IN-Vitro mutation breeding and selection for resistance to fusarium wilt in Banana

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PROBLEM

- Banana is important tropical and subtropical fruits around the word.
- Global banana production in now increasingly threatened by number of pests and diseases
- Fusarium wilt has become the major Limitation in Banana cultivation and Production.
- Fusarium oxysporum f. sp. cubense (Foc), is recognized as one of the most destructive diseases of banana worldwide.
- Race 4 not only attacks Cavendish cultivars but also cultivars susceptible to race 1 and 2.
- At present, there are no economically viable biological, chemical or cultural measures of controlling Fusarium wilt in an infected field (Ploetz 2006; Buddenhagen 2009).
- **Generally, developing the new cultivars that have resistance to fusarium wilt, is the best way of overcoming this problem**

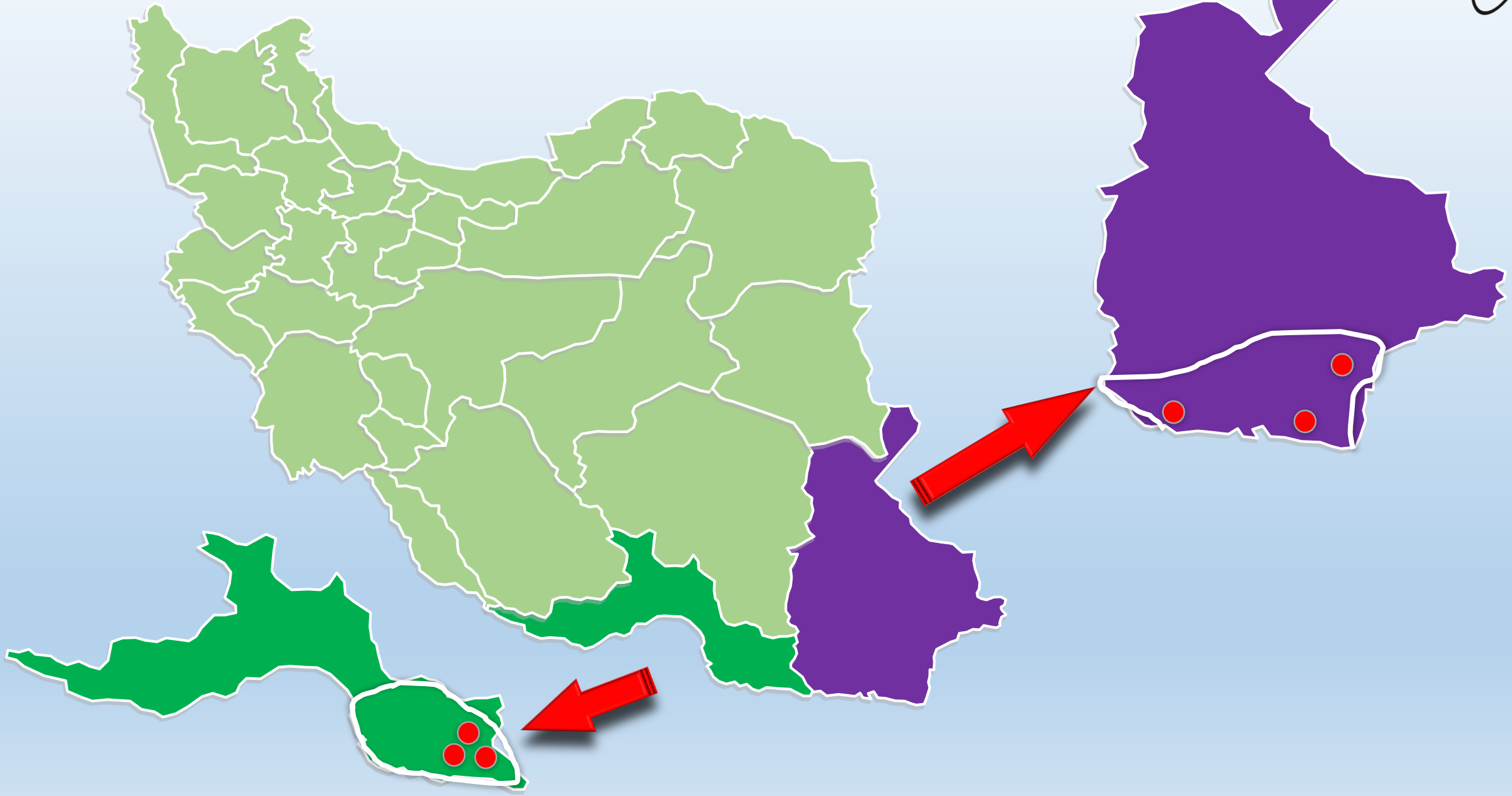
- Field screening for resistance to Fusarium wilt depends on the presence of environmental conditions conducive to disease development, and is time-consuming and expensive (Vakili 1965).
- In order to accelerate progress in banana breeding programs for resistance to Fusarium wilt, it is important to develop bioassays that can differentiate between resistant and susceptible cultivars efficiently and accurately.
- So far, the most commonly used early bioassay is a pot system (Matsumoto et al. 1995; Subramaniam et al. 2006; Weber et al. 2007; Smith et al. 2008),
- 8 week-old plants (10 to 15 cm tall) were more favorable for consistent infection than the plants less than 10 cm in the pot
- 7-8 week need to evaluation in pot system (Matsumoto et al. 1995; Smith et al. 2008).
- **In the present study, We use In-vitro selection method, as fast and early selection method for selecting resistance mutant to fusariom wilt.**

Main Provinces Producer of Banana in Iran

Hormozgan

Sistan & Balochestan

Sampling of diseased plants



Plant materials

- C.V : Cavendish
- Micropropagation :
  - Shoot-tip were isolated from suckers


a


b


c

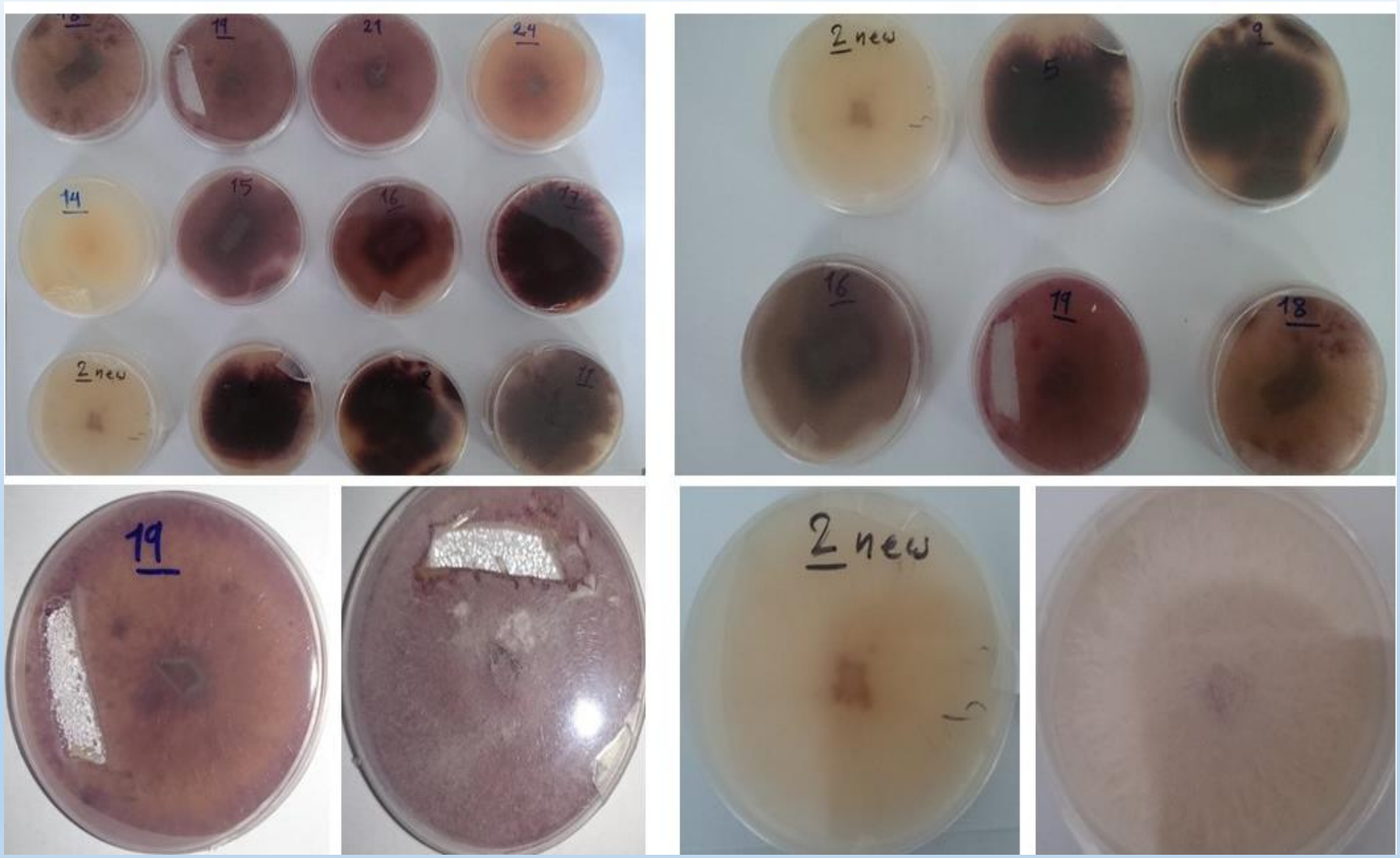

d
e

Establishment of the Radio Sensitivity Curve and Determination of LD50 Dose

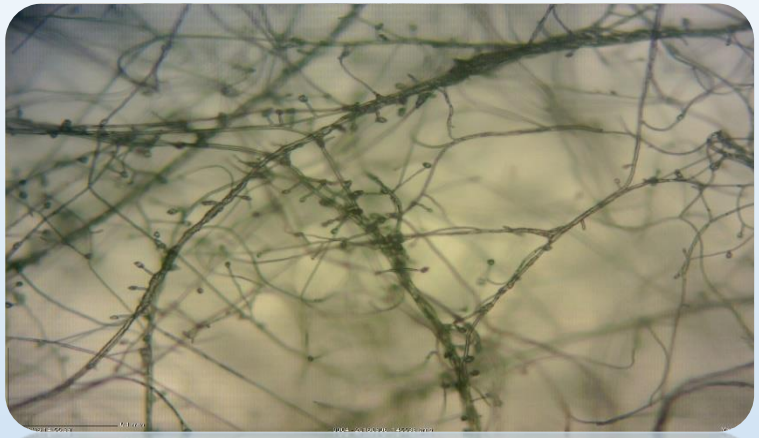
- Gamma ray doses : 0,10,15,20,25,35,45 and 60 Gy
- After 30 days %Survived Plantlet , leaves proliferation , plantlet height and fresh weight
- Fitting the generalized linear model
- Estimate the 50% lethal dose

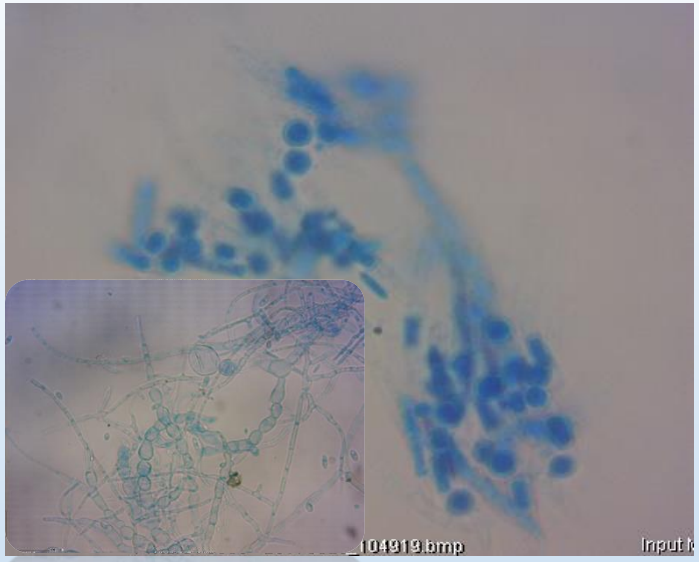
Sampling of diseased plants

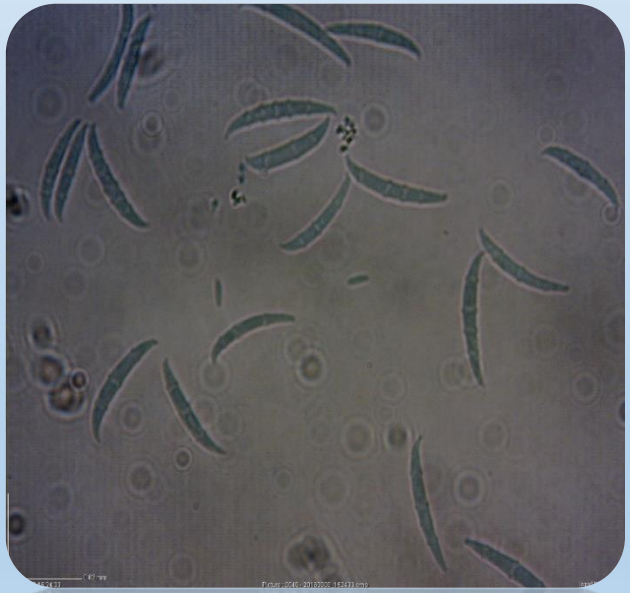
Sampling of diseased plants

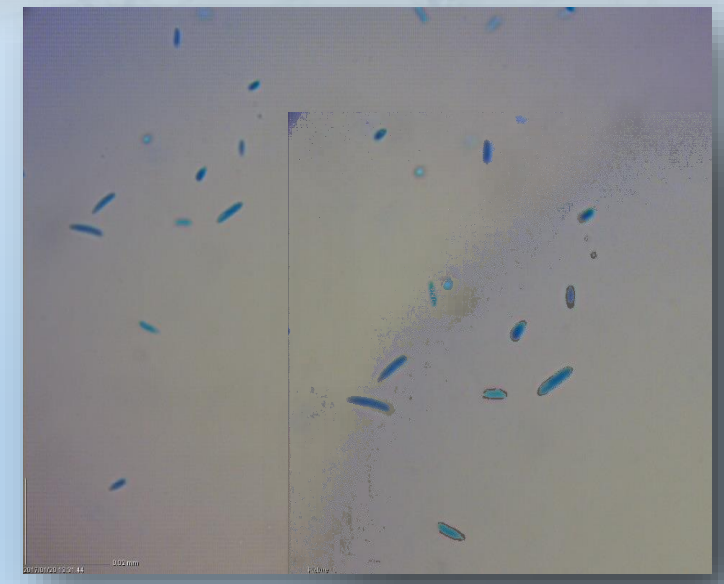


Morphological identification

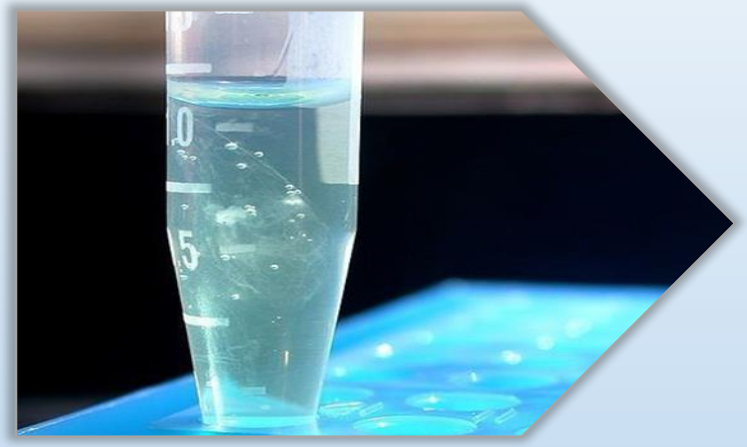








Molecular identification of *Fusarium oxysporum*



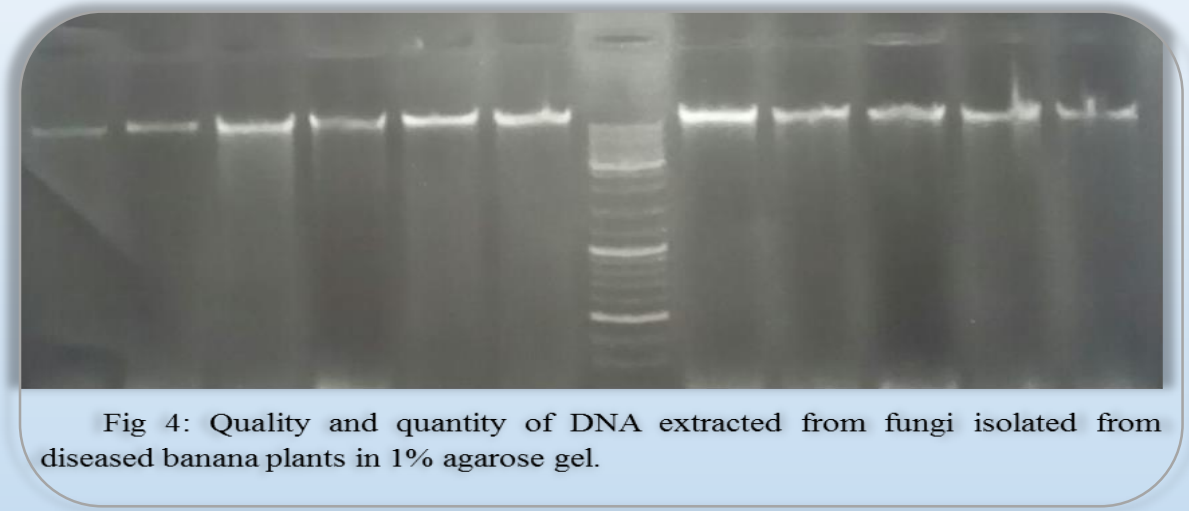


Fig 4: Quality and quantity of DNA extracted from fungi isolated from diseased banana plants in 1% agarose gel.

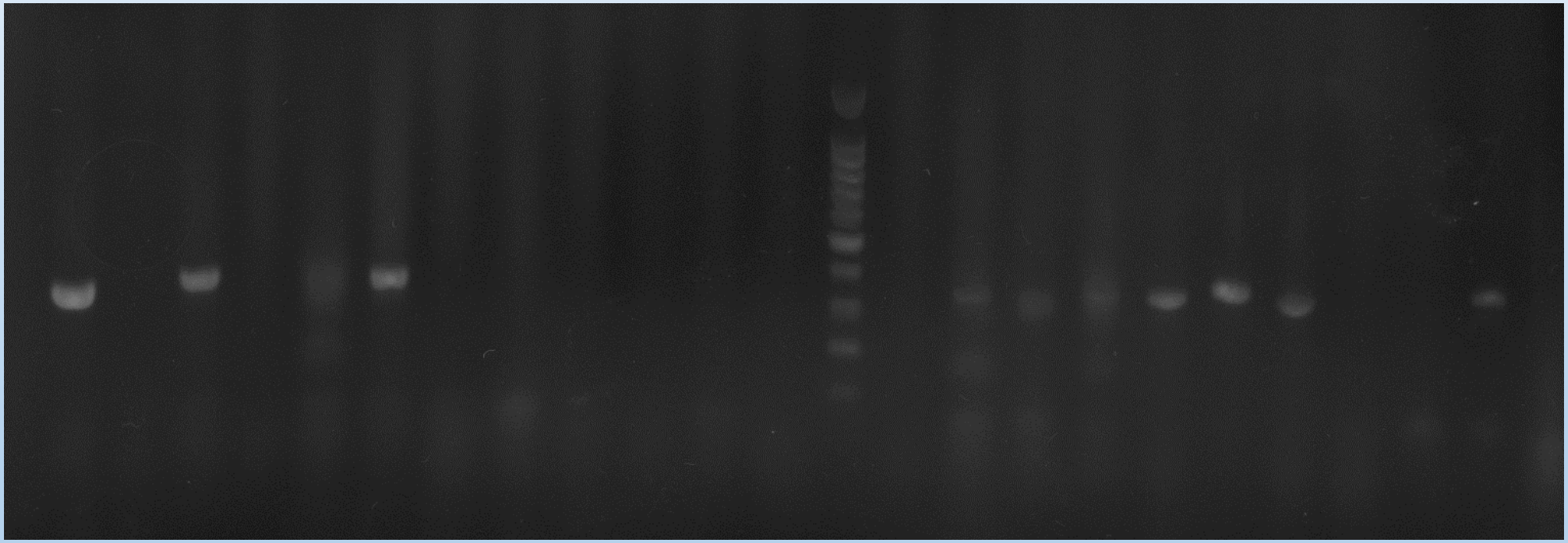
Fusarium oxysporum strains

(FOF1 5′-ACA TAC CAC TTG TTG CCT CG-3′, FOR1 5′-CGC CAA TCA ATT TGA GGA ACG-3′)

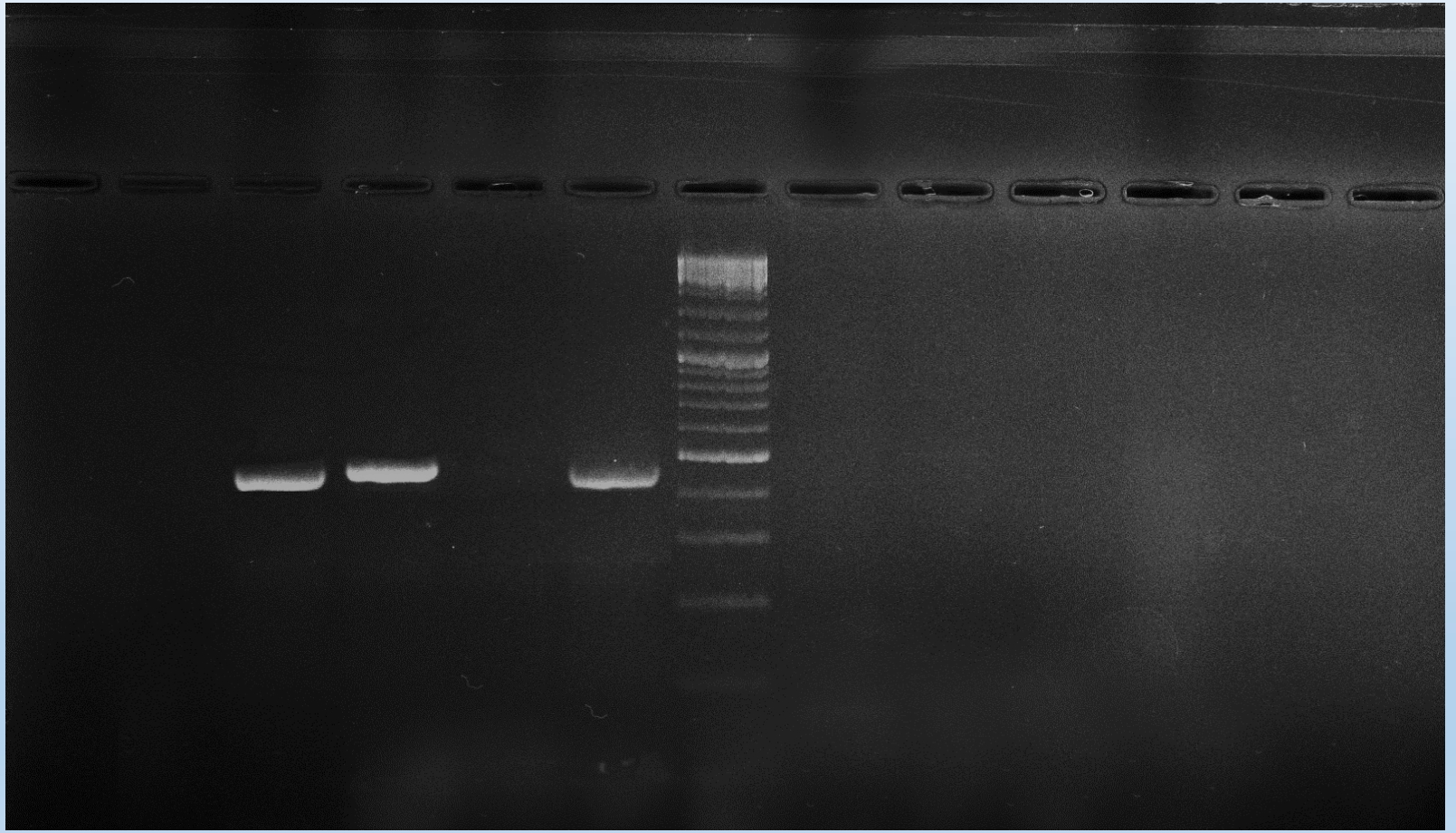
F.oxysporum f.sp.cubense

(FocTR4-F 5´-CACGTTTAAGGTGCCATGAGAG-3´, FocTR4-R 5′-GCCAGGACTGCCTCGTGA-3′)

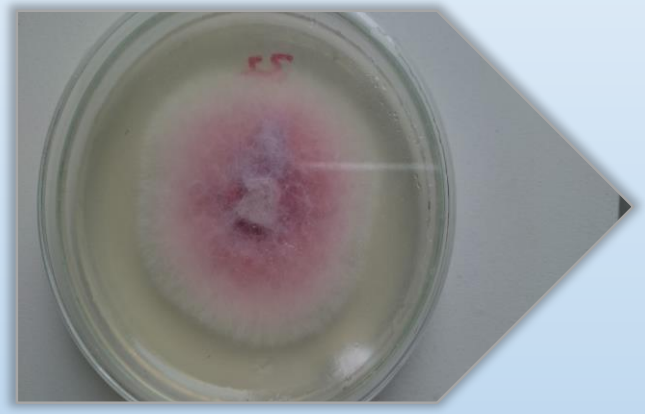
Fusarium oxysporum strains

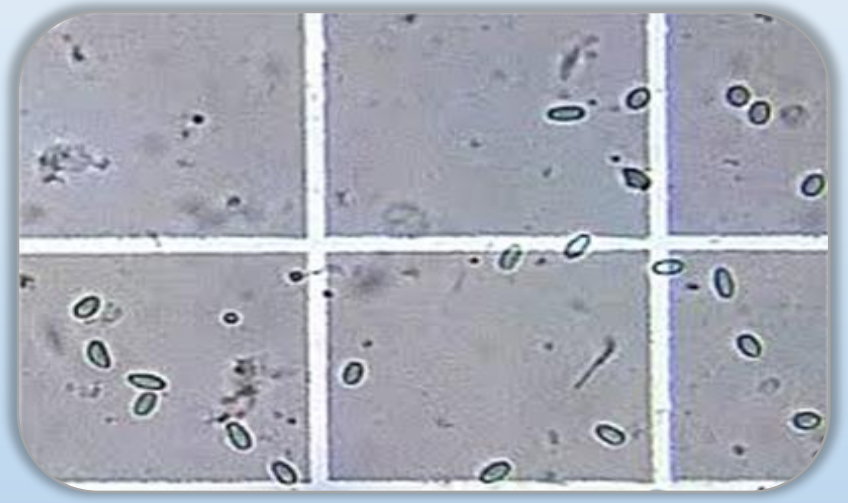


F.oxysporum f.sp.cubense



- **Inoculum**





- **Adjust concentration using**
  - deionized water

Inoculation

- In-vitro

Modified MS(½ MS salt + 1% sucrose + 0.7% agar)

Phytagel are better because of clear media

Inoculation procedures

Pathogen
Non pathogen

In-vitro

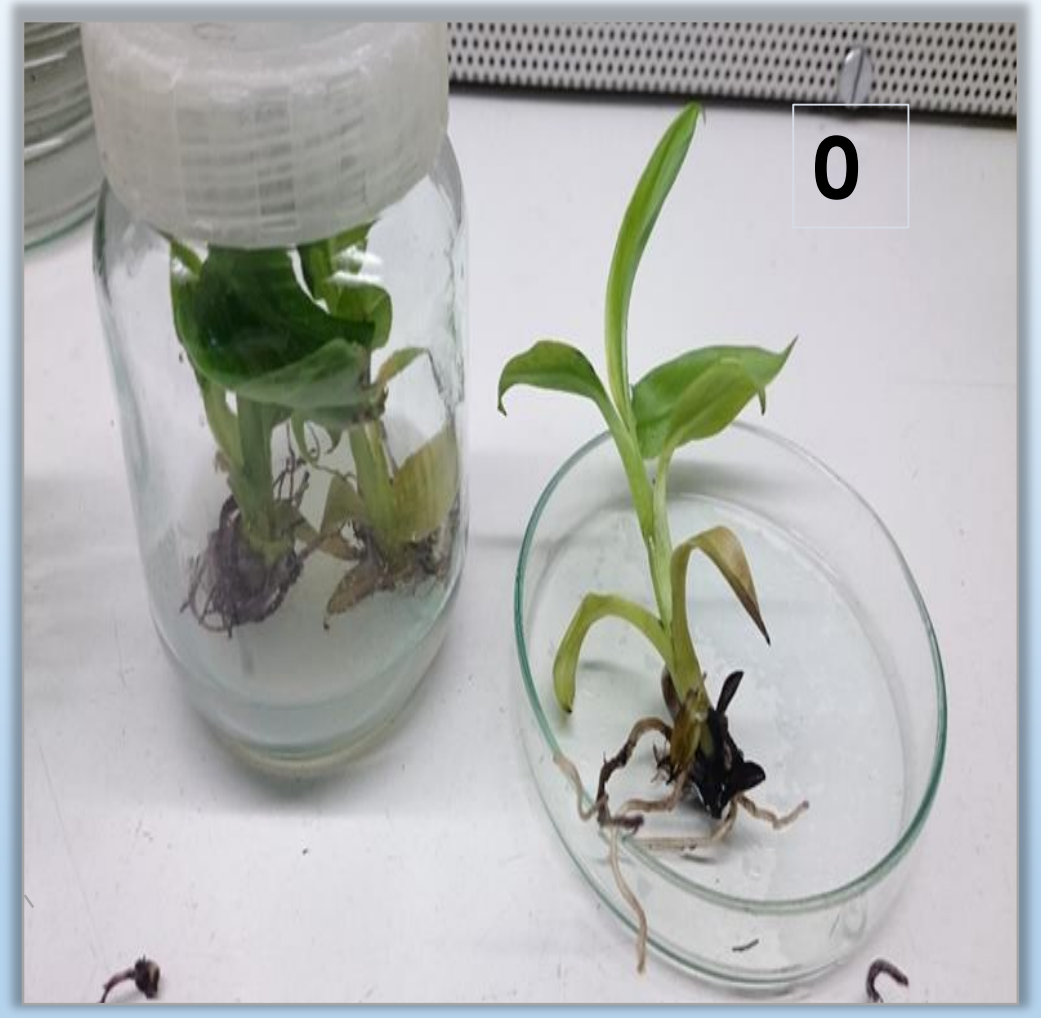
Disease severity	Disease symptoms
0	Corm completely clean, no vascular discoloration
1	the smaller leaves at the base of pseudo stem wilted, there was no discoloration the pseudo stem
2	≤1/2 the height of the pseudostem was Discolored
3	>1/2 the height of the pseudostem was discolored and (or) there was discoloration of the leaf stalk
4	≤50% of the leaves wilted or yellowed
5	>50% of the leaves wilted or yellowed
6	whole plantlet was wilted

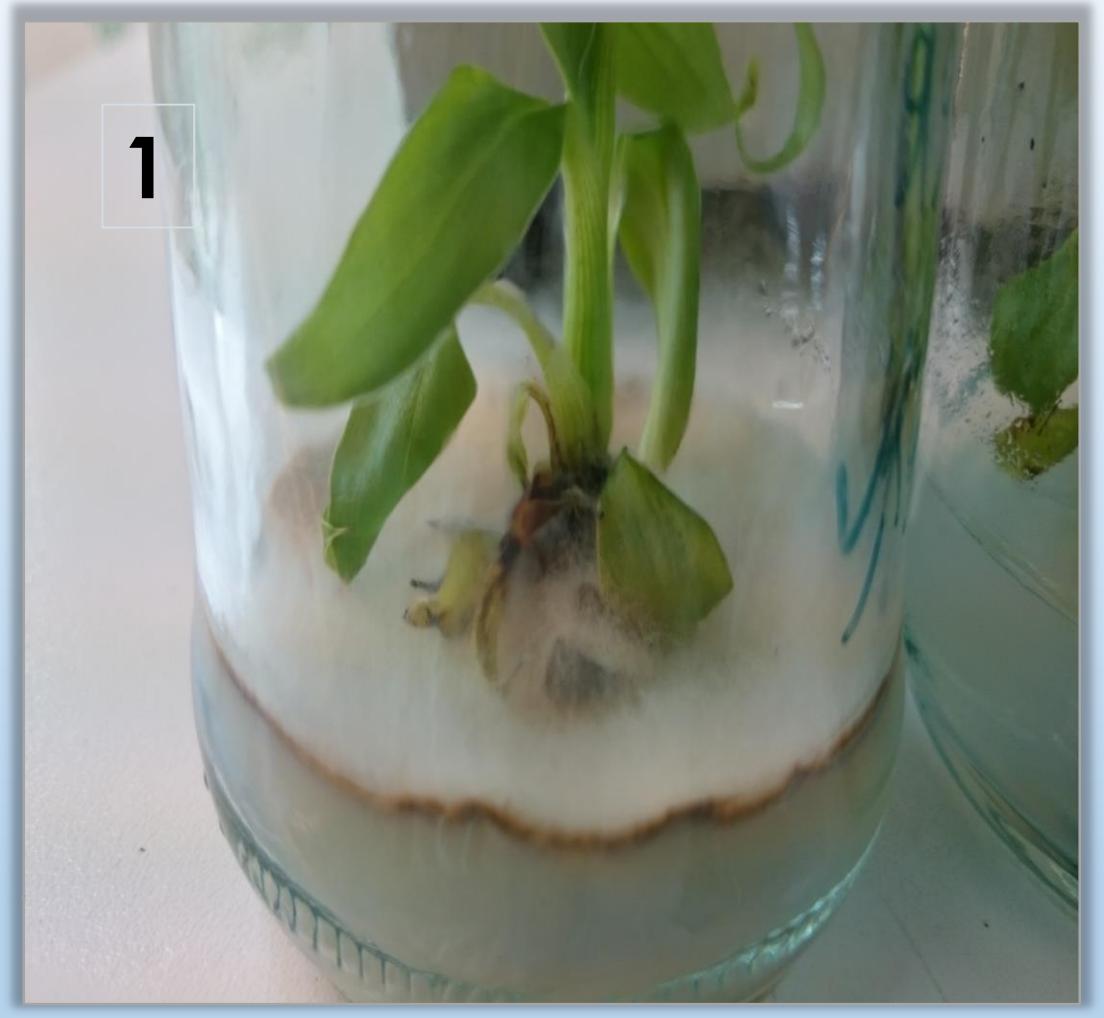






Disease severity





Disease severity

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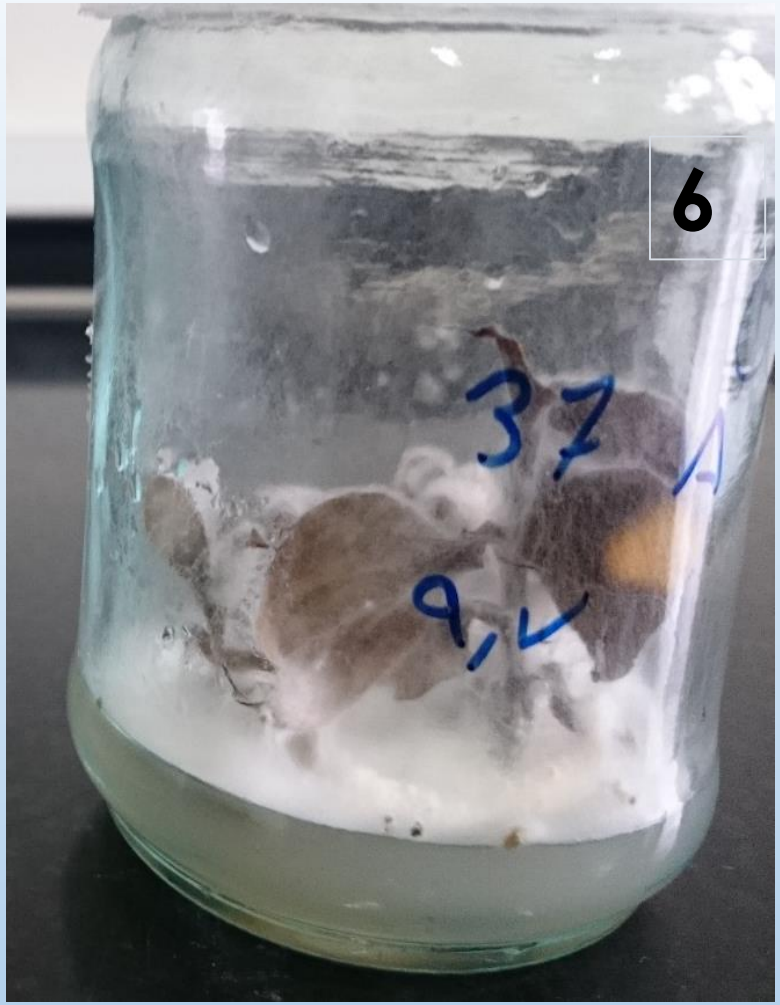
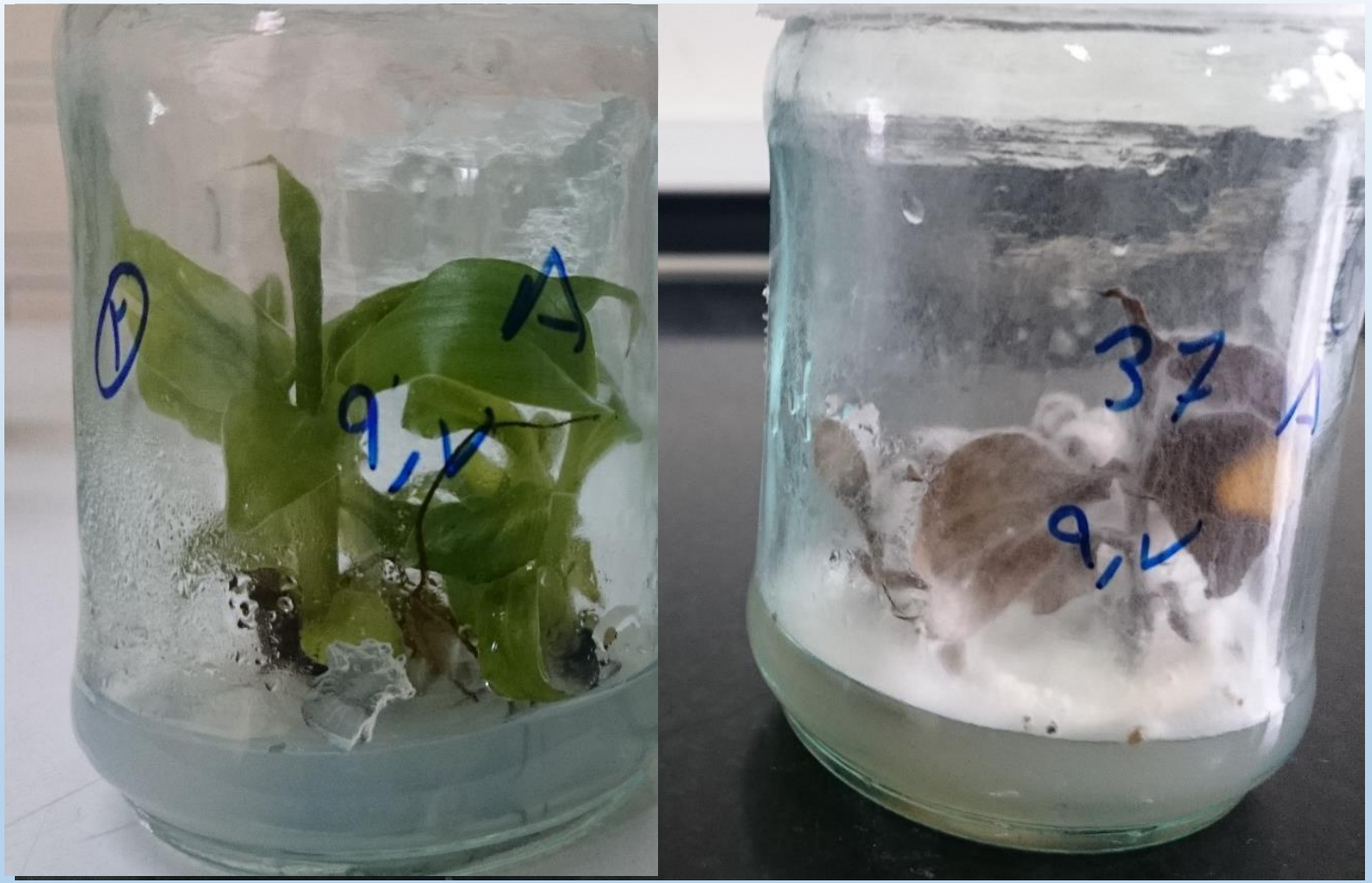
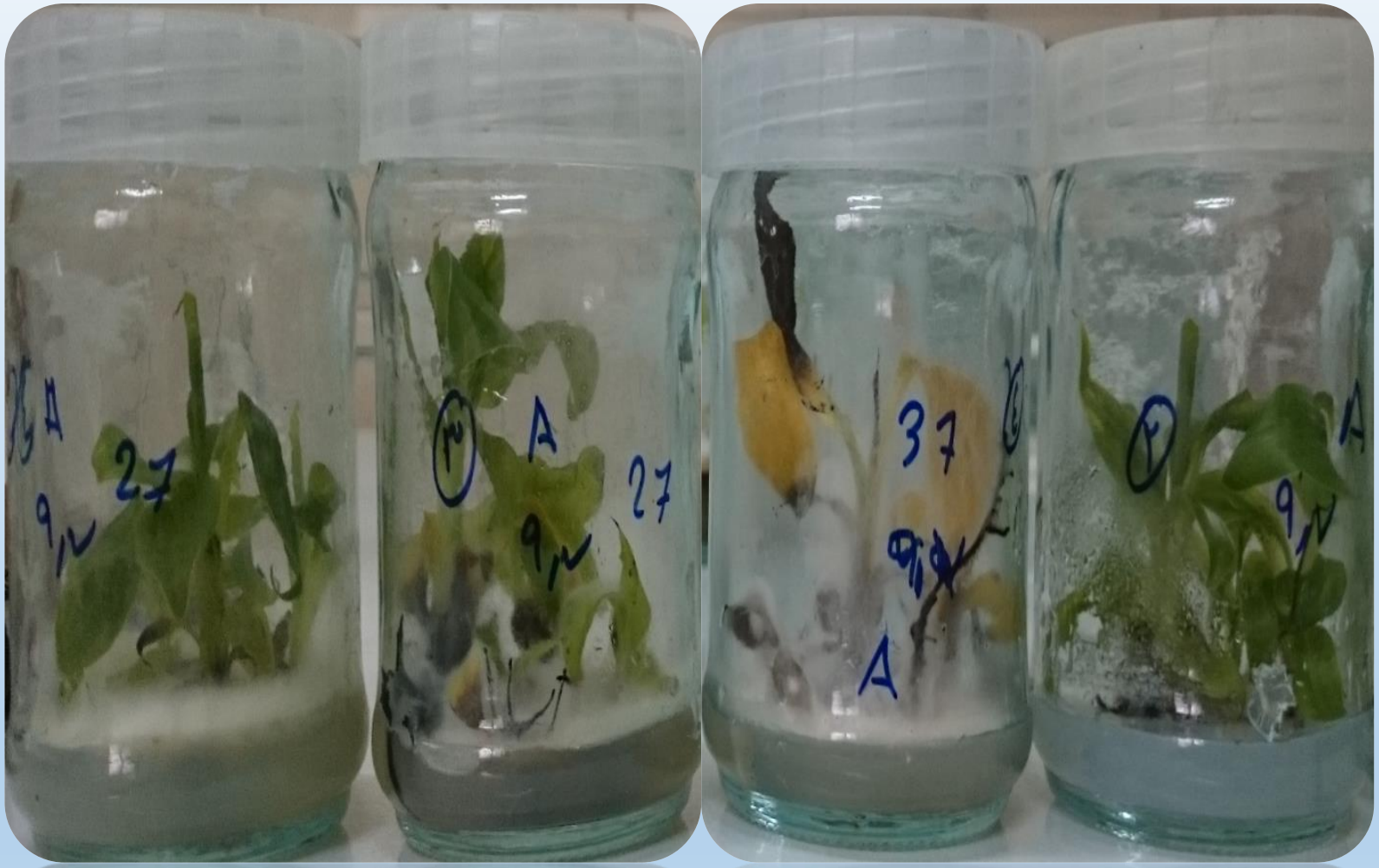
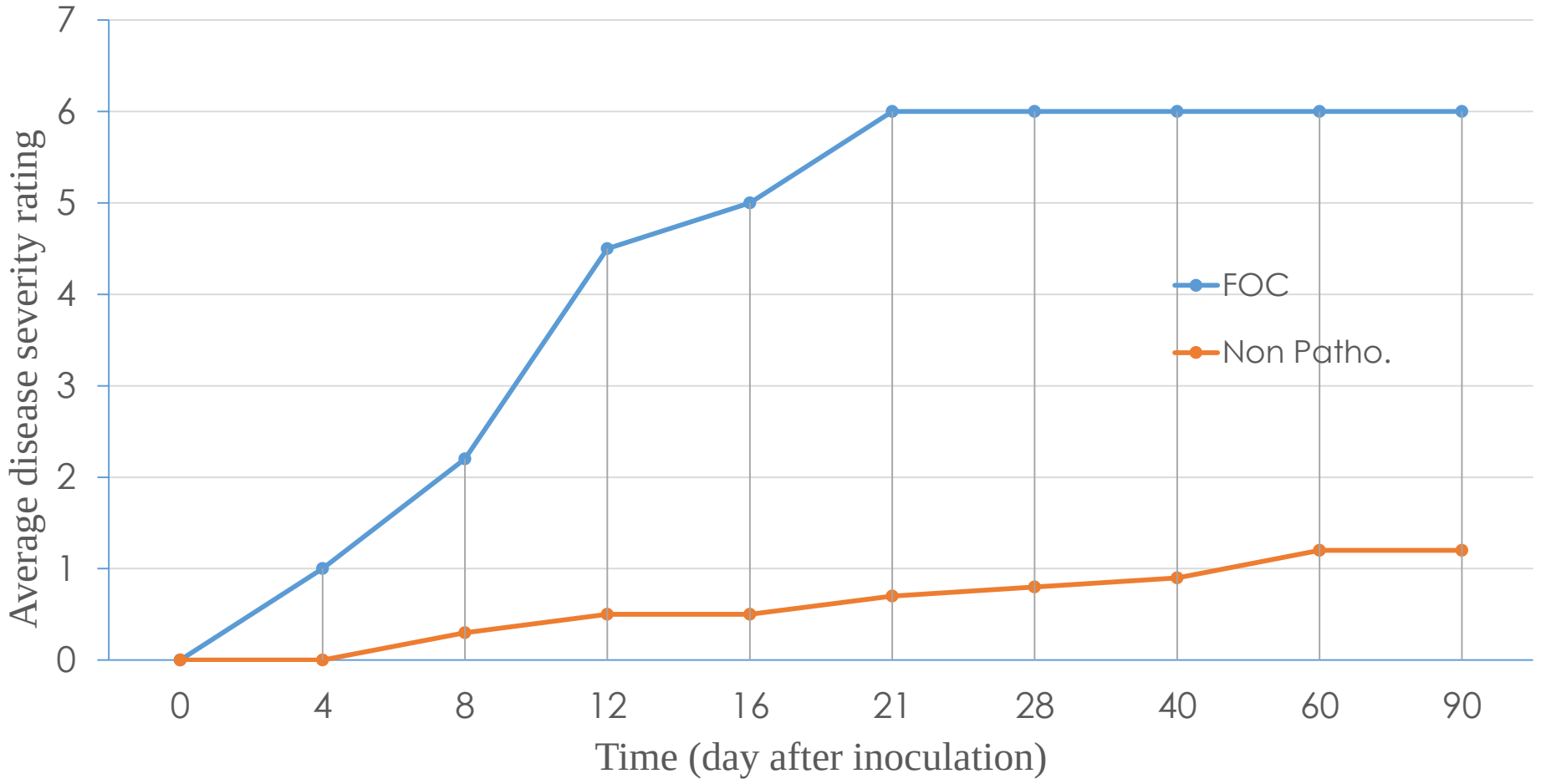


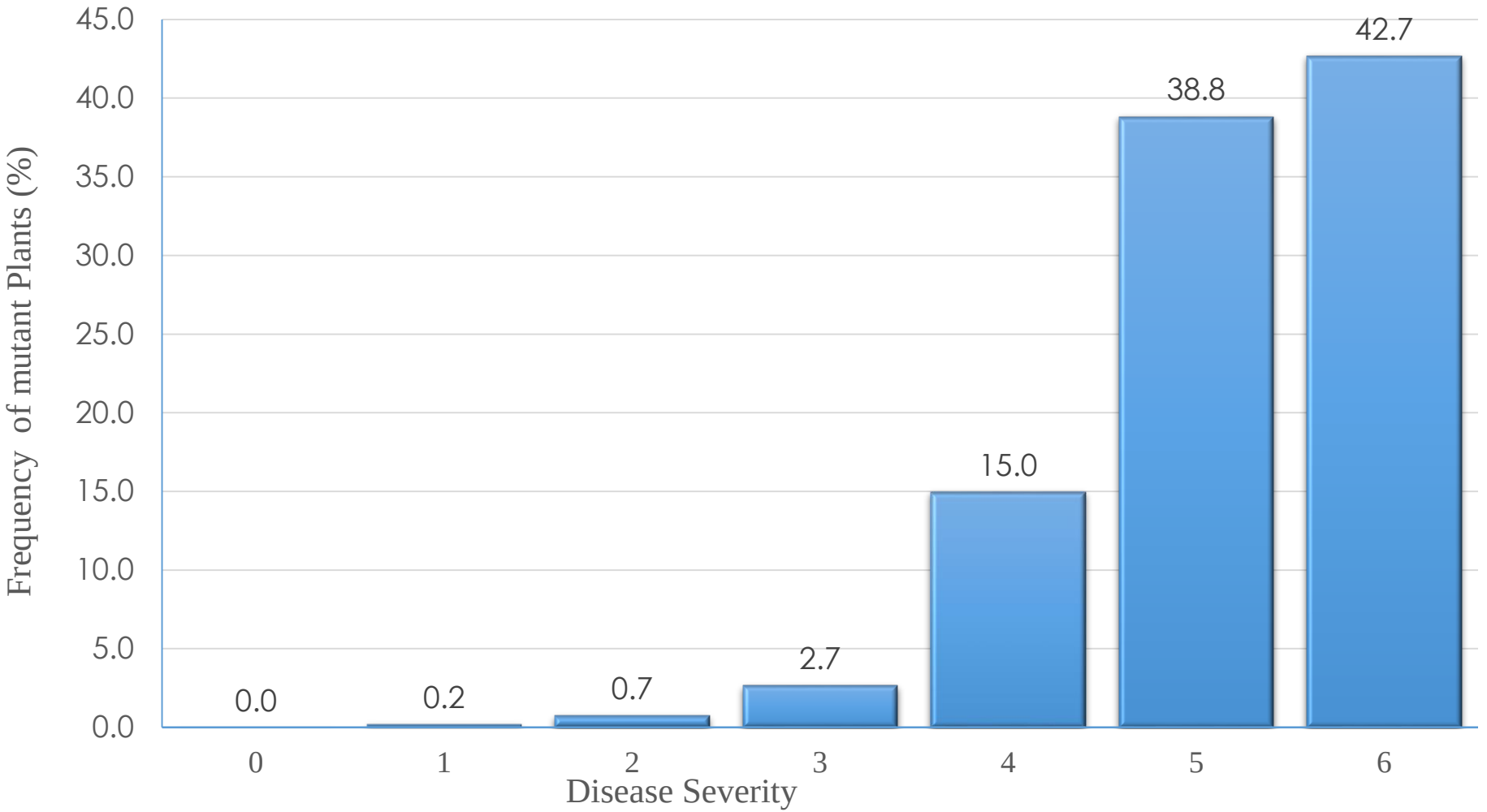






Development of Fusarium wilt on (Musa AAA Cavendish subgroup), for each cultivar, each data point represents the mean observed disease severity value, a: plantlets inoculated with Fusarium oxysporum. b: plantlets inoculated with non pathogenies Fusarium.

Symptoms caused by Fusarium oxysporum f.sp. cubense in banana plants



Frequencies of mutant Banana according to recording disease severity rating caused by Fusarium oxysporum f.sp. in Banana plants

In-vitro selection

- After 3 week all survived plants evaluated and plantlet that rated 1-2 were selected
- Selected plants transferred to pot

Conclusion

- These research showed that the in-vitro inoculation method effectively and efficiently can be used to evaluate disease resistance.
- Additionally, the procedure described in this research allows a fast resistance diagnosis of 3 to 4 weeks after inoculation with Foc tropical race 4.
- This bioassay system completely closed, So exist possibility of controlling environmental conditions, while not worrying about unwanted spread
- Since temperature is important in progress of Foc invasion and symptom development in banana, the in vitro bioassay is favorable for consistent infection by Foc.
- Since there is no acclimatization stage for plantlets used in the In-vitro selection methods, it helps to improve banana breeding efficiency.


Thank you for your time
and attention