Final report EG ISF DRT and IBEB project

Lettuce *Fusarium oxysporum* f. sp. *lactucae* (Fol: 4) resistance - 2021

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I. Background:

Discovered for the first time in 1955 and described in 1967 as a novel forma specialis, *Fusarium oxysporum* f. sp. *lactucae* (Fol) is the pathogen causing "Fusarium wilt", a disease affecting lettuce. The race 1 has been identified in 1967 in Japan and then in USA, Iran, Taiwan, south of America and Europe. The races 2 and 3 have been identified in Japan in 2001 and for the moment were also reported only in Taiwan.
The test of the resistance of lettuce to *Fusarium oxysporum* f. sp. *lactucae* is used in DUS since 2013 with the races 1 and 2. This characteristic was initially described with two UPOV notes: 1 (susceptible) and 9 (resistant) (TG/13/10 Rev).

In 2012-2014, GEVES coordinated for IBEB (International Bremia Evaluation Board) a collaborative project with 11 participating laboratories to harmonize a protocol of evaluation of the resistance of lettuce to Fol: 1. The results allowed to adapt the explanation to this characteristic (definition of symptoms indicating the intermediate level) and to define good threshold varieties to distinguish between susceptibility, intermediate resistance and resistance.
The conclusions of this project were presented by GEVES and applied to update the technical protocols for CPVO (CPVO/TP-013/6-Rev) and UPOV (TG/13/11 Rev.) with three UPOV notes: 1 (susceptible), 2 (intermediate resistant) and 3 (highly resistant).

In 2016, Giraldi et al. described a new race of *Fusarium oxysporum* f. sp. *lactucae* race 4 and used several varieties to differentiate it from the previously identified races. Therefore, the following table for differentials has been proposed at the ISF DRT WG (Table 1).

Table 1: proposition for Fol: 4 differentials to ISF WG DRT based on the publication of G. Gilardi et al.

	Race (ISF Code)			
Differential host	**1 (Fol: 1)**	**2 (Fol: 2)**	**3 (Fol: 3)**	**4 (Fol: 4)**
Patriot, Cavolo di Napoli	S	S	S	S
Costa Rica No.4	R	S	S	S
Banchu Red Fire, Lattughino biondo	S	R	S	R

S = susceptible; R = resistant (partial resistance)

Patriot, used as differential and known to be susceptible to Fol races 1, 2 and 3 had not been described in the publication of G. Giraldi et al., 2016 and was tested by one of the DRT participants and found not to be clearly susceptible.

In 2018-2019, GEVES coordinated for ISF an interlaboratory project including 14 partners to validate, the newly identified differentials and race for use in resistance claims. Several

isolates expected as races 1, 2, 3 and 4 were tested in different laboratories including the isolate from the publication of G. Giladi. The results showed that, races 1 and 2 were validated on the differentials and if race 3 was validated requiring an aggressive test to express enough symptoms and to provide conform results. Isolates tested as race 4 were found non-conform in most laboratories (Table 2). The conclusion was the description of the race 4 required further investigation.

Table 2: ISF ring test results for Fol: 4 (Isolate I, M and N)

Labs interpretation

		Isolate I						Isolate M				Isolate N			
	Expected results Fol: 4	lab 4	lab 5	lab 7	lab10	lab 11	lab14	lab1	lab8	lab 9	lab12	lab2(1)	lab3	lab6	lab13
		23/20°C (day/night)	Results not included in analysis 25°C	25°C	?		Results not included in analysis 25°C	Results not included in analysis 25°C	25°C		22/27 n/d	Results not included in analysis	25°C	25°C	Results not included in analysis
Patriot	S	S	R	IR	IR		R	R	S		S	HR	IR	IR	R
Costa Rica No. 4	S	S	S	S	S		S	IR	IR		S	HR	S	S	S
Romabella	S	S	R	IR	IR		R	IR	S		S	HR	IR	IR	R
Banchu Red Fire*	IR	S	IR	S*	not validated*		bg	IR	bg		R	HR	IR	IR	bg

not validated* = HR/HG : HR in rep 1, HG in rep 2

(1) This isolate was very "slow". We held onto it for 35 days and finally saw some disease

Lab X: lab who provided the isolate
R: resistant; S: susceptible; IR: intermediate resistant; Bg: bad germination

II. ISF EG DRT / IBEB project 2021:

As the new race of *Fusarium* creates a serious threat for growers and a challenge for breeders, a harmonisation action was needed on short term. That is why ISF DRT / IBEB members decided to engage a new project coordinated by GEVES. The objectives were:

- To validate the ISF table for race 4
- Eventually to update the ISF table with new differentials in necessary
- To describe the resistance level of lettuce to Fol: 4
- To propose an interpretation rule and reference controls for the evaluation of the resistance to lettuce to Fol: 4.

To be able to meet these objectives, a new comparative test was organized to compare in controlled conditions different isolates for race 4 on a panel of varieties made up of differentials and varieties with different level of resistance. In parallel, it was decided to evaluate the same panel of varieties in a test in polytunnel to check as far as possible the correlation between results in controlled conditions and in field conditions.

a. Material and method

i. Comparative test in controlled conditions:

Participants:

12 Participants were involved in the comparative test in controlled conditions: BASF, Bejo, Gautier, Rijk Zwaan, Syngenta, Vilmorin, Ramiro Arnedo, Enza Zaden, ISI Sementi, Tozer, Naktuinbouw and GEVES.

Isolates:

Four isolates of Fol: 4 were selected for validation: three coming from the previous ISF project and identified as candidate isolates for race 4 and a fourth proposed by Warwick University expected with a high level of aggressivity:

- Isolate 04750888 from the Netherlands provided by AGROINNOVA (G. Gilardi et al.) (already tested in last ISF RT) coded as **isolate I**.
- 1 aggressive isolate from UK coded as **isolate W** (supplying by University of Warwick).
- 1 isolate from the Netherlands (already tested in last ISF RT) coded as **isolate M.**
- 1 isolate from the Netherlands (already tested in last ISF RT) coded as **isolate N.**

Isolates were provided by GEVES to partners. GEVES multiplied the isolates and sent an actively growing culture to each participant. Each participant produced the inoculum according to the UPOV protocol.

Panel:

During the setting up of the project, partners selected a panel (Table 3) made up of:

- Differentials to validate: Banchu Red Fire, Costa Rica, Patriot and Romabella.
- One resistant variety Ballerina and one susceptible variety Gisela
- Candidate controls with different levels of resistance: Cobham green, Palmos, Lomeria and 3 varieties non denominated (var_3, var_4 and var_7).

For the comparative test, four varieties were in duplicate indicated with the status "uncoded" which meant that either the denomination and the expected level of resistance were known (Banchu Red Fire and Costa Rica) or only the expected level of resistance were known (Ballerina and Gisela). These varieties were used as controls to validate the test and to help for interpretation of the other varieties.
All the varieties of the panel were also included in the comparative test, with the status "coded", which meant that the denomination and the expected level of resistance were unknown.

Table 3: panel selected for *Fusarium oxysporum* f. sp. *lactucae* race 4 comparative test

Code in test plan	Denomination	Expected	Code in test	Status
Banchu Red Fire	Banchu Red Fire lot CF676	IR	Banchu Red Fire	Uncoded
var_e	Banchu Red Fire lotCF676	IR	var_11	Coded
var_g	Ballerina	R	var_g	Uncoded
var_g	Ballerina	R	var_14	Coded
Costa Rica	Costa Rica	S	Costa Rica	Uncoded
var_b	Costa Rica	S	var_02	Coded
var_d	Gisela	S	var_d	Uncoded

var_d	Gisela	S	var_8	Coded
var_a	Patriot	S	var_05	Coded
var_c	Romabella	S	var_13	Coded
var_f	Cobham green	R	var_01	Coded
var_h		R	var_03	Coded
var_i		R	var_07	Coded
var_j	Palmos	R	var_12	Coded
var_k	Lomeria	R	var_10	Coded
var_l		R	var _04	Coded

The seeds were collected by GEVES to be sent to each partner.

Protocol:

Each laboratory has tested all isolates on the whole panel according to UPOV guidelines for Fol: 1 (annex 1). Some steps were specified:

- Method of inoculation: soaking protocol
  - Sowing of varieties in compost or vermiculite,
  - At the good stage for inoculation, lifting the plants carefully,
  - Soaking roots in adjusted spore suspension during 5 to 15min,
  - Transplant in a new container (pots or trays).
- Quantification inoculum: concentration adjusted to 10^6 sp/mL
- Substrate: compost
- Temperature: between 23°C and 26°C
- 20 plantlets tested
- Notation: when symptoms expressed on the uncoded S control are at note 3 (UPOV notation scale) at least
- Notation scale: observation of browning vessels for classes 0 and 1.

ii. Polytunnel test:

Participant:

– 1 Participant was involved in the test in polytunnel: Warwick University

Isolates:

The race 4 isolate provided by Warwick University (W) for the comparative test in controlled conditions was also used for test in polytunnel.

Panel:

The same panel of varieties used in the comparative test in controlled conditions was also used in the polytunnel test.

iii. Statistical analyses:

Several statistical tests were used for this study to compare results and to harmonize the interpretation. A small working group was defined managed by GEVES and including Enza Zaden and Syngenta to perform the statistical analyses.
The statistical tool Pathostat (available free of charge on the GEVES Website https://www.geves.fr/tools/pathostat/) was mainly used for the interpretation of varieties depending on the distribution of plants per class of observation and, in comparison with controls reaction (Cochran-Armitage test for trend). Pathostat was also used to illustrate the distribution of plants per class.
ANOVA, Tukey tests on disease index, box plots (with an LSD test) and calculation of reproducibility and accuracy were also used.

b. Results

i. Comparative test in controlled conditions:

No results were obtained for the variety Cobham green due to a lack of germination.

A first analysis of results in controlled conditions was presented by GEVES based on two interpretations. The first one was done by labs (Table 4) and a second one (Table 5) was obtained by the statistic tool (Pathostat). The interpretation done by labs showed more differences between results while interpretation with Pathostat showed less differences between labs.

Globally, differences of aggressivity were shown between isolates. The three isolates I, M, and W observed as more aggressive, and the isolate N observed as less aggressive. There was no difference of virulence between isolates and all varieties had the same interpretation regardless of the isolate.

Two differentials Patriot and Romabella confirmed the results observed in the ringtest of 2018-2019, when results were obtained that were not expected and contradicting the proposed differential table. In the present ringtest of 2021, Patriot was observed clearly resistant (intermediate resistant in few labs) when it was expected susceptible to race 4. Romabella was observed mainly intermediate resistant (sometimes resistant and rarely susceptible) when it was expected susceptible.

Table 4: results of the panel of varieties for the 4 candidate isolates of Fol: 4 with internal lab interpretation

			Isolate I											Isolate M								Isolate N				Isolate W						
	Code in test	expected	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 9	Lab 9	Lab 11	Lab 12	Lab 3	Lab 4	Lab 5	Lab 7	Lab 9	Lab 9	Lab 11	Lab 12	Lab 1	Lab 4	Lab 6	Lab 7	Lab 3	Lab 5	Lab 6	Lab 7	Lab 9	Lab 9	Lab 12
var_l	Var_04	R	IR	R	R	R	HR	R	R	R	R	R	R	R	IR	HR	R	R	R	R	R	IR	IR	R	R	R	HR	R	R	R	R	R
var_k	Var_10	R	HR	R	R	R	HR	R	R	HR	HR	R	R	R	R	HR	R	R	HR	R	R	IR	R	R	R	R	HR	IR	R	R	HR	R
var_h	Var_03	R	IR	IR?	R	R	HR	R	R	HR	HR	R	IR	IR	S	HR	R	R	HR	R	R	IR	IR	R	R	R	HR	R	R	HR	HR	R
var_j	Var_12	R	IR	IR?	IR	IR	HR	R	R	R/IR	R	R	R	IR	IR	HR	R	R	R	R	R	HR	IR	R	R	IR	HR	R	R	R	R	R
var_i	Var_07	R	IR	IR?	IR	S	HR	IR	IR	HR	R	R	IR	low IR	S	HR	IR	HR	R	R	R	HR	S	R	R	S	HR	IR	IR	R	R	R
var_g = Ballerina	var_g	R	S	S	low IR	S	IR	S	S-IR	S	S	R	S	IR	S	IR	S	S	HS	R	IR	IR	S	R	IR	IR	HR	S	IR	R/IR	S	R
Ballerina	Var_14	R	IR	S	IR	S	IR	S	R-IR	S	HS	R	S	IR	S	IR	IR	S	S	R	S	IR	IR	IR	R-IR	0	HR	S	R	R	IR	IR
var_e = Banchu	Var_11	IR	S	S	IR	S	HR	S	R	IR	IR/S	R	S	IR	IR	IR	R	IR	IR	R	R	IR	IR	R	R-IR	IR	IR	S	R	R/IR	R	R
Banchu Red Fire	Banchu Red Fire	IR	IR	IR?	IR	R/IR	HR	IR	IR	IR	IR/S	IR	IR	IR	IR	IR	R	IR	IR	IR	IR	IR	IR	R	R-IR	IR	HR	IR	R	R	R	R
var_a = Patriot	Vat_05	S	IR	IR?	IR	S	HR	IR	R	IR	IR	R	S	IR	S	HR	R	R	IR	R	IR	IR	IR	R	R	IR	HR	R	IR	R	HR/R	R
var_c = Romabella	Var_13	S	S	S	low IR	S	IR	S	R	S	IR	R	IR	IR	S	IR	IR	IR/S	IR	IR	IR	IR	IR	R	IR	IR	HR	S	R	IR	IR	R
var_b = Costa Rica	Var_02	S	S	S	low IR	S	S	S	S	HS	HS	S	S	S	S	S	S	S	HS	S	IR	S	S	IR	S	S	S	S	S	IR	S	IR
Costa Rica	Costa Rica	S	S	S	S	S	S	S	S	S/HS	HS	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	HS	HS	S
var_d	Var_08	S	S	S	S	S	S	S	S	HS	HS	S	S	S	S	S	S	HS	HS	S	S	S	S	IR	S	S	S		S	S	HS	S
Var_d = Gisela	Var_d	S	S	S	S	S	S	S	S	HS	HS	S	S	S	S	S	S	HS	HS	S	S	S	S	R	S	S	S	IR	S	S	HS	S

Table 5: results of the panel of varieties for the 4 candidate isolates of FoI: 4 with Pathostat interpretation

			Isolate I											Isolate M								Isolate N				Isolate W						
Test plan code	Code in test	expected	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 9	Lab 9	Lab 11	Lab 12	Lab 3	Lab 4	Lab 5	Lab 7	Lab 9	Lab 9	Lab 11	Lab 12	Lab 1	Lab 4	Lab 6	Lab 7	Lab 3	Lab 5	Lab 6	Lab 7	Lab 9	Lab 9	Lab 12
var_l	Var_04	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
var_k	Var_10	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
var_h	Var_03	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
var_j	Var_12	R	R	R	R	IR	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
var_i	Var_07	R	R	R	IR	IR	R	R	R	R	R	R	R	IR	S	R	IR	R	R	R	R	R	R	R	R	S	R	R	IR	R	R	R
var_g	var_g	R	IR	S	IR	S	IR	S	IR	S	IR	R	S	R	S	IR	S	S	S	R	R	IR	IR	R	IR	R	R	R	IR	IR	IR	R
var_g = Ballerina	Var_14	R	R	S	IR	S	IR	S	R	S	IR	R	S	R	S	IR	IR	S	S	R	IR	R	R	IR	IR		R	S	R	R	IR	R
var_e = Banchu	Var_11	IR	R	S	R	IR	R	S	R	R	R	R	IR	R	R	R	R	IR	R	R	R	R	R	R	IR	R	IR	R	R	IR	R	R
Banchu Red Fire	Banchu Red Fire	IR	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
var_a = Patriot	Vat_05	S	R	R	IR	IR	R	R	R	R	R	R	IR	IR	S	R	R	R	R	R	R	R	R	R	R	R	R	R	IR	R	R	R
var_c = Romabella	Var_13	S	S	S	S	IR	IR	S	R	S	IR	R	R	IR	S	IR	IR	IR	R	R	R	IR	R	R	IR	IR	R	S	R	IR	IR	R
var_b = Costa Rica	Var_02	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	IR	S	S	S	S	S	IR	S	IR
Costa Rica	Costa Rica	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
var_d	Var_08	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	IR	S	S	S		S	S	S	S
var_d = Gisela	Var_d	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	R	S	S	S	S

The analysis was repeated and extended with the results in polytunnels which were assessed later than tests in controlled conditions.
The Anova showed that in controlled conditions, there was no effect of the interaction isolate x variety. This confirms that there was no difference of virulence between candidate isolates for Fol: 4 (Table 6). Only the main effects of variety and isolate are highly significant.

Table 6: analysis of variance based on the 4 isolates and the varieties of the panel in controlled conditions

Anova

Table 5: Analysis of Variance Model

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Isolate	3	10725	3575	4.45	0.004089
Variety	14	632813	45201	56.27	1.105e-115
Isolate:Variety	42	14947	355.9	0.443	0.9992
Residuals	1001	804123	803.3		

The difference of aggressivity between isolates was confirmed (Table 7). The isolate N was the less aggressive (group A) and isolates W and M were more aggressive (group B), while the isolate I bridged the gap between the two groups of aggressivity (group AB).

Table 7: Tukey tests on effect of isolate based on the disease index in controlled conditions

Tukey : effect of isolate

Isolate	lsmean	SE	df	lower.CL	upper.CL	.group
N	43.34711	1.955845	1001	38.31403	48.38019	A
I	49.45687	1.636378	1001	45.24589	53.66785	AB
W	51.24102	1.735430	1001	46.77514	55.70689	B
M	52.45476	1.682304	1001	48.12560	56.78392	B

Moreover, with the Tukey test on effect of varieties in controlled conditions, it was possible to classify globally the varieties between three groups of level of resistance (Table 8). The varieties Costa Rica and Gisela were judged as susceptible, the varieties Ballerina, Banchu Red Fire, Patriot, var_7 and Romabella were judged as intermediate resistant and the varieties var_3, var_4, Lomeria (var_10) and Palmos (var_12) were judged as resistant.

Table 8: Tukey tests on effect of variety based on the disease index in controlled conditions

Tukey : effect of variety

Variety	lsmean	SE	df	lower.CL	upper.CL	.group	
Lomeria	11.27610	3.395353	1001	-0.2675194	22.81971	A	R
var_4	16.83237	3.395353	1001	5.2887534	28.37598	A	
var_3	22.05890	3.395353	1001	10.5152889	33.60252	A	
Palmos	23.09166	3.395353	1001	11.5480448	34.63528	AB	
var_7	39.19377	3.395353	1001	27.6501517	50.73738	BC	IR
Patriot	40.70498	3.395353	1001	29.1613650	52.24860	CD	
Banchu Red Fire	43.10326	3.395353	1001	31.5596475	54.64688	CD	
Banchu Red Fire	45.00846	3.395353	1001	33.4648464	56.55208	CD	
Romabella	50.92054	3.395353	1001	39.3769288	62.46416	CD	
Ballerina	55.81413	3.395353	1001	44.2705121	67.35774	D	
Ballerina	56.49131	3.400055	1001	44.9317070	68.05091	D	
Costa Rica	79.51242	3.416903	1001	67.8955368	91.12930	E	S
Costa Rica	82.75242	3.416903	1001	71.1355381	94.36930	E	
Gisela	83.86528	3.395353	1001	72.3216652	95.40890	E	
Gisela	86.24848	3.446315	1001	74.5316068	97.96536	E	

R= resistant, IR= intermediate resistant and S= susceptible

This interpretation was studied isolate per isolate by a Cochran Armitage test and a pairwise comparison between varieties (Figures 1 to 4). Per isolate, GEVES illustrated for each variety the repartition of plants per class.

Figure 1: interpretation of varieties in controlled conditions with isolate I

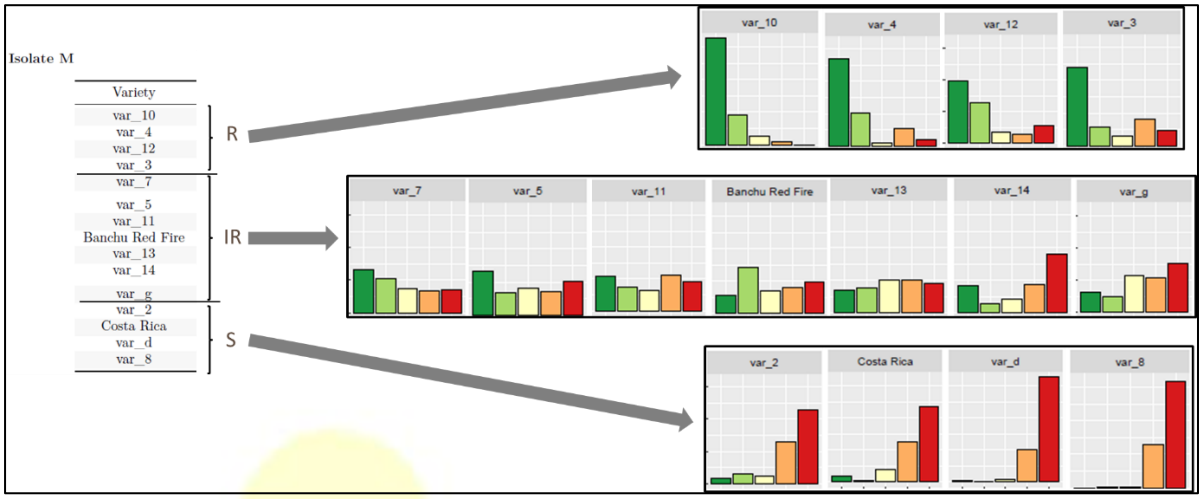

Figure 2: interpretation of varieties in controlled conditions with isolate M

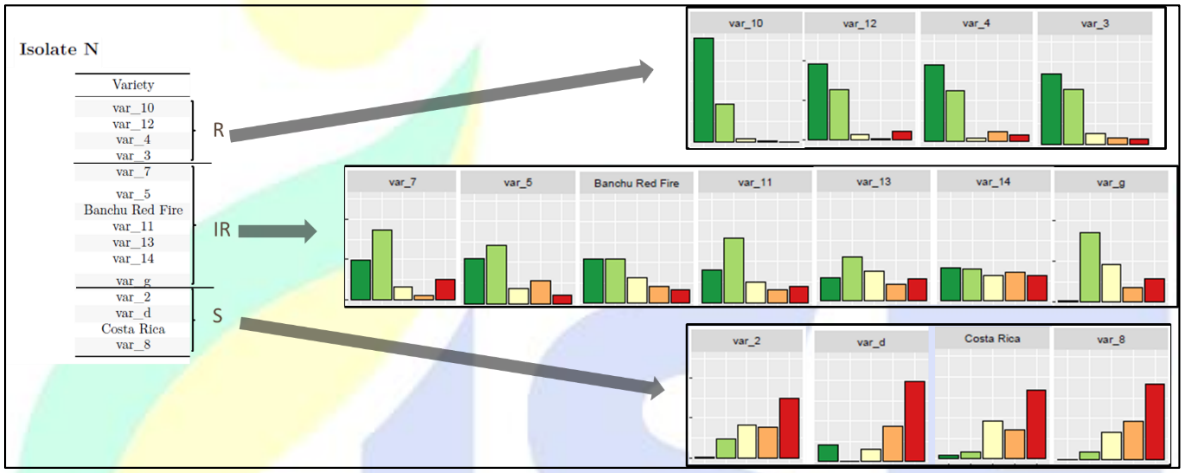

Figure 3: interpretation of varieties in controlled conditions with isolate N

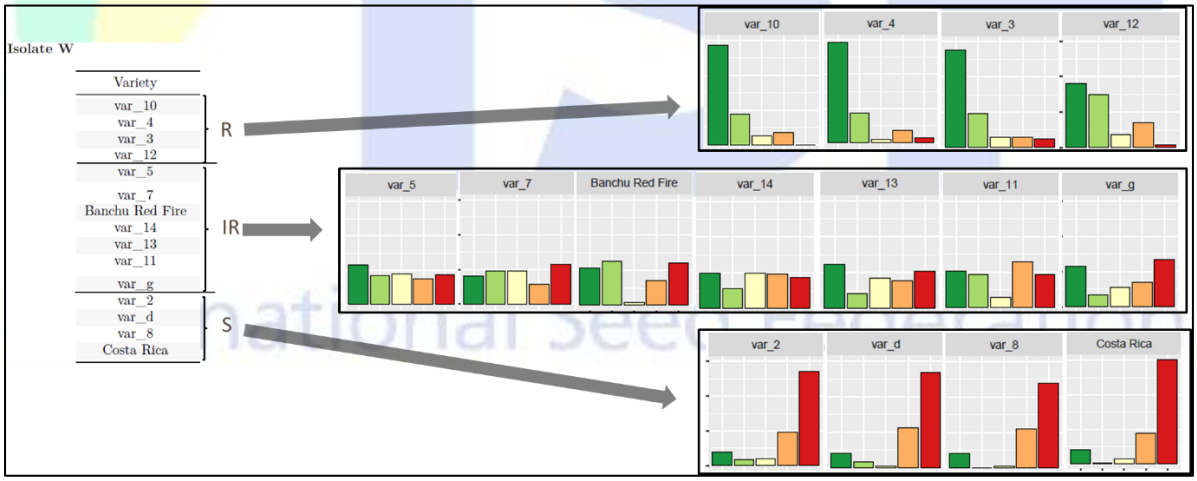

Figure 4: interpretation of varieties in controlled conditions with isolate W

The varieties had the same interpretation for the four isolates (Table 9). The distribution of plants was globally the same not depending on isolate. For resistant varieties plants were mainly in classes 0 and 1, for intermediate resistant varieties plants were in the five classes and for susceptible varieties plants were mainly in classes 3 and 4.

Table 9: interpretation of varieties in controlled condition based on the distribution of plants per class

var_d = var_8 (Gisela)	S
Costa Rica = var_2	S
var_g = var_14 (Ballerina)	IR
var_13 (Romabella)	IR
Banchu Red Fire = var_11	IR
var_5 (Patriot)	IR
var_7	IR
var_12 (Palmos)	R
var_3	R
var_4	R
var_10 (Lomeria)	R

=: same variety tested with different codes; (X): denomination of the variety when it was tested coded

ii. Polytunnel test:

The test in polytunnel (performed only with isolate W) was judged based on two symptoms: wilting on leaves and vascular browning (figure 5). Only two varieties Gisela and Costa Rica developed *Fusarium* wilt with severe symptoms for Gisela (also coded var_8) and with moderate symptoms for Costa Rica (also coded var_2). This confirmed the susceptibility of these two varieties to Fol: 4. The other varieties developed little or no wilt symptoms.
Again, only the two varieties Gisela and Costa Rica showed high level of vascular browning, while Banchu Red Fire (also coded var_11), var_13, Ballerina (also coded var_14) were observed with lower level of vascular browning. The varieties var_3, var_4, Patriot (also coded var_5), var_7, Lomeria (var_10) and Palmos (var_12) developed no or very few vascular browning

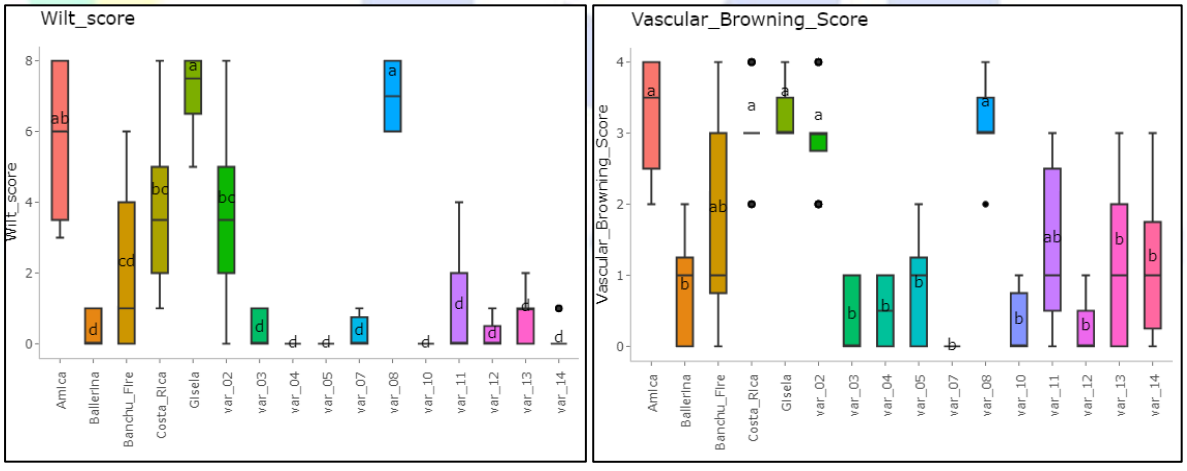

Figure 5 : *Fusarium* wilt score and vascular browning score in polytunnel test for the panel of varieties with isolate W

Analysis of results in polytunnel confirmed globally the classification of varieties in the three groups of resistance (table 10).

Table 10: classification of varieties in the resistance level depending on polytunnel results

Variety	Wilt score	Vascular browning score	Interpretarion
Costa Rica = var_2	bc	a	S
var_d = var_8 (Gisela)	a	a	S
var_g = var_14 (Ballerina)	d	b	IR
Banchu Red Fire = var_11	cd/d	ab	IR
var_13 (Romabella)	d	b	IR
var_5 (Patriot)	d	b	R
var_7	d	b	R
var_3	d	b	R
var_4	d	b	R
var_10 (Lomeria)	d	b	R
var_12 (Palmos)	d	b	R

=: same variety tested with different codes; (X): denomination of the variety when it was tested coded; grey: varieties with a different interpretation between tests in controlled condition and polytunnel.

Only the varieties Patriot and var_7, judged as intermediate resistant in controlled conditions, were judged differently resistant in polytunnel test. That confirmed the "sufficient" correlation with results obtained in controlled conditions with the four isolates.

The importance of symptoms of brown vessels for market was discussed and it appeared that it has not the same importance depending on companies. If only wilt symptoms are considered, it was possible to distinguish only two levels of resistance (susceptible and resistant). But taking into account of vascular browning and results in controlled conditions, three levels of resistance were observable with the presence of intermediate resistance (low level of vascular browning in polytunnel or distribution of plants between the classes in controlled conditions).

c. Scenarios of decision rules

Based on the results, different scenarios of interpretation were discussed by partners to evaluate if an interpretation in two notes (susceptible and resistant) or in three notes (susceptible, intermediate resistant and resistant) could be proposed for a DUS protocol.
The data of the comparative test were used by GEVES to continue the analysison two scenarios of decision rules selected by partners (Figure 9). It was decided to test the scenario with candidates of intermediate resistant and resistant controls with different levels of resistance to obtain interpretations from the less to the more restrictive.

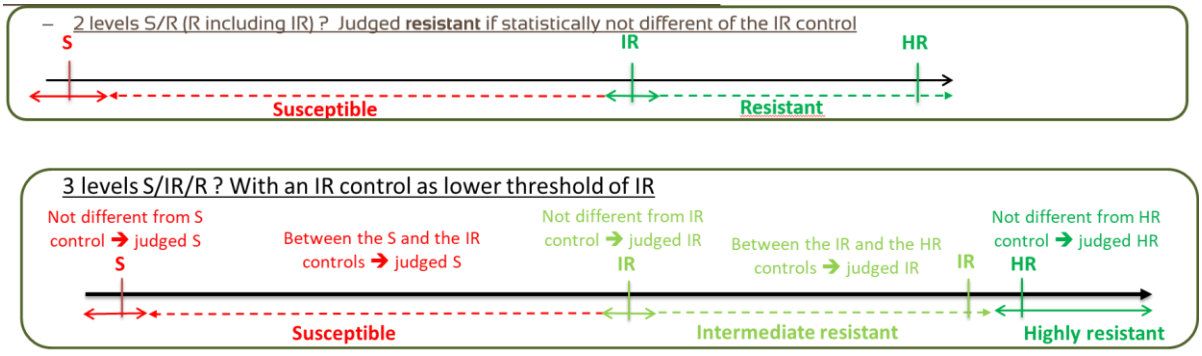

Figure 6: scenarios of decision rules to compare for Fol: 4

For the first scenario with only two levels Susceptible and Resistant, one susceptible candidate control (Gisela), three intermediate resistant candidate controls (lower border of resistance) (Ballerina, Banchu Red Fire and Patriot), and two resistant candidate controls (Palmos and Lomeria) were chosen by partners. The interpretation of the varieties according to the first scenario and with the comparison of all the combinations of candidate controls was defined with the Pathostat tool (detailed results in annex 2).
For all the combinations of controls tested:

- A good correlation between labs was observed with a reproducibility around 0.8.
- The four varieties expected as resistant (var_3, var_4, Lomeria and Palmos) were well interpreted as resistant.
- The interpretation of varieties expected with an intermediate resistant level were judged differently depending on the intermediate resistant and the resistant controls selected. Ballerina being the intermediate resistant control given the most of varieties interpreted as resistant. And Patriot the one given the most of varieties interpreted as susceptible.
- Lomeria and Palmos had less effect than lab conditions.

For the second scenario, with three level of resistance (Susceptible, intermediate resistant and highly resistant) it was decided to keep Gisela as susceptible candidate control, to include two intermediate resistant control: Ballerina (with the lower level of intermediate resistance) and var_7 (with the higher level of intermediate resistance) and to compare two highly resistant candidate controls (Palmos and Lomeria). The interpretation of the varieties according to the second scenario and with the comparison of the two highly resistant candidate controls was defined with the Pathostat tool (detailed results in annex 3). For both combinations, isolate I showed the higher level of accuracy and the best reproducibility between laboratories (around 0.6).

d. Decisions and conclusion

During the meeting of presentation of results of the different scenarios, GEVES and ISF organized a system of pool to simplify the decisions to answer to the objectives of the project. The vote of partners not present during the meeting was collected by GEVES before the meeting and included in the answers. The principle of the majority was retained for the selection of the decision rule and the reference material (isolate and controls) for the evaluation of the resistance of lettuce to Fol: 4 (Figure 10) and for the validation of the differential table (Table11).

Interpretation rule: the second scenario with **three levels of resistance (**susceptible, intermediate resistance and highly resistance) was retained by the majority.

Reference isolate for *Fusarium oxysporum* f. sp. *lactucae* race 4: the **isolate I,** isolate 04750888 provided by G. Gilardi (AGROINNOVA) was unanimously selected.

Reference controls:

- **Gisela** was selected as susceptible reference control. This variety is expected to be susceptible to the four races of *Fusarium* but it has to be validated on races 2 and 3.
- **Ballerina** was selected as lower level of intermediate resistant control.
- **Patriot** was selected as higher level of intermediate resistant control
- **Lomeria and Palmos** were selected as highly resistant controls.

Figure 7: interpretation rule and controls for evaluation of resistance of lettuce to Fol: 4

During the discussion, Naktuinbouw made a point of noting that the selection of an interpretation in 3 classes (S, IR, and HR) made by the partners of the project is for market claims, and that the CPVO protocol may still have only 2 classes (S and R) (proposal supported by Naktuinbouw). This simplification proposal, which does not correspond to the decision made in this project and the choice of a lot of breeding companies regarding market claims, will have to be discussed at CPVO level by the examination offices at the time of the proposal to add the Fol: 4 characteristics in the Technical protocol for DUS.

Differentials table: the selected controls were added to the differentials table (Table 11). The interpretations of Patriot and Romabella for race 4 were updated (from susceptible to intermediate resistant) following the results of the project. The resistant level observed during the project was identified as HR (highly resistant) in the differentials table to comply with the terminology.

Table 11: Table of differentials selected and validated in the project

Differential host	**Fol: 1**	**Fol: 2**	**Fol: 3**	**Fol: 4**
Gisela	S	ND	ND	S
Patriot	S	S	S	IR
Costa Rica N°4	HR	S	S	S
Romabella	HR	HR	S	IR
Banchu Red Fire	S	HR	S	IR
Ballerina	S	ND	ND	IR
Lomeria	S	ND	ND	HR
Palmos	HR	ND	ND	HR

S: susceptible, IR: intermediate resistant, HR: highly resistant, ND: no data

Follow up this project, complementary tests will be conducted by two partners and GEVES to complete the differentials table for missing results for races 2 and 3. If Gisela was found not to be susceptible to all races, a new search for a susceptible control to all races should

be carried out. Moreover, a choice between the two varieties Lomeria and Palmos could be done depending on results on races 2 and 3 not yet known.

The availability of the reference material (differentials, controls and isolate I) was checked with the owner which confirmed that this material can be used for DUS and the material will be integrated in MATREF.

Partners discussed about the communication to plan with the conclusion of this project. The differential table was proposed for validation to ISF website. Partners will share the conclusions by social media, Newsletter, etc… An abstract has been accepted for the IHC 2022 congress (poster) and an article will be written by John Clarkson (University of Warwick).

GEVES and all the partners of the ISF project wish to thank Giovanna Gilardi and AGROINNOVA for the supplying of *Fusarium oxysporum* f. sp. *lactucae* race 4 material and for the confirmation of the availability of the reference isolate for a future use for disease resistance testing.

Organisation of the project

Preparation, participation, and coordination of the project

GEVES coordinated this project.

Participants:

Table 12: Participants in the ISF lettuce/Fol: 4 project

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Meetings of the project:

Kick-off meeting online on 01 July 2021

Meeting on controlled condition results online on 06 October 2021

Working group meeting on analysis of results on 18 November 2021

Final meeting online on 15 December 2021

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Annex 1

Ad 53: Resistance to *Fusarium oxysporum* f.sp. *lactucae* (Fol) race 1

1. Pathogen	*Fusarium oxysporum* f.sp. *lactucae*
2. Quarantine status	EPPO alert list
3. Host species	lettuce - *Lactuca sativa* L.
4. Source of inoculum	NIAS Genebank[7] (JP), CREA-SCS[8] (IT), Naktuinbouw[9] (NL), GEVES[10] (FR)
5. Isolate	Fol: 1
6. Establishment isolate identity	use microscope and inoculation to lettuce susceptible standard
7. Establishment pathogenicity	use lettuce susceptible standard
8. Multiplication inoculum	
8.1 Multiplication medium	inoculation by sowing on contaminated soil: Wheat bran-soil medium inoculation by soaking seedlings: on synthetic liquid medium (e.g. Potatoes Dextrose Broth)
8.6 Harvest of inoculum	inoculation by sowing on contaminated soil: 7-10 day-old culture inoculation by soaking seedlings: 15 days
9. Format of the test	
9.1 Number of plants per genotype	at least 30, in case of doubt 60
9.2 Number of replicates	at least 2
9.3 Control varieties	susceptible: Cobham Green, Patriot (Cobham Green is slightly less susceptible than Patriot) moderately resistant: Affic, Fuzila, Natexis (Natexis is the lower level of moderate resistance) resistant: Costa Rica No.4, Romasol
9.4 Test design	include control varieties
9.5 Test facility	greenhouse or climate room
9.6 Temperature	25-28 °C (day) / 20 °C (night)
9.7 Light	under natural day length
10. Inoculation	two methods can be used for inoculation:

	sowing seeds on contaminated soil	**soaking seedlings**
10.1 Preparation inoculum	wheat bran-soil medium culture mixed with sterilized soil	soaking of roots and of hypocotyl axis for 5 to 15 min in the inoculum suspension
10.2 Quantification inoculum	soil : culture = 20 : 1	spores are harvested and adjusted to 10^6 to 10^7 sp/ml
10.3 Plant stage at inoculation	seeds stimulated to emerge (remark: avoid seeds rotted by factors other than pathogen)	cotyledons to 2 or 3 leaves appearing

10.4 Inoculation method	two methods can be used, as described above
10.5 First observation	7- 10 days post inoculation
10.6 Second observation	14 days post inoculation
10.7 Final observations	20-25 days post inoculation (sowing or soaking). One or two of these 3 observations may be sufficient. The observation for inoculation by soaking is destructive since stems are cut for the observation of vessels.
11. Observations	
11.1 Method	visual and/or counting number of plants with symptom; as information calculate a disease index.

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11.2 Observation scale

inoculation by sowing seeds on contaminated soil	inoculation by soaking seedlings
0: healthy	0: plant without symptoms and healthy vessels 
1: slightly stunting, growing reduction	1: plant with brown vessels only below the cotyledon without yellowing and wilting 
2: severely stunting	2: plant with brown vessels above the cotyledon, without yellowing and wilting 
3: die	3: plant yellowing and wilting, brown vessels 
	4: dead plant 

11.3 Validation of test

results should be compared with results of controls and are depending of the aggressiveness of the test and the distribution of the plants over the categories.
a disease index may be helpful (example for the method of inoculation by soaking seedlings: DI= (0A + 1B + 2C + 3D + 4E) / (A + B + C + D + E), where A to E are number of plants in each category).

12. Interpretation of data in terms of UPOV characteristic states

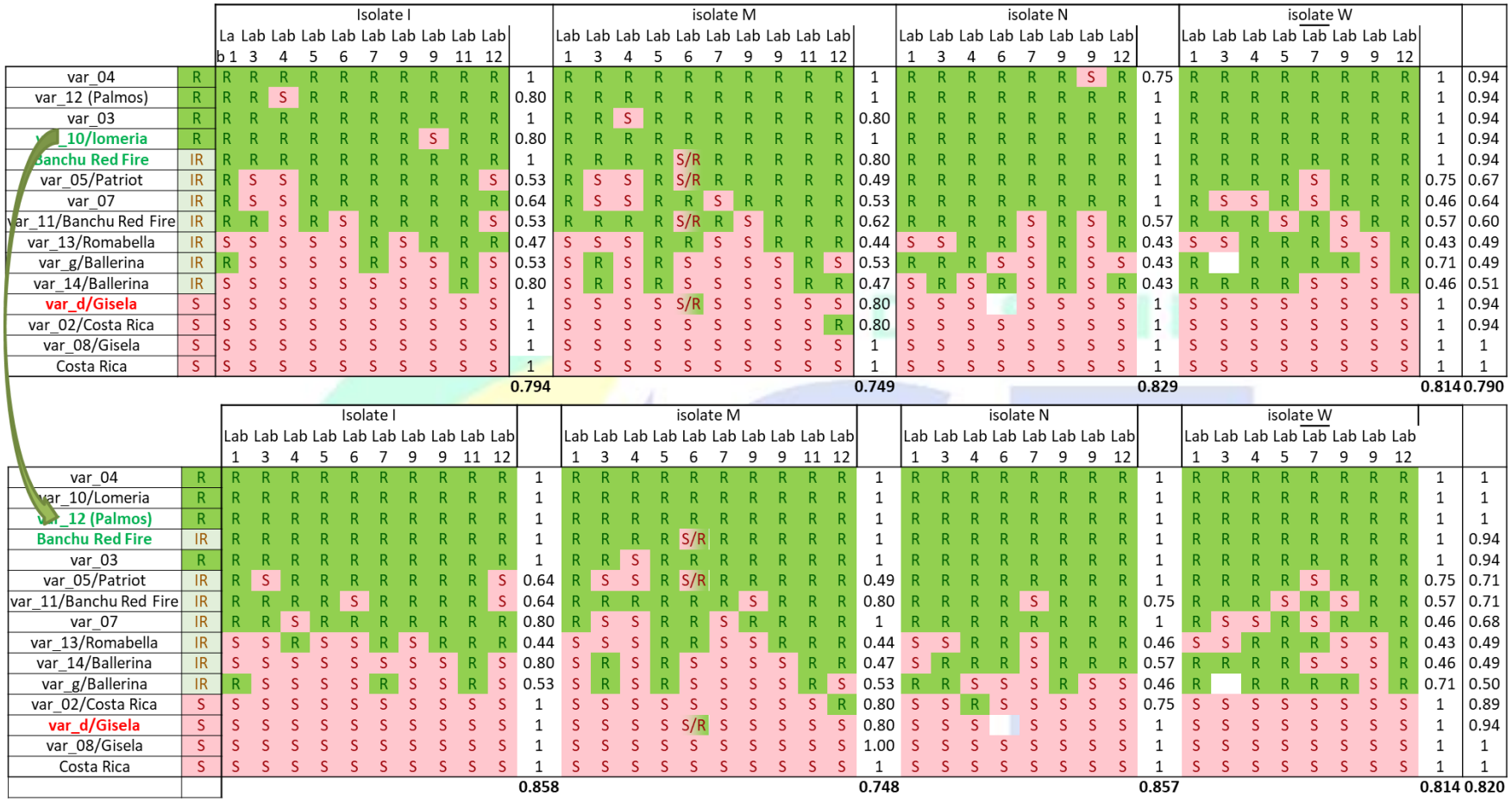
compare the distribution over the categories with the result of the controls.

Annex 2: interpretation of the varieties done by Pathostat based on the first scenario of decision rule with two levels of interpretation

		Isolate I												isolate M												isolate N												isolate W												Global reproducibility
	observed	Lab 1	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 9	Lab 9	Lab 11	Lab 12			Lab 1	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 9	Lab 9	Lab 11	Lab 12			Lab 1	Lab 3	Lab 4	Lab 6	Lab 7	Lab 9	Lab 9	Lab 12					Lab 1	Lab 4	Lab 5	Lab 7	Lab 9	Lab 9	Lab 12					
var_10/Lomeria	R	R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	1				1
var_04	R	R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	1				1
var_03	R	R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	1				1
var_12/Palmos	R	R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	1		R	R	R	S	R	R	R	0.71				0.94
var_05/Patriot	IR	R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	S/R	R	R	R	R	R	0.80		R	R	R	R	R	R	R	R	1		R	R	R	S	R	R	R	0.71				0.89
var_g/ Ballerina	IR	R	R	R	R	S/R	R	R	R	R	R	0.80		S/R	R	S/R	R	S/R	R	R	R	R	R	0.47		R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	1				0.78
var_07	IR	R	R	R	R	R	S	R	R	R	R	0.80		R	R	R	R	R	R	R	R	R	R	1.00		R	R	R	R	R	R	R	R	1		R	S	R	S	R	R	R	0.52				0.84
var_11/Banchu Red Fire	IR	R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	S/R	R	R	R	R	R	0.80		R	R	R	R	R	R	R	R	1		R	R	S	R	S	R	S	0.43				0.79
Banchu Red Fire	IR	R	R	R	R	R	S	R	R	S	R	0.64		R	R	R	R	S/R	R	R	R	R	R	0.80		R	R	R	R	R	R	R	R	1		S	R	R	S	R	R	S	0.43				0.70
var_13/Romabella	IR	R	S	R	R	S	R	R	R	R	R	0.64		S	R	R	R	R	R	R	R	R	R	0.80		R	S	R	R	S	R	R	R	0.57		S	R	R	R	S	R	R	0.52				0.67
var_14/Ballerina	IR	R	R	S	S	S/R	S	R	R	R	S	0.36		S	S	S	R	S	S	R	S	R	R	0.47		S	R	S	R	S	R	S	R	0.43		R	R	R	S	S	S	R	0.43				0.46
var_02/Costa Rica	S	R	S	S	S	S	S	S	S	S	R	0.64		S	R	S	S	S	R	S	S	R	S	0.53		S	S	S	R	S	R	S	S	0.57		S	S	S	S	S	S	R	0.71				0.64
Costa Rica	S	R	S	S	S	S	S	S	S	S	R	0.64		S	S	S	S	S	R	S	S	S	S	0.82		S	S	S	S	S	R	S	S	0.75		S	S	S	S	S	S	S	1				0.79
var_08/Gisela	S	R	S	S	S	S	S	S	S	S	S	0.80		S	S	S	S	S	S	R	S	S	S	0.80		S	S	S	S	S	S	S	S	1		S	S	S	S	S	S	S	1				0.89
var_d/Gisela	S	S	S	S	S	S	S	S	S	S	S	1		S	S	S	S	S	S	S	S	S	S	1		S	S	S	R	S	S	S	S	0.75		S	S	S	S	S	S	S	1				0.94
												0.810												**0.806**											**0.862**											**0.748**		**0.809**

		Isolate I												isolate M												isolate N												isolate W												Global reproducibility
	observed	Lab 1	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 9	Lab 9	Lab 11	Lab 12			Lab 1	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 9	Lab 9	Lab 11	Lab 12			La b 1	Lab 3	Lab 4	Lab 6	Lab 7	Lab 9	Lab 9	Lab 12					Lab 1	Lab 4	Lab 5	Lab 7	Lab 9	Lab 9	Lab 12					
var_03	R	R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	1				1
var_04	R	R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	1				1
var_10/Lomeria	R	R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	1				1
var_12/Palmos	R	R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	1				1
var_05/Patriot	IR	R	R	R	R	R	R	R	R	R	R	1		R	S	R	R	S/R	R	R	R	R	R	0.62		R	R	R	R	R	R	R	R	1		R	R	R	S	R	R	R	0.71				0.84
var_11/Banchu Red Fire	IR	R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	S/R	R	R	R	R	R	0.80		R	R	R	R	R	R	R	R	1		R	R	S	R	S	R	R	0.52				0.84
var_g/ Ballerina	IR	R	R	R	R	S/R	R	R	R	R	R	0.80		S/R	R	S/R	R	S/R	R	R	R	R	R	0.47		R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	1				0.78
Banchu Red Fire	IR	R	R	R	R	R	S	R	R	S	R	0.64		R	R	R	R	S/R	R	R	R	R	R	0.80		R	R	R	R	R	R	R	R	1		S	R	R	R	R	R	R	0.71				0.79
var_07	IR	R	R	R	R	R	S	R	R	R	R	0.80		R	S	R	R	R	R	R	R	R	R	0.80		R	R	R	R	R	R	R	R	1		R	S	R	S	R	R	R	0.52				0.79
var_13/Romabella	IR	S	S	R	R	S	R	R	R	R	R	0.53		S/R	S	R	R	S/R	R	R	R	R	R	0.47		R	S	R	R	S	R	R	R	0.57		S	R	R	R	S	R	R	0.52				0.55
var_14/Ballerina	IR	S	R	S	S	S/R	S	S	R	R	S	0.40		S/R	S	S/R	R	S/R	S	R	S	R	R	0.20		S	R	R	R	S	R	R	R	0.57		R	R	R	S	S	S	R	0.43				0.38
var_02/Costa Rica	S	S	S	S	S	S	S	S	S	S	R	0.80		S/R	S	S/R	S	S/R	S	R	S	S	R	0.24		S	S	R	R	S	S	S	S	0.57		S	S	S	S	S	S	R	0.71				0.57
Costa Rica	S	S	S	S	S	S	S	S	S	S	R	0.80		S/R	S	S/R	S	S/R	S	R	S	S	S	0.33		S	S	S	S	S	S	S	S	1		S	S	S	S	S	S	S	1				0.73
var_d/Gisela	S	S	S	S	S	S/R	S	S	S	S	S	0.80		S/R	S	S/R	S	S/R	S	S	S	S	S	0.47		S	S	S	R	S	S	S	S	0.75		S	S	S	S	S	S	S	1				0.73
var_08/Gisela	S	S	S	S	S	S	S	S	S	S	S	1		S/R	S	S/R	S	S/R	S	S	S	S	S	0.47		S	S	S	S	S	S	S	S	1		S	S	S	S	S	S	S	1				0.83
												0.839												**0.644**											**0.898**											**0.810**		**0.789**

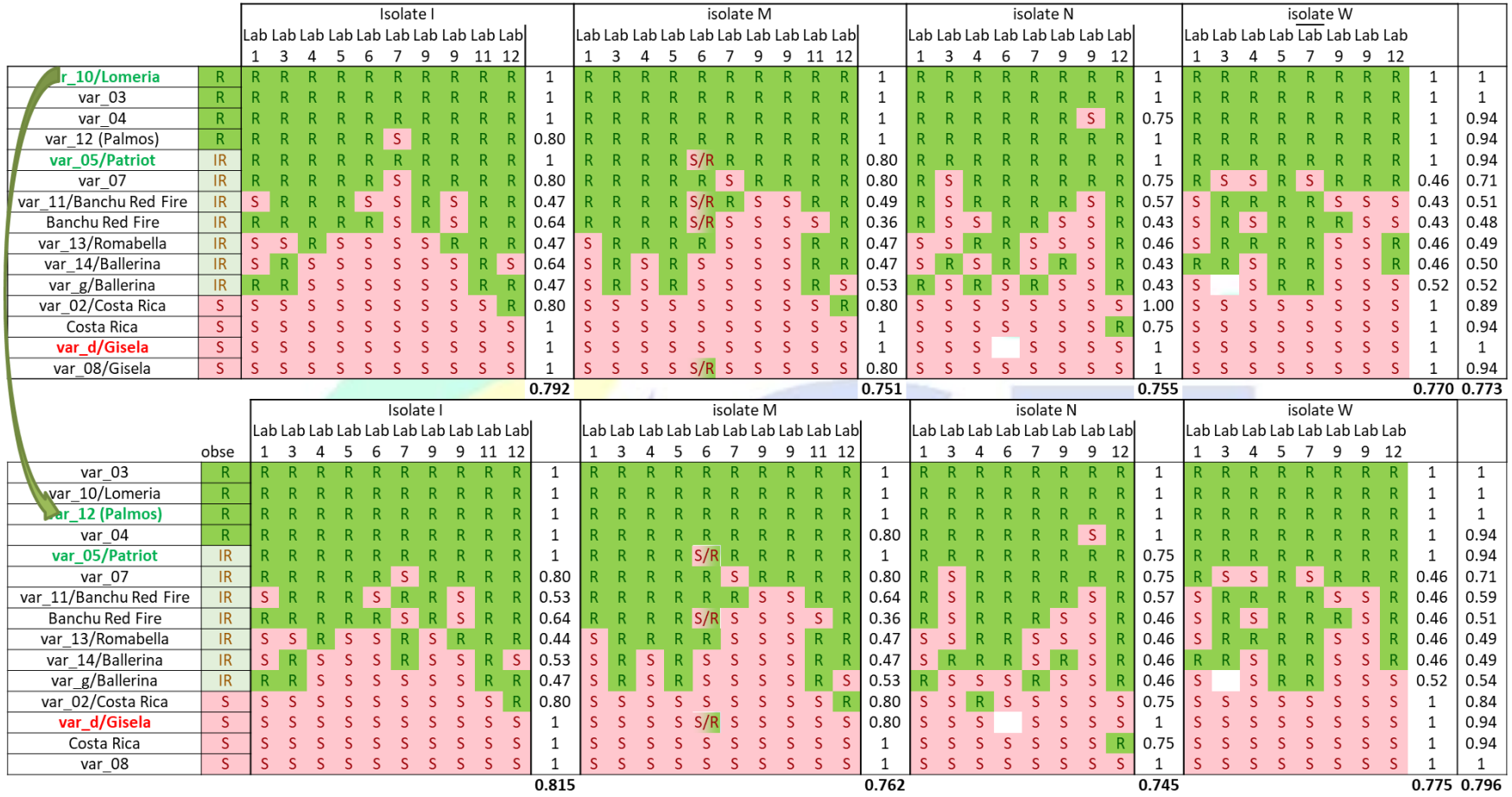
Figure 8: interpretation with Balleria as intermediate resistant candidate control and Lomeria (var_10) (top table) and Palmos (var_12) (bottom table) used as candidate controls



		Isolate I												isolate M												isolate N												isolate W													
		La b	Lab 1	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 9	Lab 9	Lab 11	Lab 12		Lab 1	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 9	Lab 9	Lab 11	Lab 12		Lab 1	Lab 3	Lab 4	Lab 6	Lab 7	Lab 9	Lab 9	Lab 12		Lab 1	Lab 3	Lab 4	Lab 5	Lab 7	Lab 9	Lab 9	Lab 12			
var_04	R	R	R	R	R	R	R	R	R	R	R	1	R	R	R	R	R	R	R	R	R	R	1	R	R	R	R	R	R	S	R	0.75	R	R	R	R	R	R	R	R	1	0.94	
var_12 (Palmos)	R	R	R	S	R	R	R	R	R	R	R	0.80	R	R	R	R	R	R	R	R	R	R	1	R	R	R	R	R	R	R	R	1	R	R	R	R	R	R	R	R	1	0.94	
var_03	R	R	R	R	R	R	R	R	R	R	R	1	R	R	S	R	R	R	R	R	R	R	0.80	R	R	R	R	R	R	R	R	1	R	R	R	R	R	R	R	R	1	0.94	
var_10/lomeria	R	R	R	R	R	R	R	R	S	R	R	0.80	R	R	R	R	R	R	R	R	R	R	1	R	R	R	R	R	R	R	R	1	R	R	R	R	R	R	R	R	1	0.94	
Banchu Red Fire	IR	R	R	R	R	R	R	R	R	R	R	1	R	R	R	R	S/R	R	R	R	R	R	0.80	R	R	R	R	R	R	R	R	1	R	R	R	R	R	R	R	R	1	0.94	
var_05/Patriot	IR	R	S	S	R	R	R	R	R	R	S	0.53	R	S	S	R	S/R	R	R	R	R	R	0.49	R	R	R	R	R	R	R	R	1	R	R	R	R	S	R	R	R	0.75	0.67	
var_07	IR	R	S	S	R	R	R	R	R	R	R	0.64	R	S	S	R	R	S	R	R	R	R	0.53	R	R	R	R	R	R	R	R	1	R	S	S	R	S	R	R	R	0.46	0.64	
var_11/Banchu Red Fire	IR	R	R	S	R	S	R	R	R	R	S	0.53	R	R	R	R	S/R	R	S	R	R	R	0.62	R	R	R	R	S	R	S	R	0.57	R	R	R	S	R	S	R	R	0.57	0.60	
var_13/Romabella	IR	S	S	S	S	S	R	S	R	R	R	0.47	S	S	S	R	R	S	S	R	R	R	0.44	S	S	R	R	S	R	S	R	0.43	S	S	R	R	R	S	S	R	0.43	0.49	
var_g/Ballerina	IR	R	S	S	S	S	R	S	S	R	S	0.53	S	R	S	R	S	S	S	S	R	S	0.53	R	R	R	S	S	R	S	S	0.43	R		R	R	R	R	S	R	0.71	0.49	
var_14/Ballerina	IR	S	S	S	S	S	S	S	S	R	S	0.80	S	R	S	R	S	S	S	S	R	R	0.47	S	R	S	R	S	R	S	R	0.43	R	R	R	R	S	S	S	R	0.46	0.51	
var_d/Gisela	S	S	S	S	S	S	S	S	S	S	S	1	S	S	S	S	S/R	S	S	S	S	S	0.80	S	S	S		S	S	S	S	1	S	S	S	S	S	S	S	S	1	0.94	
var_02/Costa Rica	S	S	S	S	S	S	S	S	S	S	S	1	S	S	S	S	S	S	S	S	S	R	0.80	S	S	S	S	S	S	S	S	1	S	S	S	S	S	S	S	S	1	0.94	
var_08/Gisela	S	S	S	S	S	S	S	S	S	S	S	1	S	S	S	S	S	S	S	S	S	S	1	S	S	S	S	S	S	S	S	1	S	S	S	S	S	S	S	S	1	1	
Costa Rica	S	S	S	S	S	S	S	S	S	S	S	1	S	S	S	S	S	S	S	S	S	S	1	S	S	S	S	S	S	S	S	1	S	S	S	S	S	S	S	S	1	1	
												0.794											**0.749**									**0.829**										**0.814**	**0.790**

		Isolate I												isolate M												isolate N												isolate W													
		Lab 1	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 9	Lab 9	Lab 11	Lab 12			Lab 1	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 9	Lab 9	Lab 11	Lab 12			Lab 1	Lab 3	Lab 4	Lab 6	Lab 7	Lab 9	Lab 9	Lab 12			Lab 1	Lab 3	Lab 4	Lab 5	Lab 7	Lab 9	Lab 9	Lab 12					
var_04	R	R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	1	1	
var_10/Lomeria	R	R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	1	1	
var_12 (Palmos)	R	R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	1	1	
Banchu Red Fire	IR	R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	S/R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	1	0.94	
var_03	R	R	R	R	R	R	R	R	R	R	R	1		R	R	S	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	1	0.94	
var_05/Patriot	IR	R	S	R	R	R	R	R	R	R	S	0.64		R	S	S	R	S/R	R	R	R	R	R	0.49		R	R	R	R	R	R	R	R	1		R	R	R	R	S	R	R	R	0.75	0.71	
var_11/Banchu Red Fire	IR	R	R	R	R	S	R	R	R	R	S	0.64		R	R	R	R	R	R	S	R	R	R	0.80		R	R	R	R	S	R	R	R	0.75		R	R	R	S	R	S	R	R	0.57	0.71	
var_07	IR	R	R	S	R	R	R	R	R	R	R	0.80		R	S	S	R	R	S	R	R	R	R	1		R	R	R	R	R	R	R	R	1		R	S	S	R	S	R	R	R	0.46	0.68	
var_13/Romabella	IR	S	S	R	S	S	R	S	R	R	R	0.44		S	S	S	R	R	S	S	R	R	R	0.44		S	S	R	R	S	R	R	R	0.46		S	S	R	R	R	S	S	R	0.43	0.49	
var_14/Ballerina	IR	S	S	S	S	S	S	S	S	R	S	0.80		S	R	S	R	S	S	S	S	R	R	0.47		S	R	R	R	S	R	R	R	0.57		R	R	R	R	S	S	S	R	0.46	0.49	
var_g/Ballerina	IR	R	S	S	S	S	R	S	S	R	S	0.53		S	R	S	R	S	S	S	S	R	S	0.53		R	R	R	S	S	R	S	R	0.46		R		R	R	R	R	S	R	0.71	0.50	
var_02/Costa Rica	S	S	S	S	S	S	S	S	S	S	S	1		S	S	S	S	S	S	S	S	S	R	0.80		S	S	R	S	S	S	S	S	0.75		S	S	S	S	S	S	S	S	1	0.89	
var_d/Gisela	S	S	S	S	S	S	S	S	S	S	S	1		S	S	S	S	S/R	S	S	S	S	S	0.80		S	S	S		S	S	S	S	1		S	S	S	S	S	S	S	S	1	0.94	
var_08/Gisela	S	S	S	S	S	S	S	S	S	S	S	1		S	S	S	S	S	S	S	S	S	S	1.00		S	S	S	S	S	S	S	S	1		S	S	S	S	S	S	S	S	1	1	
Costa Rica	S	S	S	S	S	S	S	S	S	S	S	1		S	S	S	S	S	S	S	S	S	S	1		S	S	S	S	S	S	S	S	1		S	S	S	S	S	S	S	S	1	1	
												0.858											**0.748**									**0.857**										**0.814**	**0.820**

Figure 9: interpretation with Banchu Red Fire as intermediate resistant candidate control and Lomeria (var_10) (top table) and Palmos (var_12) (bottom table) used as candidate controls



		Isolate I												isolate M												isolate N												isolate W													
		Lab 1	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 9	Lab 9	Lab 11	Lab 12			Lab 1	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 9	Lab 9	Lab 11	Lab 12			Lab 1	Lab 3	Lab 4	Lab 6	Lab 7	Lab 9	Lab 9	Lab 12					Lab 1	Lab 3	Lab 4	Lab 5	Lab 7	Lab 9	Lab 9	Lab 12				
r_10/Lomeria	R	R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	1				R	R	R	R	R	R	R	R	1		1	
var_03	R	R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	1				R	R	R	R	R	R	R	R	1		1	
var_04	R	R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	S	R	0.75				R	R	R	R	R	R	R	R	1		0.94	
var_12 (Palmos)	R	R	R	R	R	R	S	R	R	R	R	0.80		R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	1				R	R	R	R	R	R	R	R	1		0.94	
var_05/Patriot	IR	R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	S/R	R	R	R	R	R	0.80		R	R	R	R	R	R	R	R	1				R	R	R	R	R	R	R	R	1		0.94	
var_07	IR	R	R	R	R	R	S	R	R	R	R	0.80		R	R	R	R	R	S	R	R	R	R	0.80		R	S	R	R	R	R	R	R	0.75				R	S	S	R	S	R	R	R	0.46		0.71	
var_11/Banchu Red Fire	IR	S	R	R	R	S	S	R	S	R	R	0.47		R	R	R	R	S/R	R	S	S	R	R	0.49		R	S	R	R	R	R	R	S	0.57				S	R	R	R	R	S	S	S	0.43		0.51	
Banchu Red Fire	IR	R	R	R	R	R	S	R	S	R	R	0.64		R	R	R	R	S/R	S	S	S	S	R	0.36		R	S	S	R	R	S	S	R	0.43				S	R	S	R	R	R	S	S	0.43		0.48	
var_13/Romabella	IR	S	S	R	S	S	S	S	R	R	R	0.47		S	R	R	R	R	S	S	S	R	R	0.47		S	S	R	R	S	S	S	R	0.46				S	R	R	R	R	S	S	R	0.46		0.49	
var_14/Ballerina	IR	S	R	S	S	S	S	S	S	R	S	0.64		S	R	S	R	S	S	S	S	R	R	0.47		S	R	S	R	S	R	S	R	0.43				R	R	S	R	R	S	S	R	0.46		0.50	
var_g/Ballerina	IR	R	R	S	S	S	S	S	S	R	R	0.47		S	R	S	R	S	S	S	S	R	S	0.53		R	S	R	S	R	S	S	R	0.43				S		S	R	R	S	S	S	0.52		0.52	
var_02/Costa Rica	S	S	S	S	S	S	S	S	S	S	R	0.80		S	S	S	S	S	S	S	S	S	R	0.80		S	S	S	S	S	S	S	S	1.00				S	S	S	S	S	S	S	S	1		0.89	
Costa Rica	S	S	S	S	S	S	S	S	S	S	S	1		S	S	S	S	S	S	S	S	S	S	1		S	S	S	S	S	S	S	R	0.75				S	S	S	S	S	S	S	S	1		0.94	
var_d/Gisela	S	S	S	S	S	S	S	S	S	S	S	1		S	S	S	S	S	S	S	S	S	S	1		S	S	S		S	S	S	S	1				S	S	S	S	S	S	S	S	1		1	
var_08/Gisela	S	S	S	S	S	S	S	S	S	S	S	1		S	S	S	S	S/R	S	S	S	S	S	0.80		S	S	S	S	S	S	S	S	1				S	S	S	S	S	S	S	S	1		0.94	
												0.792												**0.751**										**0.755**												**0.770**	**0.773**	

		Isolate I												isolate M												isolate N												isolate W													
	obse	Lab 1	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 9	Lab 9	Lab 11	Lab 12			Lab 1	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 9	Lab 9	Lab 11	Lab 12			Lab 1	Lab 3	Lab 4	Lab 6	Lab 7	Lab 9	Lab 9	Lab 12					Lab 1	Lab 3	Lab 4	Lab 5	Lab 7	Lab 9	Lab 9	Lab 12				
var_03	R	R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	1				R	R	R	R	R	R	R	R	1		1	
var_10/Lomeria	R	R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	1				R	R	R	R	R	R	R	R	1		1	
var_12 (Palmos)	R	R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	1				R	R	R	R	R	R	R	R	1		1	
var_04	R	R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	R	R	S	R	1				R	R	R	R	R	R	R	R	1		0.94	
var_05/Patriot	IR	R	R	R	R	R	R	R	R	R	R	1		R	R	R	R	S/R	R	R	R	R	R	1		R	R	R	R	R	R	R	R	0.75				R	R	R	R	R	R	R	R	1		0.94	
var_07	IR	R	R	R	R	R	S	R	R	R	R	0.80		R	R	R	R	R	R	S	R	R	R	0.80		R	S	R	R	R	R	R	R	0.75				R	S	S	R	S	R	R	R	0.46		0.71	
var_11/Banchu Red Fire	IR	S	R	R	R	S	R	R	S	R	R	0.53		R	R	R	R	R	R	S	S	R	R	0.64		R	S	R	R	R	R	S	R	0.57				S	R	R	R	R	S	S	R	0.46		0.59	
Banchu Red Fire	IR	R	R	R	R	R	S	R	S	R	R	0.64		R	R	R	R	S/R	S	S	S	S	R	0.36		R	S	R	R	R	S	S	R	0.46				S	R	S	R	R	R	S	R	0.46		0.51	
var_13/Romabella	IR	S	S	R	S	S	R	S	R	R	R	0.44		S	R	R	R	R	S	S	S	R	R	0.47		S	S	R	R	S	S	S	R	0.46				S	R	R	R	R	S	S	R	0.46		0.49	
var_14/Ballerina	IR	S	R	S	S	S	R	S	S	R	S	0.53		S	R	R	R	S	R	S	S	R	R	0.47		S	R	R	S	R	S	R	R	0.46				R	R	R	S	R	S	S	R	0.46		0.49	
var_g/Ballerina	IR	R	R	S	S	S	S	S	S	R	R	0.47		S	R	S	R	S	S	S	S	R	S	0.53		R	S	S	S	R	S	S	R	0.46				S		S	R	R	S	S	S	0.52		0.54	
var_02/Costa Rica	S	S	S	S	S	S	S	S	S	S	R	0.80		S	S	S	S	S	S	S	S	S	R	0.80		S	S	R	S	S	S	S	S	0.75				S	S	S	S	S	S	S	S	1		0.84	
var_d/Gisela	S	S	S	S	S	S	S	S	S	S	S	1		S	S	S	S	S/R	S	S	S	S	S	0.80		S	S	S		S	S	S	S	1				S	S	S	S	S	S	S	S	1		0.94	
Costa Rica	S	S	S	S	S	S	S	S	S	S	S	1		S	S	S	S	S	S	S	S	S	S	1		S	S	S	S	S	S	S	R	0.75				S	S	S	S	S	S	S	S	1		0.94	
var_08	S	S	S	S	S	S	S	S	S	S	S	1		S	S	S	S	S	S	S	S	S	S	1		S	S	S	S	S	S	S	S	1				S	S	S	S	S	S	S	S	1		1	
												0.815												**0.762**										**0.745**												**0.775**	**0.796**	

Figure 10: interpretation with Patriot as intermediate resistant candidate control and Lomeria (var_10) (top table) and Palmos (var_12) (bottom table) used as candidate controls

Annex 3: interpretation of the varieties done by Pathostat based on the second scenario of decision rule with three levels of interpretation

		Isolate I												isolate M												isolate N												isolate W												
		Lab 1	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 9	Lab 9	Lab 11	Lab 12			Lab 1	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 9	Lab 9	Lab 11	Lab 12			Lab 1	Lab 3	Lab 4	Lab 6	Lab 7	Lab 9	Lab 9	Lab 12					Lab 1	Lab 3	Lab 4	Lab 5	Lab 7	Lab 9	Lab 9	Lab 12				Accuracy
var_10/Lomeria	R	R	R	R	R	R	S	R	R	R	R	0.90		R	R	R	R	R	R	R	R	R	R	1.00		R	R	R	R	R	R	R	R	1.00		R	R	R	R	R	IR/R	R	R	0.88		0.94
var_04	R	IR	R	R	R	R	R	IR	IR	R	R	0.70		IR	R	IR	IR/R	R	R	IR/R	IR	R	R	0.50		IR/R	R	IR	IR/R	R	IR/R	IR	R	0.38		IR	R	R	IR/R	R	IR/R	IR	R	0.50		0.53
var_12 (Palmos)	R	IR	IR	IR	R	R	R	IR	IR	IR	R	R	0.40	R	IR	IR	IR/R	IR	R	IR/R	IR	R	R	0.40		IR/R	R	IR	IR/R	R	IR/R	IR	R	0.38		R	IR	IR	IR/R	IR	IR/R	IR	R	0.25		0.36
var_03	R	IR	R	R	R	R	R	R	R	R	IR	0.80		IR	IR	IR	IR/R	IR	R	IR/R	R	R	R	0.40		IR	R	IR	IR/R	R	R	IR	R	0.50		IR	R	IR	IR/R	R	R	R	R	0.63		0.58
var_07	IR	IR	IR	IR	IR/R	IR	IR	IR	IR	R	IR	0.47		IR	IR	IR	IR/R	IR	IR	IR/R	IR	R	IR	0.44		IR/R	IR	IR	IR/R	IR	IR/R	IR	IR	0.38		IR	IR	S/IR	IR/R	IR	IR/R	IR	IR	0.38		0.42
var_05/Patriot	IR	IR	IR	IR	R	IR	R	IR	IR	IR	IR	0.50		IR	IR	IR	IR/R	S/IR	R	IR	IR	IR	IR	0.44		IR	IR	IR	IR/R	IR	IR/R	IR	R	0.42		IR	IR	IR	IR/R	IR	IR/R	IR	IR	0.43		0.45
var_11/B R F	IR	IR	IR	IR	R	IR	IR	IR	IR	R	IR	0.50		IR	IR	IR	IR/R	S/IR	IR	IR	IR	IR	IR	0.44		IR	IR	IR	IR/R	IR	R	IR	R	0.45		IR	IR	IR	IR	R	S	IR	IR	0.46		0.47
Banchu Red Fire	IR	IR	IR	IR	R	IR	IR	IR	IR	S	IR	0.47		IR	IR	IR	IR/R	S/IR	IR	IR	IR	IR	IR	0.44		IR	IR	IR	IR/R	IR	IR	IR	R	0.46		S	IR	IR	IR/R	IR	IR/R	IR	IR	0.38		0.44
var_13/Romabella	IR	S	S	IR	IR	S	IR	IR	IR	IR	IR	0.41		S/IR	IR	IR	IR/R	IR	IR	IR	IR	IR	IR	0.44		IR	S	IR	IR/R	S	IR	IR	R	0.36		S	IR	IR	IR/R	IR	S	IR	IR	0.38		0.41
var_g/ Ballerina	IR	IR	IR	IR	IR	S/IR	IR	IR	IR	IR	IR	0.47		S/IR	IR	S	IR/R	S/IR	IR	IR	IR	IR	IR	0.38		IR	IR	IR	IR	IR	IR	IR	IR	0.50		IR		IR	IR/R	IR	IR/R	IR	IR	0.42		0.44
var_14/Ballerina	IR	S	IR	S	S	S/IR	S	S	IR	IR	S	0.23		S	IR	S	IR/R	S	S	IR	S	R	IR	0.25		S	IR	S	IR/R	S	IR/R	IR	R	0.22		IR	IR	IR	IR/R	IR	S	S	IR	0.38		0.28
var_02/Costa Rica	S	S	S	S	S	S	S	S	S	S	IR	0.90		S/IR	S	S	S	S	S	IR	S	S	IR	0.70		S	S	S	IR	S	S	S	IR	0.75		S	S/IR	S/IR	S	S	S	S	IR	0.71		0.77
Costa Rica	S	S	S	S	S	S	S	S	S	S	IR	0.90		S	S	S	IR	S	S	IR	S	S	S	0.80		S	S	S	S	S	S	S	IR	0.88		S	IR	S	S	S	S	S	S	0.88		0.86
var_08/Gisela	S	S	S	S	S	S	S	S	S	S	S	1.00		S	S	S	S	S	S	S	S	S	S	1.00		S	S	S	S	S	S	S	S	1.00		S	IR	S/IR	S	S	S	S	S	0.75		0.94
var_d/Gisela	S	S	S	S	S	S	S	S	S	S	S	1.00		S	S	S	S	S	S	S	S	S	S	1.00		S	S	S	R	S	S	S	S	0.88		S	S	S	S	S	S	S	S	1.00		0.97
												0.644												0.576										0.570										0.562	0.592	

		Isolate I												isolate M												isolate N												isolate W												
	observ	La b 1	La 3	La 4	Lab 5	Lab 6	La b 7	Lab 9	Lab 9	Lab 11	Lab 12			La b 1	La 3	Lab 4	Lab 5	Lab 6	La b 7	Lab 9	La b 9	Lab 11	Lab 12			La b 1	La 3	Lab 4	Lab 6	La b 7	Lab 9	La b 9	Lab 12					La b 1	Lab 3	Lab 4	Lab 5	Lab 7	Lab 9	Lab 9	Lab 12				Accuracy
var_10/Lomeria	R	R	R	R	R	IR	R	R	IR/R	R	0.80		R	R	R	R	R	R	IR	R	R	R	0.90		R	R	IR/R	R	R	IR/R	R	R	0.75		R	R	R	R	IR/R	R	R	0.88		0.83
var_03	R	R	R	R	R	R	R	R	IR/R	IR	0.80		IR	R	IR	IR/R	R	R	IR	R	R	R	0.60		IR	R	IR/R	IR/R	R	R	R	R	0.63		R	R	R	IR/R	R	R	R	R	0.88		0.72
var_04	R	R	R	R	R	R	IR/R	IR/R	IR/R	R	0.70		IR	R	R	IR/R	R	R	IR	IR	R	R	0.60		IR/R	R	IR/R	IR/R	R	IR/R	IR	R	0.38		R	R	R	IR/R	R	IR/R	IR/R	R	0.63		0.58
var_12 (Palmos)	R	R	R	R	R	R	IR/R	IR/R	IR/R	R	0.70		R	R	R	IR/R	R	R	IR/R	IR	R	R	0.70		IR/R	R	IR/R	IR/R	R	IR/R	R	R	0.50		R	R	R	IR/R	IR/R	IR/R	IR/R	IR/R	0.38		0.58
var_05/Patriot	IR	IR	IR	R	R	IR	R	IR	IR	IR/R	IR	0.46		IR	IR	IR	IR/R	S/IR	R	IR	IR	IR	IR	0.44		IR	IR	IR	IR/R	IR	IR/R	R	R	0.40		IR	IR	IR	IR/R	IR	IR/R	IR/R	IR/R	0.33		0.41
var_07	IR	IR	IR	IR	IR/R	IR	IR	IR/R	IR/R	IR/R	IR	0.38		IR	IR	IR	IR/R	IR	IR	IR/R	R	IR	IR	0.44		IR/R	IR	IR/R	IR/R	IR	IR/R	IR	IR	0.33		IR	S/IR	S/IR	IR/R	IR	IR/R	IR/R	IR/R	0.20		0.35
var_g/ Ballerina	IR	IR	IR	IR	IR	S/IR	IR	IR	IR/R	IR	0.44		S/IR	IR	S/IR	IR/R	S/IR	IR	IR	IR	IR	IR	0.38		IR	IR	IR	IR	IR	IR	IR	IR	0.50		IR		IR	IR/R	IR/R	IR/R	IR	IR	0.36		0.43
var_11/B R F	IR	IR	R	R	R	IR	R	IR/R	IR	IR/R	IR	0.40		IR	R	IR	IR/R	IR	IR	IR	IR	IR	IR	0.47		IR	IR	IR/R	IR/R	IR	R	IR	R	0.40		IR	R	IR	IR	R	S	IR	IR	0.45		0.44
Banchu Red Fire	IR	IR	R	IR	R	IR	R	IR	IR	S	IR	0.46		IR	R	R	IR/R	S/IR	IR	IR	IR	IR	IR	0.43		IR	IR	IR	IR/R	IR	IR	IR	R	0.46		S	R	IR	IR/R	IR	IR/R	IR	IR	0.36		0.43
var_13/Romabella	IR	S	S	IR	IR	S	R	IR	IR	IR/R	IR	0.36		S/IR	IR	IR	IR/R	IR	IR	IR	IR	IR	IR	0.44		IR	S	IR	IR/R	S	IR	IR	R	0.36		S	IR	IR	IR/R	IR/R	S	IR	IR/R	0.27		0.37
var_14/Ballerina	IR	S	IR	S	S	S/IR	S	S	IR	IR/R	S	0.17		S/IR	IR	S/IR	IR/R	S/IR	S	IR	S	R	IR	0.25		S	IR	IR	IR/R	S	IR/R	IR	R	0.30		IR	R	IR	IR/R	IR	S	S	IR/R	0.30		0.25
var_02/Costa Rica	S	S	S	S	S	S	S	S	S	S	IR	0.90		S/IR	S	S/IR	S	S/IR	S	IR	S	S	IR	0.50		S	S	IR	IR	S	S	S	IR	0.63		S	S	S/IR	S	S	S	S	IR	0.75		0.69
Costa Rica	S	S	S	S	S	S	S	S	S	S	IR	0.90		S/IR	S	S/IR	IR	S/IR	S	IR	S	S	S	0.50		S	S	S	S	S	S	S	IR	0.88		S	IR	S/IR	S	S	S	S	S	0.75		0.75
var_d/Gisela	S	S	S	S	S	S	S	S	S	S	S	1.00		S/IR	S	S/IR	S	S/IR	S	S	S	S	S	0.70		S	S	S	R	S	S	S	S	0.88		S	S/IR	S/IR	S	S	S	S	S	0.75		0.83
var_08/Gisela	S	S	S	S	S	S	S	S	S	S	S	1.00		S/IR	S	S/IR	S	S/IR	S	S	S	S	S	0.70		S	S	S	S	S	S	S	S	1.00		S	S/IR	S/IR	S	S	S	S	S	0.75		0.86
												0.631												0.53 6										0.55 9										0.536	0.569	

Figure 11: interpretation with Lomeria (var_10) (top table) and Palmos (var_12) used as resistant candidate controls