



Available Online at EScience Press

Plant Protection

ISSN: 2617-1287 (Online), 2617-1279 (Print)
http://esciencepress.net/journals/PP

Research Article

Induction of Hairy Roots in Tomato (*Solanum lycopersicum* L.) and Assessment of Tolerance to *Fusarium oxysporum* Culture Filtrates

^aBadia Abdul Razzaq Malla Obaida, ^bNadeem Ahmed Ramadan

^a College of Science, Department of Biology, University of Mosul, Mosul, Iraq.

^b College of Technical Health and Medicine, Al-Hadbaa University, Mosul, Iraq.

ARTICLE INFO

Article history

Received: 15th January, 2026

Revised: 2nd April, 2026

Accepted: 4th April, 2026

Keywords

Agrobacterium rhizogenes

Hairy root transformation

Fusarium oxysporum

Tomato

Solanum lycopersicum L.

Fungal culture filtrate

tolerance

ABSTRACT

Hairy root cultures induced by *Agrobacterium rhizogenes* are widely used as efficient systems for genetic transformation and studying plant-pathogen interactions. This study aimed to establish a reliable protocol for hairy root induction in tomato and to evaluate the response of transformed roots to *Fusarium oxysporum* filtrates. Surface sterilization of tomato seeds using 70% ethanol and 6% sodium hypochlorite achieved 95% decontamination, producing healthy, contamination-free seedlings. Stem segments inoculated with *A. rhizogenes* R1601 via direct injection showed rapid hairy root induction within 4-7 days. A high transformation efficiency of 94.28% was recorded, with an average of 3.0 roots per explant, while no root formation occurred in control samples. Hairy roots were successfully maintained on MS medium supplemented with 400 mg/L cefotaxime, which effectively eliminated residual bacterial contamination. The addition of *F. oxysporum* filtrate to the growth medium resulted in a significant, concentration-dependent inhibition of hairy root development ($p < 0.001$). At lower concentrations (5%), minimal effects were observed, whereas higher concentrations (15-20%) markedly reduced root survival, with complete inhibition in non-transformed roots at 20%. Strong negative correlations ($r = -0.97$ to -0.98) confirmed a dose-response relationship. Transformed hairy roots exhibited relatively greater tolerance, likely due to enhanced metabolic activity and activation of defense-related pathways associated with *rol* genes. In conclusion, the developed protocol is highly efficient for hairy root induction in tomato, and transformed roots demonstrate improved tolerance to fungal filtrates, highlighting their potential utility in stress biology and phytoremediation studies.

Corresponding Author: Badia Abdul Razzaq Malla Obaida

Email: badia.jamal@uomosul.edu.iq

© 2026 EScience Press. All rights reserved.

Introduction

In plant biotechnology, genetic transformation methods are critical for producing varieties with better farming characteristics, such as resistance to pests, biotic and abiotic stresses. *Agrobacterium rhizogenes*-mediated hairy root systems are among the most effective

techniques for studying gene expression and plant-pathogen interactions. The T-DNA fragment of the Ri plasmid is transferred into the genome of the host plant by virulence genes, through which the resultant hairy roots grow rapidly, are extensively branched, and are genetically stable (Lacroix and Citovsky, 2019; Ebrahim

et al., 2025). The hairy root system has extensive applications in functional genomics research, genetic engineering, secondary metabolite production, and plant responses to biological stress (Al-Sahuki and Abdel Basset, 2020). Several studies have successfully induced and genetically modified hairy roots in several plant crops, including black cumin (*Nigella sativa*), chickpeas, fenugreek, corn, and lavender, which has enabled the study of gene functions and the improvement of important agricultural traits (Georgiev et al., 2012; Aggarwal et al., 2018; Chakrabarty et al., 2020; Garagounis et al., 2020; Mohammed and Masyab, 2020; Al-Taie, 2022). Recent studies have also shown that hairy root systems represent a suitable model for studying plant-pathogen interactions, as well as their applications in fields such as phytoremediation and plant molecular biology (Al-Sanjari, 2022).

Tomato (*Solanum lycopersicum* L.) is the most significant vegetable crop in the world; however, owing to various fungal diseases, it is most severely infected by *Fusarium* wilt caused by the fungus *Fusarium oxysporum*. This disease causes significant economic losses in tomato production because of its impact on the root system and vascular tissues of the plant. Therefore, understanding the mechanisms underlying plant resistance to this pathogen is crucial for developing effective strategies for improving crop resistance.

Hairy root systems induced by *A. rhizogenes* are very useful in experimental studies on root responses to biotic stress factors, such as interactions with fungal pathogens. These systems can be established very easily, developed quickly, and utilized as a point of learning about plant resistance mechanisms in the laboratory (Mohamed et al., 2015). Accordingly, this study aimed to induce hairy roots in tomatoes using *A. rhizogenes* strain R1601 and to evaluate the response of these roots to fungal stress caused by *F. oxysporum*. This study is expected to contribute to the development of an experimental system for studying the interaction between tomatoes and *Fusarium* wilt, which may support future research aimed at improving disease resistance in this crop.

Materials and Methods

Source of the Fungus

The pure isolate of the fungus *F. oxysporum*, previously isolated from infected pea plants, *Pisum sativum* L., was obtained from the strain bank in the Department of Life

Sciences, College of Science, University of Mosul and grown on solid nutrient medium, Potato Dextrose Agar (PDA).

Source of the bacterial vector vaccine: *A. rhizogenes* R1601

The genetically modified strain of *A. rhizogenes* R1601 was obtained from the University of Washington (Professor E.W. Nester, Washington University, USA) and carried genetic markers for resistance to the antibiotics kanamycin (Kana.^{Res+}) and carbenicillin (Carb.^{Res+}). The bacteria were maintained on APM (Morgan et al., 1987) solid media prepared by dissolving 5 g Yeast extract, 0.5 g Casamino acid, 8 g Mannitol, 2 g (NH₄)₂SO₄, 5 g NaCl and 15 g Agar in 1 liter of distilled water and maintaining with the two mentioned above antibiotics at a concentration of 100 mg/L⁻¹ each.

Antibiotics used and their sources

Kanamycin and carbenicillin used in this study were supplied as powders containing the active ingredients by the General Company for Pharmaceutical Industries and Medical Supplies, Samarra, Iraq. These were added to the APM medium to culture *A. rhizogenes* R1601 and to prepare the inoculum. Cefotaxime, also supplied by the same source, was used to eliminate these bacteria from hairy root cultures.

Preparation of toxic fungal filtrates

The standard method for extracting toxic fungal filtrates was as follows. The mushrooms were grown on Zabec-Dux liquid nutrient medium, specifically formulated for mycotoxin production, prepared by dissolving 2.0 g KNO₃, 1 g KH₂PO₄, 0.5 g MgSO₄, 0.5 g KCl, 0.01 g FeSO₄, and 15 g glucose in 1 liter of distilled water. The pH was adjusted to 6, and the medium was placed in 250 ml glass flasks in 50 ml steps. The flasks were autoclaved at 121°C and a pressure of 1.5 kg/cm² for 20 min. The flasks were inoculated with two 0.8 cm diameter discs of 10-day-old fungal cultures grown on PDA solid nutrient medium. The flasks were incubated at 27 ± 2°C for 20 days. The flasks were filtered through gauze, and the filtrate was cooled and centrifuged at 4°C and 12,000 rpm for 20 min to remove any remaining spores and fungal mycelia. It was then filtered using 0.2µm Millipor Filter microfilters, and the filtrate was stored in glass bottles at -10°C until use (Kidd et al., 2016).

Seed sterilization and seedling preparation

Tomato seeds were surface-sterilized using a 70% ethanol solution (2 min), and then a sodium hypochlorite solution (6%NaOCl) (1:1 v/v) was applied

to the samples (10 min). The seeds were washed in sterile water three times (3 min each) (Saeed et al., 2019) and placed on Murashige and Skoog (MS) medium solidified on agar surfaces (Murashige and Skoog, 1962). Cultures were kept in the dark at $25 \pm 2^\circ\text{C}$ and transferred to a 16h light and 8 h dark cycle at 1500 lx.

Stem cuttings inoculated with bacteria using the injection method

Healthy 20-day-old seedling stems were cut into 2.5 cm pieces and injected with 5-10 ml of a 1×10^8 cell/ ml bacterial suspension using a sterile microneedle with the tip of the needle submerged to puncture the stem pieces at their upper ends. The inoculated pieces (3 pieces per flask) were then vertically implanted into the solid MS medium. The samples were then incubated under light at 25°C (Mohamed et al., 2015).

Establishment of hairy root cultures

Emerged hairy roots (3-4 cm long) were cut at the point of inoculation and moved to 25ml agarified solid MS medium. Three or four subcultures were performed in MS medium with cefotaxime (300-400 mg/L) to eliminate the remaining bacteria. Each subculture took 20 days under identical incubation conditions.

Fungal filtrate treatment

Sterile fungal filtrates were added to the MS medium at concentrations of 5, 10, 15, and 20% (v/v). Twenty-five milliliters of each medium were dispensed into Petri dishes, and ten hairy root segments (1 cm long) were placed in each dish. The control samples consisted of non-inoculated roots. The cultures were incubated at $25 \pm 2^\circ\text{C}$ under photoperiodic conditions for 20 days. Surviving root segments were re-cultured three consecutive times at the same filtrate concentration. Resistant hairy roots were propagated in 100 ml beakers containing 30 ml of MS medium (two segments per beaker) and subcultured every 30 days.

Statistical analysis

All experimental data were subjected to statistical analysis using SPSS software (version 25.0). Differences between treatments (inoculated vs. control) were

evaluated using an independent samples *t*-test. For experiments involving multiple concentrations of fungal filtrate, one-way analysis of variance (ANOVA) was performed to determine the significance of treatment effects on hairy root development. When ANOVA indicated significant differences, mean comparisons were interpreted accordingly.

The relationship between fungal filtrate concentration and root development was assessed using Pearson's correlation coefficient (*r*) to determine the strength and direction of linear association. In addition, Spearman's rank correlation was applied to validate the consistency of the dose-response relationship under non-parametric conditions.

All statistical tests were conducted at a significance level of $p \leq 0.05$, while highly significant differences were considered at $p < 0.001$.

Results and Discussion

Seed Sterilization efficiency

Sterilization of tomato seeds with 70% ethanol and 6% NaOCl resulted in a high rate of decontamination (95%) and clean seedlings with no microbial contamination (Figure 1-A). The results of these studies are similar to those of earlier studies (Abdullah, 2000), which supports the effectiveness of this protocol with regard to aseptic seedling preparation.

Inoculation of stem segments using the direct bacterial injection method

A high response was observed in the stem segments of sterile tomato seedlings inoculated with *A. rhizogenes* R1601, in which hairy roots appeared 4-7 days post-inoculation. The roots grew rapidly with negative geotropism and white coloration (Figure 1-B). Control segments laced with distilled water lost their sterility and did not grow into roots. The efficiency of transformation was 94.28% and the average number of hairy roots per segment was 3.0, indicating that the tomato genotype was very compatible with the bacterial strain (Table 1).

Table 1. Hairy root formation in inoculated vs control stem segments.

Sample Type	No. of Segments	Responsive Segments	% Hairy Root Formation	Average Roots/Segment	p-value
Inoculated	70	66	94.28	3.0	< 0.001
Control*	25	0	0	0	< 0.001

*Control samples were inoculated with sterile distilled water.

The induction of hairy root formation was highly significant ($p < 0.001$) when bacterial inoculation was performed, and the rate of transformation was 94.28% and zero percent in controls.

Infliction of wounds is an important step in the process of genetic transformation, as it stimulates the formation of many compounds such as acetosyringone, which act as chemical attractants for these bacteria. Through these wounds, a piece of bacterial plasmid DNA called T-DNA is transferred to plant cells and integrated into their genetic makeup (Tzfira and Citovsky, 2007). Researchers have attributed the reason for the formation of hairy roots to the fact that a piece of T-DNA transferred into the cell of the host plant contains a group of genes *iaaM*, *iaaH* and *tmr* responsible for the formation of enzymes that build auxins and cytokinins, which leads to a hormone imbalance that stimulates cell division and elongation at those sites (Meyer et al., 2000). The purpose of the inoculation with *A. rhizogenes* was to obtain genetically transformed seedlings. The first sign of its success is the appearance of hairy roots, which indicate compatibility between bacterial strains and plants that undergo genetic transformation (Peterson et al., 2021). The direct injection method has achieved the same success in many plant species, including bright eyes (Bahari et al., 2020), broccoli (Sultan and Mohammed, 2020), and onions (Wang et al., 2025).

Preparation of bacteria-free hairy root cultures

Hairy roots were successfully maintained by consecutive subcultures in MS medium containing 400 mg/L cefotaxime. This antibiotic successfully eliminated the remaining *A. rhizogenes* to achieve bacteria-free cultures (Figure 1-C). Removal of bacteria from hairy root cultures aims to prove their survival and eliminate their effects on genetically transformed plant tissues. Cefotaxime is effective in removing bacteria from transformed hairy root cultures of many plants, such as broccoli (Abdullah et al., 2021), chickpeas (Mohamed et al., 2015), and fenugreek (Mohammed, 2020), which might be due to its effect on the synthesis pathways of proteins, enzymes, and nucleic acids (Yassin et al., 2005), as well as its effect on the osmolarity of cell membranes (Venkatachalam et al., 2011).

Effect of the addition of fungal filtrate to the growth medium of hairy root explants

The addition of fungal filtrate to the nutrient medium used for the growth of hairy roots increased their ability to cut hairy roots previously inoculated with bacteria. *A. rhizogenes* increased with increasing concentrations of

fungal filtrate added to the medium compared to the un-supplemented samples (Table 2). It was noted from the data that the 5% concentration had a limited effect and was similar to the comparison sample grown on a medium free of filtrates, as most of the overgrown cuttings continued to grow. Several planted cuttings showed tolerance to a concentration of 15% leachate, indicating their continued ability to grow. This effect was clear at a concentration of 20%, indicating that only three cuttings continued to grow (Figure 1-D). The fact that the hairy roots tolerate fungal filtrates is attributable to a variety of physiological and molecular processes involved in the nature of the hairy roots that are a product of transformation by *A. rhizogenes*. The transformation process results in the movement of a part of the DNA, called T-DNA, from the root plasmid into the plant genome. T-DNA contains *rol* genes responsible for the morphological and physiological changes that are characteristic of hairy roots. These genes influence the regulation of hormonal balance within the plant, leading to increased metabolic activity and growth rate as well as enhanced adaptability to biotic stresses (Guillon et al., 2006). Hairy roots are also characterized by their high capacity to produce defensive secondary compounds such as phenols and flavonoids, which contribute to reducing the toxic effects of mycotoxins and enzymes present in fungal filtrates. This is coupled with the stimulation of some defense pathways, which include the enhancement of some defense enzymes, such as peroxidase (POD), polyphenol oxidase (PPO), and phenylalanine ammonia-lyase (PAL), which fortify the cell wall and elevate the defense response of the plant toward invaders (Georgiev et al., 2012). Furthermore, hairy roots exhibit a higher physiological capacity than normal roots, including greater efficiency in nutrient uptake and increased metabolic activity, which enhances the ability of plants to withstand biotic stresses, such as mycotoxins or fungal filtrate (Shanks and Morgan, 1999). Based on the above, the increased tolerance observed in this study can be interpreted as a result of the combined effect of both the high physiological capacity of hairy roots and the metabolic changes associated with the expression of translocated *rol* genes within T-DNA, which enhances plant defense mechanisms and reduces the harmful effects of toxic compounds found in the fungal filtrate. Therefore, the success of phytoremediation using genetically modified hairy roots stems from their increased branching, which provides a larger surface area for contact with pollutants and pathogens (Bahramnejad et al., 2019; Fan et al., 2020).

Table 2. Effect of various concentrations of sterilized filtrate of *F. oxysporum* added to the hairy root growth medium after 20 days.

Samples	No. of developing root pieces					Pearson r	p- value
	Conc. of fungal filtrate (%)						
	0	5	10	15	20		
Hairy roots inoculated with bacteria	30	29	14	9	3	−0.9700	< 0.001
Hairy roots not inoculated with bacteria	30	25	10	6	0	−0.9778	< 0.001

When the concentration of *F. oxysporum* filtrate was increased, it led to a significant dose-dependent inhibition of hairy root development (ANOVA, $p < 0.001$ in both groups). At a 20% filtrate concentration, the non-inoculated group showed an inhibition of 0% survival. Pearson and Spearman correlations were used to verify that the dose-response relationship was almost perfect ($r = -0.97$ to -0.98 , $0 = -1.00$). However, there was no statistically significant difference in the resistance to fungal filtrate in cases of bacterial inoculation at any of the concentrations tested.

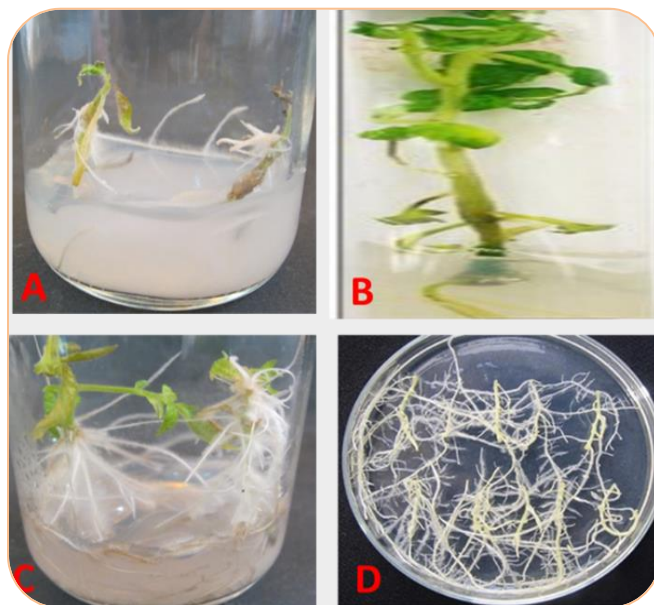


Figure 1. Morphological and physiological characteristics of transgenic hairy roots induced in tomato stem segments by *A. rhizogenes* R1601. A: Emergence of hairy roots from inoculated stem segments after 4-7 days, showing rapid growth and negative geotropism. B: Stem segments of control, inoculated with sterile distilled water, had no root formation. C: Hairless root cultures with no bacteria growing in MS media and 400 mg /L cefotaxime and showed intense branching and light brown color. D: Hairy roots that were placed in 20% *F. oxysporum* filtrate and the surviving segments showed resistance to fungi stress.

Conclusion

This study demonstrated that genetic modification of the tomato plant was possible through the bacterium *A. rhizogenes* R1601, which induced the development of hairy roots with unique morphological features. These roots also showed a high rate of growth, extensive branching, and aversion to the ground, and were also able to tolerate *F. oxysporum* extracts at specific levels and thus possible genetic changes due to the transformation process. These findings suggest that the hairy root system may be utilized at a convenient laboratory scale for plant-pathogen interaction studies. Nevertheless, determining the contribution of such changes in providing real resistance at the whole-plant level requires additional research, such as whole-plant and gene expression of resistance-related genes.

Acknowledgements

The authors thank the University of Mosul and Al-Hadba University College for their technical support.

Author's contributions

BARMO carried out the experimental work, analyzed the data, and drafted the initial version of the manuscript. NAR conceptualized and supervised the study, provided guidance on the experimental design, and critically reviewed and revised the manuscript for intellectual rigor. Both BARMO and NAR contributed to the critical assessment of the research, participated in editing and refining the manuscript, and enhanced its scientific clarity and overall presentation. All authors have read and approved the final version of the manuscript.

Research Funding

This research did not receive any grant from funding agencies.

Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 2: Zero Hunger

SDG 3: Good Health and Well-being

SDG 12: Responsible Consumption and Production

Policy Reference

Food and Agriculture Organization Sustainable Crop Production and Protection Policy; Convention on Biological Diversity (CBD) – Biotechnology and Biosafety Framework.

Publisher's Note

The publisher remains neutral regarding jurisdictional claims and institutional affiliations. The views and data presented are solely those of the authors and do not necessarily reflect those of *Plant Protection* or its publisher, who assumes no responsibility for errors or consequences arising from the use of this information.

References

- Abdullah, A., 2000. Formation of some plants resistant to fusarium wilt, *Fusarium oxysporum* and *Fusarium solani*, from calluses of different parts of the genetic tomato plant, *Lycopersicon esculentum* Mill. University of Al Mosul.
- Abdullah, R.M., Salih, S.M., Al-Nema Qutaiba, S., 2021. Plant regeneration from transformed tissues of broccoli (*Brassica oleracea* var. *italica*) by *Agrobacterium rhizogenes* ATCC13332 and their retention of rol-genes. *European Academic Research* 9(9), 6006-6017.
- Aggarwal, P.R., Nag, P., Choudhary, P., Chakraborty, N., Chakraborty, S., 2018. Genotype-independent *Agrobacterium rhizogenes*-mediated root transformation of chickpea: a rapid and efficient method for reverse genetics studies. *Plant Methods* 14(1), 55.
- Al-Sahuki, M., Abdel Basset, A., 2020. Plant genomics and breeding. University of Baghdad.
- Al-Sanjari, A.M.H.S., 2022. Recruitment of copper oxide nanoparticles by tissues of sunflower plants transgenic with the vector *Agrobacterium rhizogenes* ATCC 15834. University of Mosul, Mosul, Iraq.
- Al-Taie, A.A.R., 2022. *Agrobacterium rhizogenes* R1601 is a natural vector for genetic transformation of *Solanum nigrum* L. University of Al Mosul.
- Bahari, Z., Sazegari, S., Niazi, A., Afsharifar, A., 2020. The application of an *Agrobacterium*-mediated in planta transformation system in *Catharanthus roseus*, a medicinal plant. *Czech Journal of Genetics and Plant Breeding* 56(1), 34-41.
- Bahramnejad, B., Naji, M., Bose, R., Jha, S., 2019. A critical review on use of *Agrobacterium rhizogenes* and their associated binary vectors for plant transformation. *Biotechnology* 37(7), 107405.
- Chakrabarty, S., Jin, M., Wu, C., Chakraborty, P., Xiao, Y., 2020. *Bacillus thuringiensis* vegetative insecticidal protein family Vip3A and mode of action against pest Lepidoptera. *Pest Management Science* 76(5), 1612-1617.
- Ebrahim, H.S., El Ashry, A.A., Rabie, S.A.A., Ismail, I.A., El-Bahr, M.K., Gabr, A.M.M., 2025. Establishment of hairy root cultures by *Agrobacterium rhizogenes*-mediated transformation of *Gardenia jasminoides* variegata for enhancing phenolic and antioxidant accumulation capacity. *Egyptian Pharmaceutical Journal* 24(1), 131-140.
- Fan, Y., Zhang, X., Zhong, L., Wang, X., Jin, L., Lyu, S., 2020. One-step generation of composite soybean plants with transgenic roots by *Agrobacterium rhizogenes*-mediated transformation. *BMC Plant Biology* 20(1), 208.
- Garagounis, C., Georgopoulou, M.-E., Beritza, K., Papadopoulou, K.K., 2020. An *Agrobacterium rhizogenes*-mediated hairy root transformation protocol for fenugreek. *MethodsX* 7, 101098.
- Georgiev, M.I., Agostini, E., Ludwig-Müller, J., Xu, J., 2012. Genetically transformed roots: from plant disease to biotechnological resource. *Trends in Biotechnology* 30(10), 528-537.
- Guillon, S., Tremouillaux-Guiller, J., Pati, P.K., Rideau, M., Gantet, P., 2006. Hairy root research: recent scenario and exciting prospects. *Current Opinion in Plant Biology* 9(3), 341-346.
- Kidd, S., Halliday, C., Alexiou, H., Ellis, D., 2016. Descriptions of medical fungi. CABI.
- Lacroix, B., Citovsky, V., 2019. Pathways of DNA transfer to plants from *Agrobacterium tumefaciens* and related bacterial species. *Annual Review of Phytopathology* 57(1), 231-251.
- Meyer, A.D., Tempé, J., Costantino, P., 2000. Hairy root: a molecular overview. *Plant-Microbe Interactions* 5, 1-39.
- Mohamed, R.Y., Al-Mallah, M.K., Ramadan, N.A., 2015. A protocol for the production of chickpea plants resistant to *Fusarium* wilt from genetically transformed hairy roots. *European Academic*

- Research 3(3), 2833-2842.
- Mohammed, A.A.-H., 2020. Uptake of copper oxide nanoparticles by different tissues of fenugreek (*Trigonella foenum-graecum* L.) plant. Plant Archives 20(2), 8121-8126.
- Mohammed, A.A.-H., Masyab, H.M., 2020. Genetic transformation of *Nigella sativa* L. plants with *Agrobacterium rhizogenes* 35S GUS R1000 and estimation of thymoquinone level in transformed hairy root cultures. Plant Archives 20 (Supplement 1), 3649-3652.
- Morgan, A.J., Cox, P.N., Turner, D.A., Peel, E., Davey, M.R., Gartland, K.M.A., Mulligan, B.J., 1987. Transformation of tomato using an Ri plasmid vector. Plant Science 49(1), 37-49.
- Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15(3), 473-497.
- Peterson, A., Kishchenko, O., Zhou, Y., Vasylenko, M., Giritch, A., Sun, J., Borisjuk, N., Kuchuk, M., 2021. Robust *Agrobacterium*-mediated transient expression in two duckweed species (Lemnaceae) directed by non-replicating, replicating, and cell-to-cell spreading vectors. Frontiers in Bioengineering and Biotechnology 9, 5.
- Saeed, W., Naseem, S., Gohar, D., Ali, Z., 2019. Efficient and reproducible somatic embryogenesis and micropropagation in tomato via novel structures-rhizoid tubers. PLoS ONE 14(5), e0215929.
- Shanks, J.V., Morgan, J., 1999. Plant 'hairy root' culture. Current Opinion in Biotechnology 10(2), 151-155.
- Sultan, S.J., Mohammed, A.A., 2020. Genetic transformation of broccoli (*Brassica oleracea* var. *italica*) by plasmids of *Agrobacterium rhizogenes* R1601 and their molecular detection. Biochemical and Cellular Archives 20(2), 5491-5496.
- Tzfira, T., Citovsky, V., 2007. *Agrobacterium*: from biology to biotechnology. Springer Science and Business Media.
- Venkatachalam, L., Lokesh, V., Bhagyalakshmi, N., 2011. A rare event of *Agrobacterium rhizogenes*-assisted genetic transformation of 'Silk' banana (genotype AAB). Journal of Microbial and Biochemical Technology 3(1), 013-017.
- Wang, D., Liu, Y., Zhang, Y., Huang, X., Wang, J., Wang, Y., Liu, Y., Yan, C., Lv, B., Jia, Y., 2025. Establishment of transient transformation systems in Welsh onion (*Allium fistulosum* L.): hairy root induction and protoplast transformation. Plants 14(17), 2664.
- Yassin, A., Fredrick, K., Mankin, A.S., 2005. Deleterious mutations in small subunit ribosomal RNA identify functional sites and potential targets for antibiotics. Proceedings of the National Academy of Sciences 102(46), 16620-16625.