detected	none detected
9	17-sep	Kaligedang	Granola	Virus	none detected	none detected
10	17-sep	Kaligedang	Granola	Virus	none detected	none detected
11	17-sep	Kaligedang	Granola	Virus	none detected	PVY
13a	17-sep	Kaligedang	Granola	Virus	none detected	none detected
14	17-sep	Kaligedang	Granola	Virus	none detected	none detected
15	17-sep	Kaligedang	Granola	Virus	none detected	none detected
16	17-sep	Kaligedang	Granola	Virus	none detected	none detected

					Virus	
Sample	**Date**	**Location**	**Cultivar**	**Observed symptoms**	Ewindo	WUR
7	27-11-2018	Pangalengan	unknown	virus	-	PVY
8	27-11-2018	Pangalengan	unknown	virus	-	none detected
16	5-12-2018	Ampelgading	Granola	virus	-	none detected
17	5-12-2028	Ampelgading	Granola	virus	-	none detected
18	5-12-2018	Ampelgading	Granola	virus	-	none detected
20	6-12-2018	Ampelgading	Granola	virus	-	none detected
21	6.12.2018	Ampelgading	Granola	virus	-	none detected
22	6-12-2018	Ampelgading	Granola	virus	-	none detected
23	6-12-2018	Ampelgading	Granola	virus	-	none detected
24	6-12-2018	Ampelgading	Granola	virus	-	none detected
25	6-12-2028	Ampelgading	Granola	virus	-	none detected
26a	6-12-2028	Ampelgading	Granola	virus	-	none detected
29	6-12-2018	Ampelgading	Granola	virus	-	none detected

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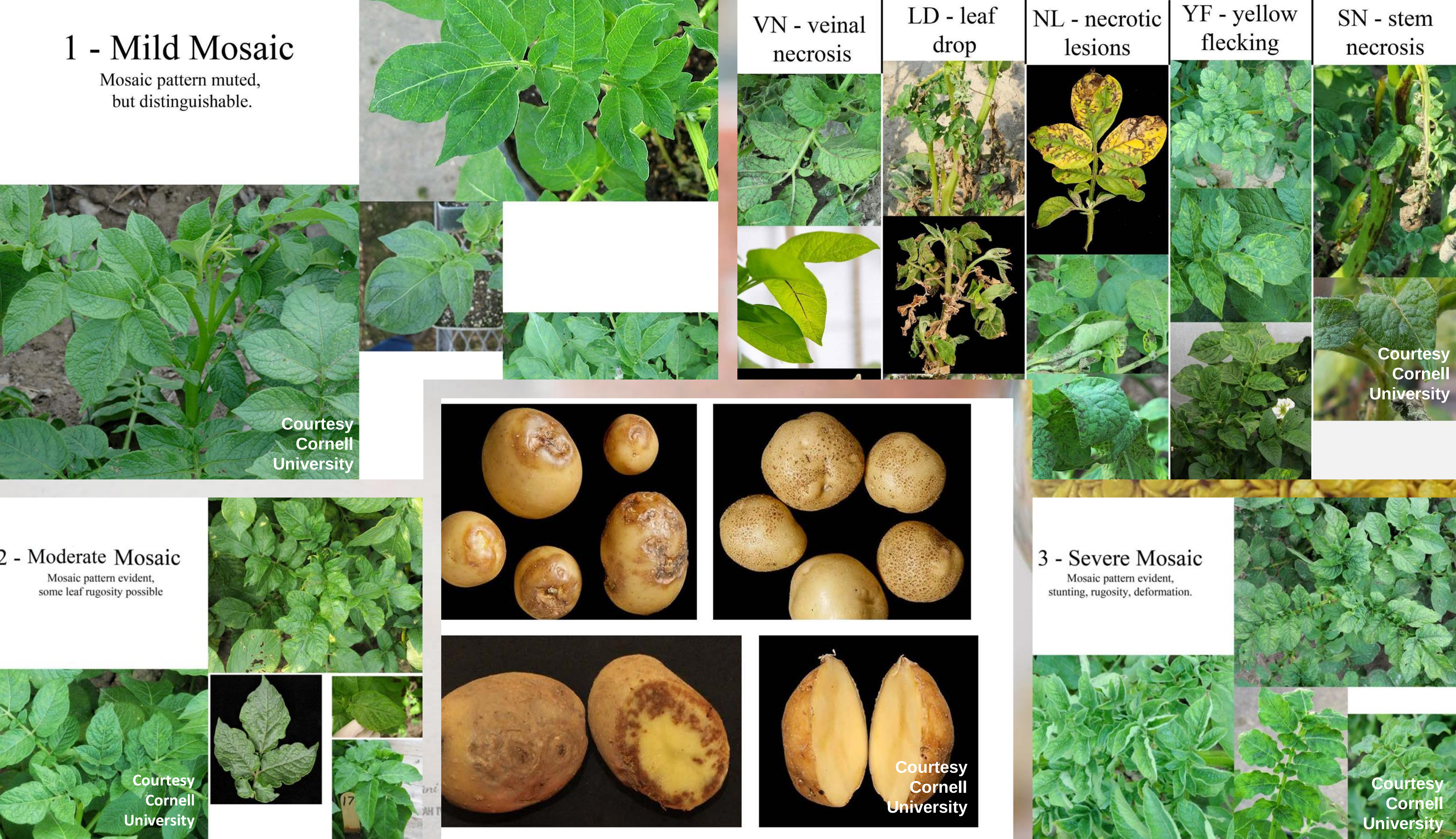
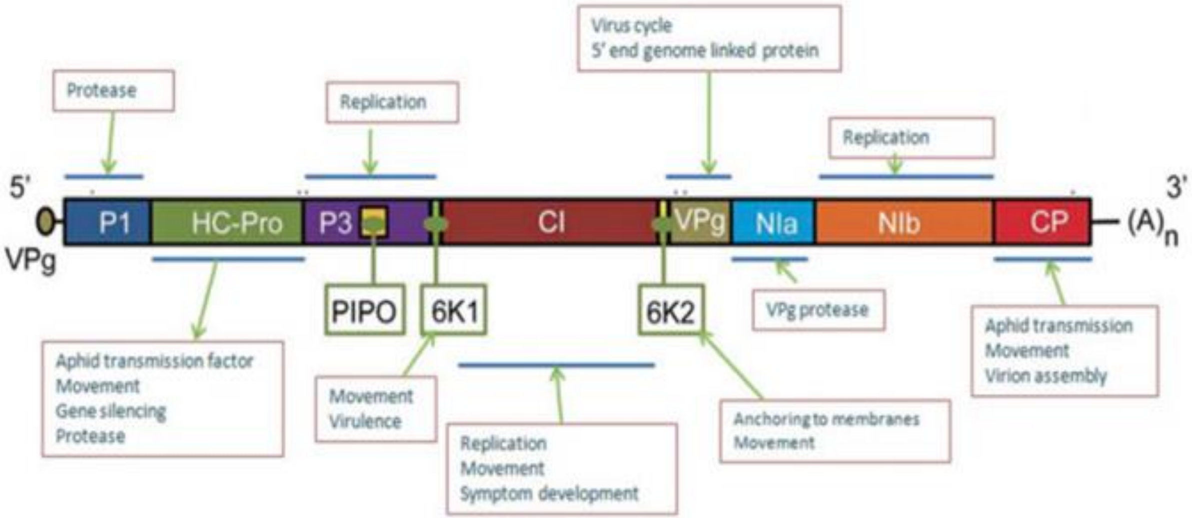
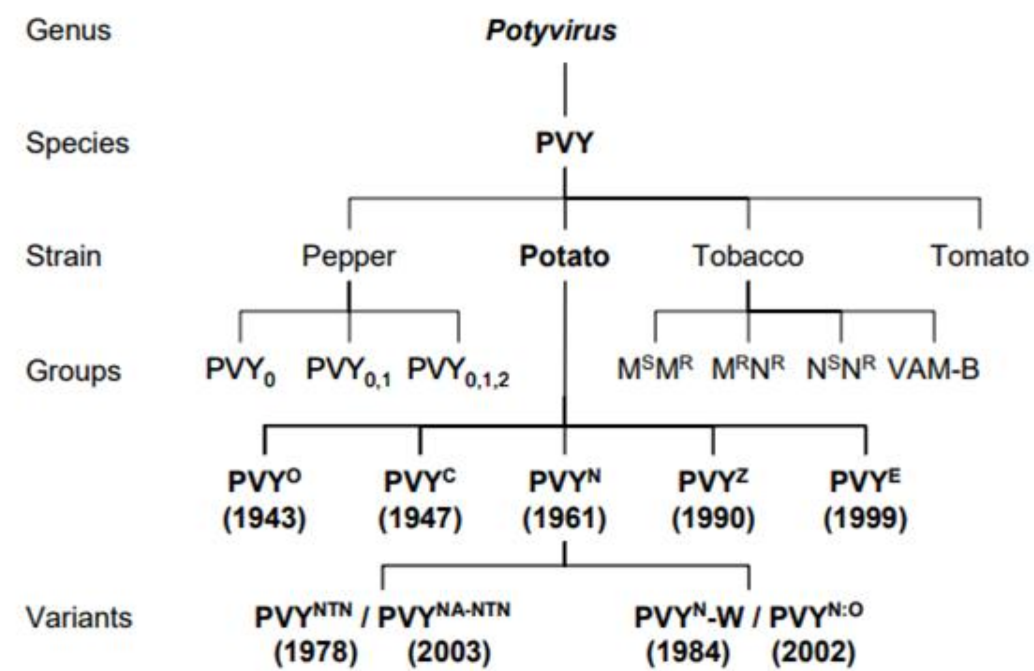


1 - Mild Mosaic
Mosaic pattern muted,
but distinguishable.
Courtesy
Cornell
University
VN - veinal
necrosis
LD - leaf
drop
NL - necrotic
lesions
YF - yellow
flecking
SN - stem
necrosis
Courtesy
Cornell
University
2 - Moderate Mosaic
Mosaic pattern evident,
some leaf rugosity possible
Courtesy
Cornell
University
Courtesy
Cornell
University
3 - Severe Mosaic
Mosaic pattern evident,
stunting, rugosity, deformation.
Courtesy
Cornell
University





	Host species[a]						
	Tobacco	Potato				Chenopodium	Physalis
	Veinal leaf necrosis 	Local lesions[b] 			Tuber necrosis 	Leaf local lesion 	Leaf necrosis 
		Nc	*Ny$_{tbr}$*	*Nz*			
PVY^{O}	-	-	+	-	-	+	+
PVY^{C}	-	+	-	-	-	+	+
PVY^{N}	+	-	-	-	-[c]	-	-
PVY^{NTN}	+	-	-	-	+	-	-
PVY^{Z}	-	-	-	+	-	ND	ND
PVY^{E}	-	-	-	-	-	ND	ND

Following the principles of IPM for Virus Diseases:

Prevention

- Check crop field and pest history;
- Produce seed potatoes in favourable conditions (low vector pressure, isolated from other fields);
- Crop rotation;
- Use healthy certified seed;
- Use virus tolerant varieties;
- Net nursery for seedlings;
- Use reflective mulches;
- Attract beneficial insects by planting flowering plants;
- Eliminate infection source: infected plants, weeds, alternate hosts
- Disinfection of equipment.

Monitoring

- Regular monitoring of the field.
- Look at the underside of the leaves for insect vectors;
- Check distorted and curled leaves;
- Use sticky yellow and blue (thrips) traps to monitor for virus vectors.
- Take sample on FTA card and send to Ewindo lab for diagnostic tests

Direct measures

- The Technical Guide provides several insecticides to control aphids, thrips and white fly;
- The MyAgri APP provides botanical and chemical insecticides to control aphids and thrips that are available/registered in Indonesia;
- Use selective insecticides that do not harm the beneficial insects

CONCLUSION

- The finding bacterial diseases *P. Brasiliense, R. solanacearum* and *P. parmentieri showed bacterial diseases* are serious problem, they can survive in the soil for years so it should be much better managed

- Virus disease like PVY not so very common, current IPM program works sufficiently well.

- Potato Diseases monitoring and identification is important:
    - for seed production farmer to produce healthy seed and avoid tuber-borne pathogen spread to clean area
    - for commercial farmers to get high yield and high-quality potato for consumption

- Effective IPM depends on effective roguing and thus on the accuracy of pathogen recognition:
    - Train people to (better) recognize the visual diseases symptoms to improve roguing
    - Use FTA cards and diagnostics to confirm or reject visual diagnostics in the field


thank you