Research Topics and Seminar
(501)
Dr. Hassan Al-Haddad

Improving T-DNA Transfer to *Tamarix hispida* by Adding Chemical Compounds During *Agrobacterium tumefaciens* Culture

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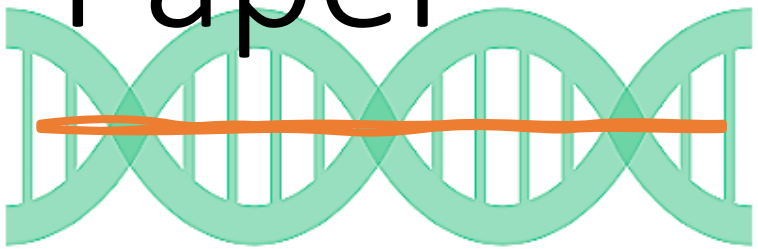
Paper



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Improving T-DNA Transfer to *Tamarix hispida* by Adding Chemical Compounds During *Agrobacterium tumefaciens* Culture

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Agrobacterium tumefaciens-mediated gene transfer is the most commonly used method for plant genetic engineering. However, during the period of *A. tumefaciens* culture, the effects of *Agrobacterium* culture before inoculation on genetic transformation are poorly understood. In the present study, we investigated the factors that affect the genetic transformation efficiency during *Agrobacterium* culture using *Tamarix hispida* as transgenic plant material. *Agrobacterium* treatment with spermidine (Spe), azacitidine (5-AzaC), dithiothreitol (DTT), or acetosyringone (AS) alone all significantly improved the efficiency of T-DNA transfer. Treatment with 5-AzaC reduced DNA methylation in *Agrobacterium* to induce the expression of virulence (*vir*) family genes, including *vir*A, *vir*B1, *vir*C1, *vir*D2, *vir*D4 *vir*E2, and *vir*G. Spe treatment significantly induced the expression of all the studied genes, including *vir*A, *vir*B1, *vir*C1, *vir*D1, *vir*D2, *vir*D4, *vir*E2, and *vir*G. DTT treatment decreased reactive oxygen species accumulation. AS treatment activated the expression of the genes *vir*A, *vir*B1, *vir*C1, *vir*D1, *vir*D2, *vir*D4 and *vir*G. All these effects resulted in increased T-DNA transfer. Additionally, combined Spe, 5-AzaC, DTT, and AS treatment improve *Agrobacterium* infection to a greater extent compared with their use alone, increasing T-DNA transfer by more than 8-fold relative to no treatment. Therefore, to improve genetic transformation, pretreatment of *Agrobacterium* during the culture period is important for improving genetic transformation efficiency.

Keywords: *Agrobacterium*-mediated transformation method, spermidine (Spe), azacitidine (5-AzaC), dithiothreitol (DTT), acetosyringone (AS), T-DNA transfer, *Agrobacterium* infection

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INTRODUCTION

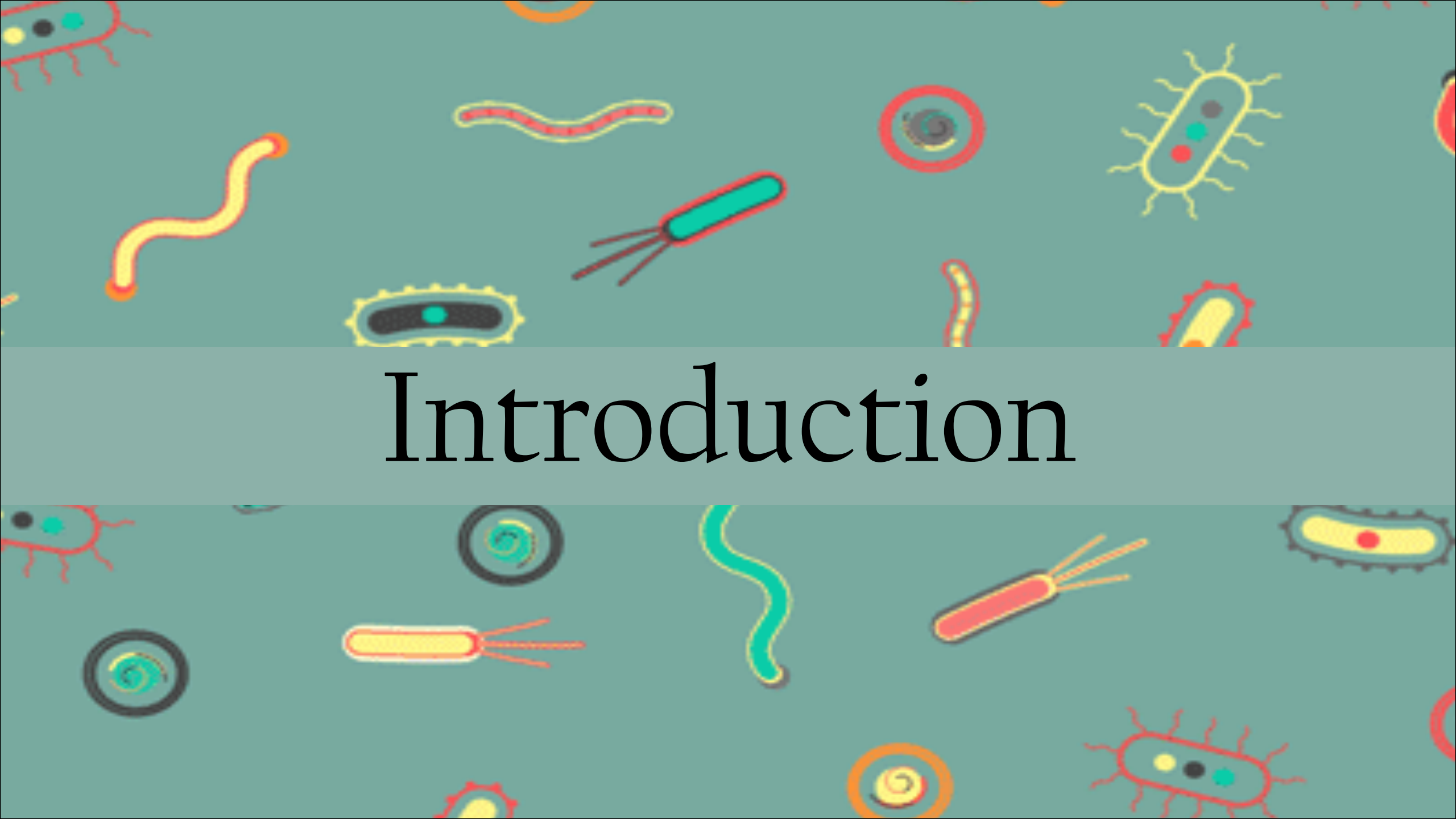
Genetic transformation is a method that transfers DNA of interest into the cell of an organism resulting in genetic alteration, and has been used in many areas of biotechnology, such as studies of gene function, genetic improvement, and molecular breeding. To deliver foreign genes into host plants, three methods are mainly used: *Agrobacterium tumefaciens*- or *Agrobacterium rhizogenes*-mediated plant transformation (Hinchee et al., 1988), protoplast transformation (Fischer and Hain, 1995), and particle bombardment (McCabe et al., 1988). Among these methods, *Agrobacterium*-mediated plant genetic transformation has low cost, is the best choice for plant transformation, and is

Outline

- Introduction

1. Method of production of transgenic plant
2. Description about *Agrobacterium* and Ti plasmid.

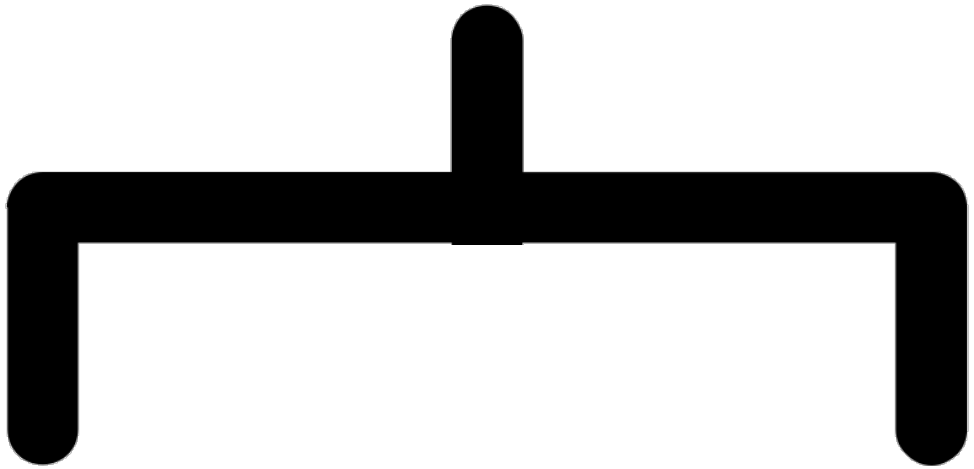
- Objective
- Material and methods
- Result
- Discussion
- Conclusion



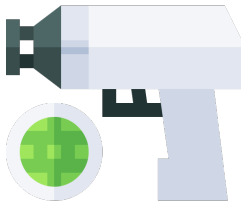
Introduction

Transgenic plant

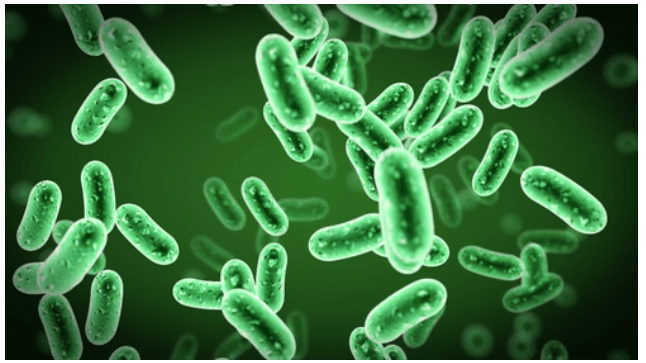
To produce a transgenic plant there are many methods



Microprojectile Bombardment



Ti Plasmid of *A. tumefaciens*

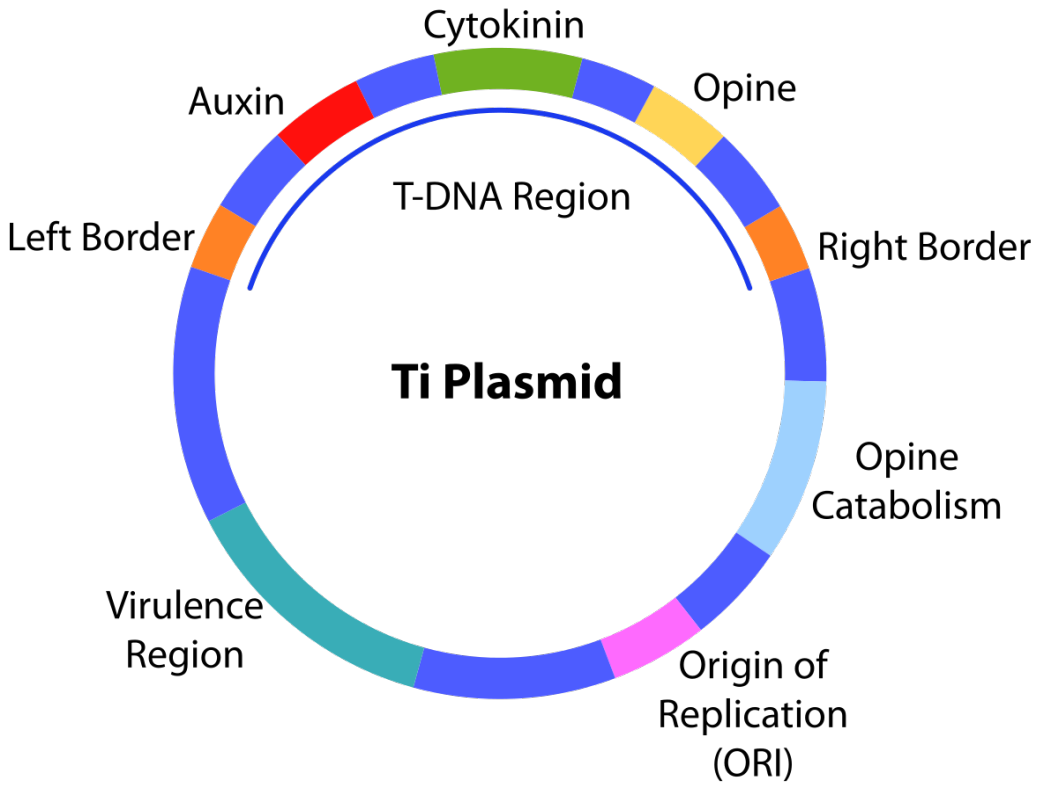


Agrobaterium tumefaciens

- A. tumefaciens is a gram negative bacterium and it is a is a phytopathogen that can cause infect plant and transfer genes into host plants and this result in tumors.
- It is the reason behind crown gall disease.

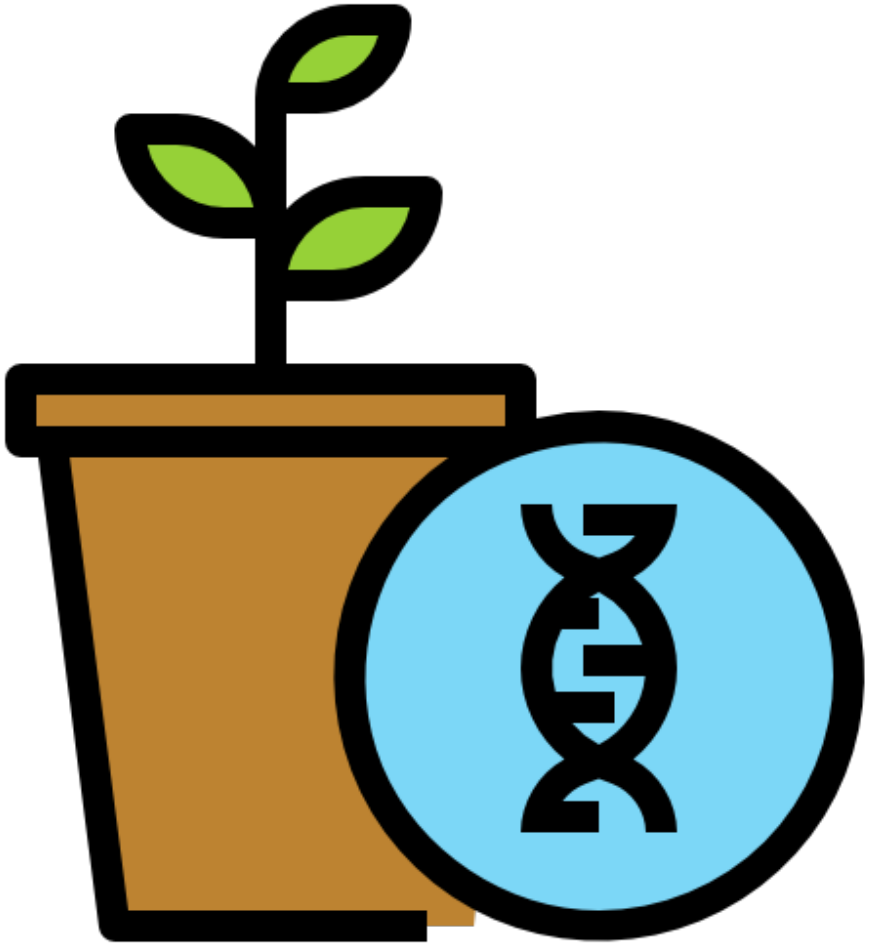
Ti Plasmid

- Agrobacterium contains a special plasmid which called Ti (tumor inducing) plasmid.
- Ti plasmid consists of T-DNA region which is 10 to 30 kb in length.
- The T-DNA is defined by its left and right borders and includes genes for the biosynthesis of auxin, cytokinin, and an opine.



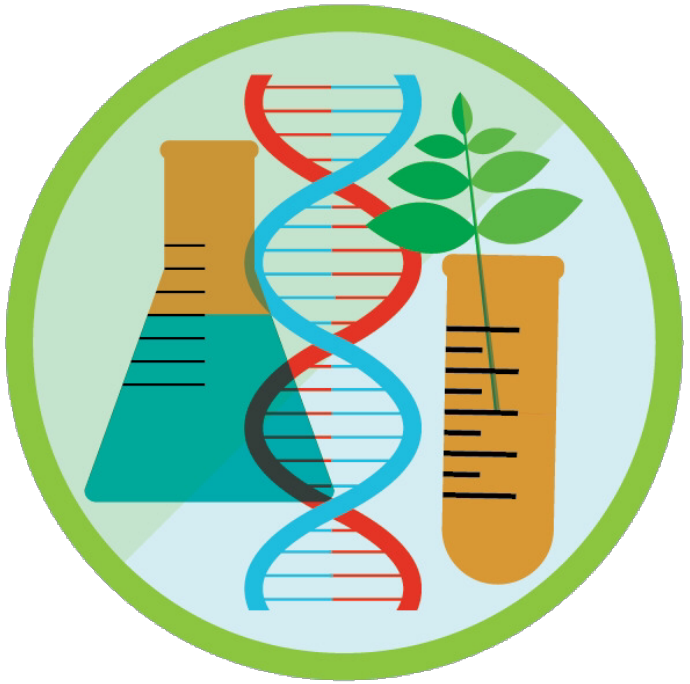
Agrobacterium and crops

- cotton
- rice
- Maize, potato
- tomato
- cabbage,



The reasons behind making transgenic plant

- To make it disease resistant towards the insect, bacteria and viruses.
- To enhance the production of crop
- To increase the nutritional value of the crops



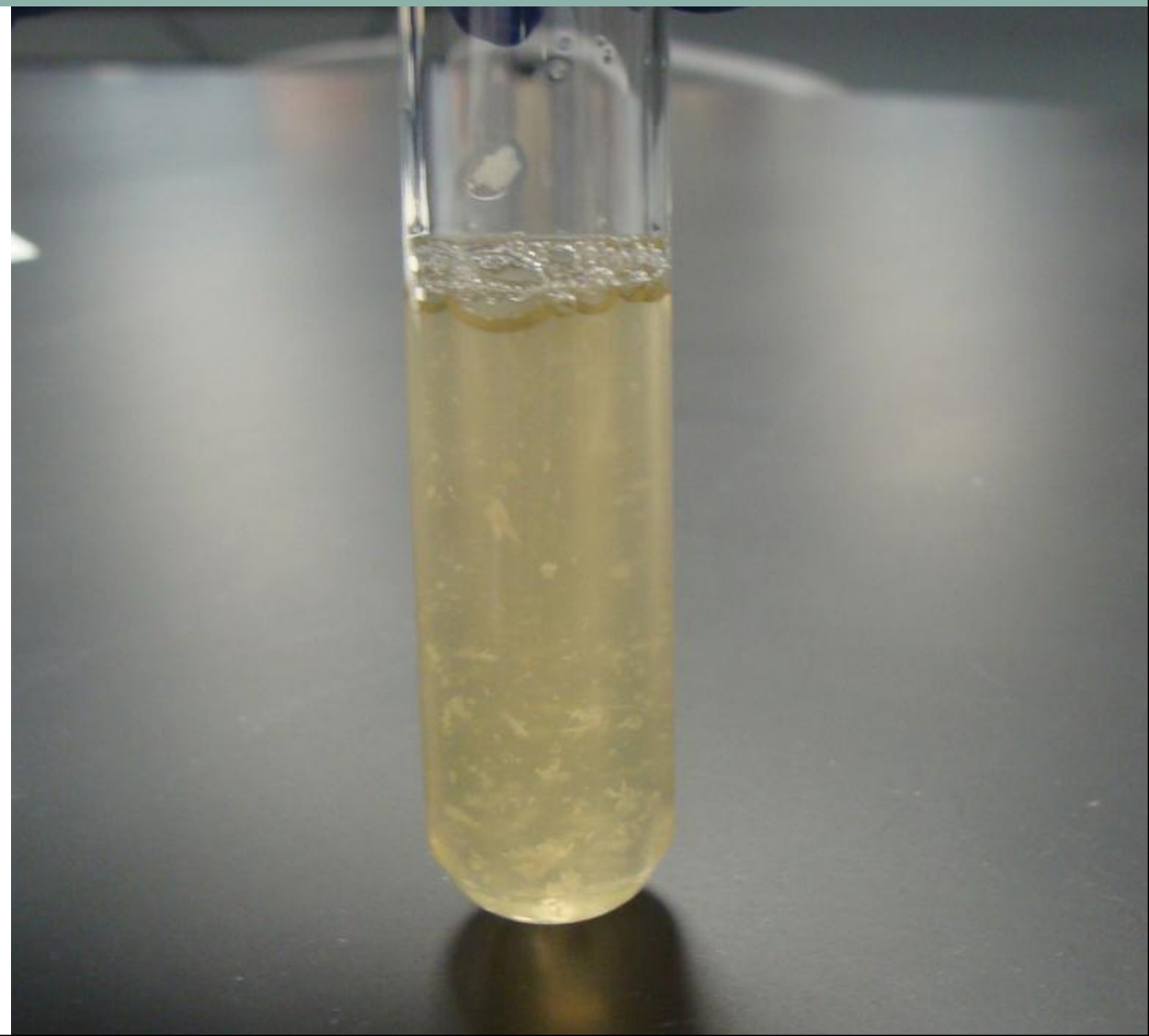
Objective

- To identify the factors affecting the infection capability of Agrobacterium.

Material and methods

Treatment of of Agrobacterium tumefaciens

- A specific strain of *Agrobacterium* tumefacins was picked and cultured in LB liquid culture supplied with several antibiotics such as rifampicin and Kanamycin.

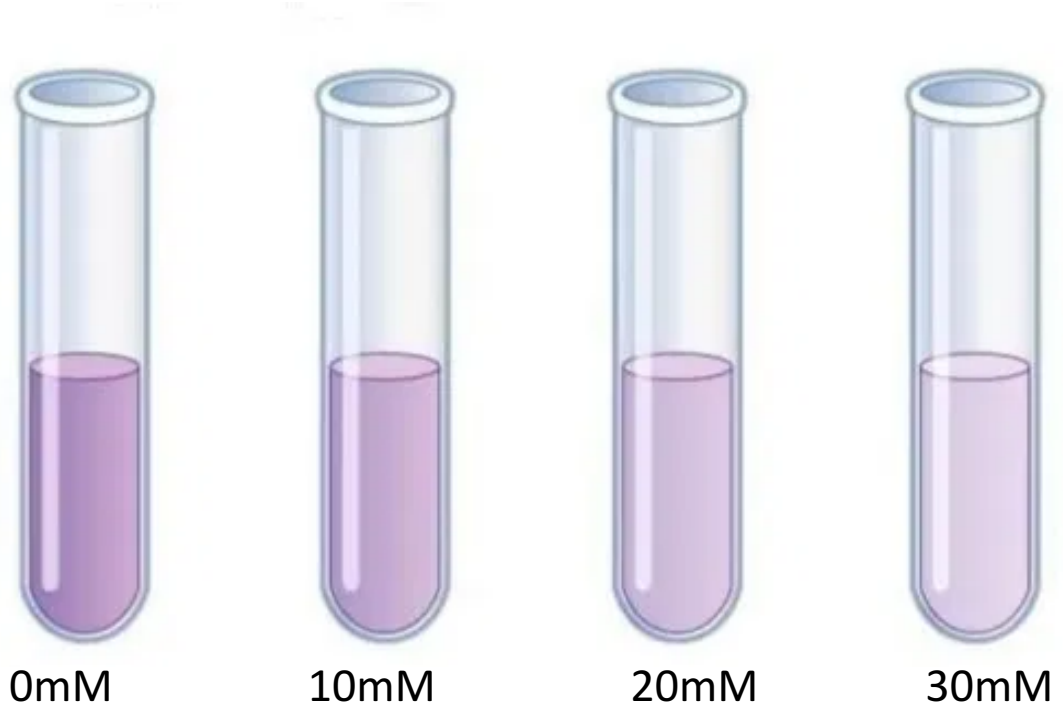


Addition of chemical compounds

- Spermidine was added to the medium in different concentrations.

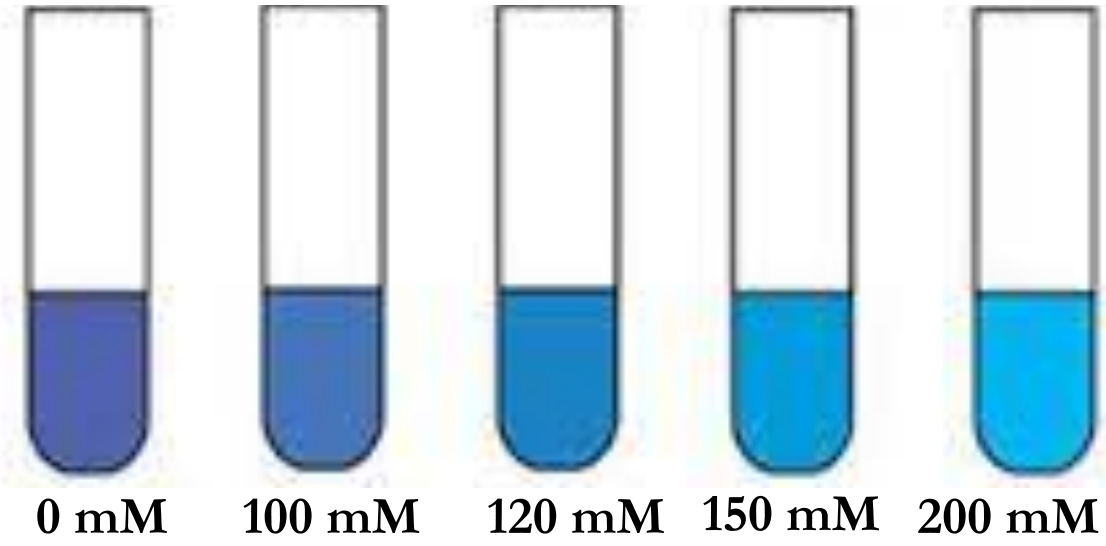
Addition of chemical compounds

- AzaC treatment, the diluted cultures were added different concentrations of 5-AzaC



Addition of chemical compounds

- Acetosyringone was also added to the medium in different concentrations.



Addition of chemical compounds

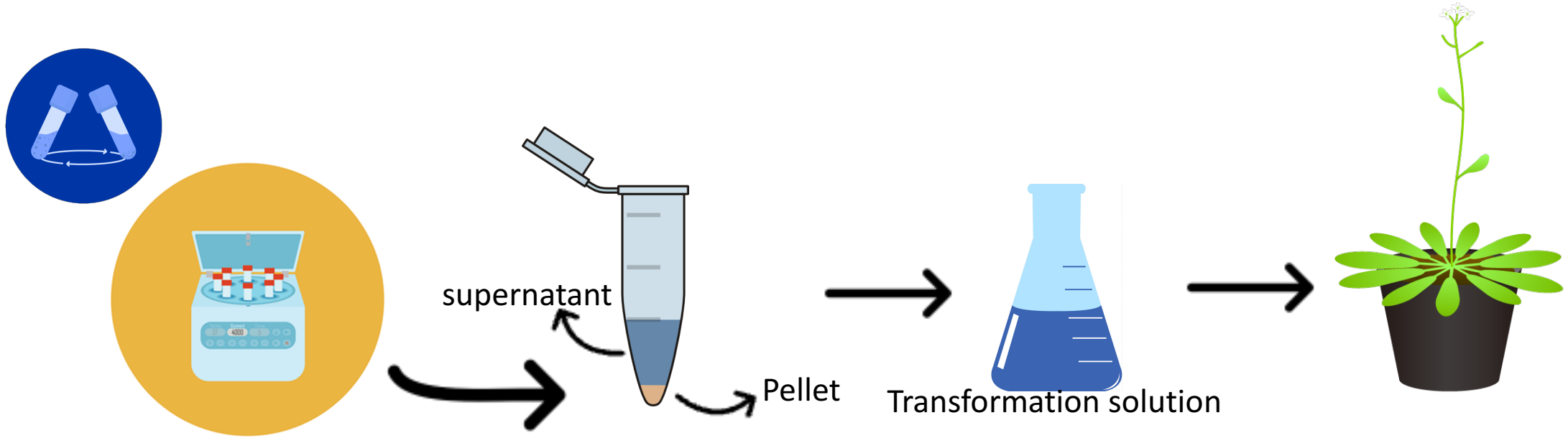
The diluted cultures were supplied with different concentrations of dithiothreitol (DTT)

Cultivation

- Bacteria were cultivated for 7 to 8 hours at 28c

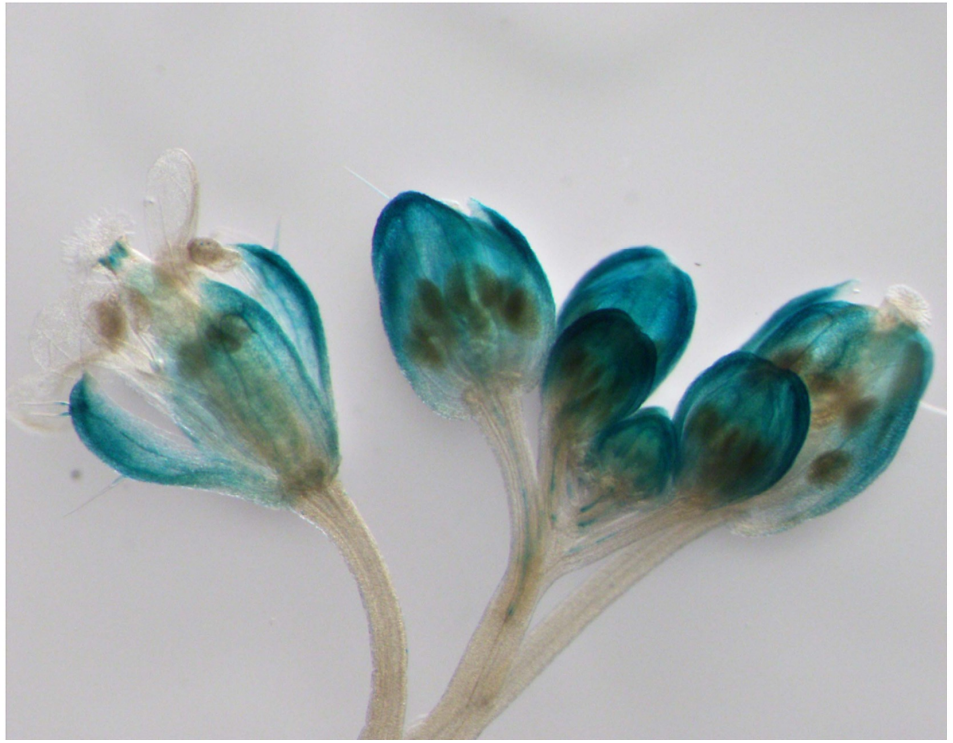
Transformation step

- The plants were soaked in the transformation solution with shaking at 90 rpm for 2 h at 25°C



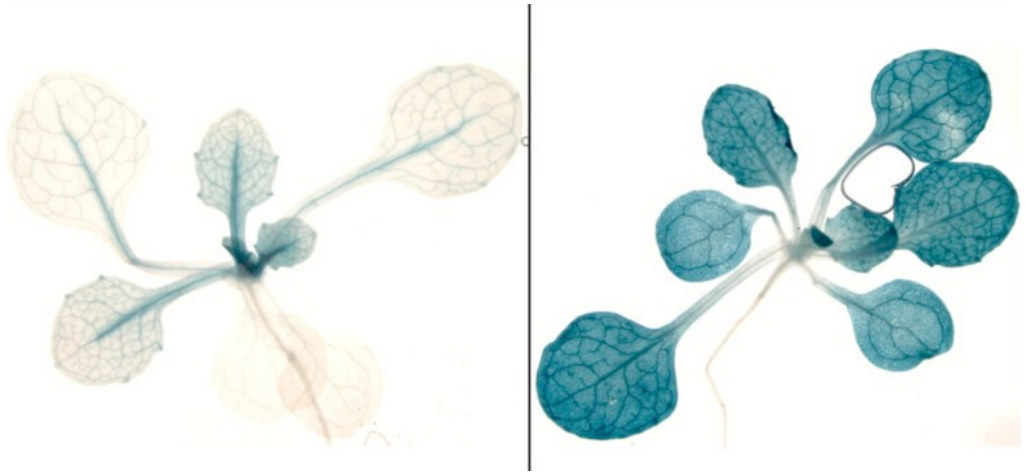
Determination of (GUS) Activity

- The samples were ground into a fine powder under liquid nitrogen
- After that the homogenized in an extraction buffer in which the b-d-glucuronide (MUG) was added.
- enzyme reaction was performed at 37°C and it was stopped by adding Sodium carbonate



Calculation of the Gus activity

- The last step was to calculate the GUS activity and this is was done by Bradford assay.

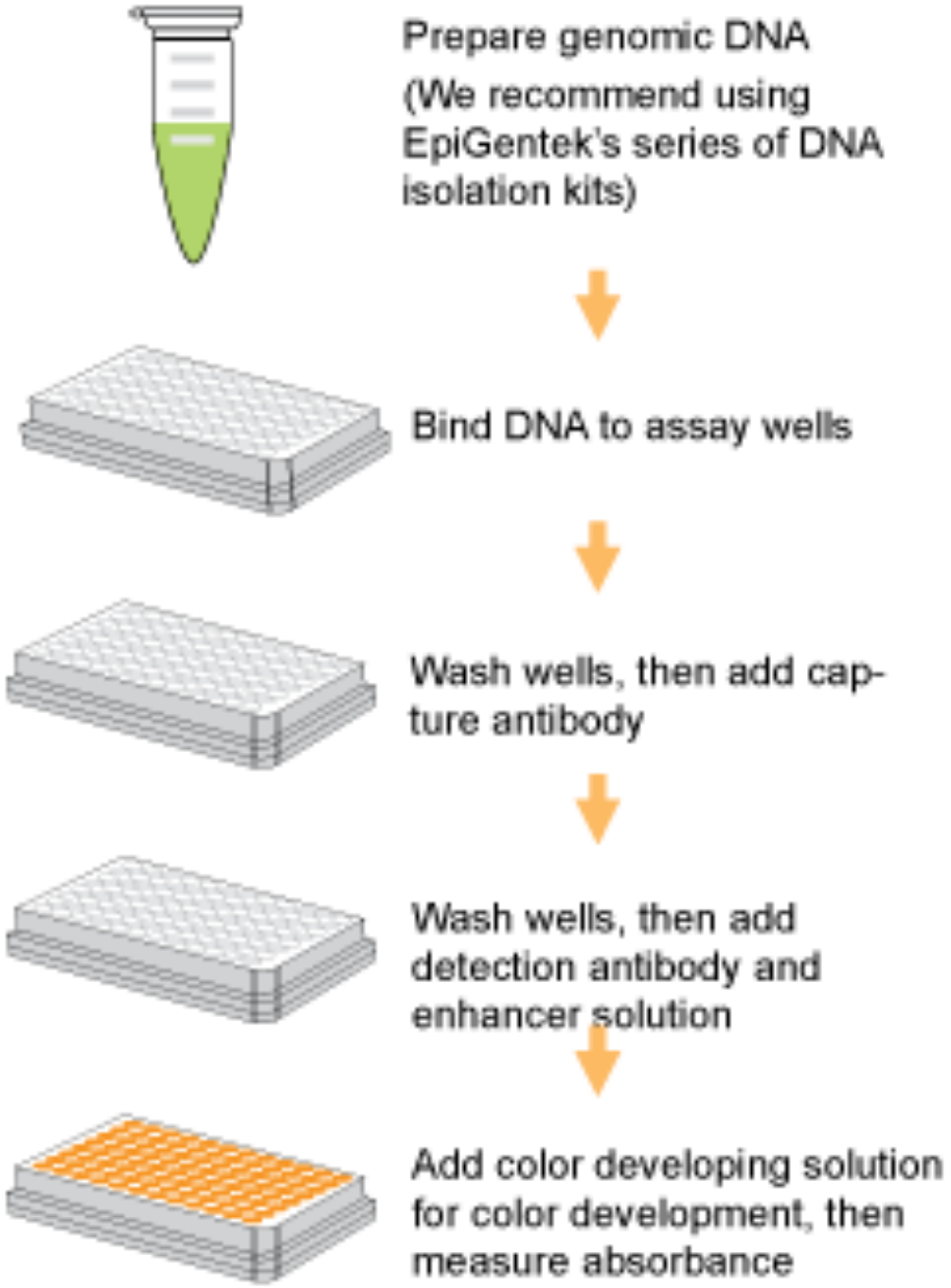


Determination of the Soluble ROS Content

- The ROS content was determined using a Hydrogen Peroxide Content measuring kit.

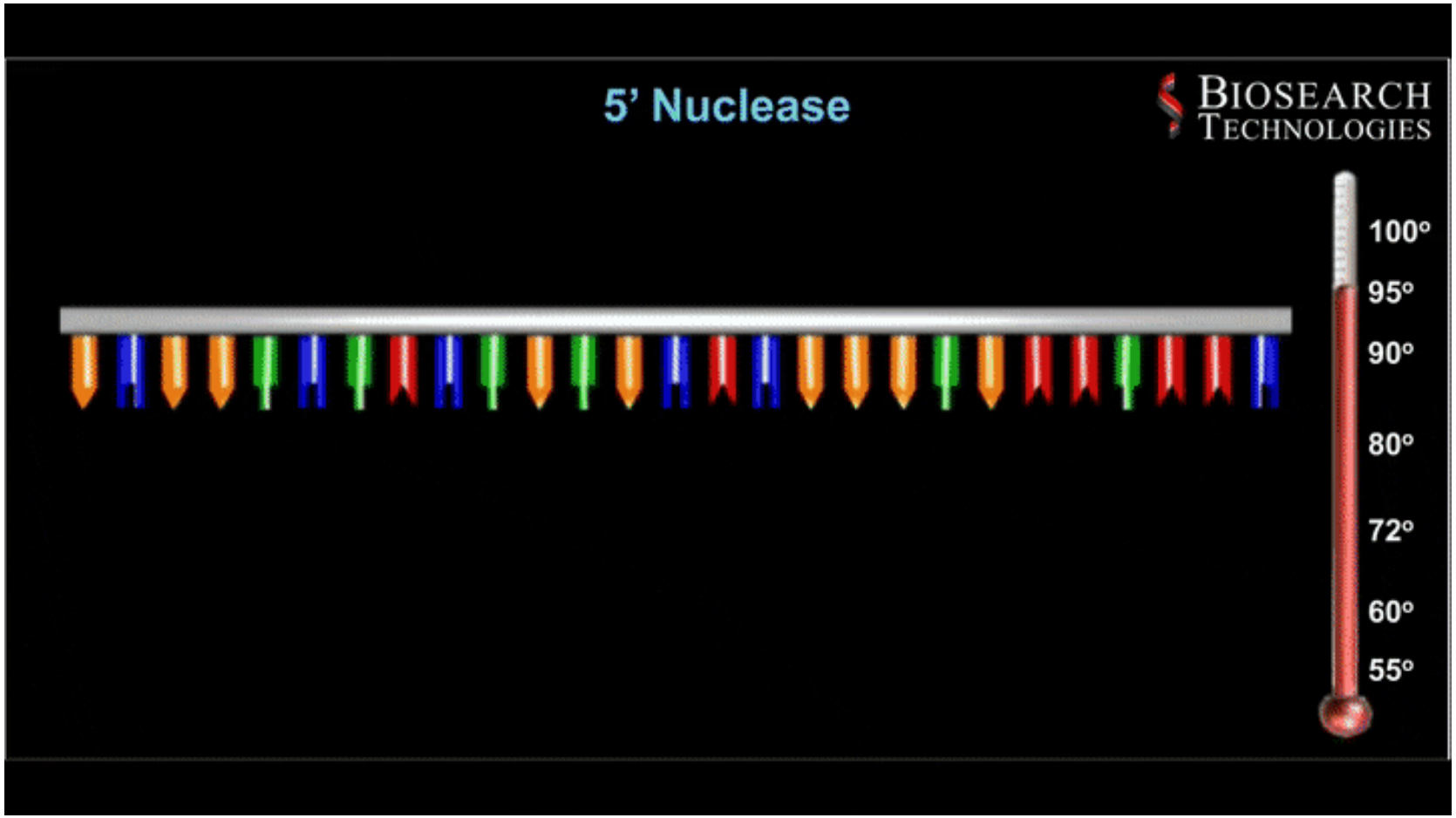
Determination of DNA Methylation Level

- The DNA methylation content was determined using Methylated DNA Quantification Kit.



qRT-PCR

It is the process which convert mRNA to DNA.



Statistical Analysis

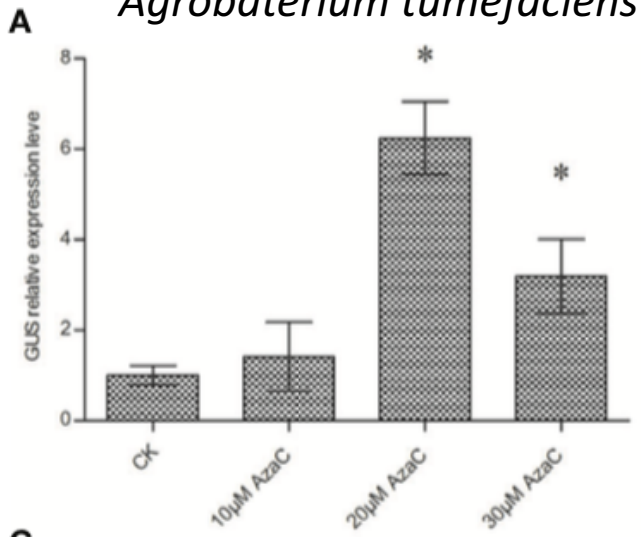
It was carried out by the SPSS 16.0 software package

Result

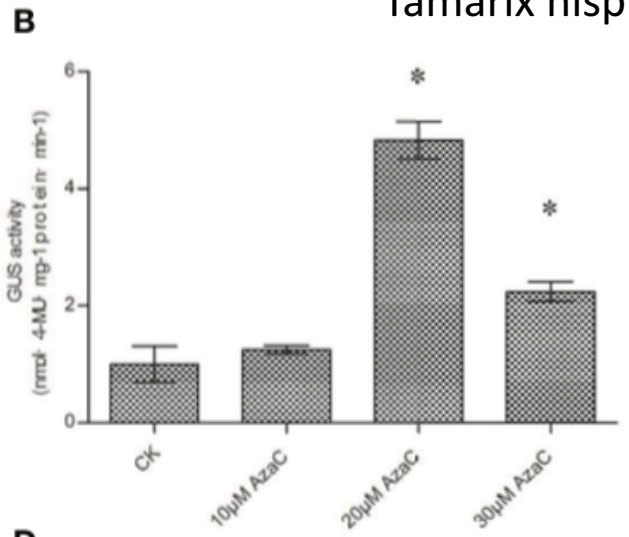
The Effects of (5-AzaC) on T-DNA Transfer and other activity

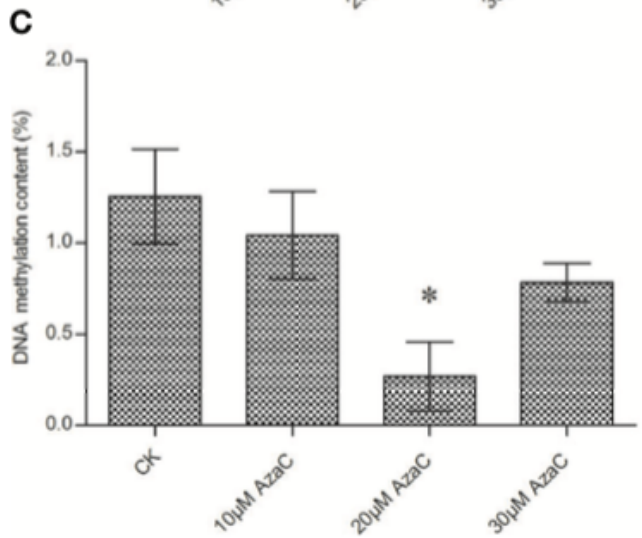
- Treating with the 5-AzaC increased b-glucuronidase (GUS) expression.
- 5-AzaC is a demethylation agent.
- Demethylation of the gene means gene activation.

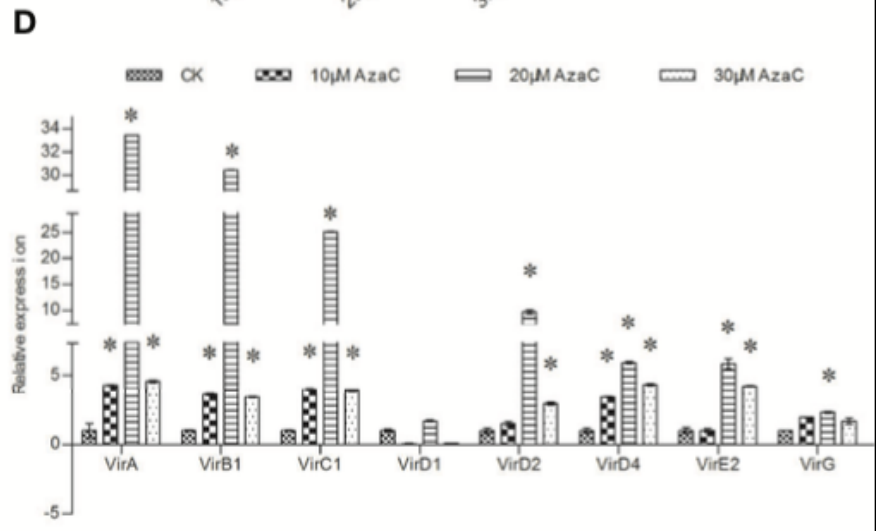
GUS expression in *Agrobaterium tumefaciens*



GUS expression in Tamarix hispida

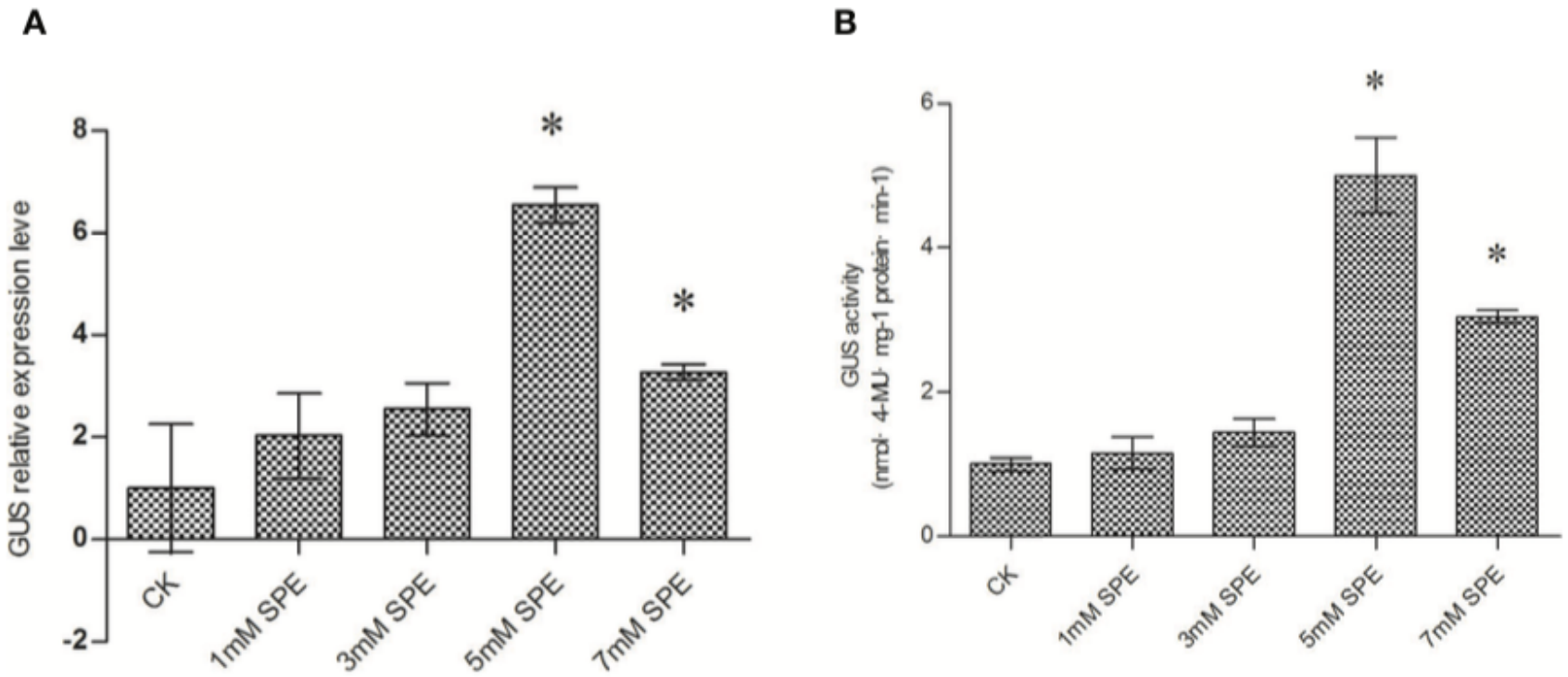






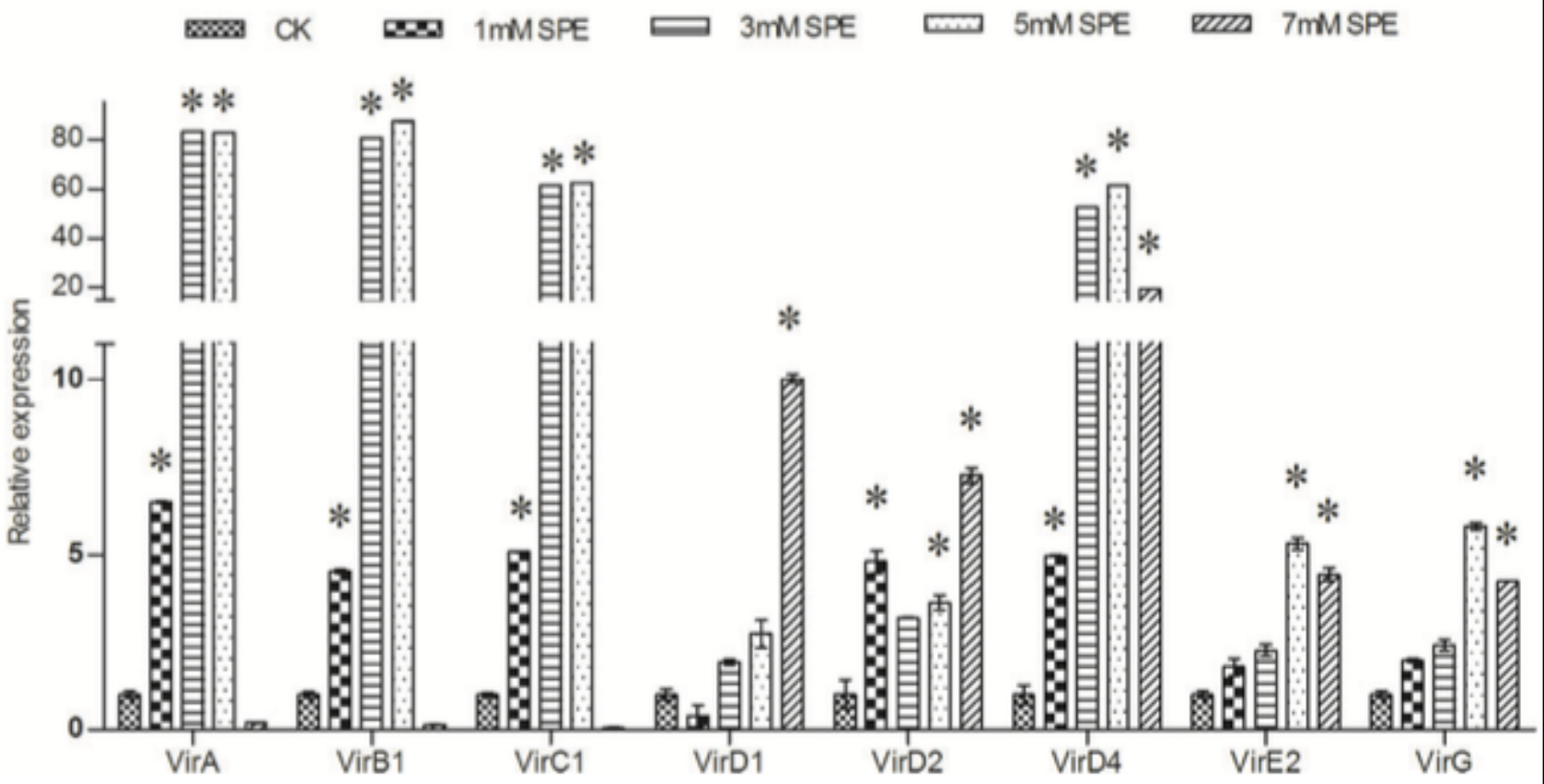
The Effects of (Spe) on T-DNA Transfer

- The results showed that the supplementation with Spe can significantly improve genetic transformation.



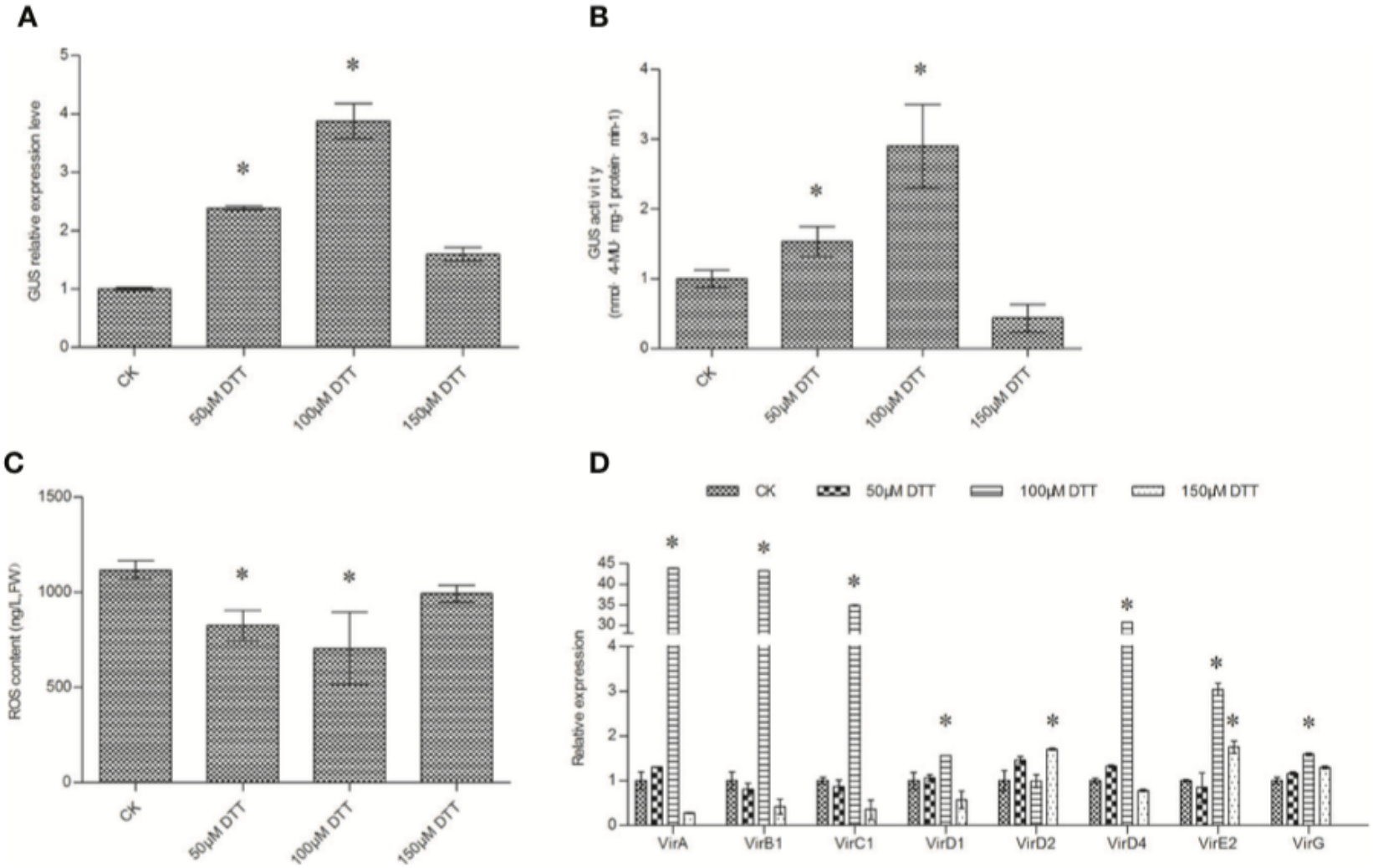
The Expression of vir Family Genes

- The relative expression of vir genes in Agrobacterium cells when treated with different concentrations of Spe.



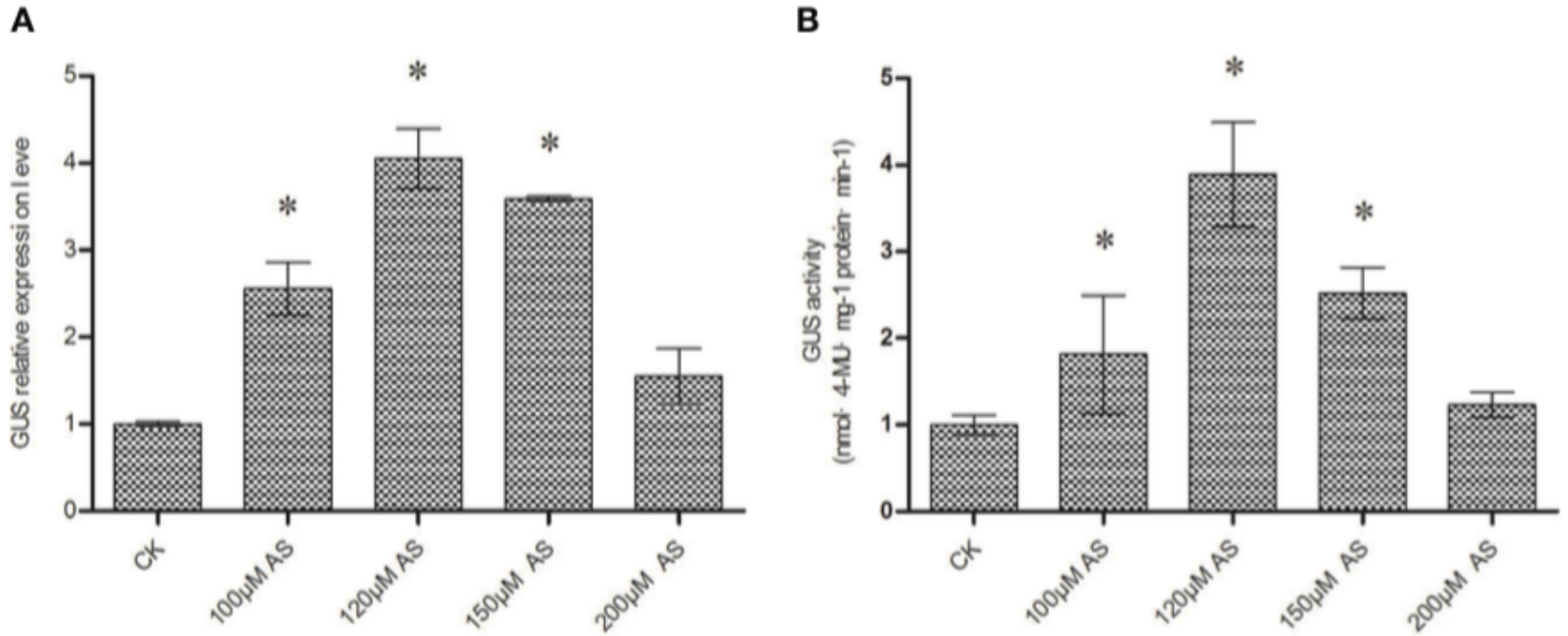
The Effects of DTT on T-DNA Transfer and ROS Accumulation

- GUS expression and activity measurements showed that supplementation with DTT could significantly improve T-DNA transfer.
- It has been found that DTT with different concentrations reduced ROS.



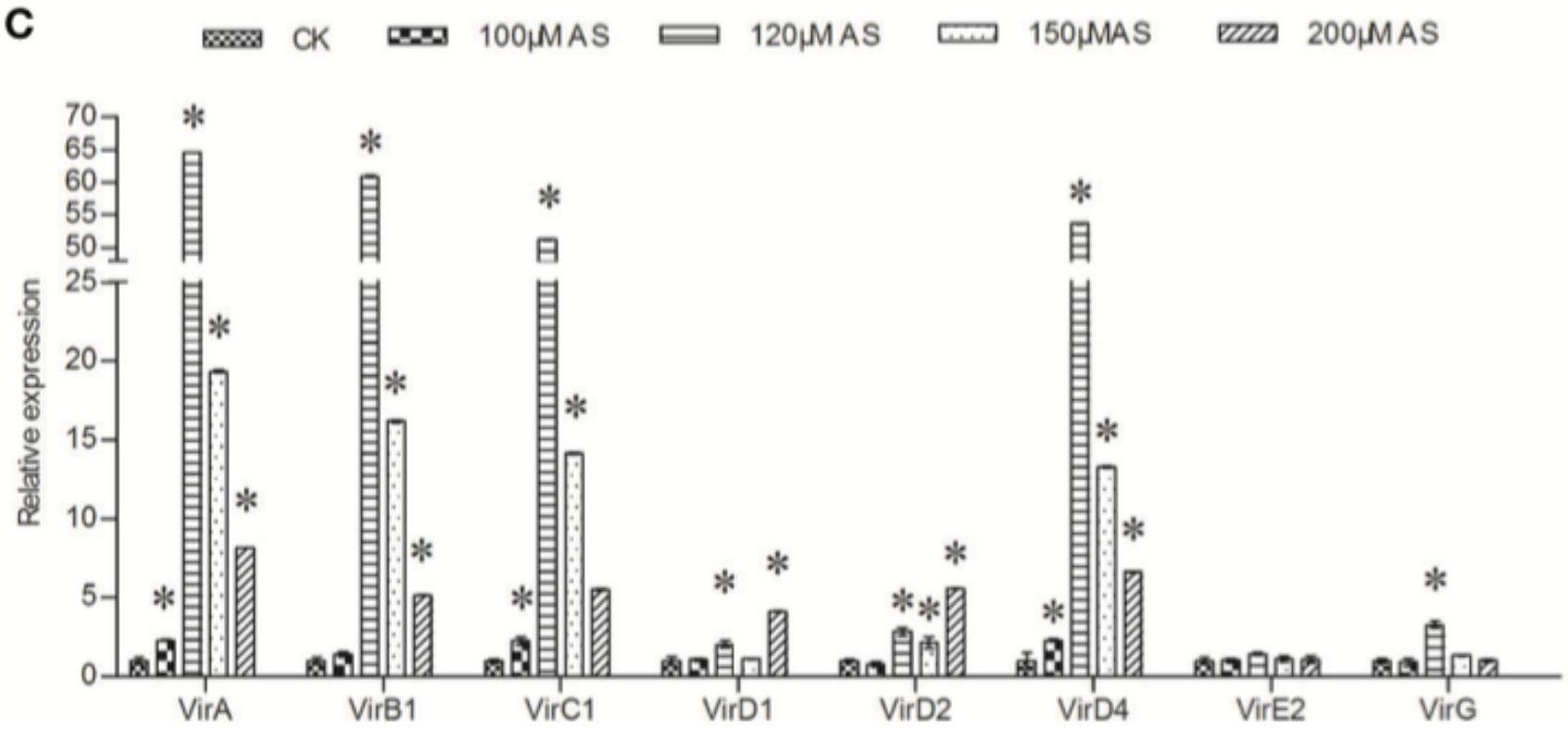
Acetosyringone (AS) Treatment and T-DNA Transfer

- The expression of vir genes were induced by adding acetosyringone and this also improve T-DNA transfer.



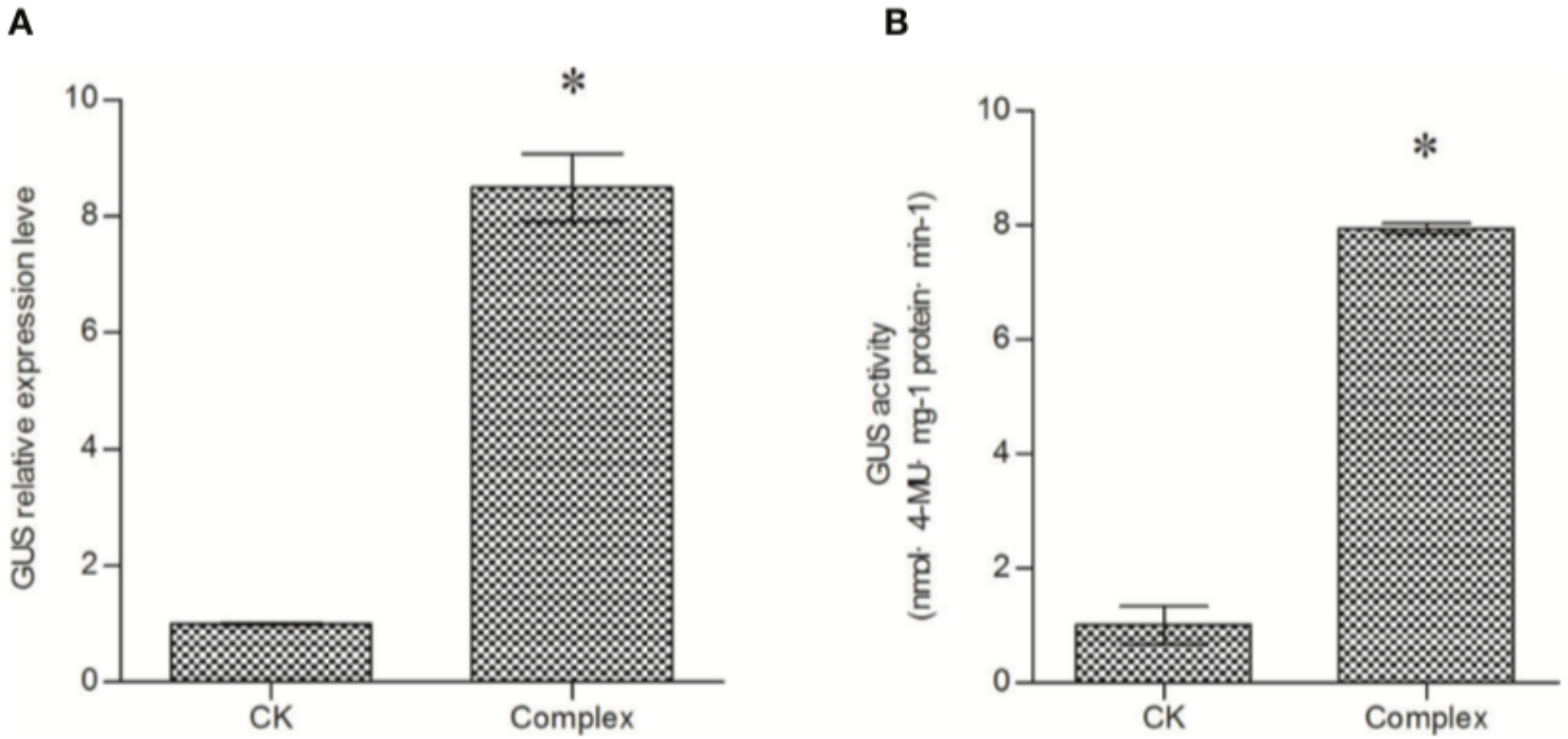
Acetosyringone (AS) Treatment and T-DNA Transfer

- A S treatment could significantly induce the expression of all vir genes, except virE2.



The Combined Effects of Chemical Compounds on T-DNA Transfer

- The result shows that both GUS expression and Gus activity was higher in the case of using the combination compounds.
- the efficiency of T-DNA transfer is greater than any of the reagents used alone.



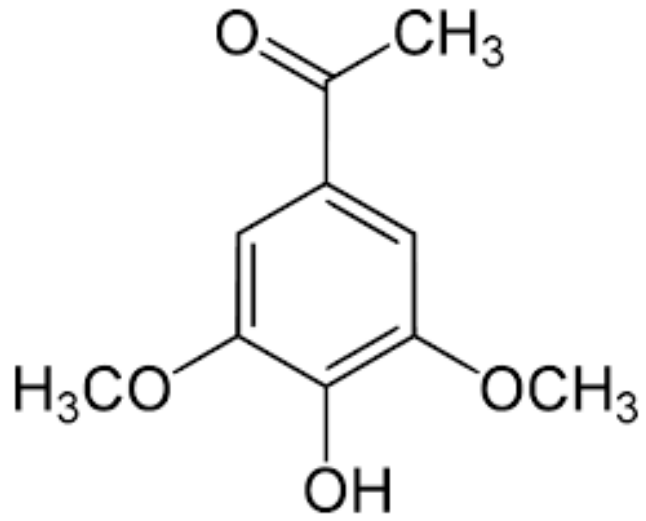
Discussion

Discussion

- In this study, the results showed that during the period of Agrobacterium cell culture and specifically with the treatment with 5-AzaC, Spe, and AS all vir gene expression are highly induce , suggesting that these factors might improve T-DNA transfer by directly activating the vir genes.

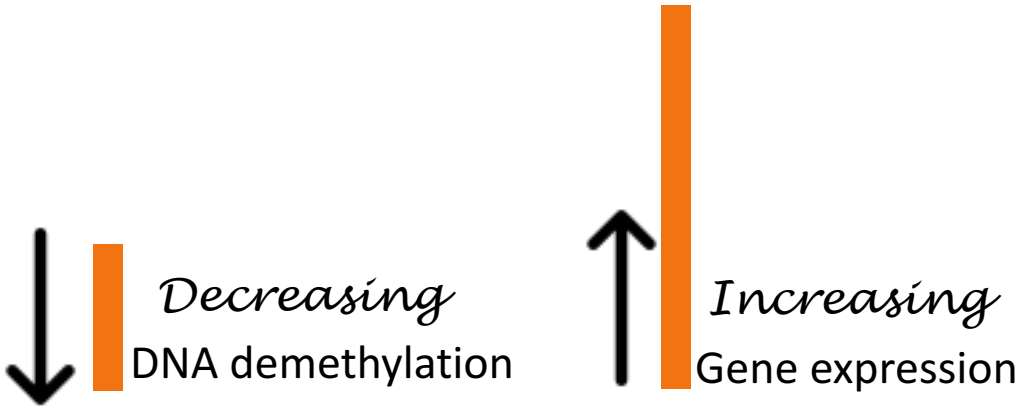
acetosyringone

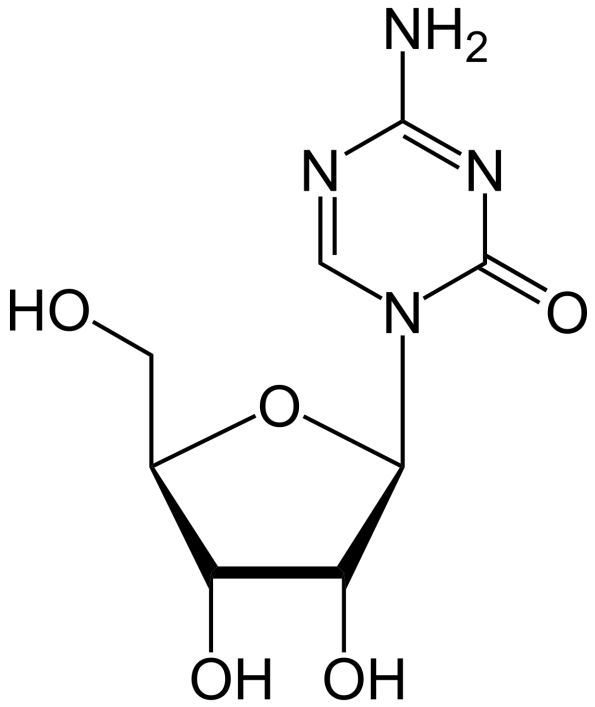
- The result shows AS treatment during Agrobacterium cell culture could induce the expression of vir genes to improve T-DNA transfer.



Azacytidine

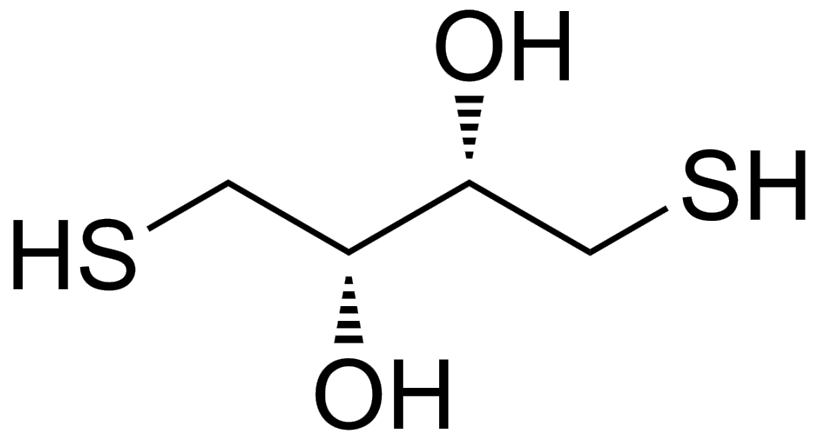
- Azacytidine is a DNA demethylating agent that can reduce or inhibit DNA methylation





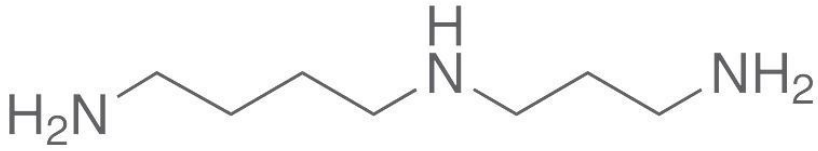
Spermidine

- It has been found that all the studied vir genes (virA, virB1, virC1, virD1, virD2, virD4, virE2, and virG gene) were significantly induced by Spe and this is done by improving T-DNA transfer.



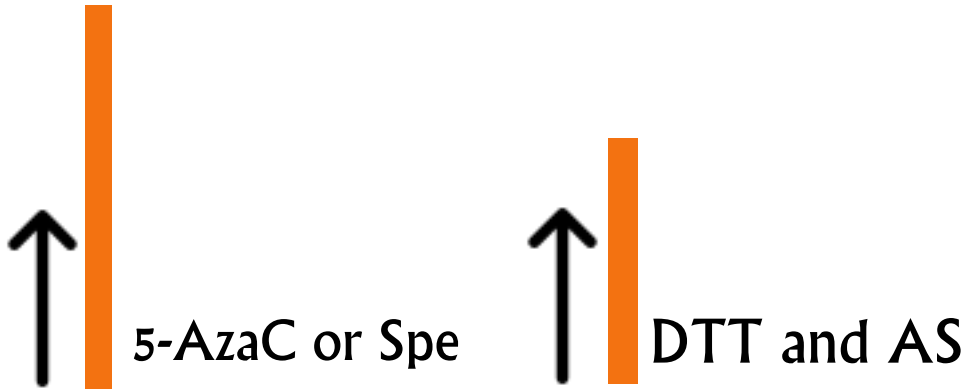
Dithiothreitol

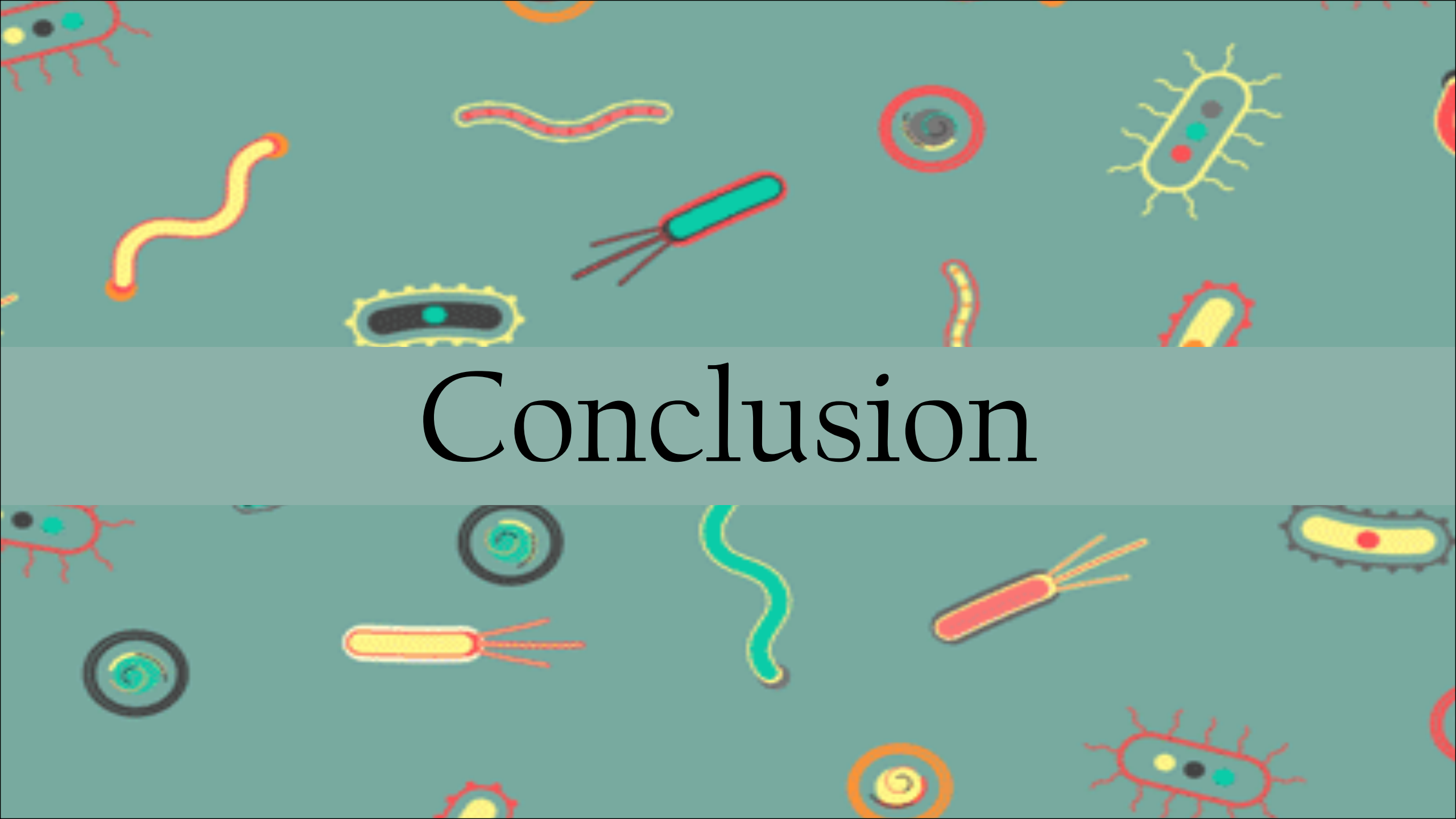
- reducing ROS accumulation by DTT treatment would improve T-DNA transfer



T-DNA transfer

- The results showed that treatment with 5-AzaC or Spe increased T-DNA transfer compared with treatments DTT and AS





Conclusion

Conclusion

- This results show an improvements of T-DNA transfer when Adding Chemical Compounds.
- The Combined Effects of Chemical Compounds has an improvement more than the individual compounds.

Reference

- Zhao, H., Jia, Y., Cao, Y. and Wang, Y., 2020. Improving T-DNA Transfer to Tamarix hispida by Adding Chemical Compounds During Agrobacterium tumefaciens Culture. *Frontiers in Plant Science*, 11.
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