Cihan University
College of Science
Biology Department

Transformation Efficiency with Three Different *Agrobacterium Tumefaciens* Strains: C58C1, EHA105 And LBA4404

Prepare by : Ali M.Hussein
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Outlines:

- **Cotton Germination and Explant Isolation**
- **Bacterial Culture and Transformation Media**
- **Growth Bacterial Strains & Binary Plasmid Used Electroporation of pTJK136 to Agrobacteria**
- **Gene Transfer to Cotton Tissues and Transient Expression Analysis**

Introduction

Transgenic Cotton:

- Gene transfer is a transfer one or several genes and their inclusion in a host organism, and that are transferred naturally or by the number of Techniques genetic engineering.
- There are many techniques to conduct gene transfer, where the most common methods in the laboratory is through by ***Agrobacterium tumefaciens.***

Agrobacterium tumefaciens:

- ***Agrobacterium tumefaciens*** causes **crown gall** disease of a wide range of dicotyledonous (broad-leaved) plants, especially members of the rose family such as apple, pear, peach, cherry, almond, raspberry and roses.
- The microbe ***Agrobacterium tumefaciens*** is harmful to plants and useful to scientists for the same reason: It transfers DNA into plant genomes. Found in soil worldwide, *A. tumefaciens* causes disease in plants by transferring its own DNA into plant cells. But in the laboratory, the ability to move all sorts of genes into plants has made the microbe the standard tool for investigating plant genetics and modifying crops.

Aim

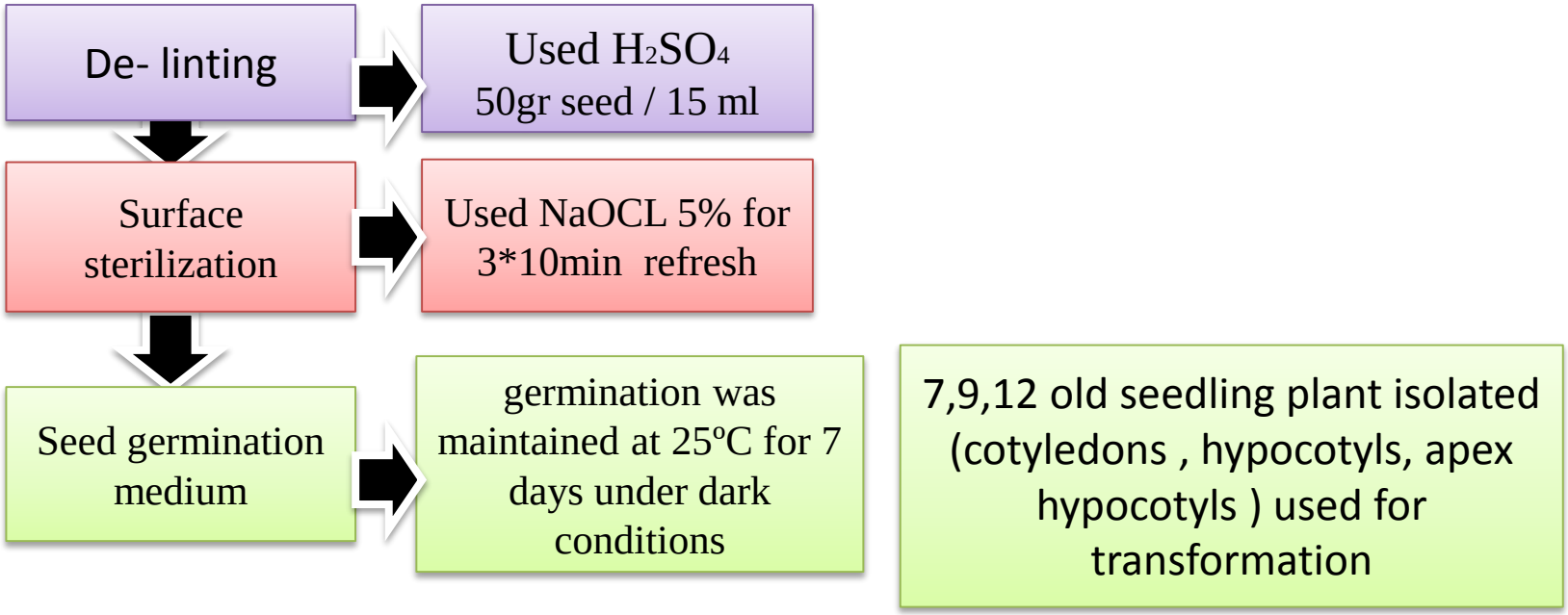
The purpose of this study is to evaluate the transformation ability of four different *Agrobacterium tumefaciens* strains (KYRT1 , C58C1, LBA4404 and EHA105) on cotton (*G. hirsutum* cv. Coker 312) hypocotyl , cotyledon and apex hypocotyl explants.

Methodology

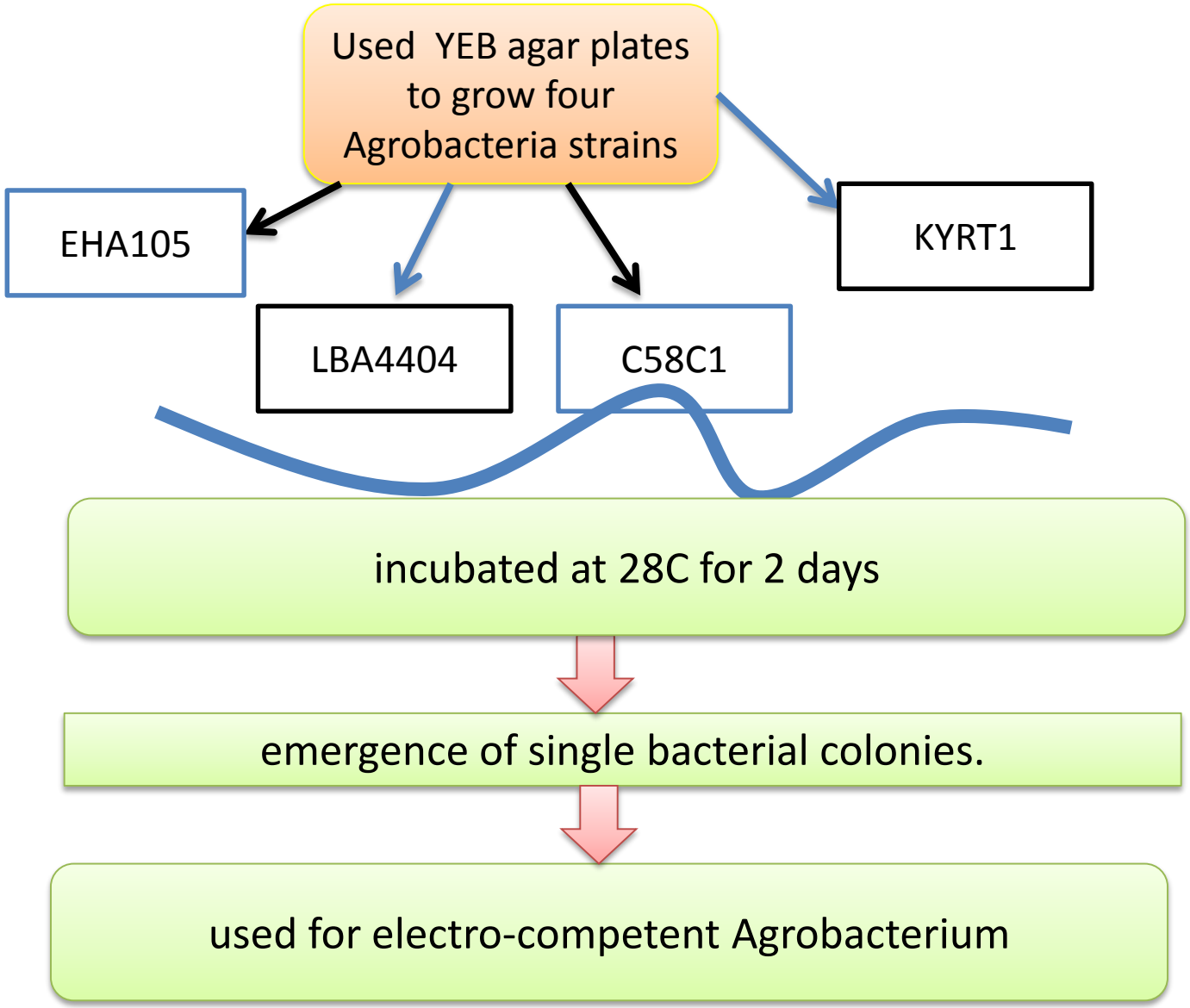
- Throughout this study the binary transformation vector **pTJK136** will be transformed in to these agrobacterium strains.
- These strains was analyzed for the transformation vector.
- Then these strains was cultured for transformation of cotton hypocotyl , cotyledon , apex hypocotyl tissues.
- Finally transformed tissues was subjected to transgene expression. Evaluation of this transgene expression was used for quantification of efficiency of transformation with the four different agrobacterium strain.

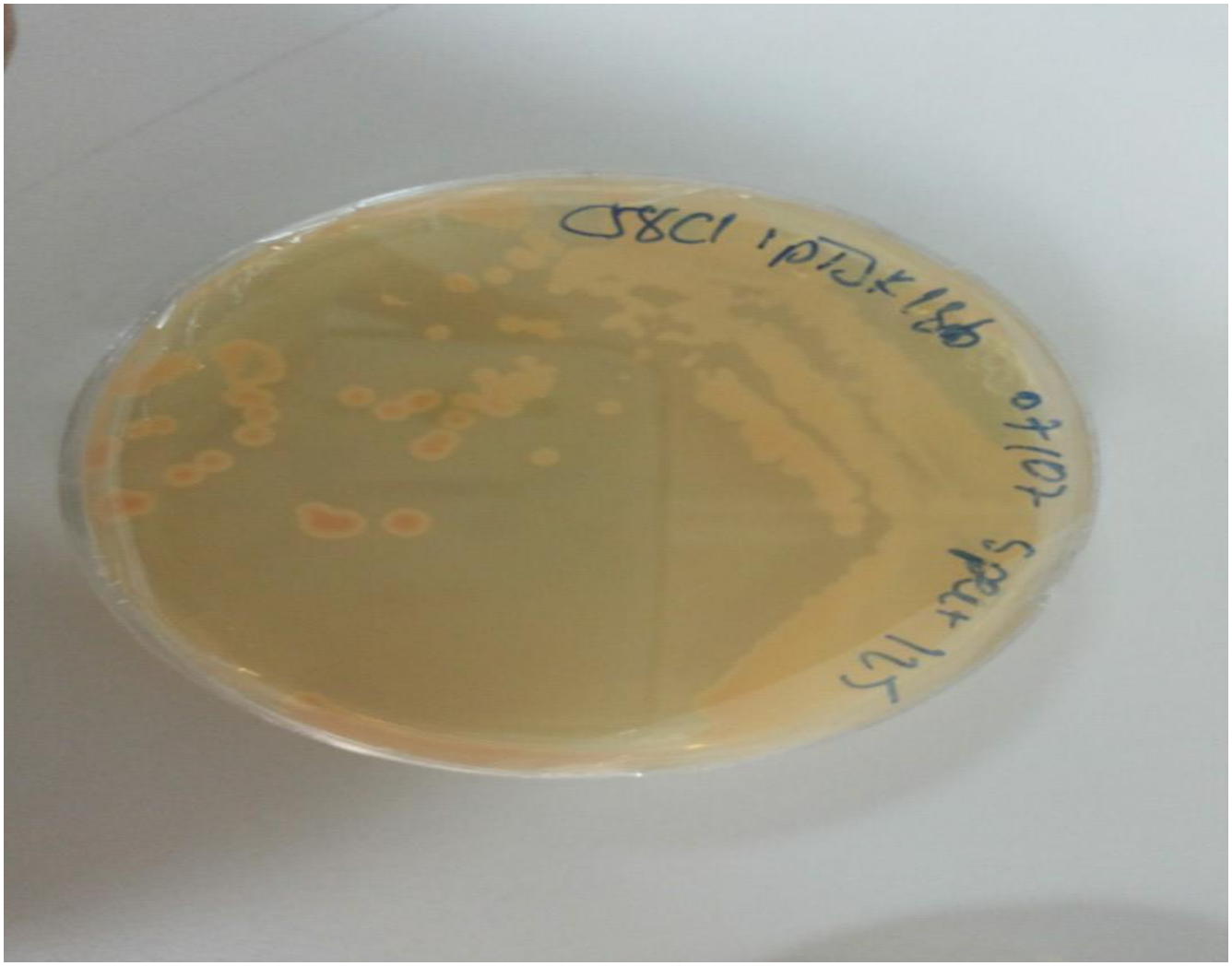
MATERIALS AND METHODS:

1.Cotton Germination and Explant Isolation

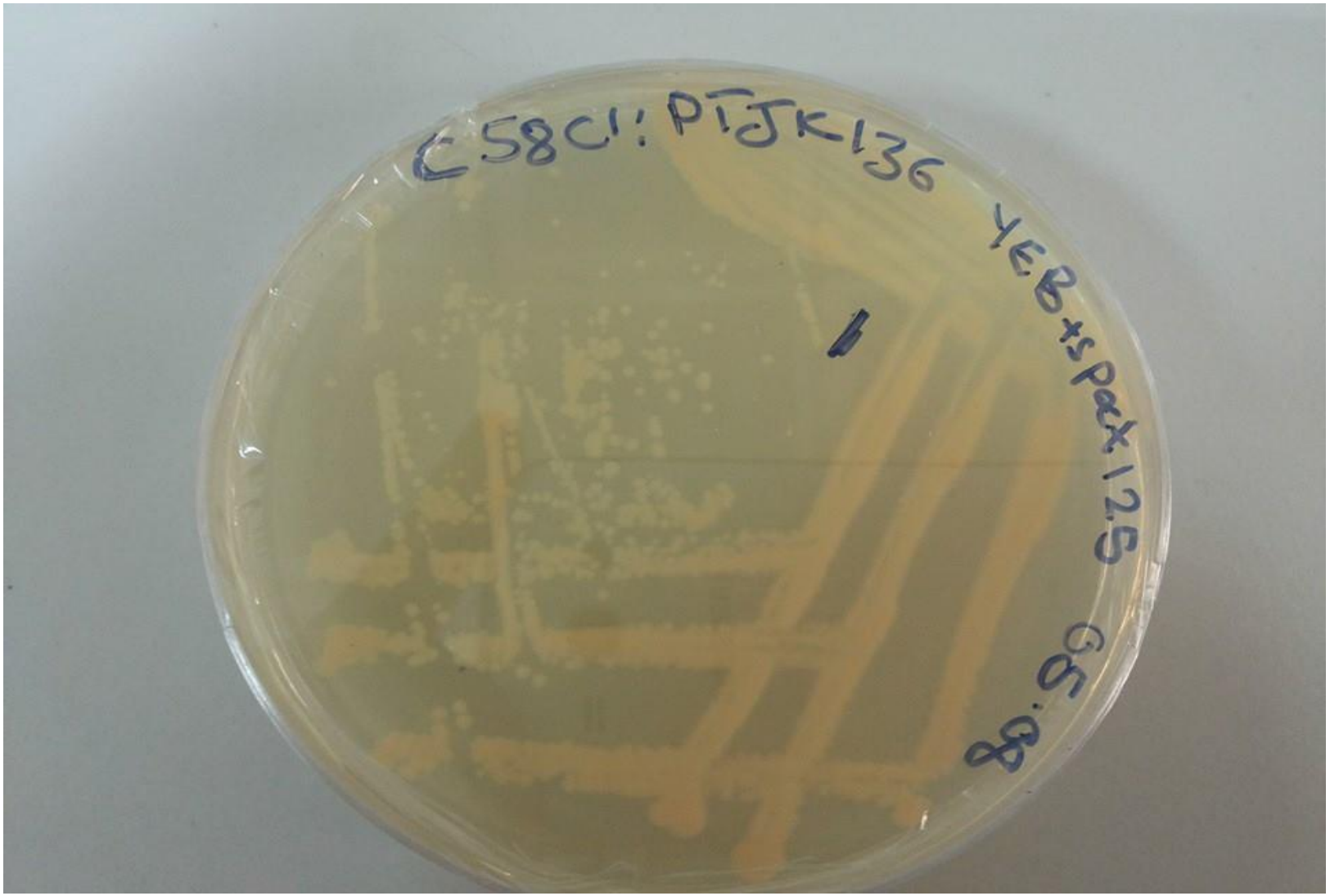


Growth Bacterial Strains

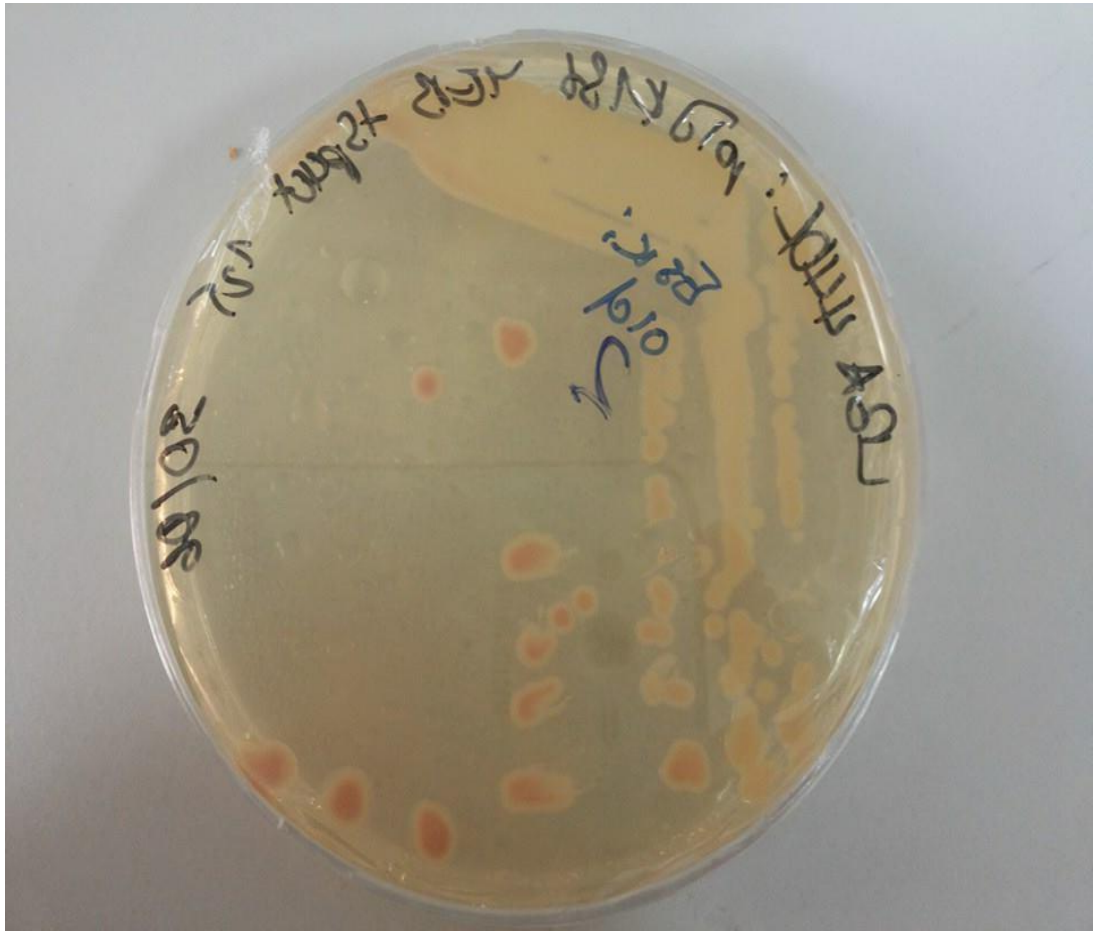




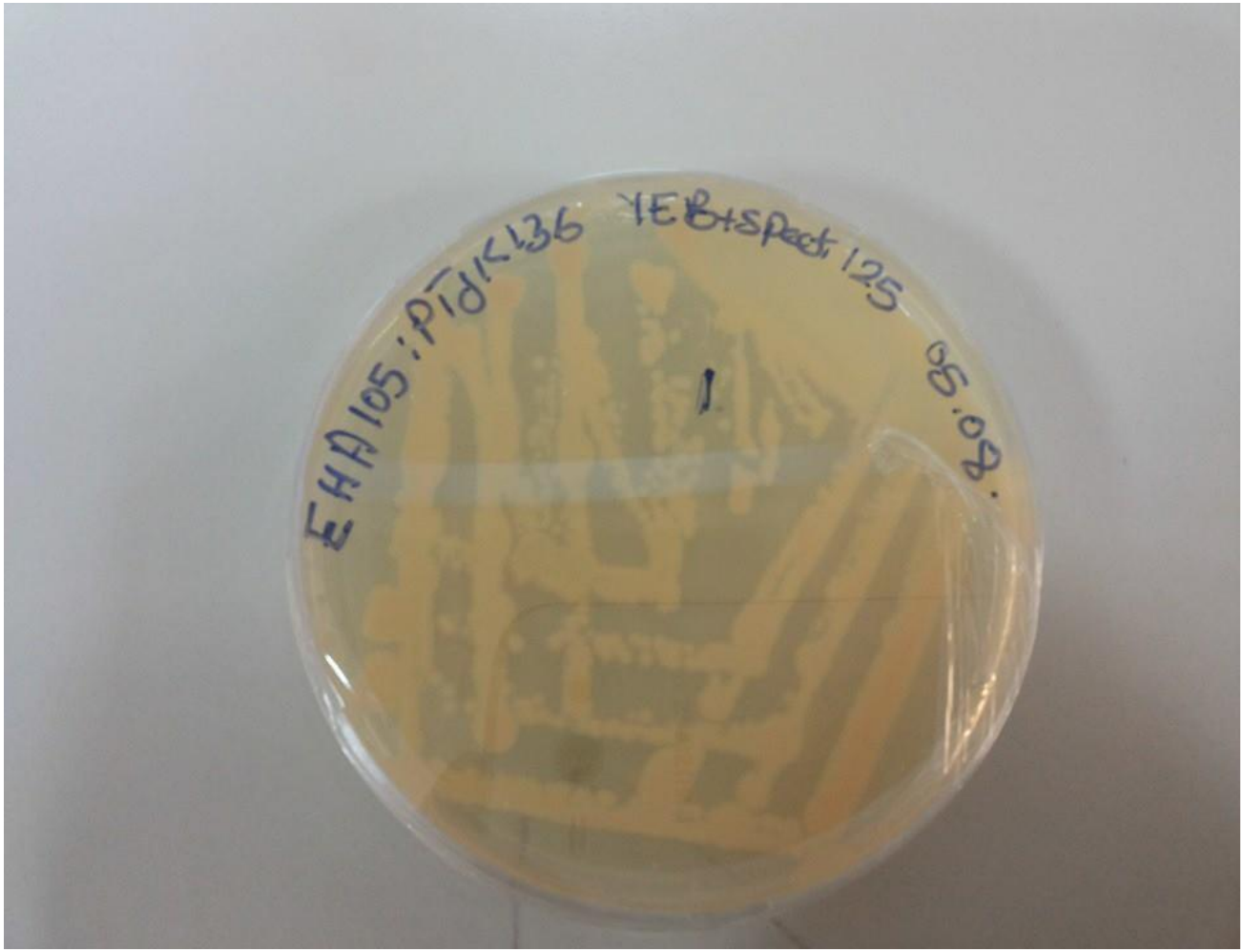
C58C1agro



C58C1agro

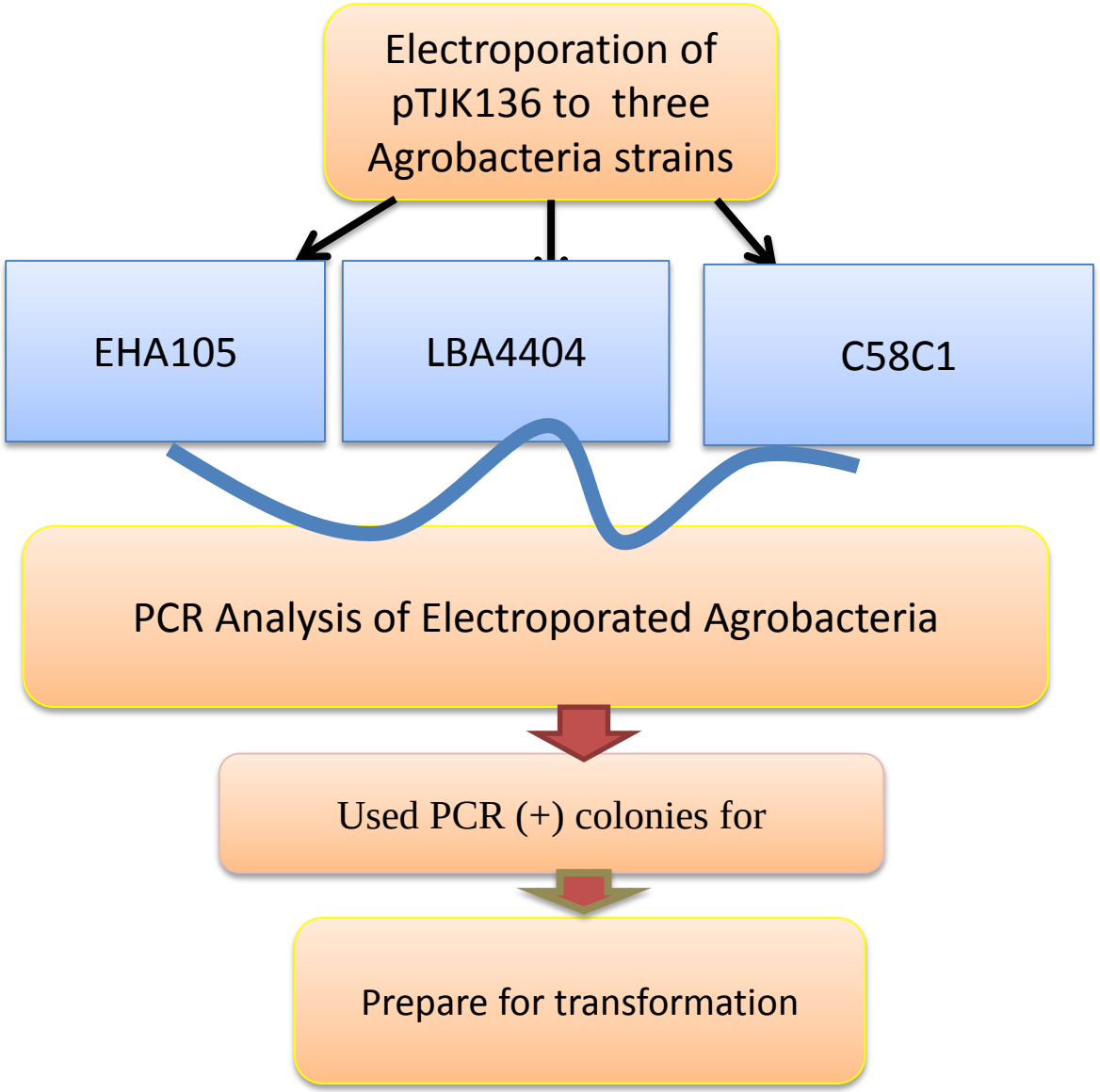


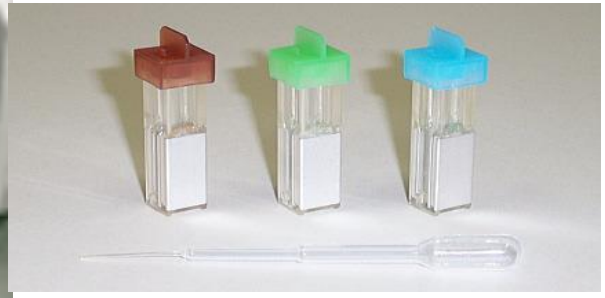
LBA4404 agro

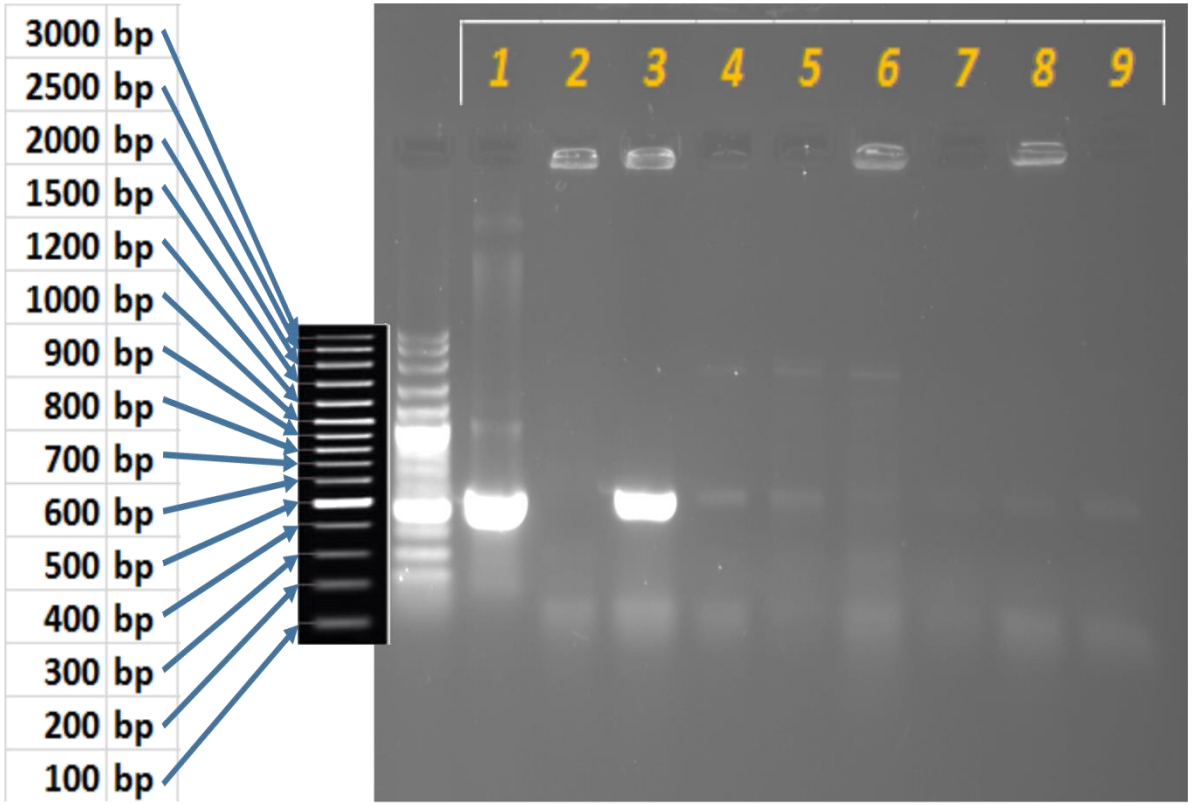


EHA105 agro

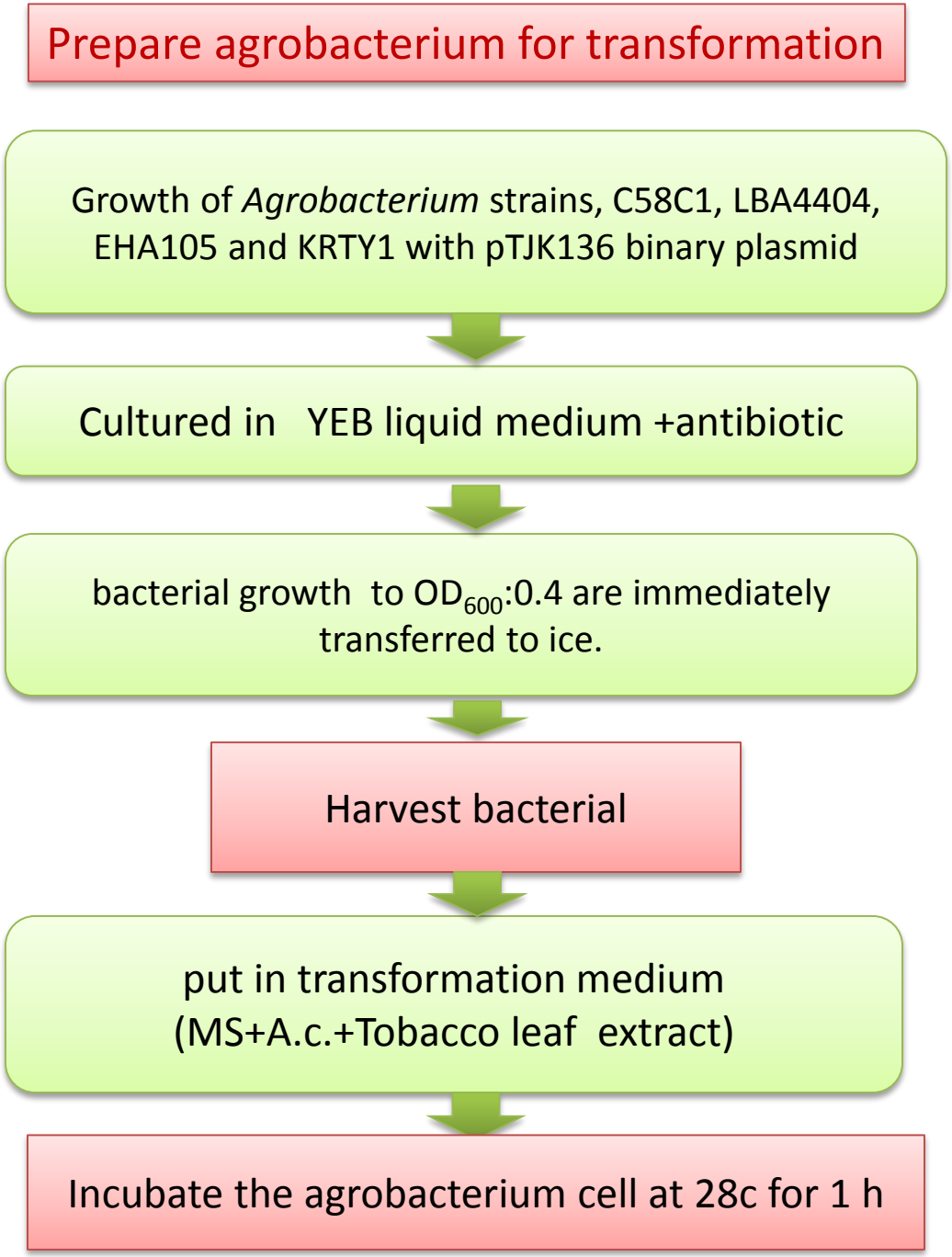
Bacterial Culture and Transformation Media
Electroporation of pTJK136



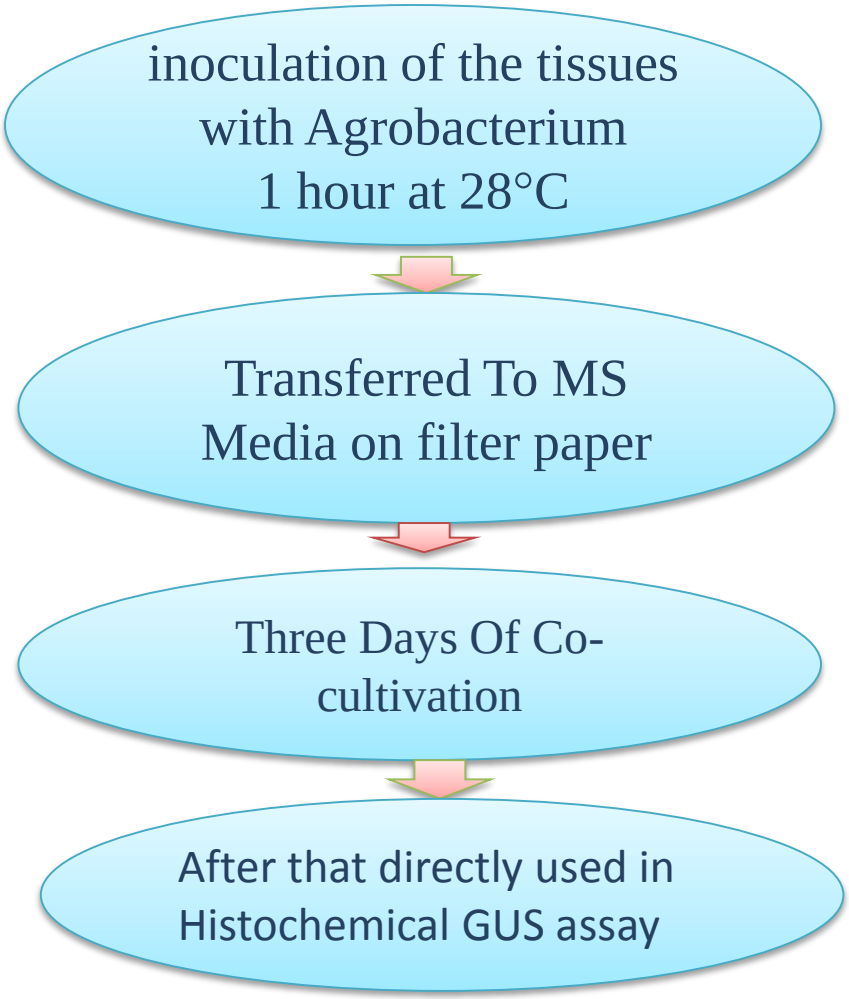




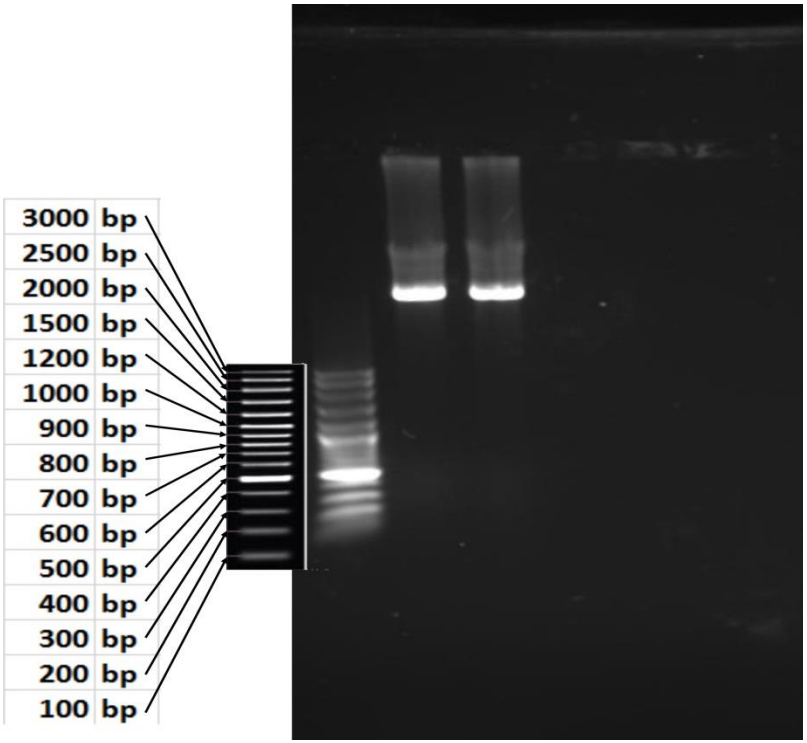
Agarose gel electrophoresis image of P35S PCR (540 bps) from pTJK136. Vivantis 100bp plus ladder was used as marker


Prepare agrobacterium for transformation
Growth of *Agrobacterium* strains, C58C1, LBA4404, EHA105 and KRTY1 with pTJK136 binary plasmid
Cultured in YEB liquid medium +antibiotic
bacterial growth to OD_{600}:0.4 are immediately transferred to ice.
Harvest bacterial
put in transformation medium (MS+A.c.+Tobacco leaf extract)
Incubate the agrobacterium cell at 28c for 1 h

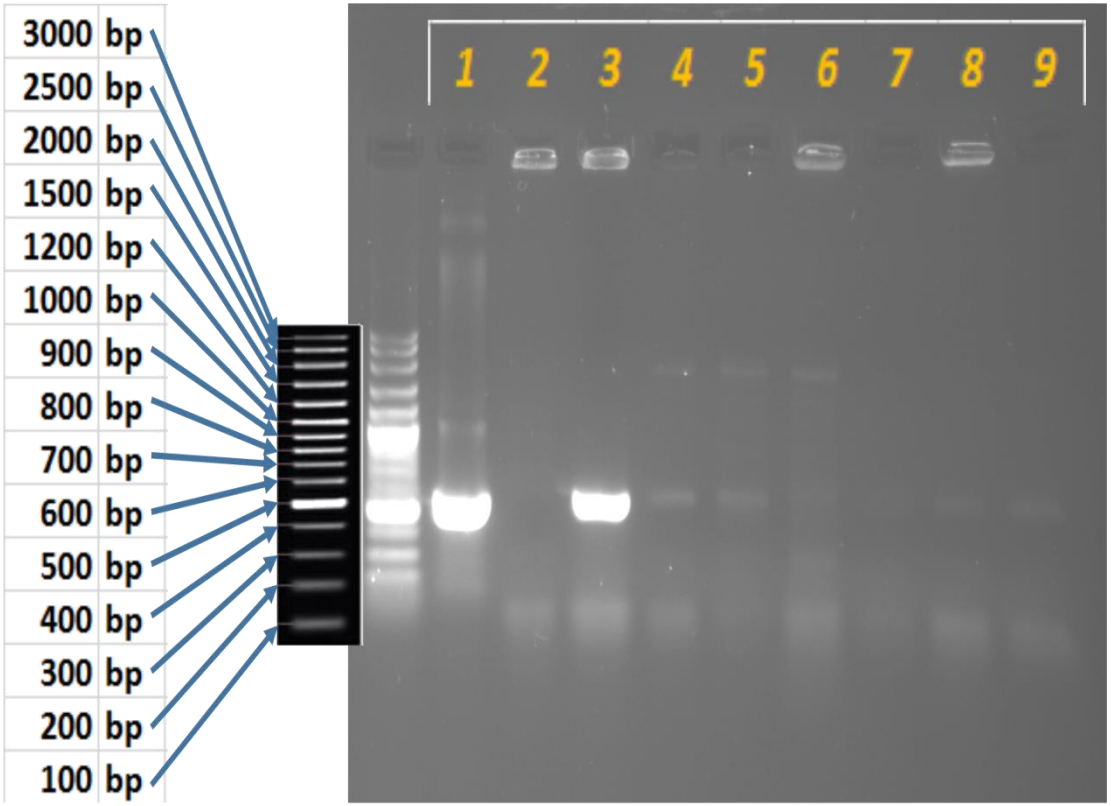
Transformation of the Explants with Agrobacterium Strains



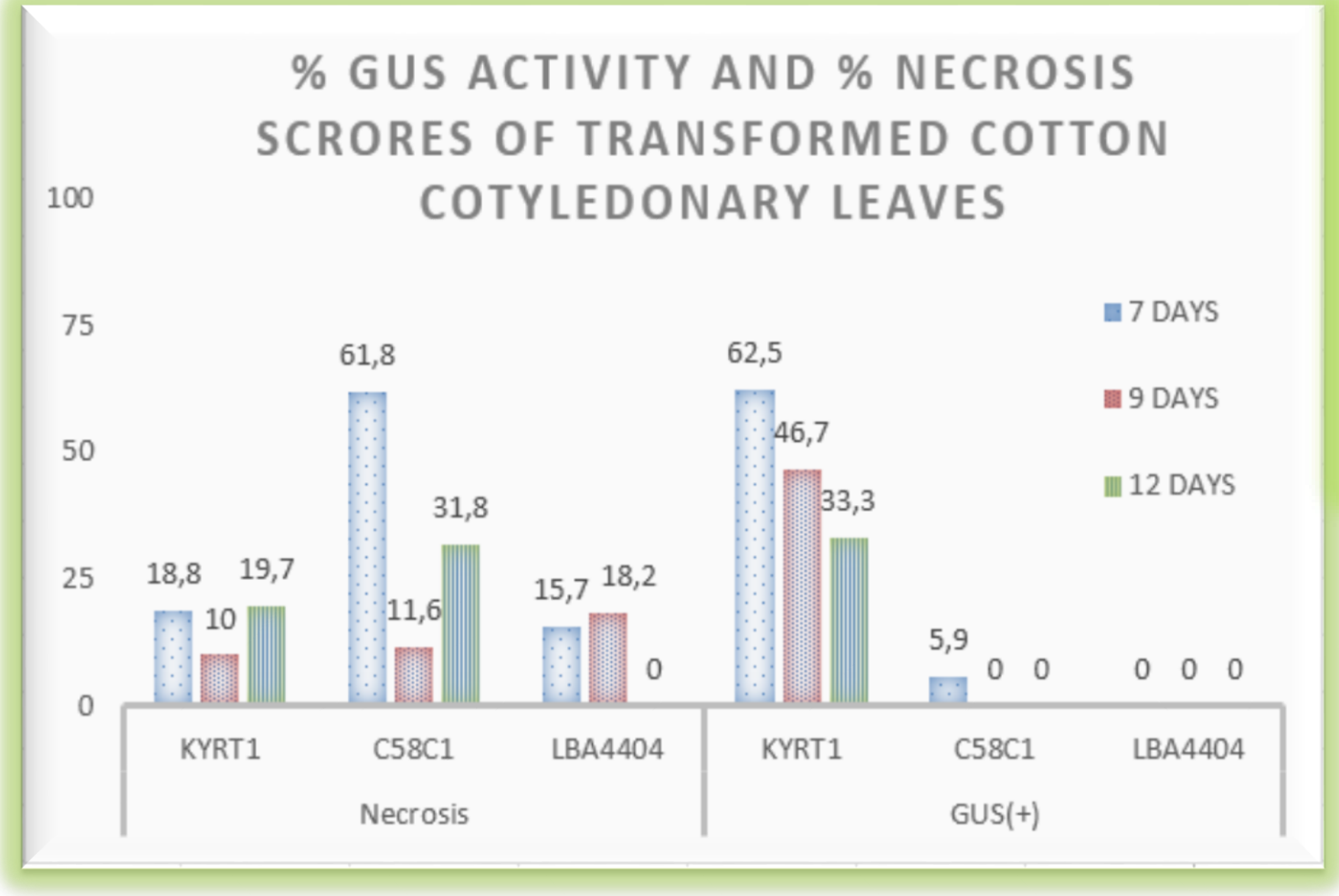
RESULTS AND DISCUSSION

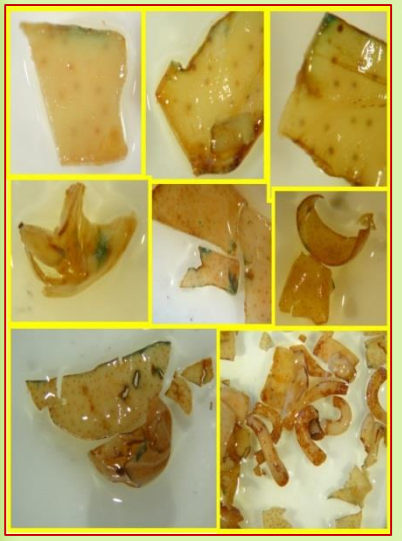


Figuer 1:- Agarose gel electrophoresis image of pTJK136. Plasmid was electrophoresed in 0.7% agarose gel prepared with 0.5X TBE buffer at 7V/ cm voltage rating for 30 minutes



Agarose gel electrophoresis image of P35S PCR (540 bps) from pTJK136.
Vivantis 100bp plus ladder was used as marker. Wells designed as W1 through W9. W1 is amplification from the intact isolate plasmid pTJK136. W2 is amplification from (-) control which was LBA4404 without pTJK136. W3 is amplification from (+) control which was KYRT1 with pTJK136. W4-W7 were four different colonies from C58C1 electro-transformed with pTJK136. W8 and W9 were P35S amplifications from single colonies of LBA4404 and EHA105





Figuer 3: The highest rate of necrosis was score with C58C1 on 7 days old seedling cotyledonary leaves (61.8 %) while that of KYRT1 and LBA4404 was similar with % scores of 18.8 and 15.7 respectively. Necrotic behavior of the cotyledonary leaves excised from 7 days old

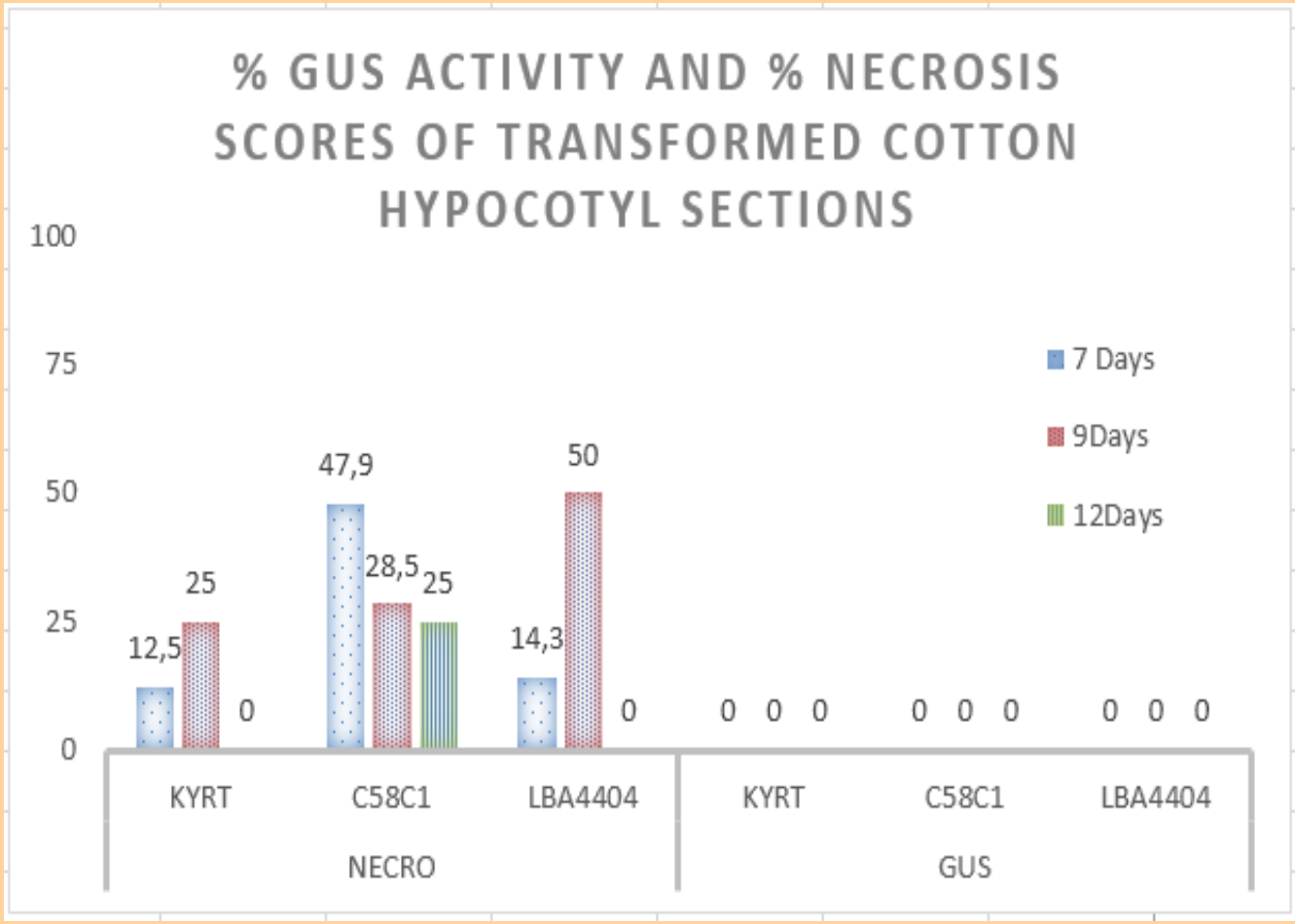
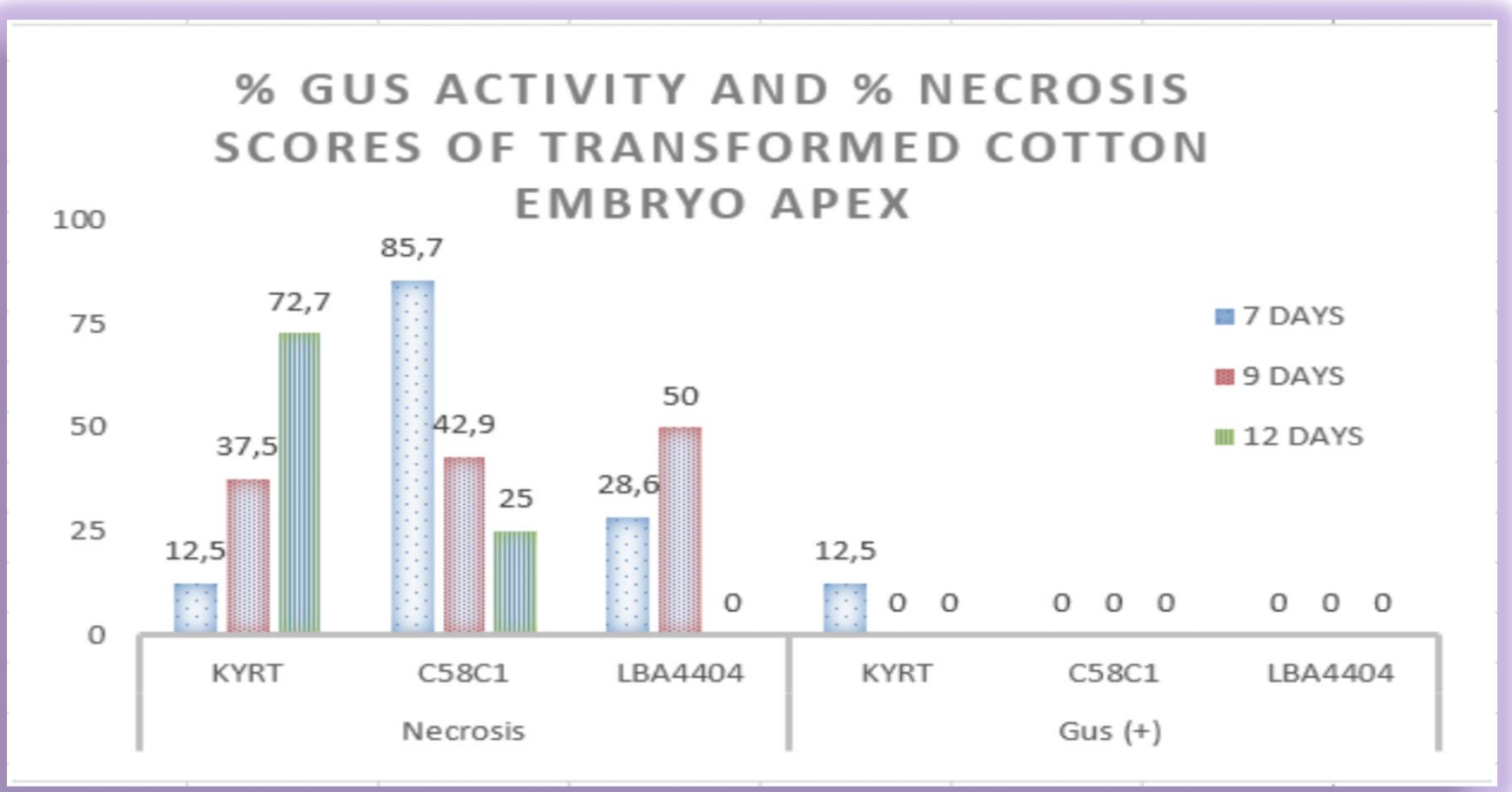
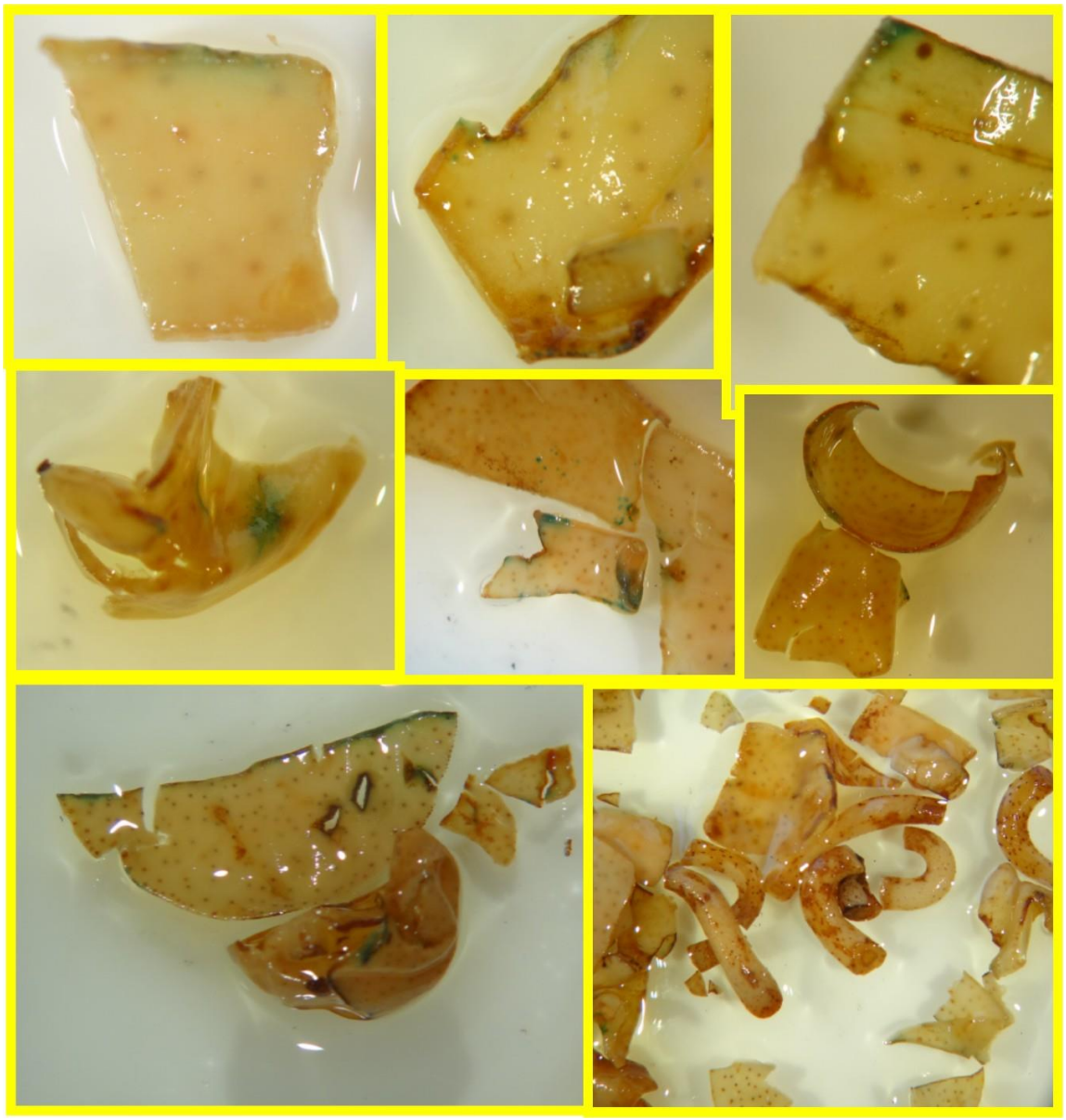


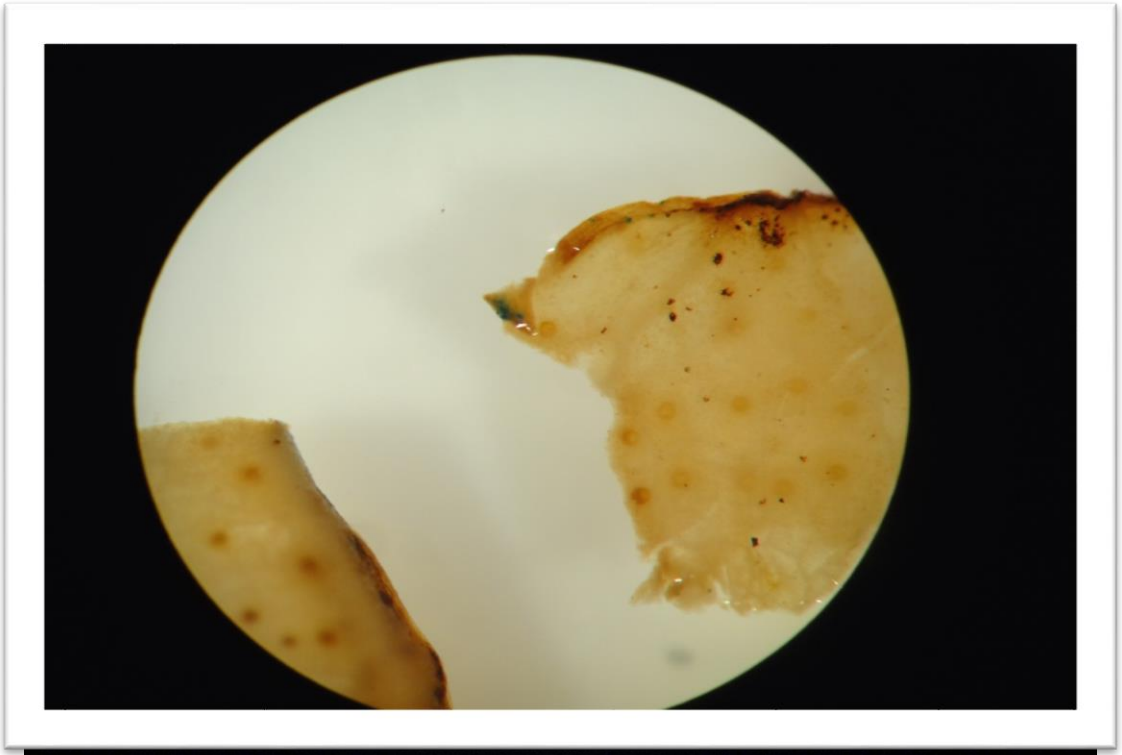
Figure : Hypocotyl tissue on the other hand did not gave any GUS (+) score for any of the bacterial strains used and for any of the seedling germination dates. On the other hand the same trend of necrosis scores was achieved with that of embryo shoot apical meristem except the necrosis rate for the hypocotyl tissues from 12 days old seedlings (0%).

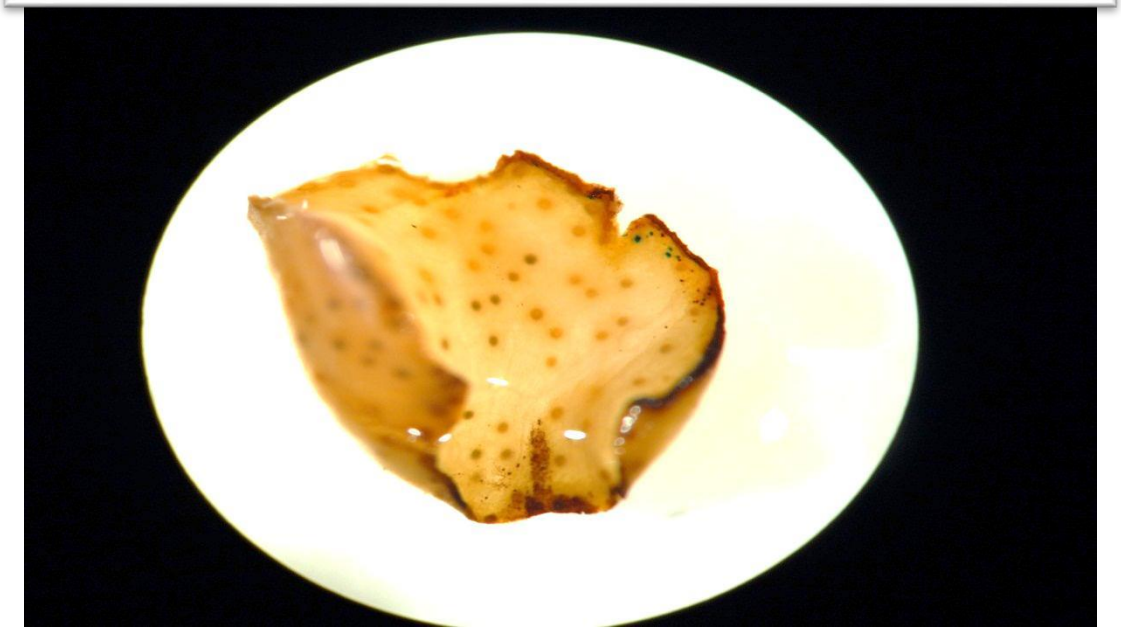


Necrosis scores and GUS activity scores for cotton embryo apical meristem is given in figure 12. Interestingly almost similar necrosis trends were achieved with that of cotyledonary leaf scores but in this section discrepancy of necrosis rates were more clearly from 7 to 12 days old germinates for all of the Agrobacterium strains. On the other had the only GUS (+) score was taken from KYRT1 with embryo apex tissue taken from 7 days old seedlings



GUS (+) pictures of cotyledonary leaf explants transformed with KYRT1:pTJK136





GUS (+) pictures of cotyledonary leaf explants transformed with c58c1:pTJK136

Conclusion :

We did transformation with three agrobacterium strains (KYRT1,C58C1, and LBA4404) and the binary plasmid is PTJK136
With three different part of explant (cotyledon , hypocotyl , and apex hypocotyl) and the best result is (KYRT1) with (cotyledon) 62.5%


THANK
YOU