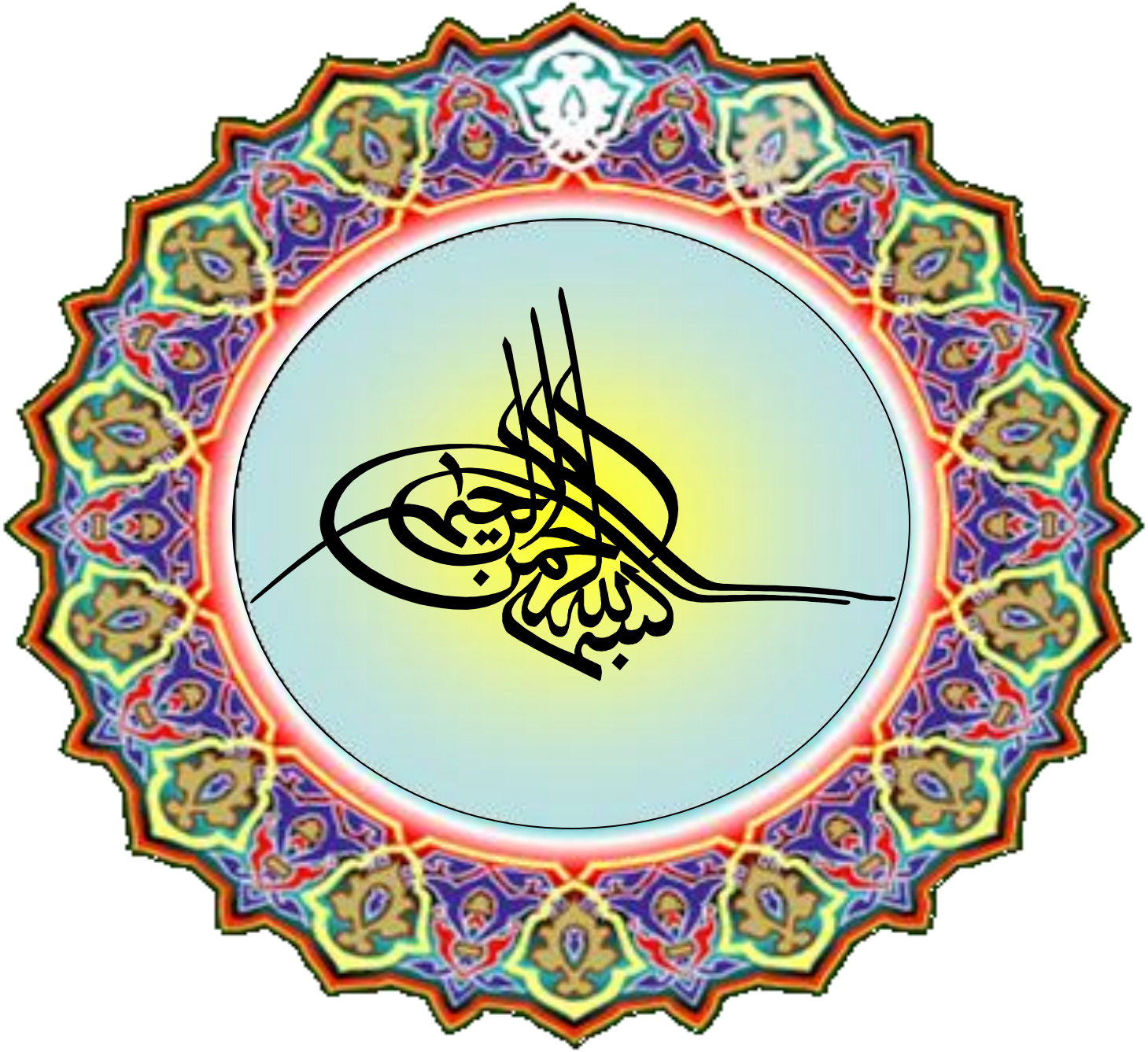

بسم الله الرحمن الرحيم

SCBPDC

First Report of *Leifsonia xyli* subsp. *xyli*, Causal Agent of Ratoon Stunting of Sugarcane in Iran

k. Taher-khani[1], A. Chinea Martin[2], H. Hashemi Sohi[3]and N. Nassir-pour[1]

1- Institute of Sugarcane Research & Training, Sugarcane Development & By-product Co., Ahwaz, Iran

2- National Sugarcane Research Institute (INICA), Sugar Ministry, Havana, Cuba

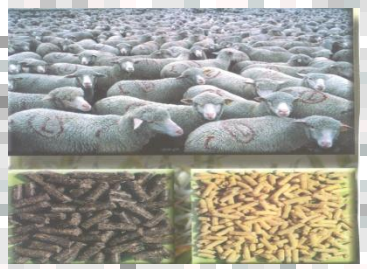
3-National Research Center for Genetic Engineering & Biotechnology, Tehran, Iran

10th PATHOLOGY WORKSHOP 7-11, NANNING, CHINA


Iran has designed a development of the sugarcane industry
to obtain its demand for sugar and its By - products.

Sugarcane & By- Products in Khuzestan

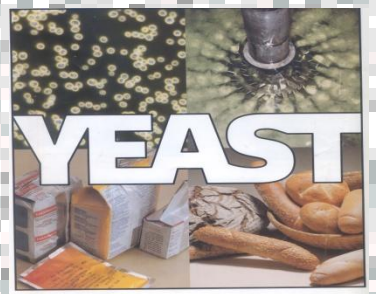
SCBPDC



8 Cattle Food



6 Paper Mills



1 Yeast



his bioethanol plant in Nebraska, USA, processes corn
produce 925 litres per day (Minnesota Corn Producers)
Photo: Vogelbusch

1 Alcohol



9 Raw Sugar



6 Sugar Refinery



3 Particle Board & MDF

What are the factors that inhibit or restrict this development?



Abiotic factors:

1- Salinity and drought



2- Freeze and frost damage



Biotic factors:

- Sugarcane are affected by 17 parasitic diseases.
- 2 viral diseases: SCMV, ScYLV
- 2 phytoplasma diseases: white leaf ,ScYP
- 4 bacterial diseases: Ratoon stunting disease (RSD) ,Leaf scald , Red streak and Red stripe
- 8 fungi diseases : Alternaria leaf spot, Cytospora leaf sheath rot, pineapple disease , pokkah boeng (knife cut) , Red rot of the leaf sheath ,Red rot in the midrib , Smut, Soty mould and wilt ,
- Nematod: 11Genus, 22 Spieces


Smut

SCMV



YLS





Nematodes





RATOON STUNTING DISEASE

RSD was reported only based on internal and external symptoms,Therefore serious study of RSD is necessary.



MATERIALES Y MÉTODOS

EVALUATION OF INCIDENCE AND INTENSITY OF RSD

- **300 cultivars were investigated for determine of external and internal symptoms of RSD.**







- **Among 60 sampled cultivar, ten mature stalks for each of them were collected randomly.**

EVALUATION OF INCIDENCE AND INTENSITY OF RSD

Percentage of functional and non-functional vessels were determined by STM

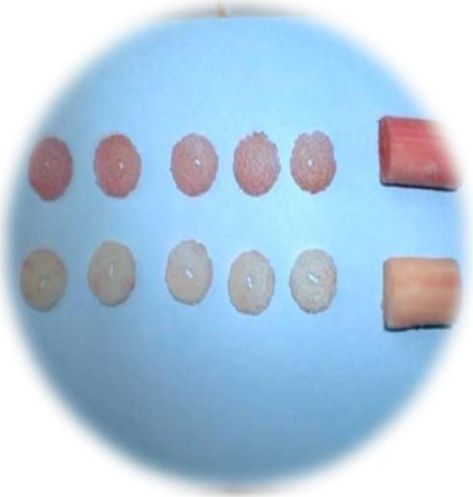
(Chagas and Tokeshi, 1994).











ISOLATION OF CAUSAL ORGANISM

1

CVs. with more than 50% of NFV were selected



2

Cutting of small part of infected tissue



3

Droplet of fibrovascular fluid was poured in M_SC (Croft et al., 1993)



4 Aerobic incubated, 28ºC

5 Typic clonies were studied morphologicaly and biochemically

ARTIFICIAL PATHOGENICITY TEST

Inoculation of single and duble buds of Cv CP50-28, suspension ($1X10^{9}$ $cell.mL^{-1}$) ,5MINS

1





1 hour after inoculation



2 Horizontal plantation



Vertical plantation

3 Control was inoculated with distiled water

4 Observation of symptoms was conducted after 6 weeks and 6 months for horizontal and vertical plantation respectively.

MOLECULAR IDENTIFICATION (PCR)

***Lxx* Ir lysated**

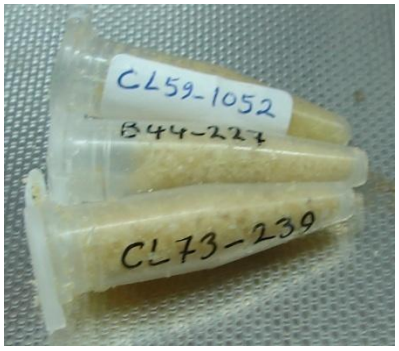
Thermic shock
(Gao et al., 2006)

PCR (Pan et al., 1998)

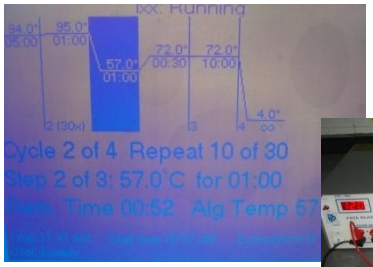
*Cxx*1 (5'-CCGAAGTGAGCAGATTGACC-3‘)
*Cxx*2 (5'-ACCCTGTGTTGTTTTCAACG-3’)

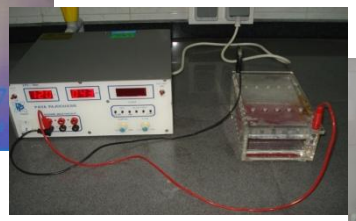
Total DNA extraction
Hot-CTAB
(Zhang, 1998; Hashemi et al., 2008), for infected and healthy CVs, in comercial plantation





N_2 liquid

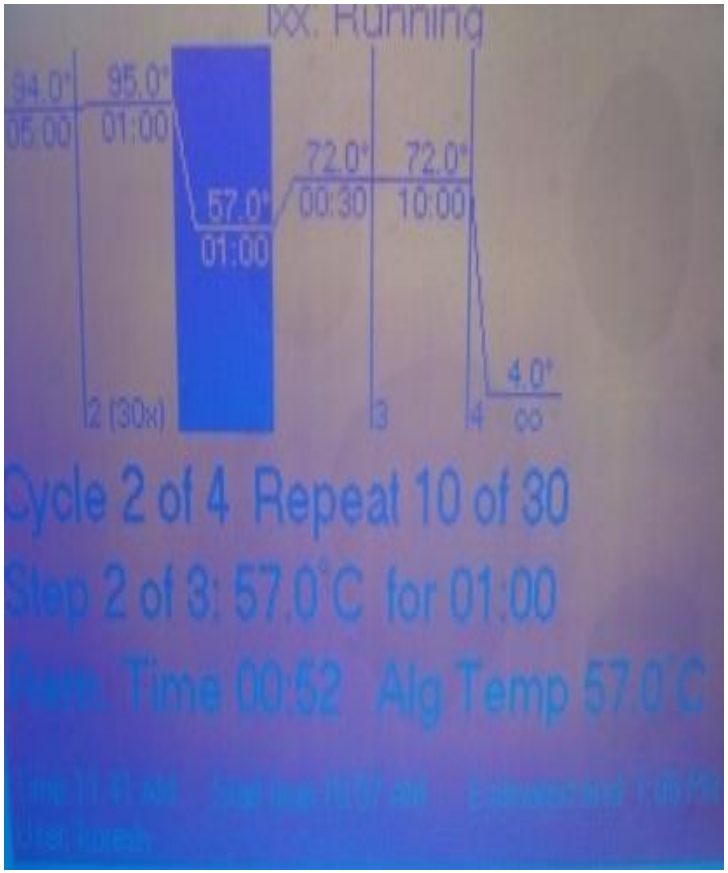






THERMOCYCLER PARAMETERS

- **Denaturization at 94°C for 5 min,**
- **30 cycles at :**
- **95°C for 1min,**
- **57°C for 1min,**
- **72°C for 30s,**
- **and final extension at 72°C for 10 min.**



PCR PRODUCT

- Las reacciones contenían 50 ng del ADN molde, 0,2 µM de cada dNTP (dATP; dTTP; dGTP; dCTP), 0,5 µM de cada cebador, 1 U de Ex-Taq ADN polimerasa, 10X Ext-Taq ADN tampón, 20 µM $MgCl_2$, en volumen total de 25 µL

SEQUENCING AND COMPARAISING

- DNA sequencing of the PCR amplicon was performed at the MWG Company,Germany by the Di-deoxy method using an ABI automated .

The resulting consensus sequence was used to search for non-redundant , homology sequence with DNA sequences available in www.ncbi.nlm.nih.gov/NCBI/ using the BLASTN program. (Altschul et al., 1990)

SEQUENCING AND COMPARAISING

DNA sequences avilable Leifsonia in IGB

No.	Accesión	Isolate	Origen	Organism	Reference
1	AF034641.1	L1A	Australia	*Lxx*	Fegan y col. (1998)
2	DQ232616.2	DNPRC108	Australia	*Lxx*	Young y col. (2008)
3	EU723209.1	-	China-Fuzhou	*Lxx*	Gao y col. (2008)
4	AF056003.1	16296	EUA	*Cxx*	Pan y col. (1998)
5	DQ232613.2	VKMAo-1401	Australia	*L. poae*	Young y col. (2008)
6	AF034642.1	TB1A	Australia	*Lxc*	Fegan y col. (1998)
7	DQ232615.2	DNPAC039	Australia	*Lxc*	Young y col. (2008)
8	AF056004.1	CXC7	EUA	*Cxc*	Pan y col. (1998)

ESTIMATION OF LOST YIELD

EXPERIMENT OF FIELD

Design: Factorial

3 repelications

Plot: 72 m^2

Factores of study

A. Saneamiento: 1. Hidrothermothrapy
2. Infected Control

B. Cultivares:
1. CP57-614
2. SP70-1143
3. NCo310
4. CP48-103
5. CP69-1062
6. IRC99-02
7. NCo293
8. Ja64-19 (Control)

Survey time : Plant , 1st and 2nd Ratoon with 15 months

Variables evaluadas: Percentage of Lost Yield

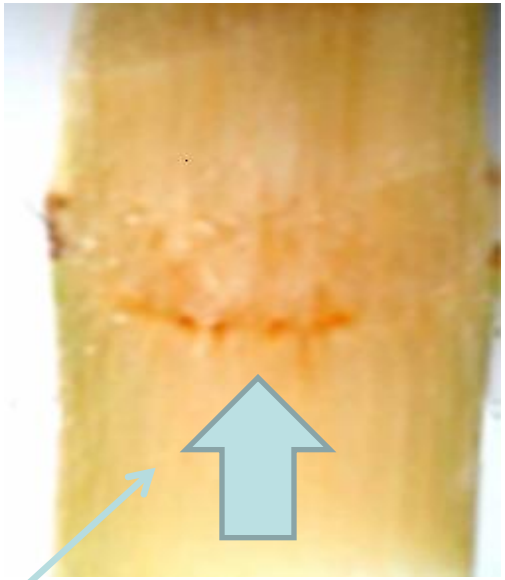


RESALTS AND DISCUSION

SYMPTOMS OF RSD



External symptoms with short internode and decrease of diameter



Internal symptoms with dots, commas and various straight from yelliw, orange, pink and red to brown in the wax band



Pink coloration of the young shoot in growing point

Unique internal symptoms of RSD exclusively (Giglioti et al., 1999)

The presence of other pathogens may cause vascular discoloration (Comstock and Lentini , 2005)

INCIDENCE AND INTENSITY OF RSD

Among 60 sampled cultivars, percentage of non-functional vessels due to plugging of xylem vessels as a result of colonization by the bacteria were between 11-69% in CP48-103 and NCo293 respectively

Cross section of
basal internode 200x



ISOLATION OF CAUSAL AGENT





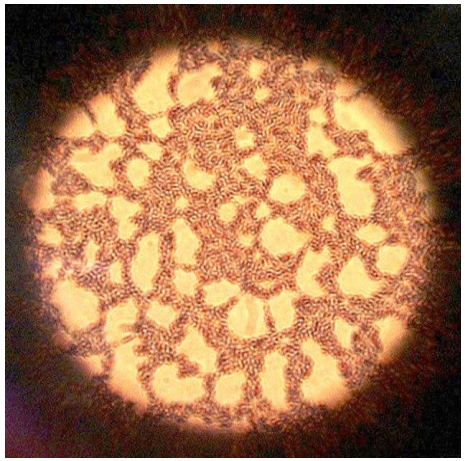


Droplet of fibrovascular liquid allowed to isolate pure culture for the first time, if a piece of infected tissue is applied from wax band area.

Typical colonies appear 2 weeks after incubation

- 0,1-0,3 mm in diameter
- Circular with entire margins
- Non- pigmented
- convex

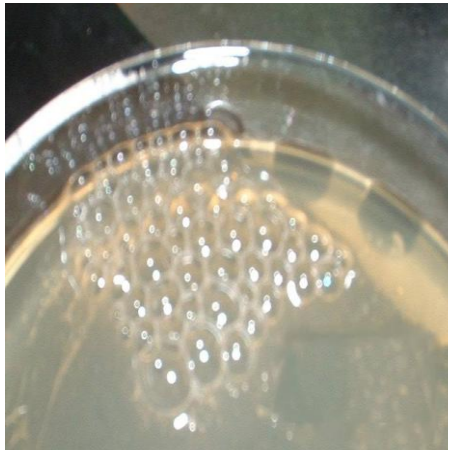
BIOCHEMICAL CHARACTERS



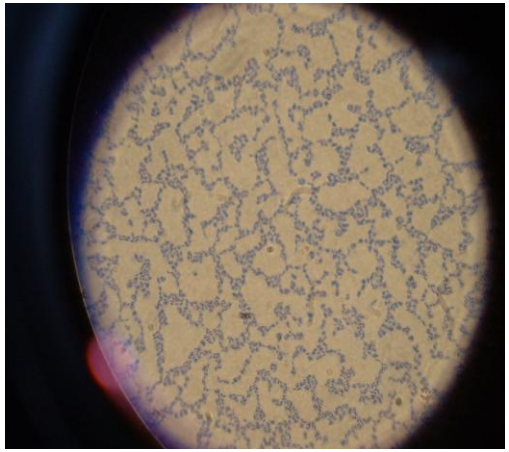
G+



Oxidas _



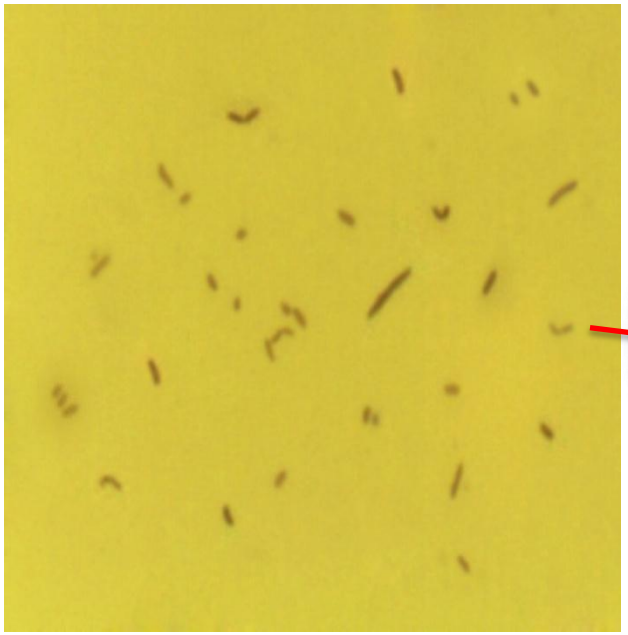
Catalas +



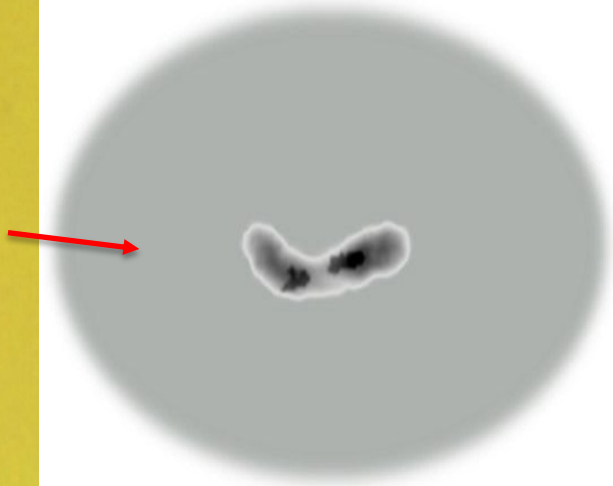
Non acid fast

- Non spore forming
  - Aerobic bacteria (28°C)

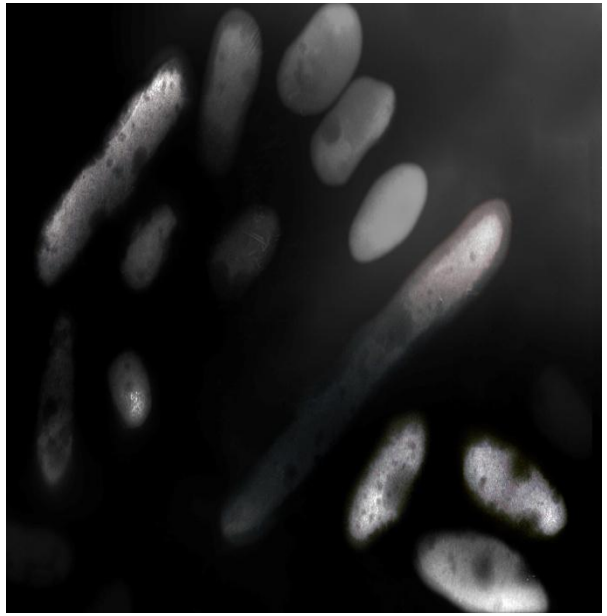
MORPHOLOGICAL CHARECTERS



Bacili form, Polymorphism
×1000



V Shape cell



Polymorphism, Mesosom
×13000

Arranged in pairs

Non-motile

1 to 4 and sometimes up to 10 µm

PATHOGENICITY TEST





Symptoms observation in young stem after 6 weeks



Internal symptoms observation in millable stalk after 6 months

Pathogenicity test was proved:

1. Effectiveness of both methods
2. Susceptibility of CP50-28 and possible use it as a standard susceptible cultivar
3. The pathogenicity of the isolate .

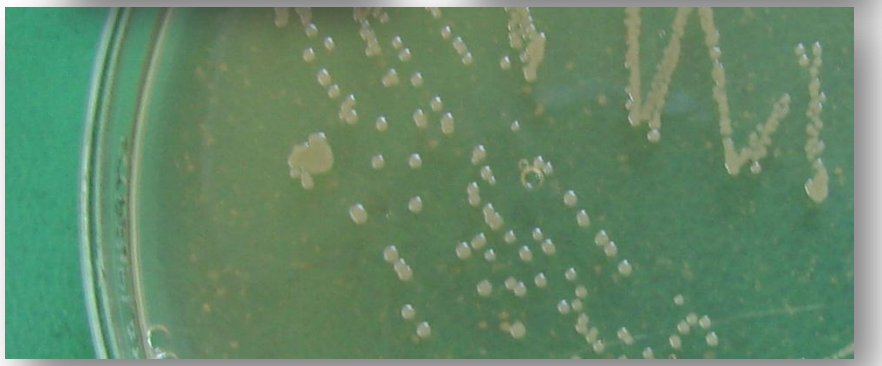
All these evidences confirm the presence of *Leifsonia xyli* subsp. *xyli* (Davis *et al.,* 1984) Evtushenko *et al. (*2000). In Iran for the first time. This isolate was named *L. xyli* subsp. *xyli* Ir.



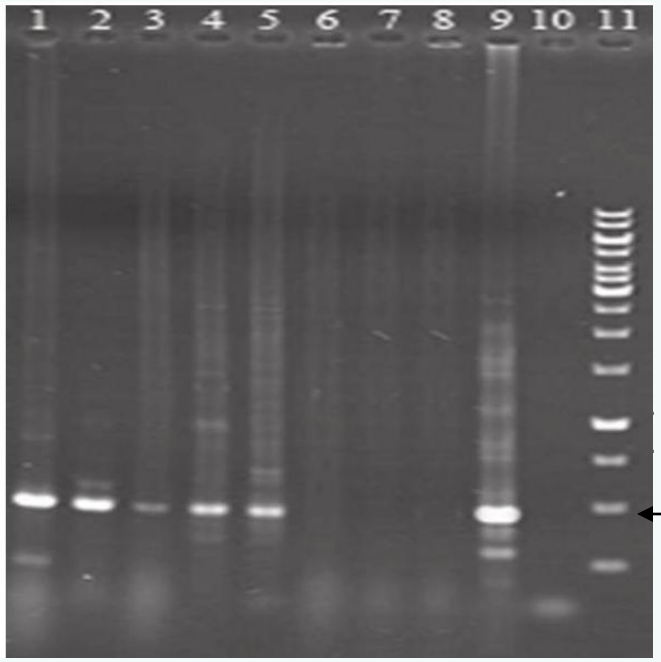








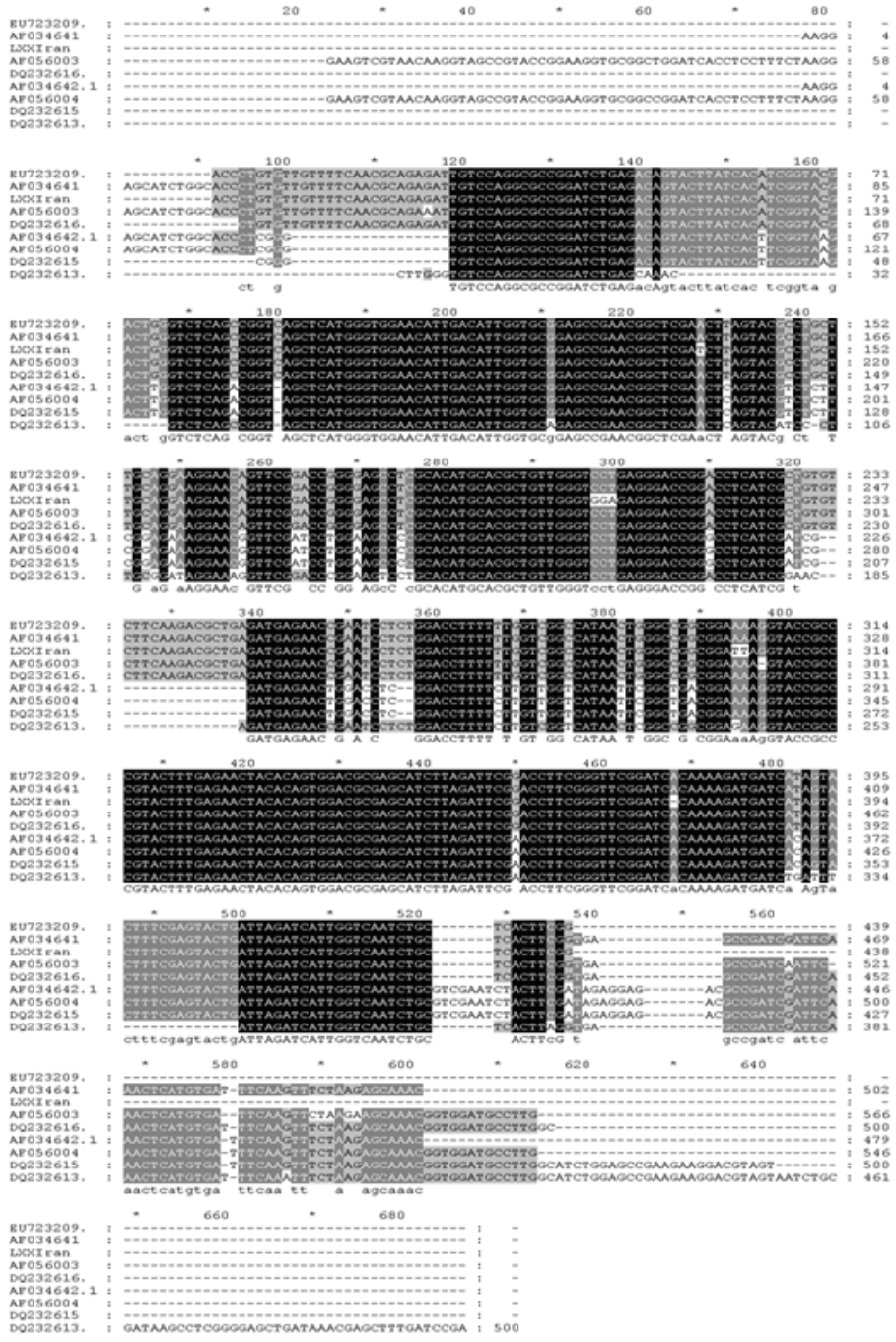
MOLECULAR IDENTIFICATION (PCR)



1. CP48-103.
2. CP63-588.
3. *L. xyli* subsp. *xyli* Ir. no lisada.
4. CP50-28.
5. CP45-3.
6. CP73-1547 asintomática.
7. L66-43 asintomática.
8. Control negativo (agua destilada).
9. *L. xyli* subsp. *xyli* Ir. lisada.
10. NCo376 asíntomática.
11. Marcador de peso molecular 1 Kpb.

← 438 pb

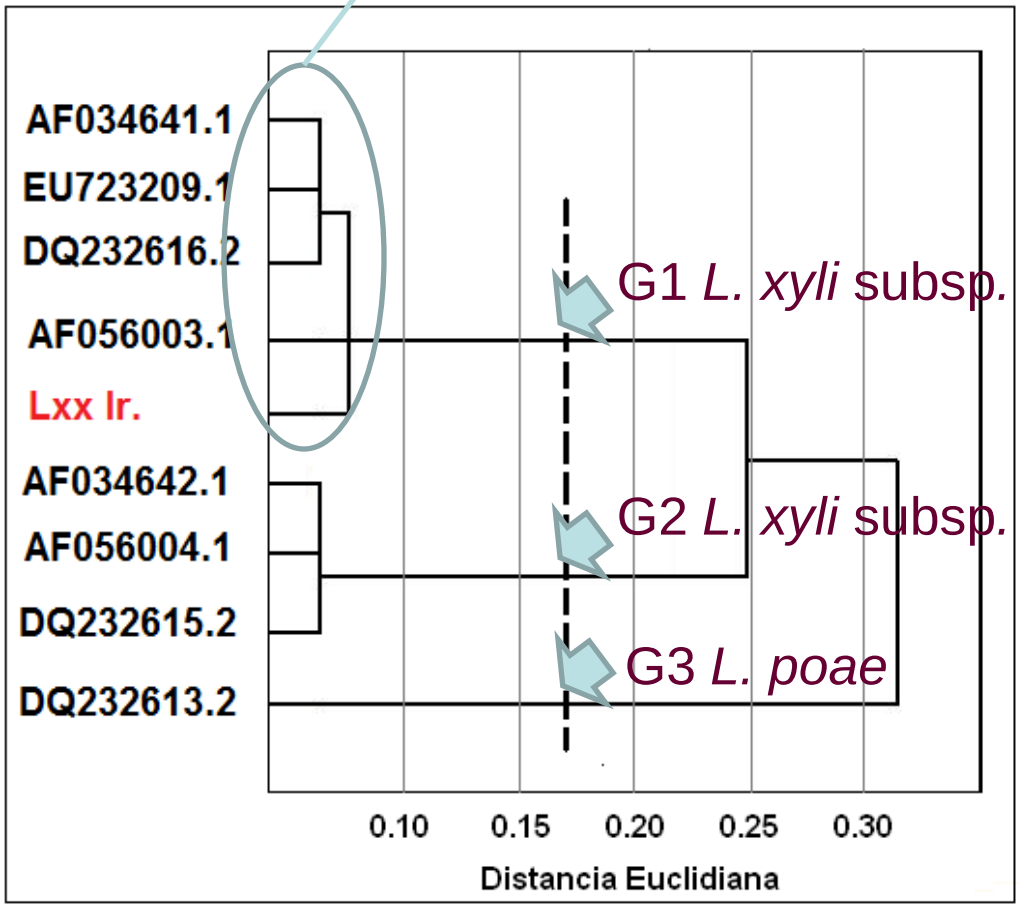
- The Cvs. With typical symptoms of RSD and positive control(*Lxx* l ysated and non lysated) were amplifaied 438bp band.
- Symptomless Cvs. And negative control didnot amplify 438bp band.
- It confirmed the presence of *Lxx* causal organism of RSD .



Alignment of the sequences of 438 bp corresponding to *L. xyli* subsp. *xyli* Ir. with other *Lxx* isolates in the International GneBank .

Sequencing and Comparising:

There are significant sequence homology of Iranian isolate (more than %98) with other isolates of *Lxx* that present in IGB.



Homology of nucleotide sequences of species of existing *Leifsonia* in IGB.

Comparison of the sequence homology of *Lxx* Ir. with others sequences present in IGB

	Porcentaje de identidad										
Divergencia		1	2	3	4	5	6	7	8	9	Accesion
	1		100	98.3	100	100	86.6	86.6	86.6	82.9	1 AF034641
	2	0.000		98.8	100	100	86.6	86.6	86.6	82.9	2 AF056003
	3	17	17		98.3	98.3	84.6	84.6	84.6	81.1	3 *Lxx* Ir
	4	0.000	0.000	17		100	86.6	86.6	86.6	82.9	4 DQ232616
	5	0.000	0.000	17	0.000		86.6	86.6	86.6	82.9	5 EU723209
	6	13.4	13.4	154	134	134		100	100	78.6	6 AF034642
	7	13.4	13.4	154	134	134	0.000		100	78.6	7 AF056004
	8	13.4	13.4	154	134	134	0.000	0.000		78.6	8 DQ232615
	9	17.1	171	189	171	171	214	214	214		9 DQ232613
		1	2	3	4	5	6	7	8	9	

WORLDWID DISTRIBUTION OF SINGLE STRAIN OF Lxx

Similarly, Gao *et al*., (2008) explained that isolate *Lxx*-Fuzhou China (EU723209) showed 100% identity with the isolates AE016822 (Vitorello Monteiro *et al*., 2004), AF034641 (Fegan *et al*., 1998) and DQ232616 (Young *et al* ., 2006) and 99% identity with the isolate AF056003 (Pan *et al*., 1998). All above mentioned, consistent with the hypothesis of genetic diversity raised by Young *et al*. (2006), about the origin of Leifsonia xyli subsp. xyli from endophytic organisms that have evolved with the wild species Saccharum spontaneum L., leading to the worldwide distribution of a single strain of *L. xyli* subsp. *xyli*, cited by Brumbley *et al.* (2006). The obtained reasult of *Lxx* Ir. Based on the genetic identity confirm once again the Young et al., (2006)

YIELD LOST DUE TO RSD

Cultivar	%Lost Yield (P)	%Lost Yield (R1)	%Lost Yield (R2)	%Average Lost Yield (P+R1+R2)
CP57-614	**20.92**	**8.23**	**1.13**	**10.1**
CP48-103	**20.68**	**38.28**	**18.82**	**25.9**
CP69-1062	**9.66**	**30.16**	**10.85**	**16.9**
SP70-1143	**24.89**	**18.96**	**12.05**	**18.6**
NCo310	**15.14**	**24.16**	**14.21**	**17.8**
IRC99-02	**20.03**	**45.11**	**31.97**	**32.4**
Ja64-19	**25.04**	**65.26**	**80.16**	**56.8**
NCo293	**1.55**	**9.7**	**12.33**	**7.9**

YIELD LOST DUE TO RSD



Infected stools (left) and healthy stools (Right) in Cv. Ja64-19

YIELD LOST DUE TO RSD



Two replications of field experiment in sth R corp


و من الله التوفيق ...


IRAN
AHWAZ
Thank you
Photos&Des : Pedram FAYAZIAN
White Bridge