Taller Internacional: Emergencia fitosanitaria en el cultivo de papa en Ecuador y sus implicancias para el Perú y la región Andina: Punta Morada, psílido de la papa y manchado interno de la papa

CENTRO INTERNACIONAL DE LA PAPA
CIP
A member of the CGIAR Consortium

PERÚ Ministerio de Agricultura y Riego

inia
Instituto Nacional de Innovación Agraria

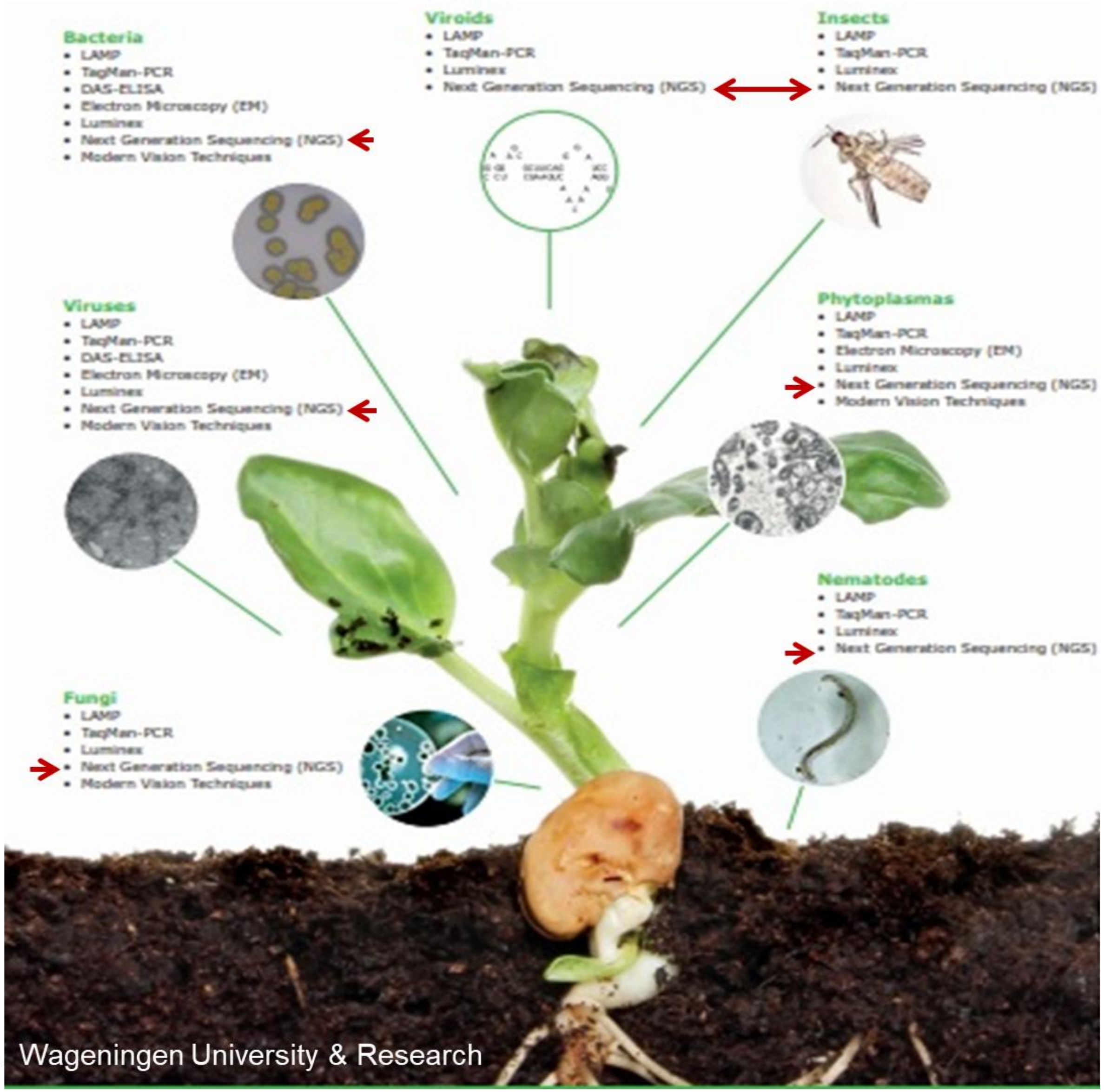
SENASA PERÚ

El uso de secuenciamiento de alto rendimiento para identificación y detección de patógenos en plantas

AÑO INTERNACIONAL DE LA SANIDAD VEGETAL 2020

Lima, 2020

Métodos para detectar patógenos de plantas



Bacteria
- LAMP
- TaqMan-PCR
- DAS-ELISA
- Electron Microscopy (EM)
- Luminex
- Next Generation Sequencing (NGS) ←
- Modern Vision Techniques

Viroids
- LAMP
- TaqMan-PCR
- Luminex
- Next Generation Sequencing (NGS) ↔

Insects
- LAMP
- TaqMan-PCR
- Luminex
- Next Generation Sequencing (NGS)

Viruses
- LAMP
- TaqMan-PCR
- DAS-ELISA
- Electron Microscopy (EM)
- Luminex
- Next Generation Sequencing (NGS) ←
- Modern Vision Techniques

Phytoplasmas
- LAMP
- TaqMan-PCR
- Electron Microscopy (EM)
- Luminex
- → Next Generation Sequencing (NGS)
- Modern Vision Techniques

Nematodes
- LAMP
- TaqMan-PCR
- Luminex
- → Next Generation Sequencing (NGS)

Fungi
- LAMP
- TaqMan-PCR
- Luminex
- → Next Generation Sequencing (NGS)
- Modern Vision Techniques

Wageningen University & Research

Que es el secuenciamiento de alta rendimiento (HTS o NGS)

Primera generación → Método SANGER
(1977)

Próxima generación (NGS)
o alta rendimiento (HTS).
(2005)

Tecnologías basadas en PCR


Roche 454 GS-FLX


Roche GS-Junior


Illumina MySeq


Illumina HiSeq 2000


Applied Biosystems (SOLiD)
5500xl Genetic Analyzer


Ion Torrent Sequencing

Tecnologías de secuenciación de moléculas individuales

Helicos
BioSciences Corporation

Oxford NANOPORE Technologies

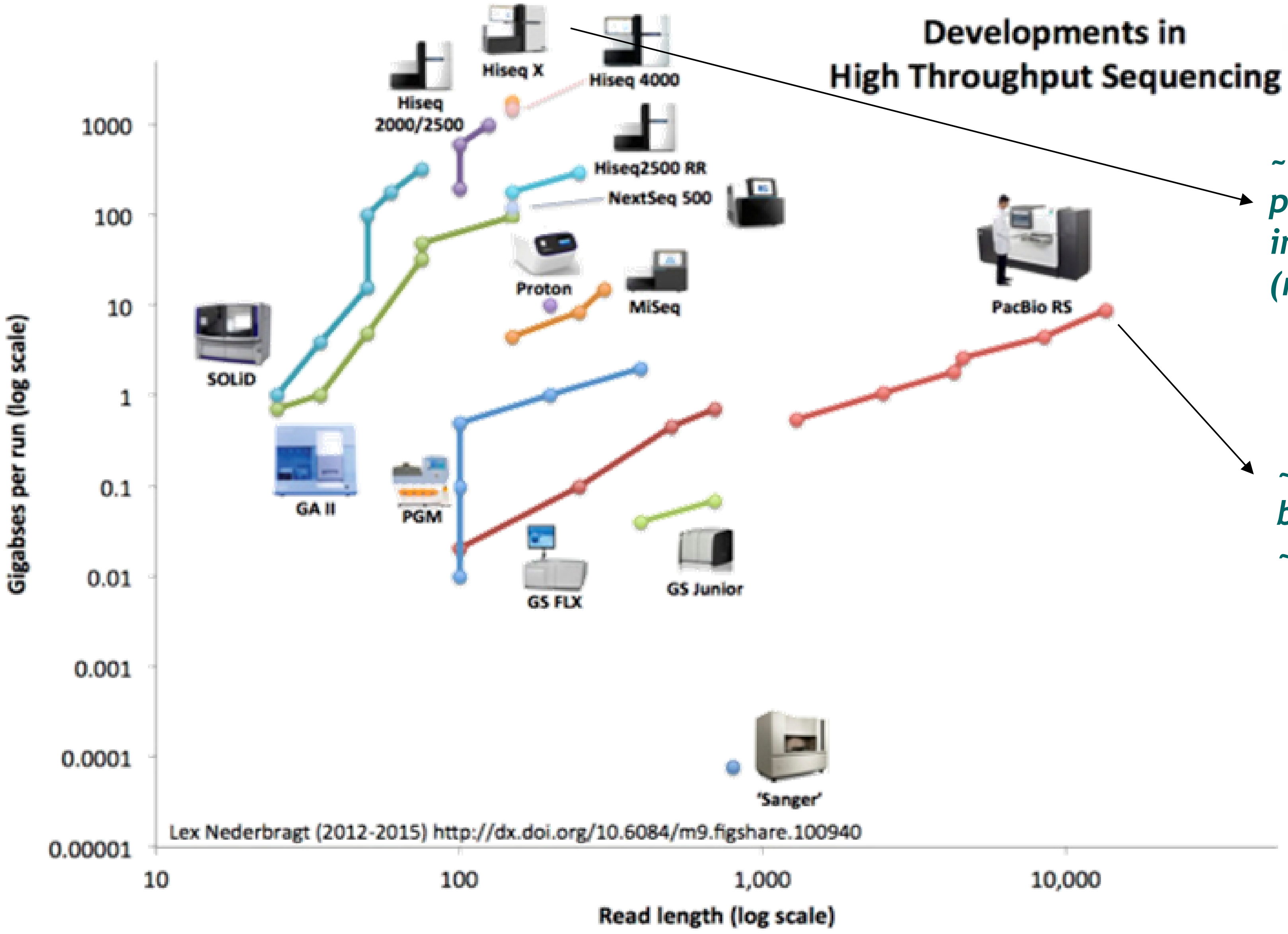
PACIFIC BIOSCIENCES

(Shokralla et al 2012)

Aunque las tecnologías HTS usan diversos procesos químicos para incorporación y detención, comparten dos pasos básicos:

- **preparación de liberarías de fragmentos/amplicones**
- **detección de los nucleótidos incorporados**

Que es el secuenciamiento de alta rendimiento (HTS o NGS)?


Developments in High Throughput Sequencing

Lex Nederbragt (2012-2015) http://dx.doi.org/10.6084/m9.figshare.100940

~500 US$/genoma de phytophthora infestans (resequencing >30x)

~300 US$/genoma de bacteria (de novo ~50x)

Contents lists available at ScienceDirect

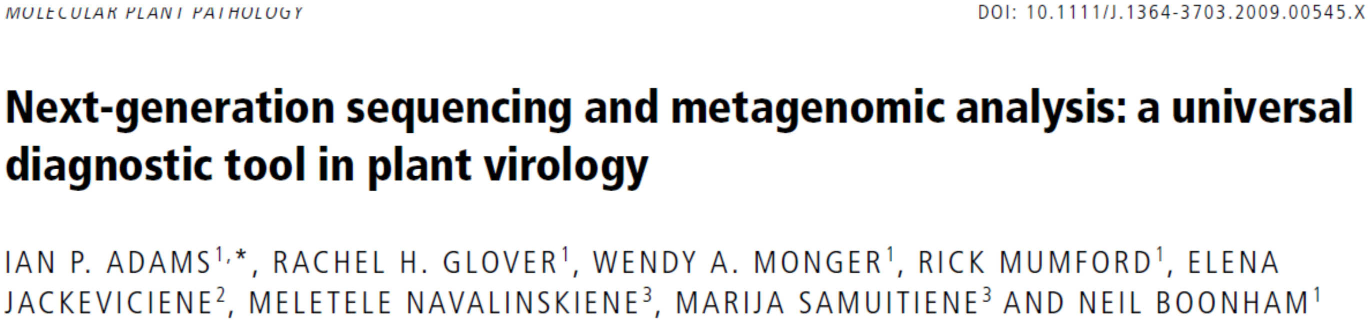
Virology

journal homepage: www.elsevier.com/locate/yviro

Rapid Communication

Complete viral genome sequence and discovery of novel viruses by deep sequencing of small RNAs: A generic method for diagnosis, discovery and sequencing of viruses

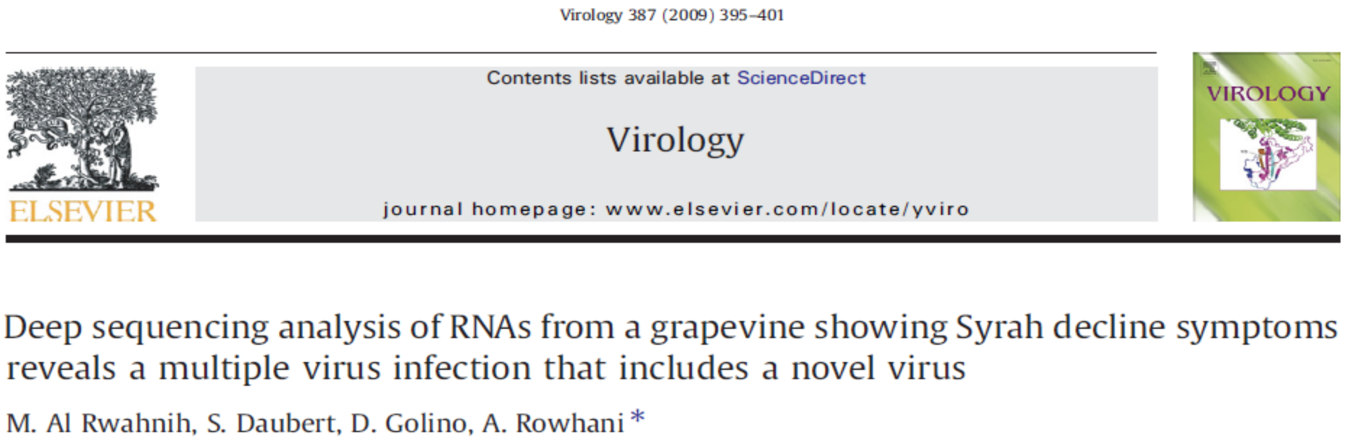
Jan F. Kreuze [a,d,*], Ana Perez [c], Milton Untiveros [a], Dora Quispe [a], Segundo Fuentes [c],
Ian Barker [c], Reinhard Simon [b]



MOLECULAR PLANT PATHOLOGY DOI: 10.1111/J.1364-3703.2009.00545.X

Next-generation sequencing and metagenomic analysis: a universal diagnostic tool in plant virology

IAN P. ADAMS[1,*], RACHEL H. GLOVER[1], WENDY A. MONGER[1], RICK MUMFORD[1], ELENA JACKEVICIENE[2], MELETELE NAVALINSKIENE[3], MARIJA SAMUITIENE[3] AND NEIL BOONHAM[1]



Virology 387 (2009) 395–401

Contents lists available at ScienceDirect

Virology

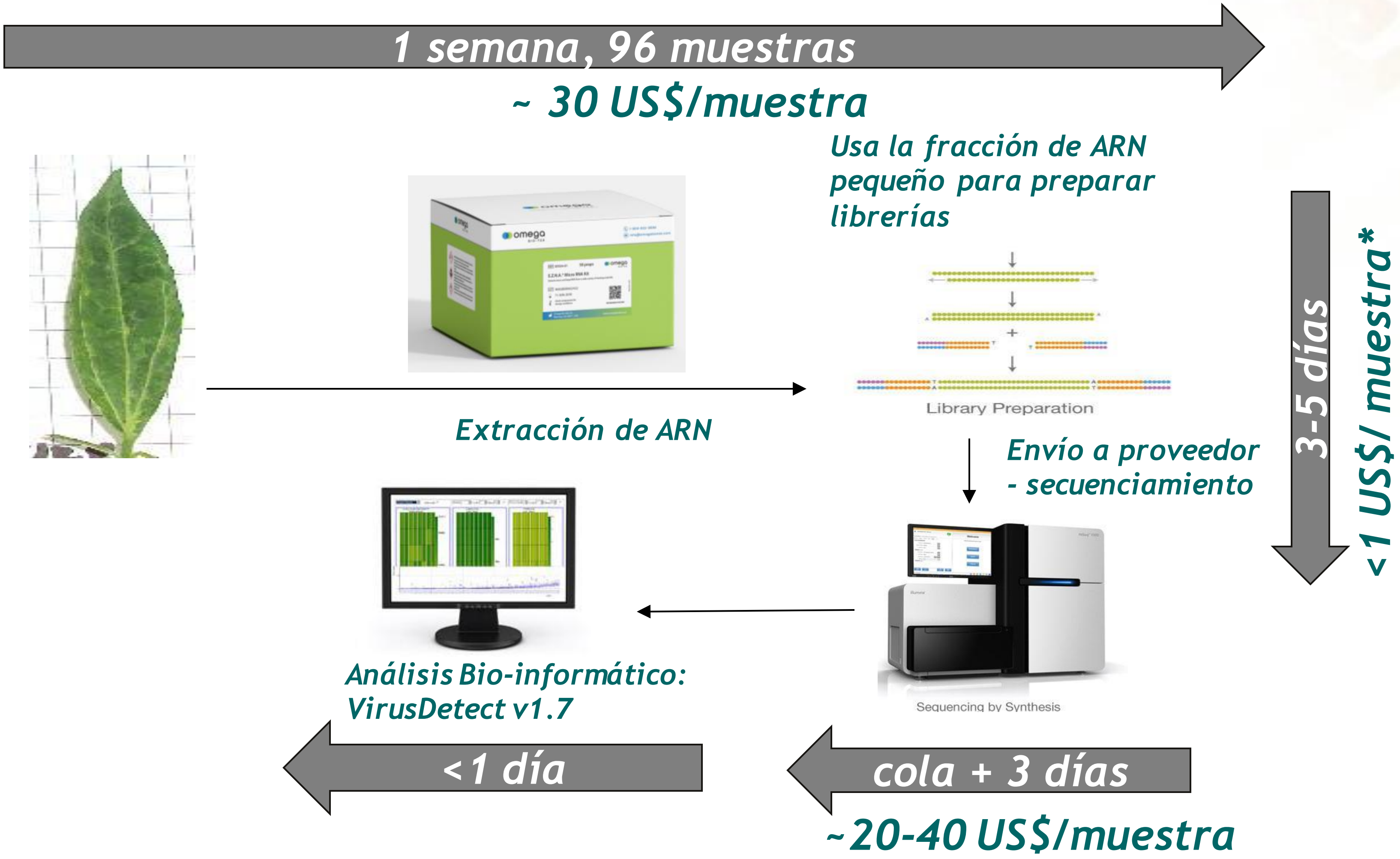
journal homepage: www.elsevier.com/locate/yviro

Deep sequencing analysis of RNAs from a grapevine showing Syrah decline symptoms reveals a multiple virus infection that includes a novel virus

M. Al Rwahnih, S. Daubert, D. Golino, A. Rowhani *

✓Amplia y gran adopción de etiología: desde 2009, >100 nuevos virus y 400+ publicaciones

Protocolo de laboratorio



1 semana, 96 muestras

~ 30 US$/muestra

Usa la fracción de ARN pequeño para preparar librerías

Extracción de ARN

Envío a proveedor - secuenciamiento

3-5 días

*<1 US$/ muestra**

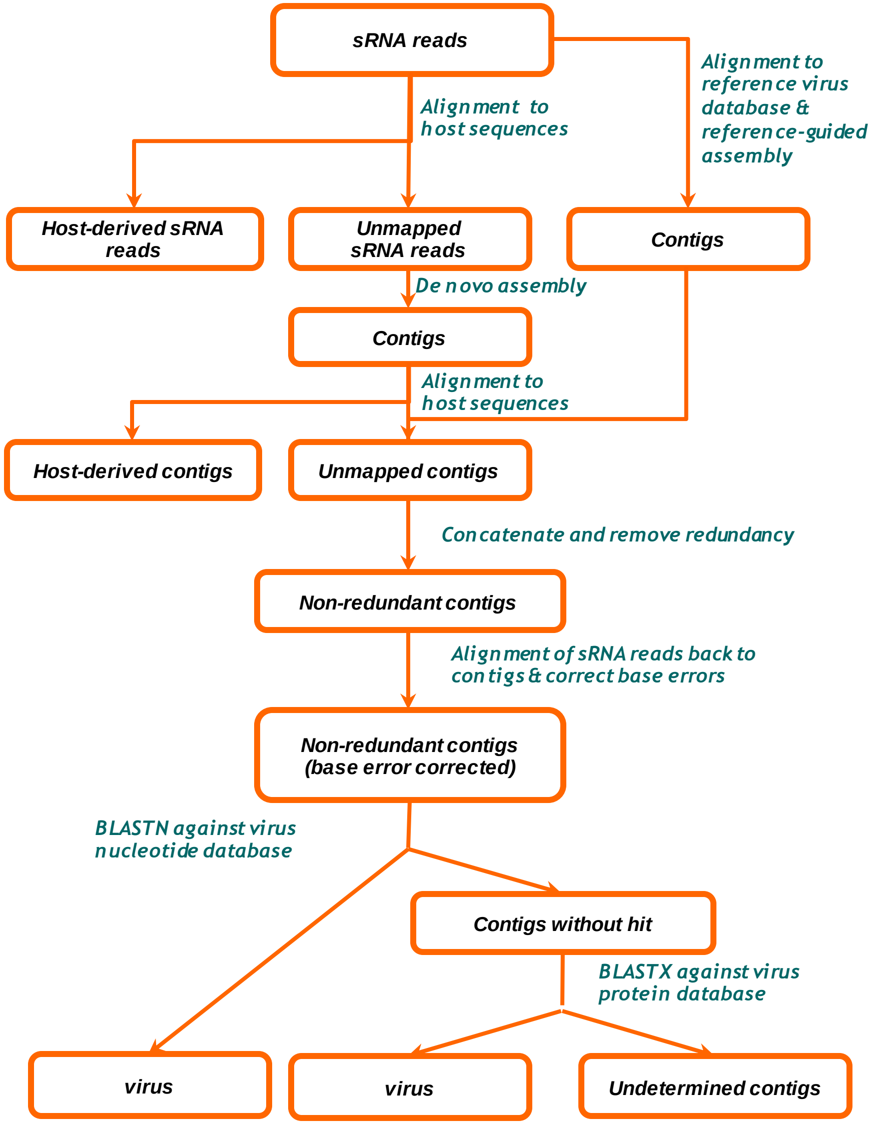
Análisis Bio-informático: VirusDetect v1.7

<1 día

cola + 3 días

~20-40 US$/muestra

Facilitando el análisis bio-informático: VirusDetect Windows



sRNA reads

Alignment to host sequences

Alignment to reference virus database & reference-guided assembly

Host-derived sRNA reads

Unmapped sRNA reads

Contigs

De novo assembly

Contigs

Alignment to host sequences

Host-derived contigs

Unmapped contigs

Concatenate and remove redundancy

Non-redundant contigs

Alignment of sRNA reads back to contigs & correct base errors

Non-redundant contigs (base error corrected)

BLASTN against virus nucleotide database

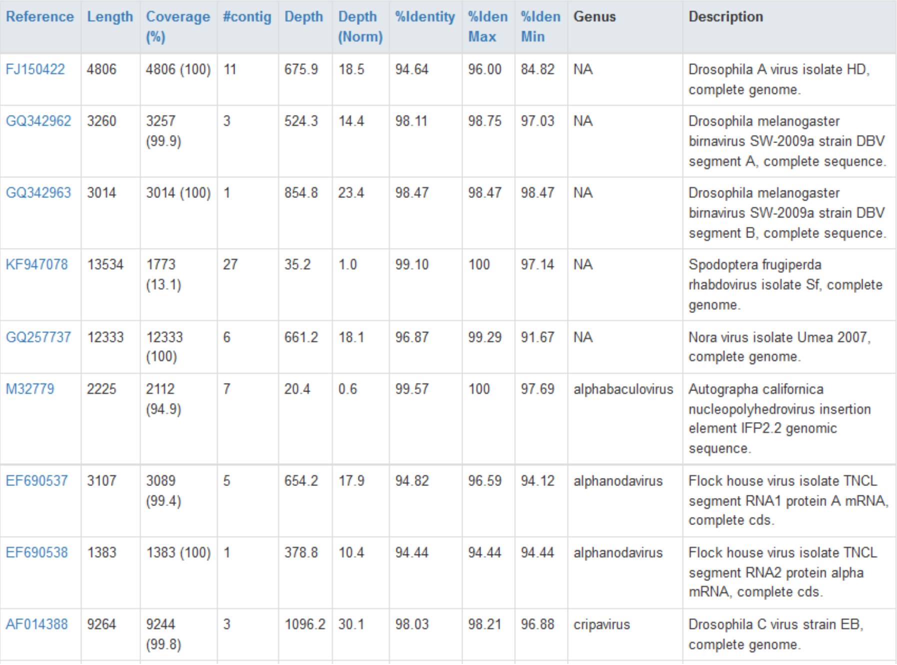
Contigs without hit

BLASTX against virus protein database

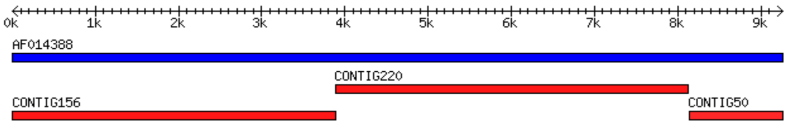
virus

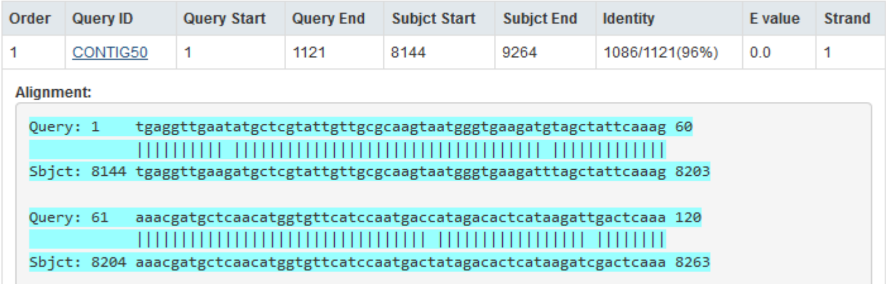
virus

Undetermined contigs



Reference	Length	Coverage (%)	#contig	Depth	Depth (Norm)	%Identity	%Iden Max	%Iden Min	Genus	Description
FJ150422	4806	4806 (100)	11	675.9	18.5	94.64	96.00	84.82	NA	Drosophila A virus isolate HD, complete genome.
GQ342962	3260	3257 (99.9)	3	524.3	14.4	98.11	98.75	97.03	NA	Drosophila melanogaster birnavirus SW-2009a strain DBV segment A, complete sequence.
GQ342963	3014	3014 (100)	1	854.8	23.4	98.47	98.47	98.47	NA	Drosophila melanogaster birnavirus SW-2009a strain DBV segment B, complete sequence.
KF947078	13534	1773 (13.1)	27	35.2	1.0	99.10	100	97.14	NA	Spodoptera frugiperda rhabdovirus isolate Sf, complete genome.
GQ257737	12333	12333 (100)	6	661.2	18.1	96.87	99.29	91.67	NA	Nora virus isolate Umea 2007, complete genome.
M32779	2225	2112 (94.9)	7	20.4	0.6	99.57	100	97.69	alphabaculovirus	Autographa californica nucleopolyhedrovirus insertion element IFP2.2 genomic sequence.
EF690537	3107	3089 (99.4)	5	654.2	17.9	94.82	96.59	94.12	alphanodavirus	Flock house virus isolate TNCL segment RNA1 protein A mRNA, complete cds.
EF690538	1383	1383 (100)	1	378.8	10.4	94.44	94.44	94.44	alphanodavirus	Flock house virus isolate TNCL segment RNA2 protein alpha mRNA, complete cds.
AF014388	9264	9244 (99.8)	3	1096.2	30.1	98.03	98.21	96.88	cripavirus	Drosophila C virus strain EB, complete genome.





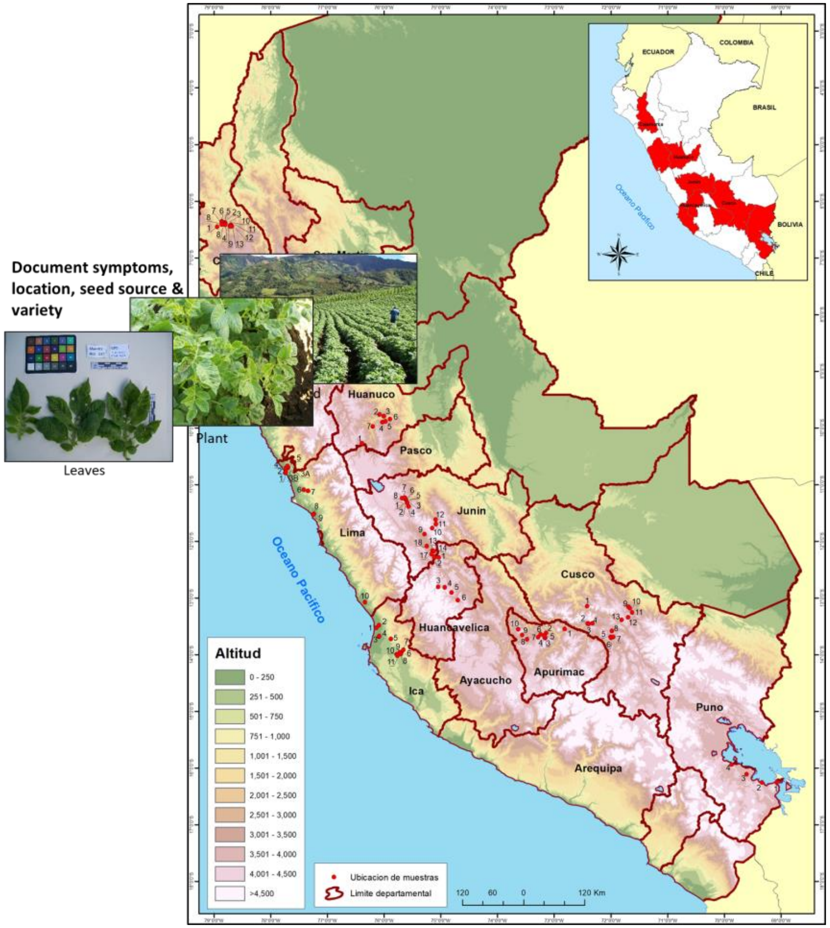
Order	Query ID	Query Start	Query End	Subjct Start	Subjct End	Identity	E value	Strand
1	CONTIG50	1	1121	8144	9264	1086/1121(96%)	0.0	1

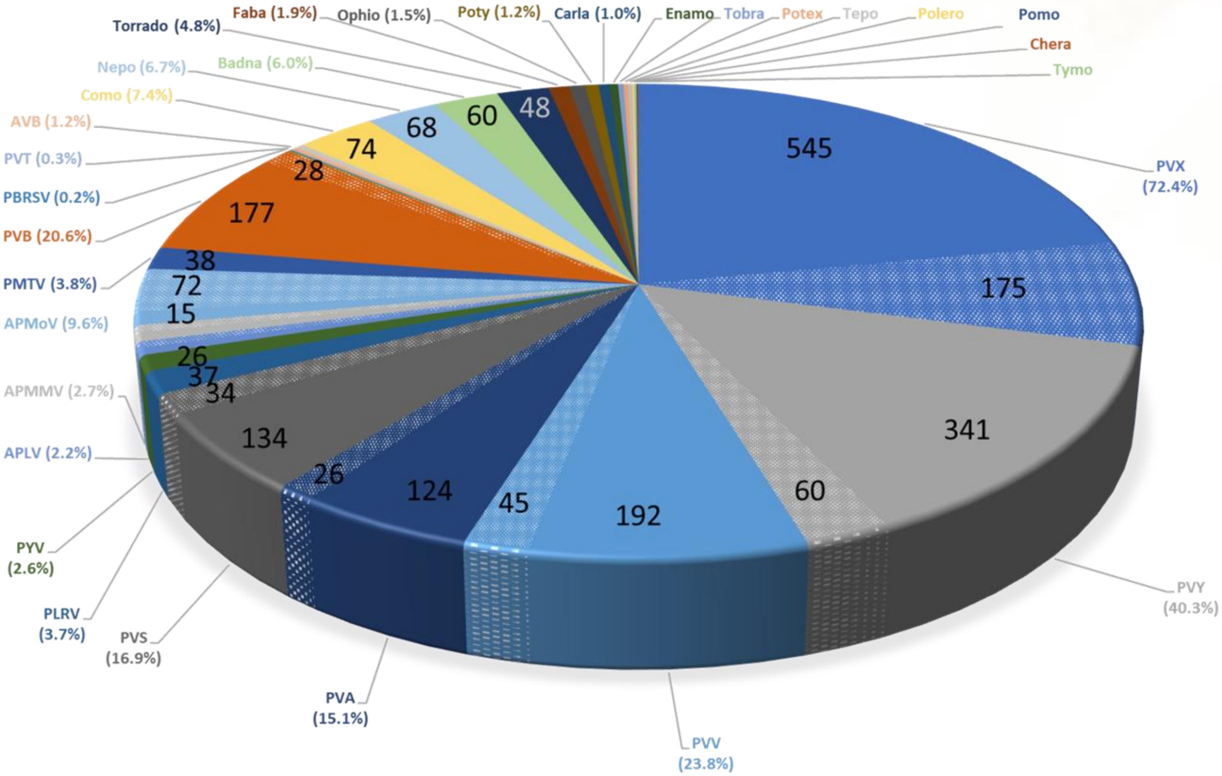
Alignment:

```
Query: 1    tgaggttgaatatgctcgtattgttgcgcaagtaatgggtgaagatgtagctattcaaag 60
            |||||||||| ||||||||||||||||||||||||||||||||||| |||||||||||||
Sbjct: 8144 tgaggttgaagatgctcgtattgttgcgcaagtaatgggtgaagatttagctattcaaag 8203

Query: 61   aaacgatgctcaacatggtgttcatccaatgaccatagacactcataagattgactcaaa 120
            ||||||||||||||||||||||||||||||||| ||||||||||||||||| ||||||||
Sbjct: 8204 aaacgatgctcaacatggtgttcatccaatgactatagacactcataagatcgactcaaa 8263
```

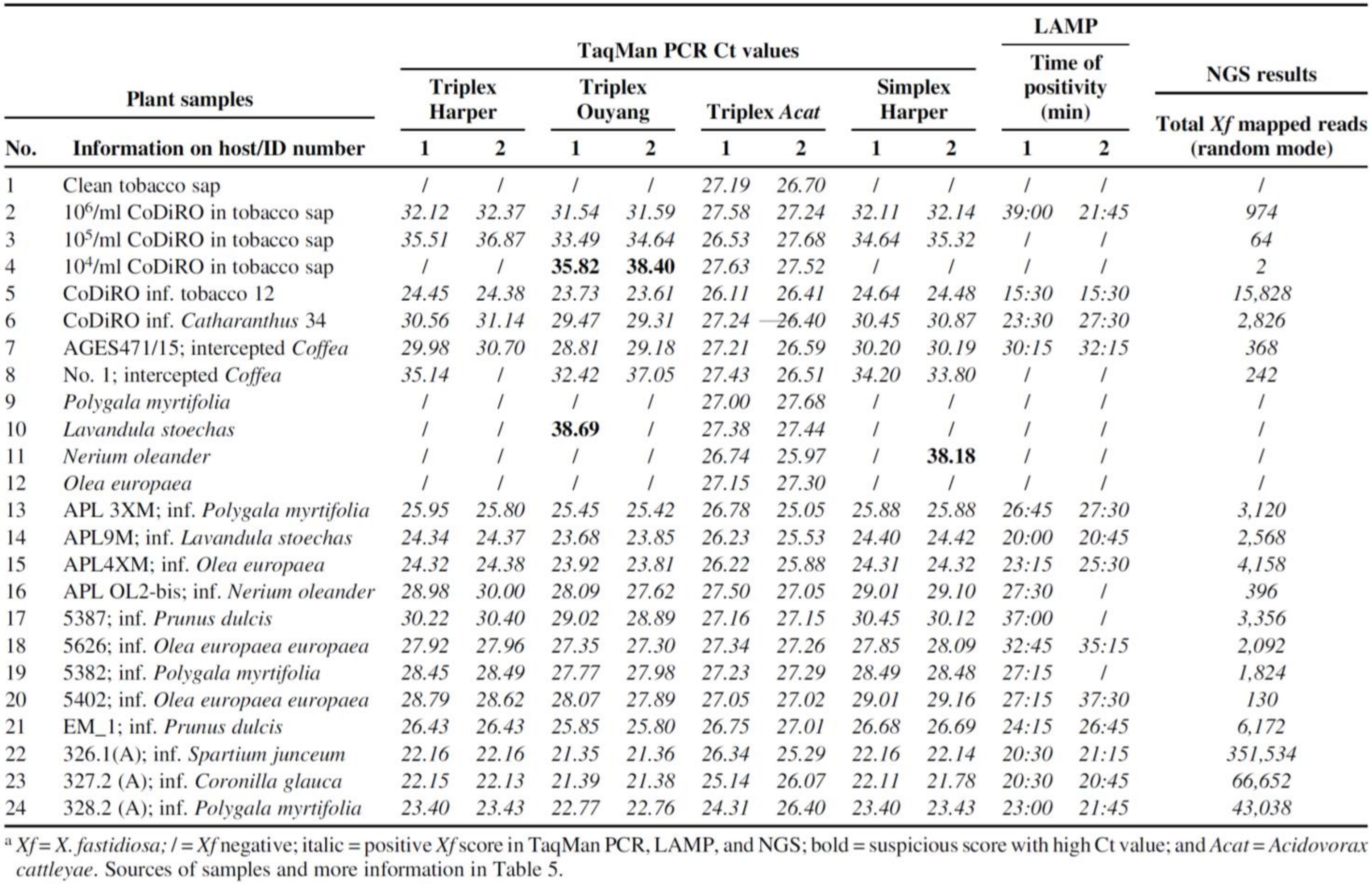
Bajo precio y análisis automático hace posible prospección de patógenos





Xylella fastidiosa

https://doi.org/10.1094/PDIS-08-18-1433-RE

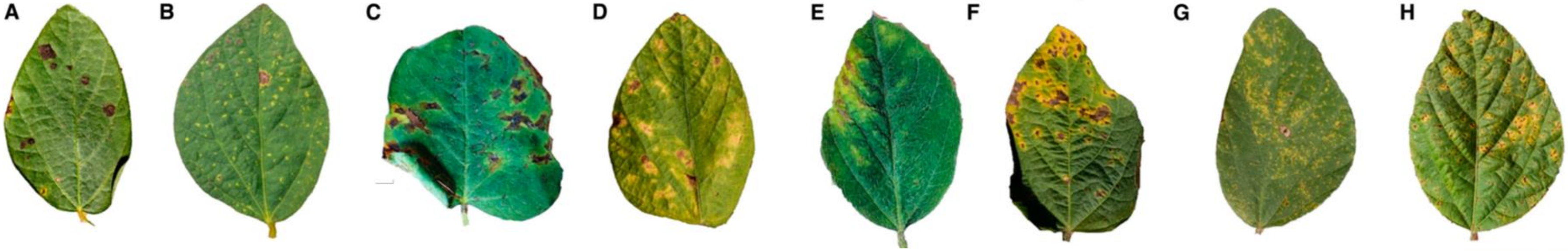


Plant samples		TaqMan PCR Ct values								LAMP		NGS results
		Triplex Harper		Triplex Ouyang		Triplex *Acat*		Simplex Harper		Time of positivity (min)		Total *Xf* mapped reads (random mode)
No.	**Information on host/ID number**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	
1	Clean tobacco sap	/	/	/	/	*27.19*	*26.70*	/	/	/	/	/
2	10^6/ml CoDiRO in tobacco sap	*32.12*	*32.37*	*31.54*	*31.59*	*27.58*	*27.24*	*32.11*	*32.14*	*39:00*	*21:45*	*974*
3	10^5/ml CoDiRO in tobacco sap	*35.51*	*36.87*	*33.49*	*34.64*	*26.53*	*27.68*	*34.64*	*35.32*	/	/	*64*
4	10^4/ml CoDiRO in tobacco sap	/	/	**35.82**	**38.40**	*27.63*	*27.52*	/	/	/	/	*2*
5	CoDiRO inf. tobacco 12	*24.45*	*24.38*	*23.73*	*23.61*	*26.11*	*26.41*	*24.64*	*24.48*	*15:30*	*15:30*	*15,828*
6	CoDiRO inf. *Catharanthus* 34	*30.56*	*31.14*	*29.47*	*29.31*	*27.24*	*26.40*	*30.45*	*30.87*	*23:30*	*27:30*	*2,826*
7	AGES471/15; intercepted *Coffea*	*29.98*	*30.70*	*28.81*	*29.18*	*27.21*	*26.59*	*30.20*	*30.19*	*30:15*	*32:15*	*368*
8	No. 1; intercepted *Coffea*	*35.14*	/	*32.42*	*37.05*	*27.43*	*26.51*	*34.20*	*33.80*	/	/	*242*
9	*Polygala myrtifolia*	/	/	/	/	*27.00*	*27.68*	/	/	/	/	/
10	*Lavandula stoechas*	/	/	**38.69**	/	*27.38*	*27.44*	/	/	/	/	/
11	*Nerium oleander*	/	/	/	/	*26.74*	*25.97*	/	**38.18**	/	/	/
12	*Olea europaea*	/	/	/	/	*27.15*	*27.30*	/	/	/	/	/
13	APL 3XM; inf. *Polygala myrtifolia*	*25.95*	*25.80*	*25.45*	*25.42*	*26.78*	*25.05*	*25.88*	*25.88*	*26:45*	*27:30*	*3,120*
14	APL9M; inf. *Lavandula stoechas*	*24.34*	*24.37*	*23.68*	*23.85*	*26.23*	*25.53*	*24.40*	*24.42*	*20:00*	*20:45*	*2,568*
15	APL4XM; inf. *Olea europaea*	*24.32*	*24.38*	*23.92*	*23.81*	*26.22*	*25.88*	*24.31*	*24.32*	*23:15*	*25:30*	*4,158*
16	APL OL2-bis; inf. *Nerium oleander*	*28.98*	*30.00*	*28.09*	*27.62*	*27.50*	*27.05*	*29.01*	*29.10*	*27:30*	/	*396*
17	5387; inf. *Prunus dulcis*	*30.22*	*30.40*	*29.02*	*28.89*	*27.16*	*27.15*	*30.45*	*30.12*	*37:00*	/	*3,356*
18	5626; inf. *Olea europaea europaea*	*27.92*	*27.96*	*27.35*	*27.30*	*27.34*	*27.26*	*27.85*	*28.09*	*32:45*	*35:15*	*2,092*
19	5382; inf. *Polygala myrtifolia*	*28.45*	*28.49*	*27.77*	*27.98*	*27.23*	*27.29*	*28.49*	*28.48*	*27:15*	/	*1,824*
20	5402; inf. *Olea europaea europaea*	*28.79*	*28.62*	*28.07*	*27.89*	*27.05*	*27.02*	*29.01*	*29.16*	*27:15*	*37:30*	*130*
21	EM_1; inf. *Prunus dulcis*	*26.43*	*26.43*	*25.85*	*25.80*	*26.75*	*27.01*	*26.68*	*26.69*	*24:15*	*26:45*	*6,172*
22	326.1(A); inf. *Spartium junceum*	*22.16*	*22.16*	*21.35*	*21.36*	*26.34*	*25.29*	*22.16*	*22.14*	*20:30*	*21:15*	*351,534*
23	327.2 (A); inf. *Coronilla glauca*	*22.15*	*22.13*	*21.39*	*21.38*	*25.14*	*26.07*	*22.11*	*21.78*	*20:30*	*20:45*	*66,652*
24	328.2 (A); inf. *Polygala myrtifolia*	*23.40*	*23.43*	*22.77*	*22.76*	*24.31*	*26.40*	*23.40*	*23.43*	*23:00*	*21:45*	*43,038*

[a] *Xf* = *X. fastidiosa*; / = *Xf* negative; italic = positive *Xf* score in TaqMan PCR, LAMP, and NGS; bold = suspicious score with high Ct value; and *Acat* = *Acidovorax cattleyae*. Sources of samples and more information in Table 5.

Patógenos foliares de soya

https://doi.org/10.1094/PDIS-05-18-0905-RE



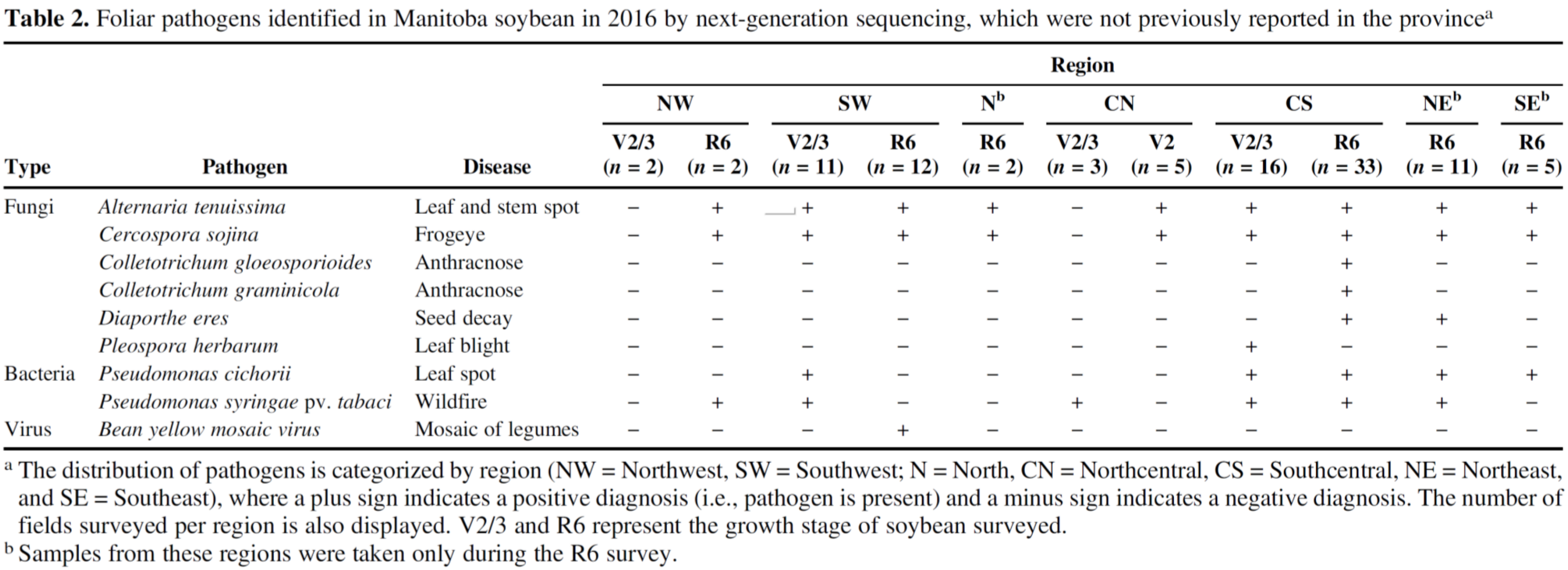


Table 2. Foliar pathogens identified in Manitoba soybean in 2016 by next-generation sequencing, which were not previously reported in the province[a]

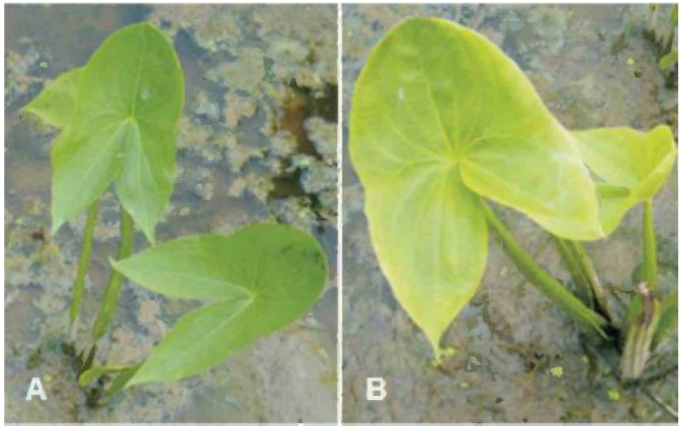
			Region										
			NW		SW		N[b]	CN		CS		NE[b]	SE[b]
Type	**Pathogen**	**Disease**	**V2/3 (*n* = 2)**	**R6 (*n* = 2)**	**V2/3 (*n* = 11)**	**R6 (*n* = 12)**	**R6 (*n* = 2)**	**V2/3 (*n* = 3)**	**V2 (*n* = 5)**	**V2/3 (*n* = 16)**	**R6 (*n* = 33)**	**R6 (*n* = 11)**	**R6 (*n* = 5)**
Fungi	*Alternaria tenuissima*	Leaf and stem spot	–	+	+	+	+	–	+	+	+	+	+
	Cercospora sojina	Frogeye	–	+	+	+	+	–	+	+	+	+	+
	Colletotrichum gloeosporioides	Anthracnose	–	–	–	–	–	–	–	–	+	–	–
	Colletotrichum graminicola	Anthracnose	–	–	–	–	–	–	–	–	+	–	–
	Diaporthe eres	Seed decay	–	–	–	–	–	–	–	–	+	+	–
	Pleospora herbarum	Leaf blight	–	–	–	–	–	–	–	+	–	–	–
Bacteria	*Pseudomonas cichorii*	Leaf spot	–	–	+	–	–	–	–	+	+	+	+
	Pseudomonas syringae pv. *tabaci*	Wildfire	–	+	+	–	–	+	–	+	+	+	–
Virus	*Bean yellow mosaic virus*	Mosaic of legumes	–	–	–	+	–	–	–	–	–	–	–

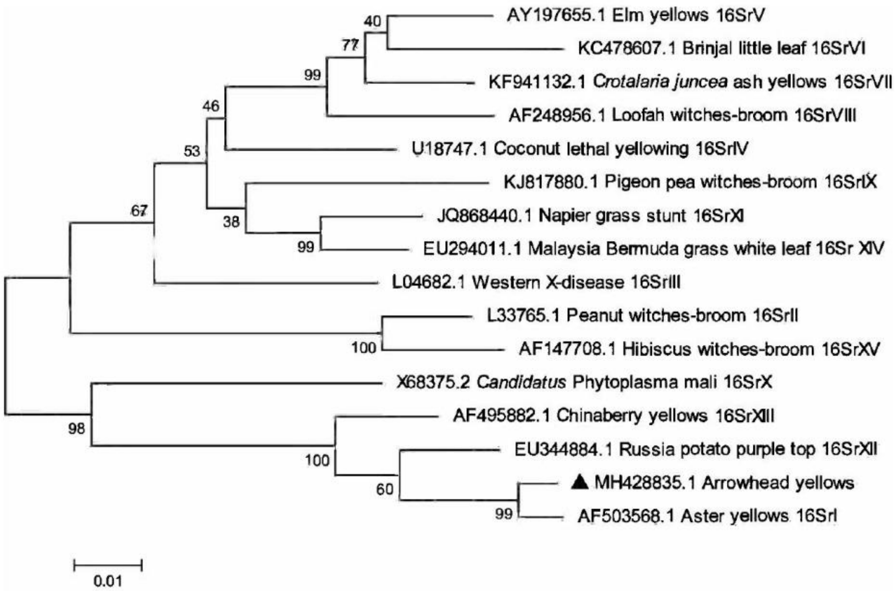
[a] The distribution of pathogens is categorized by region (NW = Northwest, SW = Southwest; N = North, CN = Northcentral, CS = Southcentral, NE = Northeast, and SE = Southeast), where a plus sign indicates a positive diagnosis (i.e., pathogen is present) and a minus sign indicates a negative diagnosis. The number of fields surveyed per region is also displayed. V2/3 and R6 represent the growth stage of soybean surveyed.

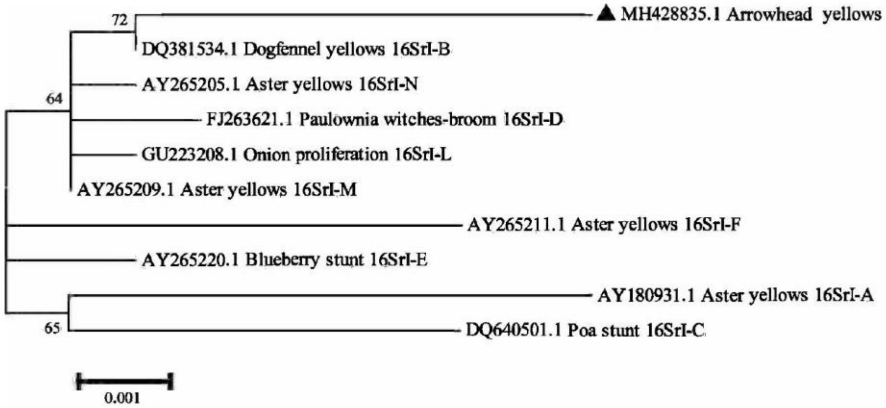
[b] Samples from these regions were taken only during the R6 survey.

Arrowhead yellows

DOI : 10.3969/j.issn.2095-1191.2019.03.19

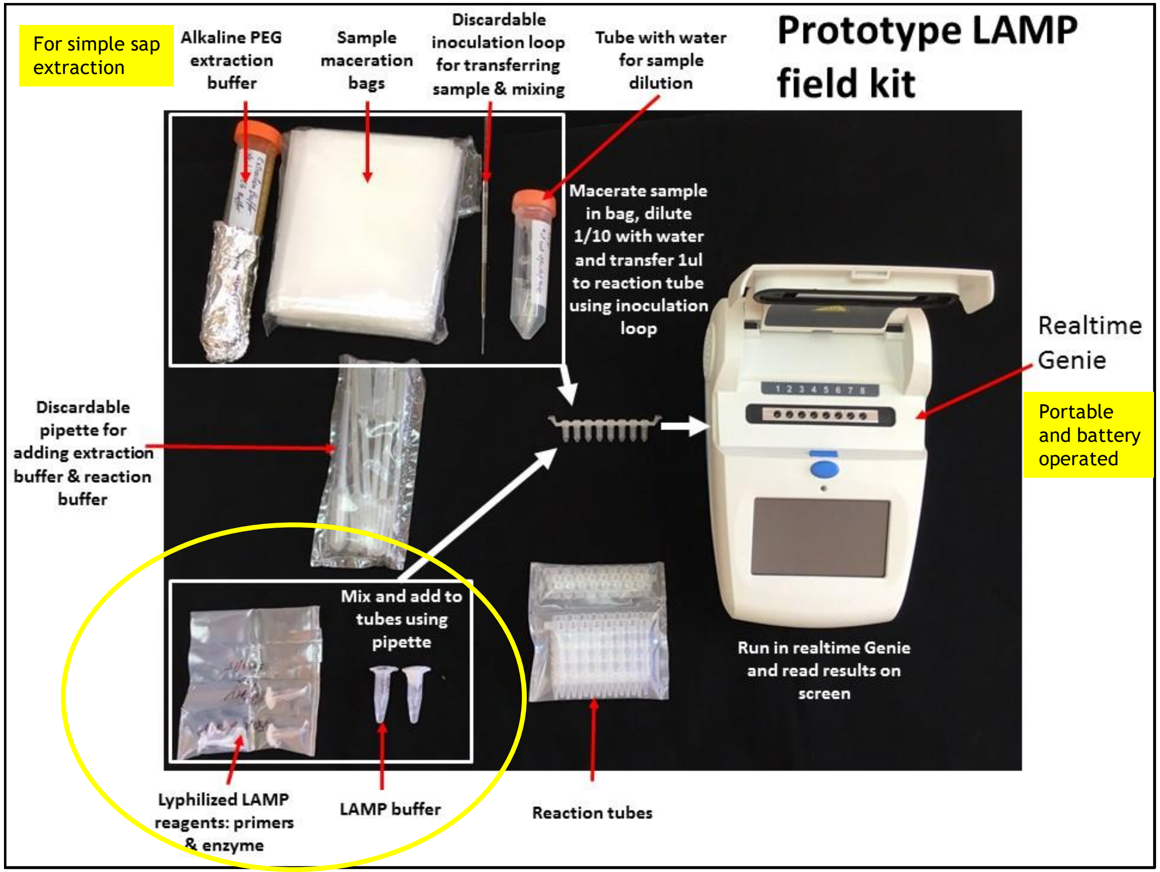






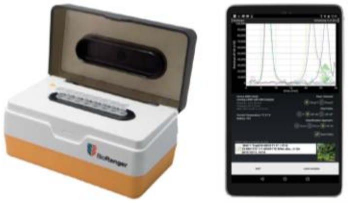
Basado en la secuencia del patógeno identificado se puede diseñar ensayos rápidos específicos

For simple sap extraction



Realtime Genie

Portable and battery operated



BioRanger

Easy to interpret

Resumen

- HTS ya es ampliamente aplicado para identificar nuevos patógenos, también en plantas
- Su sensibilidad y rapidez y aplicación genérico tienen grandes ventajas frente a otros métodos de identificación de patógenos
- Nuevos programas bioinformáticas hacen el análisis más fácil para no especialistas.

AÑO INTERNACIONAL DE LA
SANIDAD VEGETAL
2020

PROTEGER LAS PLANTAS,
PROTEGER LA VIDA