Presentation and Discussion of the Research Paper

Transgenic Tomato Lines Expressing Human Lactoferrin Show Increased Resistance to Bacterial and Fungal Pathogen

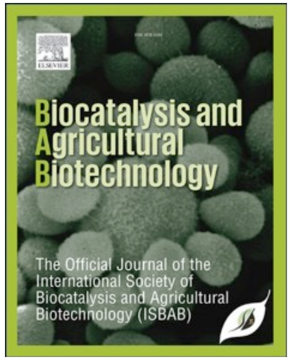
By: Anfal Ibrahem

Research Topic & Seminar (501)

Mon 18th of May 2021

Outline

Research Paper



Transgenic tomato lines expressing human lactoferrin show increased resistance to bacterial and fungal pathogens

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Agrobacterium-mediated transformation of tomato (*Lycopersicon esculentum* Mill.) plants was carried out to transfer the human lactoferrin gene (*hLf*) under control of the constitutive Cauliflower Mosaic Virus (CaMV) 35 S promoter to increase their resistance to phytopathogens. Since binary plasmid construct contained also neomycin phosphotransferase gene (*nptII*) conferring resistance to kanamycin, the selection of transgenic lines was carried out on nutrient medium supplemented with 100 mg/L kanamycin (as selective agent). PCR and Western blot analyses of transformed lines with primers specific to *hLf* gene and a monoclonal antibody against lactoferrin were performed to confirm the transgenic nature of selected tomato plants and *hLf* gene expression. The resistance of transgenic tomato lines expressing *hLf* to bacterial pathogens *Clavibacter michiganensis* subsp. *michiganensis* (causing bacterial wilt and canker of tomato), and *Ralstonia solanacearum* (causing bacterial wilt), and fungal pathogen *Phytophthora infestans* (causing late blight) was demonstrated. Although the partial resistance to bacterial wilt in transgenic tomatoes expressing modified lactoferrin has already been shown (Lee et al., 2002), here we report for the first time that the transfer and the expression of human lactoferrin gene in transgenic tomato plants leads to their enhanced resistance not only to *R. solanacearum* but also to another bacterial pathogen *C. michiganensis* and to fungal pathogen *P. infestans*. The present study demonstrates that the genetic transformation of plants with the human lactoferrin gene (*hLf*) is a promising technology to increase the resistance of plant species to certain phytopathogens.

Received 3 February 2020, Revised 29 March 2020, Accepted 4 April 2020, Available online 8 April 2020.

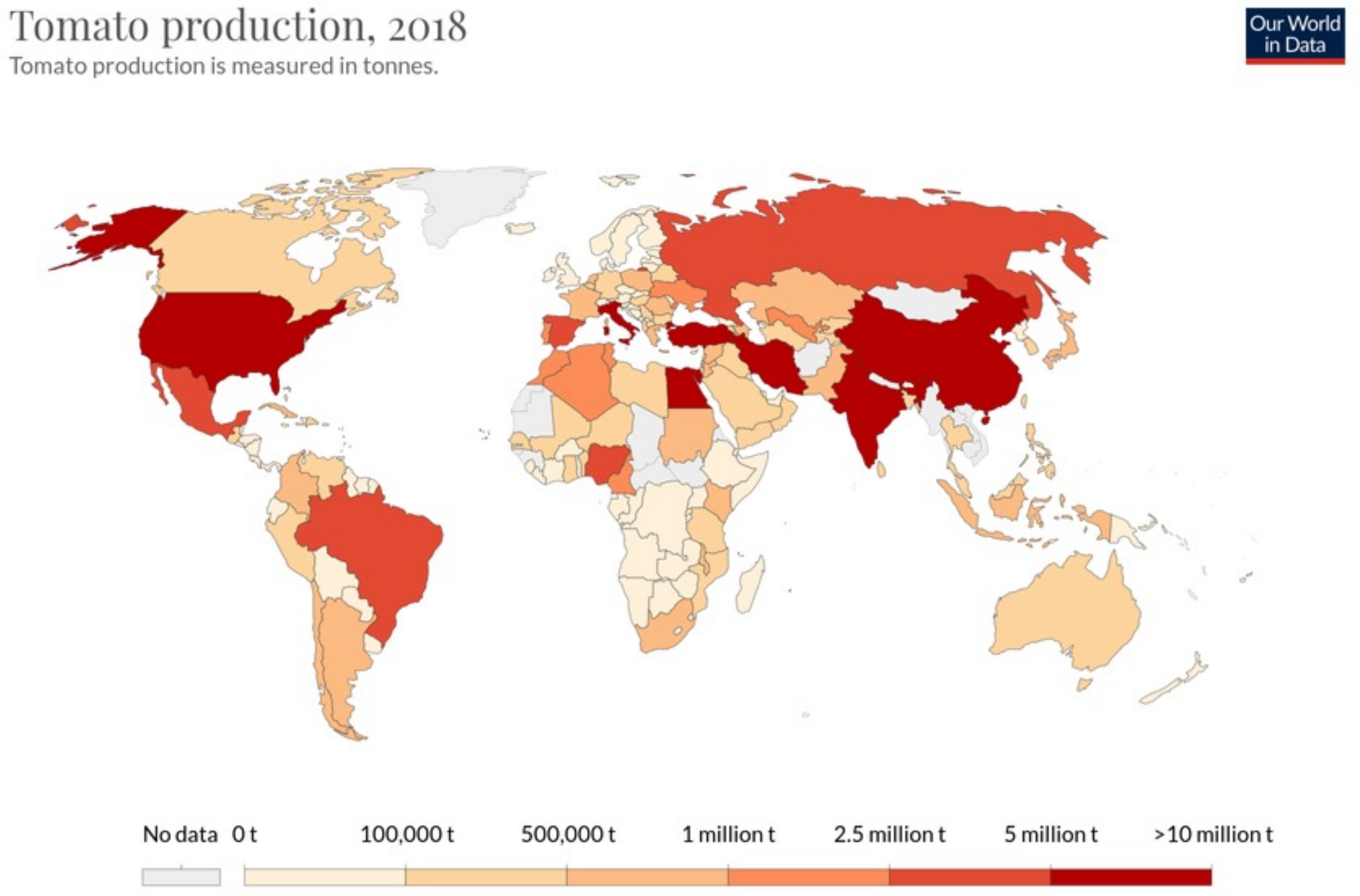


Fig.1 (Distribution of Tomato around world, which may lead to more infections)
Source: UN Food and Agriculture Organization (FAO)

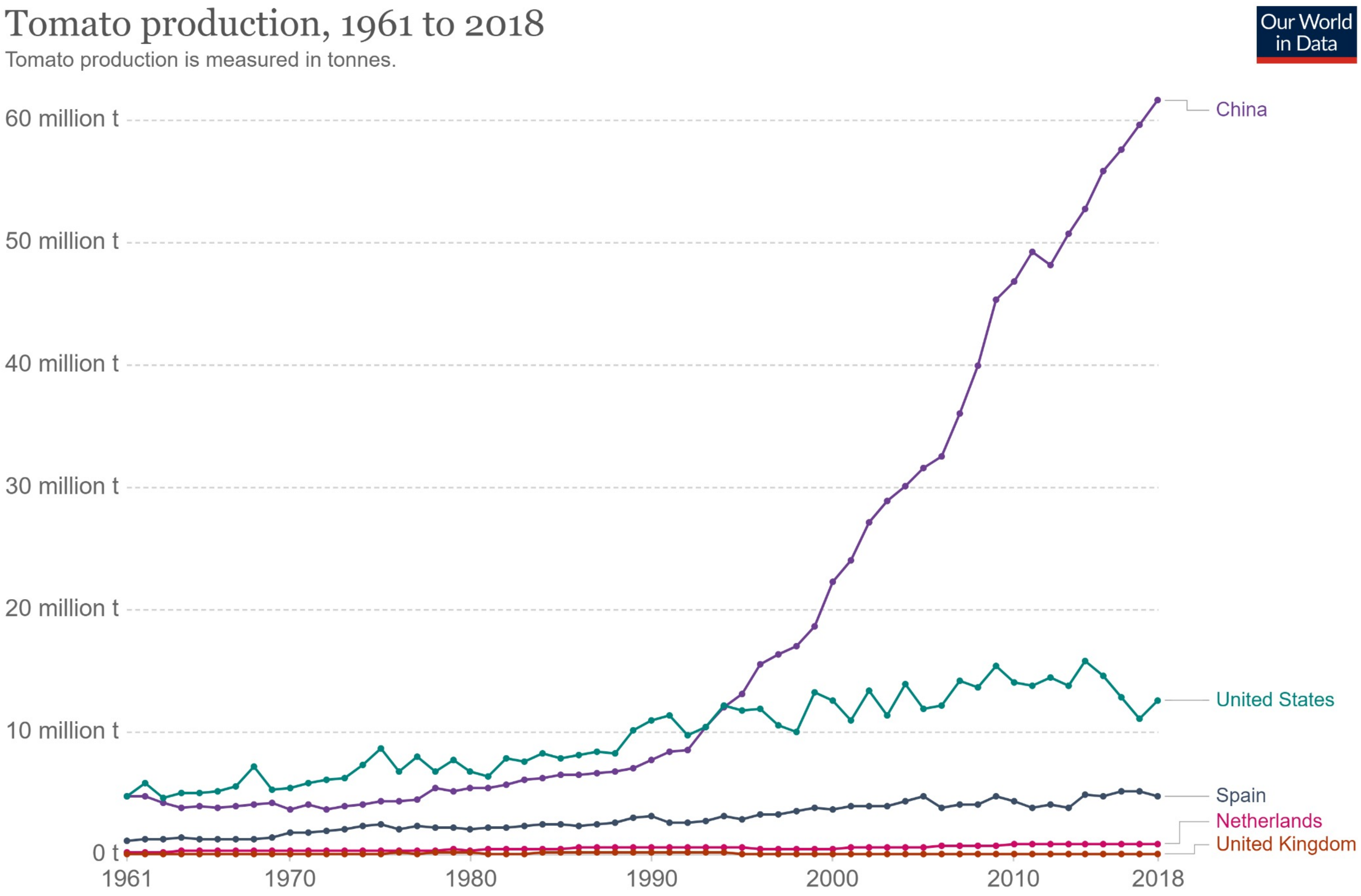


Fig.2 (Top 5 Tomato Production Countries)
Source: UN Food and Agriculture Organization (FAO)

Introduction

Phytopathogens

Causes significant loss of yield 30-80%

How Could A Tomato be so Important!

Lycopersican esculentum

Tomato Plant Diseases

Fig.3 (Bacterial Wilt)
Source: Ecoport by Jurgen Kranz

Ralstonia solanacearum

Fig.4 (Bacterial Canker)
Source: University of Minnesota Extension

Clavibater michigansis sub sp.

Fig.5 (Late Blight)
Source: Salis Bury Green House

Phytophthara infestans

Human Lactoferrin Protein

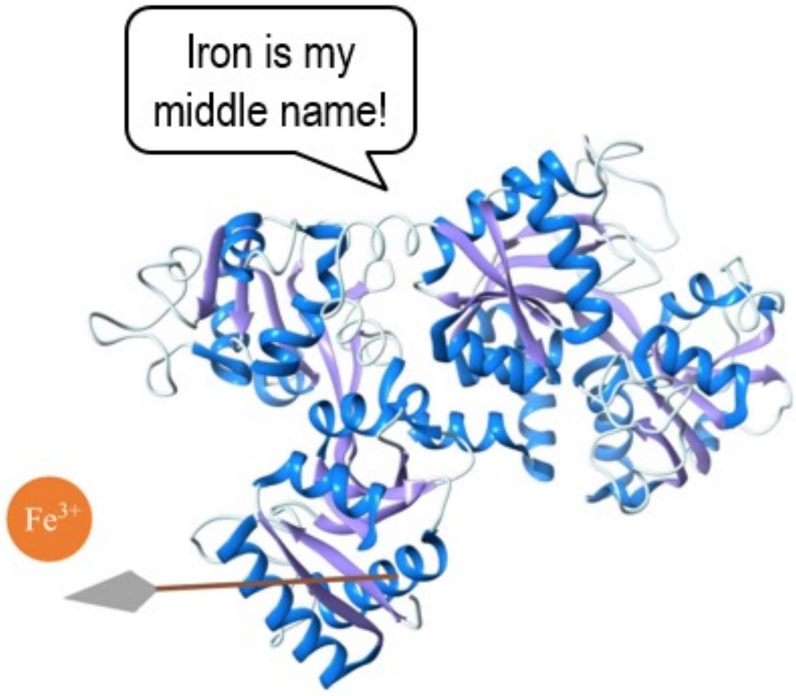


Fig.6 (Lactoferrin Lobe)
Source: Helix-pharm

Fig.7 (Red Blood Cell)
Source: Biomedical Odyssey

Fig.8 (White Blood Cell)
Source: Biomedical Odyssey

Non-Specific Immunity				
Anti-inflammatory	Anti-cancer	Anti-viral	Bactericide	fungicide

Plant Material

Sterilization → Disinfection → Germination

70% ethanol
2 min

50% Sodium hypochlorite
15 min

MST medium
24°C long day condition

Fig.10 Ukraine
Source: Google Maps

Fig.11 Tomato seeds
Source: Shutter Stock

Cotyledon of 10-12 days seedlings were used
400-500 explant for each transformation

Fig.12 Seedlings
Source: Pinterest

Genetic Transformation

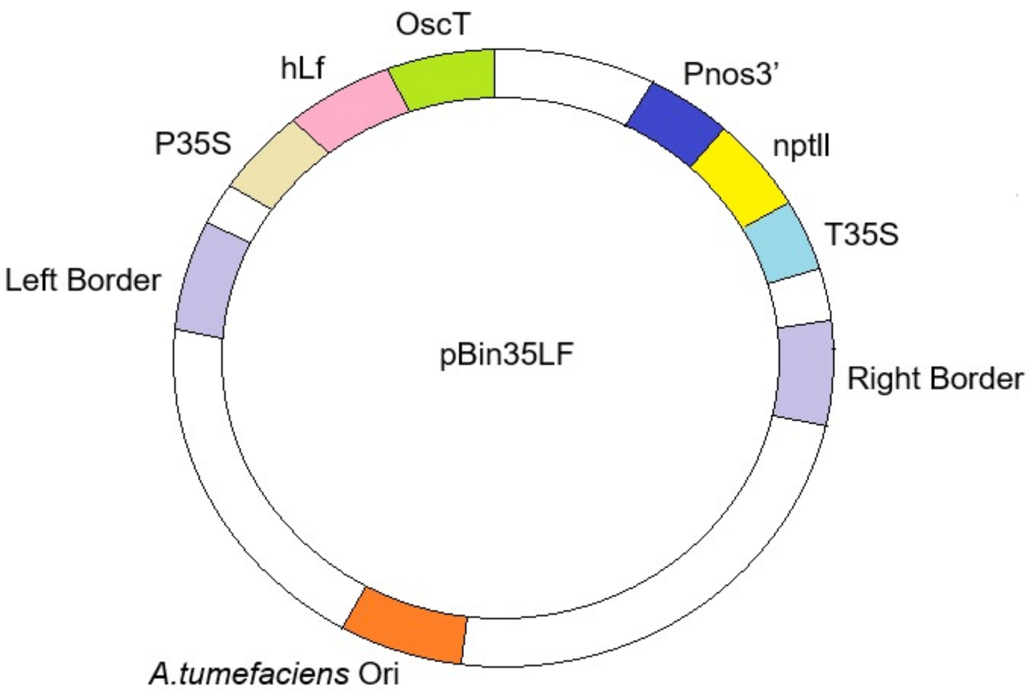


Fig.13 Binary Vector
Source: Anfal's

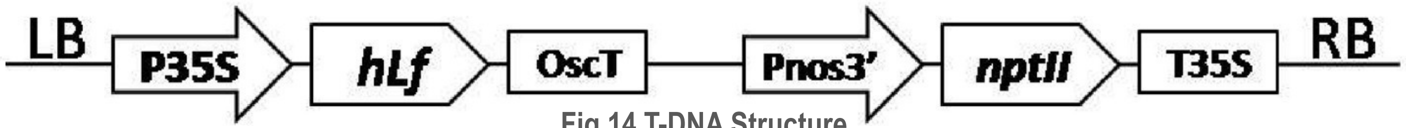
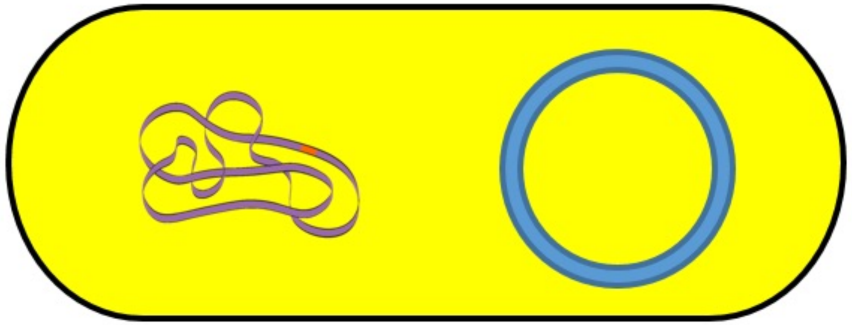


Fig.14 T-DNA Structure
Source: Biocatalysis and Agricultural Biotechnology

Insertion from LB to RB	
P35S	CaMV 35S Promoter
hLf	Human Lactoferrin Gene
OscT	Octopine Synthase Terminator
Pnos3'	Nopaline Synthase Promoter
nptII	Neomycin Phosphotransferase Gene
T35S	CaMV 35S Terminator

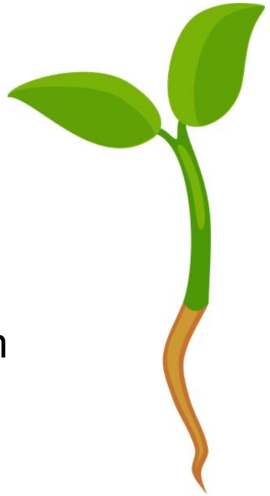
Agrobacterium tumefaciens



Cultured on LB (Lysogeny medium) + Kanamycin + Rifampicin

28°C/24h

\+



Explant

MST medium

24°C/daylong

Co-cultivation

On MST medium for Selection

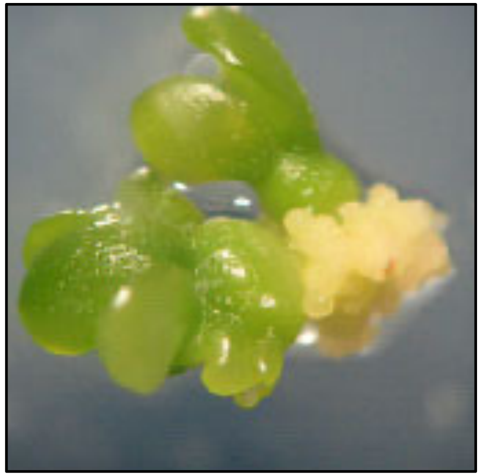


Fig.14 Cluster callus
Source: Science Alert

Transferred to fresh selective media → **Micropropagated** → **Transferred to soil**

Fig.15 Cluster callus
Source: Cossma

Fig.16 Micropropagation
Source: Cossma

Fig.17 In-vitro adaptation
Source: Cossma

Nutrient media for *in vitro* manipulations with tomato

Medium	Micro- and macrosalts MS, g/l	Sucrose, %	Nicotinic acid, mg/l	Pyridoxine, mg/l	Thiamine, mg/l	Glycine, mg/l	Zeatin, mg/l	IAA, mg/l	Kanamycin, mg/l	Cefotaxime, mg/l	Agar, g/l	pH
MST	4.3	3	0.5	0.5	1	2	–	–	–	–	10	5.7
MST-S	4.3	3	0.5	0.5	1	2	1	1	100	600	10	5.7
MST-R	4.3	3	0.5	0.5	1	2	–	–	–	600	10	5.7

Table.1 Composition of nutrient media for different in-vitro manipulations with tomato
Source: Biocatalysis and Agricultural Biotechnology

Transgenic Plant Regeneration

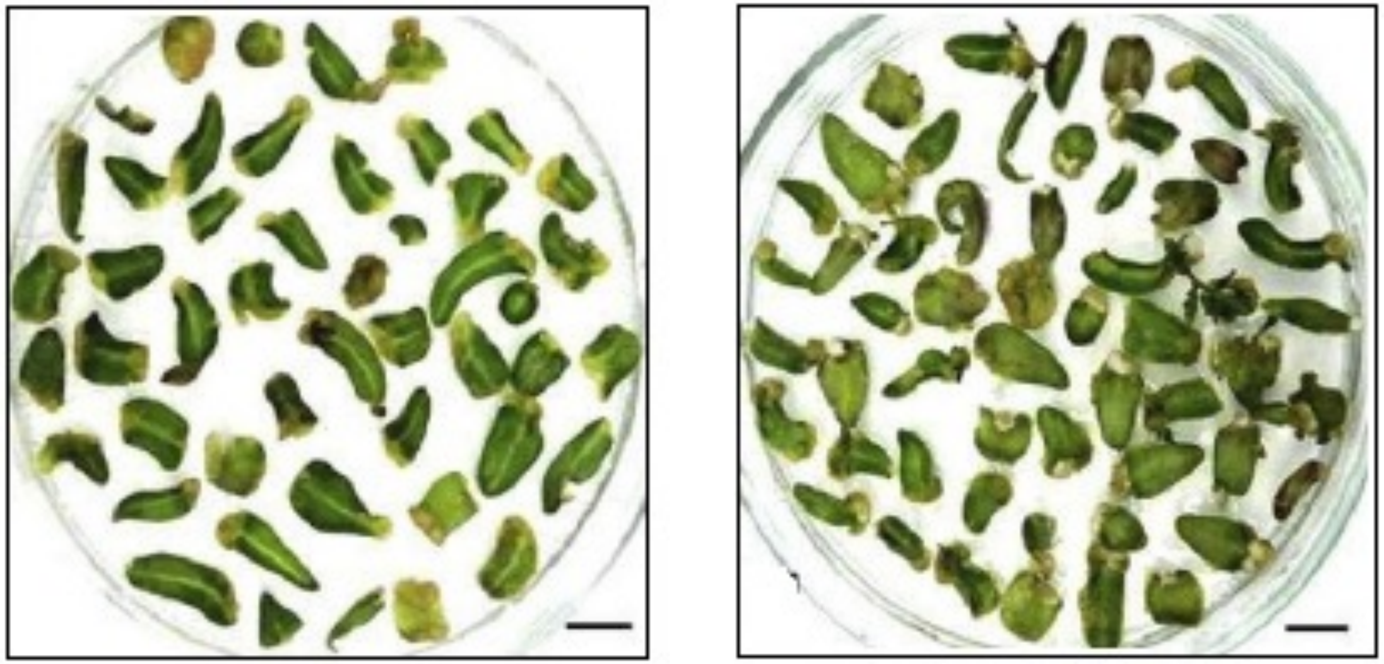


Fig.17 Cluster callus
Source: Biocatalysis and Agricultural Biotechnology

After 1 month

↓

Embryogenic callus appeared on the explant

Transgenic Plant Regeneration

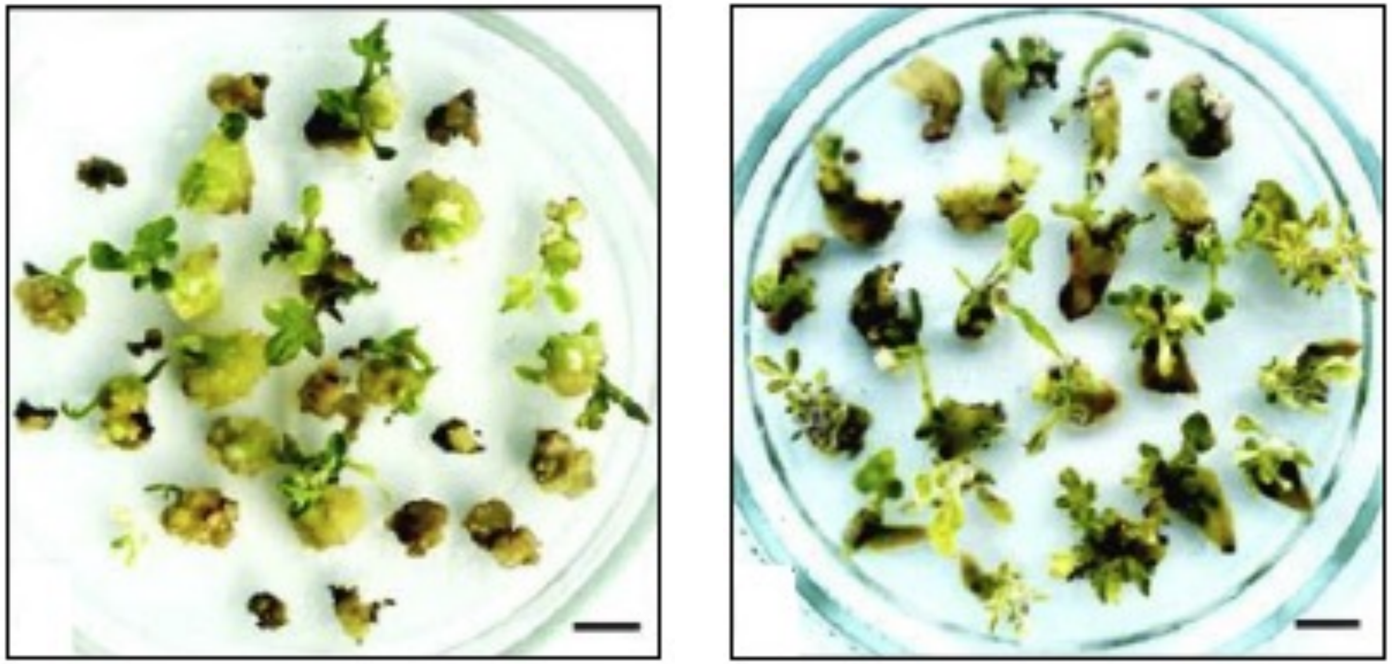


Fig.18 Shoot development
Source: Biocatalysis and Agricultural Biotechnology

After 2 month

↓

Regenerated shoots developed from calli

Transgenic Plant Regeneration

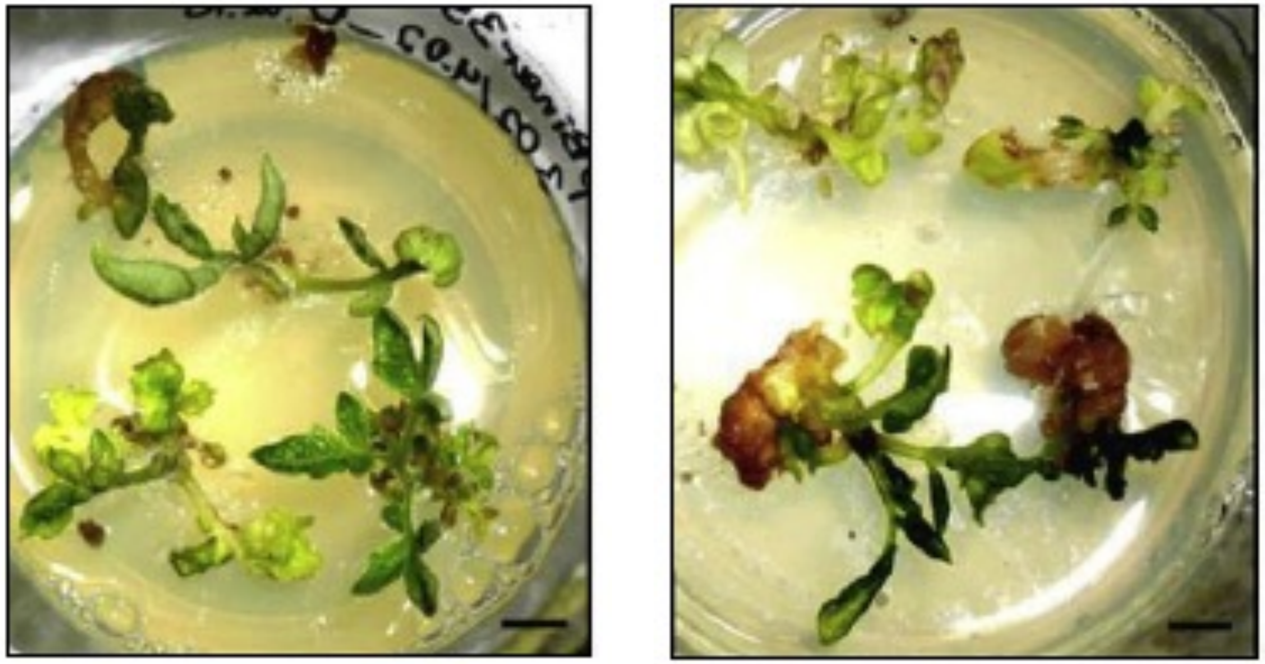


Fig.19 Shoot development more than 2-3 cm
Source: Biocatalysis and Agricultural Biotechnology

After 3 month

↓

Explant transferred to glass jars

Transgenic Plant Regeneration

Fig.20 Explant generation in MST-R medium
Source: Biocatalysis and Agricultural Biotechnology

After 4 month

↓

Regenerated shoots were isolated from the callus and transferred to the MST-R

Transgenic Plant Regeneration

Fig.21 In-vivo adaptation
Source: Biocatalysis and Agricultural Biotechnology

Further Adaptation in-vivo conditions

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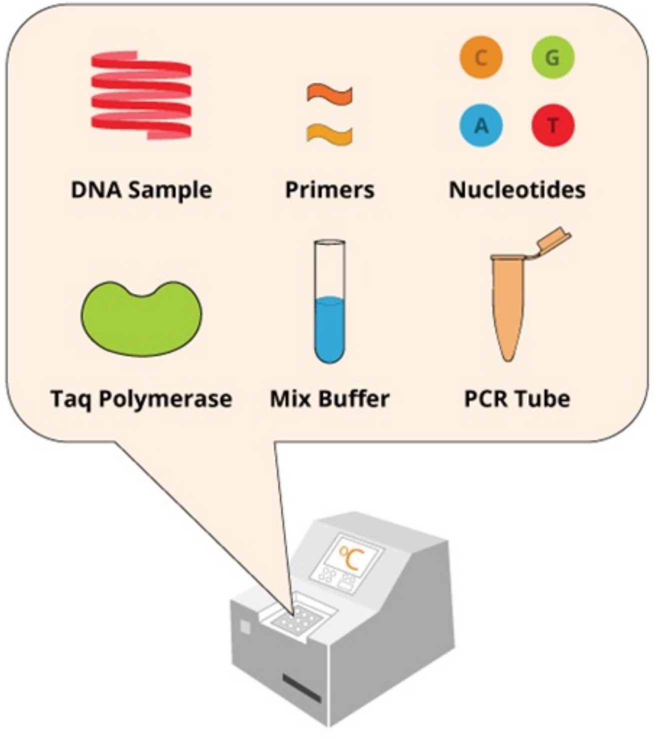
Flowering and obtaining of Seeds

PCR Analysis of Transgenic Tomato



DNA Extraction by cetyltrimethylammonium bromifde (CTAB)

↓

Primer specific for hLf gene

↓

Reaction Take in buffer containing Taq polymerase Mg^{2+} (Helicon)

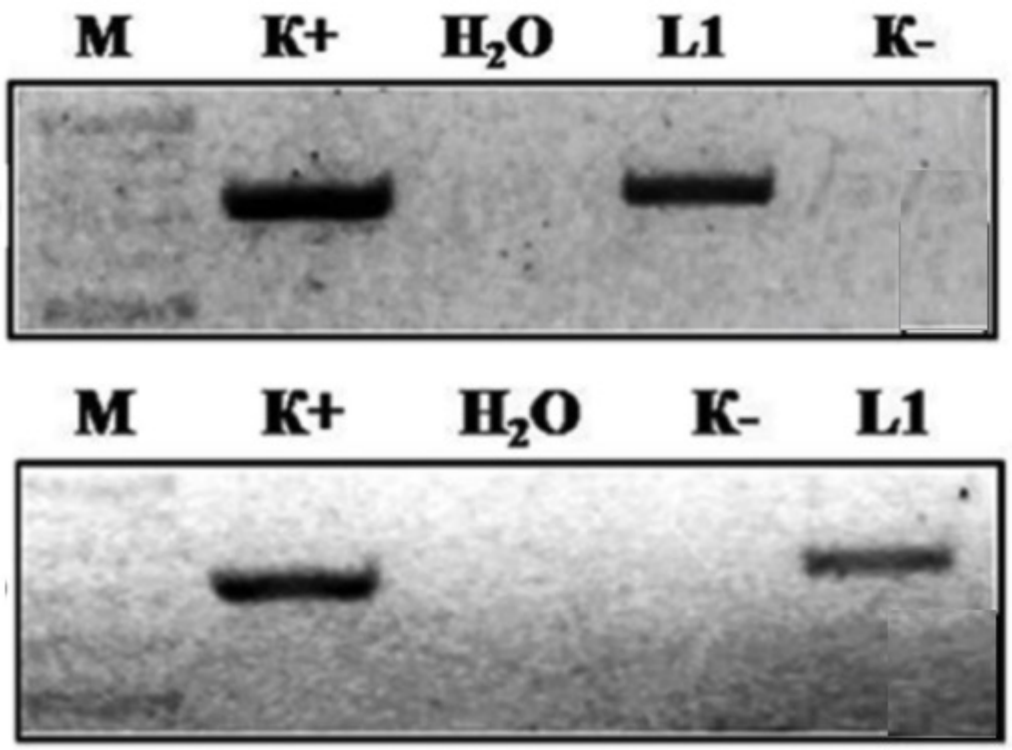


Fig.22 PCR Analysis
Source: Biocatalysis and Agricultural Biotechnology

M	(molecular mass marker)
K+	(positive control)
H_2O	(Amplified in the absence of DNA)
K-	(Negative control)
L1	(Transformed line)

To verify the integration of the hLf gene into explant genome

Size of amplification = 731b.p

Western Blot Analysis of hLf Expression in Transgenic Lines

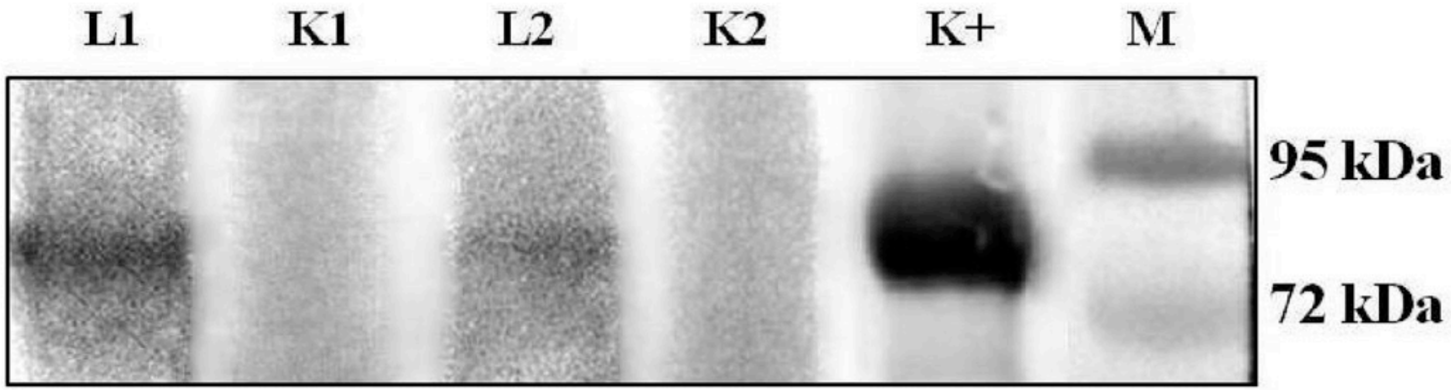


Fig.23 Western Blot of transgenic lines with monoclonal antibody against lactoferrin
Source: Biocatalysis and Agricultural Biotechnology

To confirm the expression of hLf in transgenic tomato line → Protein Separation by SDS phage

Antibacterial and Antifungal Assay

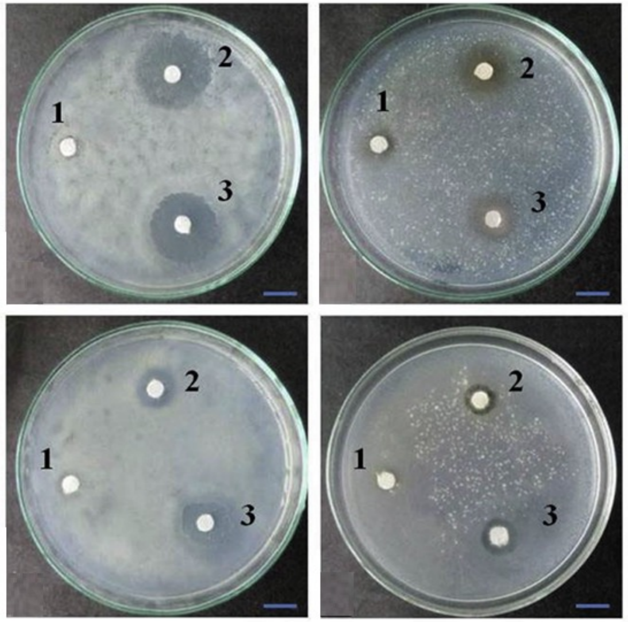


Fig.24 Anti bacterial activity of the juice isolated from transgenic tomato line
Source: Biocatalysis and Agricultural Biotechnology

Fig.25 Biotest on sensitive of Transgenic tomato to *P.infestanse*
Source: Biocatalysis and Agricultural Biotechnology

Conclusion

- Choosing of the tomato was very interesting
- Each steps were archived scussfullay although it were time consuming
- Therefore, the results of the work shows that genetic transformation of plant with human Lactoferrin gene can be promising approach for increasing the resistance of commercially valuable plant against pytophathogens.




References

- Transgenic tomato lines expressing Human lactoferrin show increased resistance to bacterial and fungal pathogens by Anastasiia Buziashvili, Lyubiv Cherednichenko, Serhii Kropyvko and Alla Yemets Biocatalysis and Agricultural Biotechnology-2020