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Bio-interaction of *Agrobacterium rhizogenes* with *Capsicum annuum* L. (sweet variety) and Establishment Hairy Roots Cultures

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Abstract

Genetic transformation using *Agrobacterium* is one of the techniques used to transfer desired genes to plants. This protocol is considered a short – cut to get transformed plants which could be an alternative method and suitable system compared with the classical methods. This study aimed to investigate the response of *C. annuum* seedlings (sweet variety) to the formation transformed hairy roots induced by *A. rhizogenes* strain R1601. Sterilized seeds of *Capsicum annuum* were inoculated with the *Agrobacterium rhizogenes* inoculum. The samples were then transferred to the surface of solidified MS medium. Hairy roots were developed at the inoculation sites and were enucleated 1.0-1.5 cm length and placed in 9.0 cm Petri-dishes containing 15 ml of agar solidified MS medium. Agropine test was performed according to the standard method. The inoculated seedlings showed a good response 90%. Transformed hairy roots were established at the injection sites within 10 days and these roots were easily grown on agar-solidified MS medium. The results are confirmed that these roots were transformed roots it in terms of positive agropine detection. The current study concluded that the biological interaction between *Agrobacterium rhizogenes* strain R1601 and *Capsicum anuum* L. seedlings, was successful. This study encourages future research to improve this plant by continuing and applying modern technologies to obtain genetically modified plants.

Keywords: *Agrobacterium rhizogenes, Hairy roots formation, Capsicum annuum, pepper*
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I. INTRODUCTION

Capsicum annuum is an economically and medicinally important crop at the same time. Pepper plants have different varieties including Bird Pepper, Chilli Pepper, Hot Pepper, Red Chilli, Spur Pepper and Tabasco Pepper (Duke, 2003). The sweet Pepper are widely used in food because they contain a high content of vitamin C (Stroev, 1989). Chilli pepper is used externally as a tonic and anti-irritant, and it is used internally as an aid to digestion and a repellent of gases (Libster, 2002). The economic and medicinal importance of this plant encourages conducting research with the aim of improving its varieties, such as conducting electrical fusion between protoplasts of two differentvarities (Al- Nema and Al- Mallah, 2020).

Green pepper is a major source of vitamin C and also contains a group of enzymes, including *peroxidase* (Cuenca *et al*., 1989). *Agrobacterium* have a unique ability to transfer parts of their genetic material and insert it into the genome of plant cell (Georgiev *et al*., 2008). The ability of this bacterium to interfere with plants in nature was exploited by using it, they were used in the process of genetic transformation into a number of dicotyledonous plants (Kifle *et al*., 1999). The ability of *Agrobacterium* strains to cause plant diseases is due to their relatively large plasmids (200-800 kbp). *A. rhizogenes* have a large root inducing plasmids so called Ri plasmids (Veena and Taylor 2007), and causes hairy root disease in infected plants (Weller *et al*. 2005).

The bacteria of this genus can be divided according to their composition of the unusual amino acids known as the opine group, as the plant cells infected with bacteria form these amino acids, which are used by the bacteria as a source of energy (Bundock and Hooykaas, 1998). This bacterium mediated gene transfer is widely used to obtain hairy roots in many plant species as in sugarbeet (Al- Mallah and Al-Nema, 2001), carrot (AL-Mallah and Mohammed, 2012), Fenugreek (AL-Mahdawe *et al.*, 2013), cowpea (Rasheed and Abdullah, 2013) and Rue (AL-Mahdawe *et al.*, 2020). The present study aims is to investigate the response of *C.*

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annuum seedlings (sweet variety) to the formation transformed hairy roots induced by *A. rhizogenes* strain R1601.

II. Materials And Methods

A. Seeds sterilization and germination

Seeds of *Capsicum annuum* (sweet variety) were surface sterilized by immersing them in a 6% of NaOCl at a concentration of (1:1, V:V) with distilled water (AL-Yozbaki, 1998). Sterilized seeds were transferred to the surface of solidified MS medium (Murashig and Skoog, 1962). Plantlets were kept under growth room condition 25±2ºC with light intensity of 1000 Lux, with a daily 16 hs. photoperiod.

B. Source and preparing of bacterial inoculum

R1601 strain of *Agrobacterium rhizogenes* was used, supplied from Professor E. W. Nester Washington University, USA, which is a genetically modified strain that has genetic markers that include resistance to the antibiotics Kanamycin (Kana. R^+) and Carbencillin (Carb. R^+). Cultures were grown on APM agar solidified medium (Morgan *et al*., 1987). The bacterial inoculum was prepared by transferring one colony of *A. rhizogenes* into a 50 ml flask containing 20 ml liquid APM medium and incubated in shaking incubator at 28±1ºC/150 rpm in dark (Al- Mallah and Al- Nema, 2001). After 24 hours of incubation, the culture was harvested by centrifugation for 15 min. at 1200 rpm. Pour off the supernatant, and re-suspend the pellet to a final OD of 1.90-2.00 for inoculation of seedlings.

C. Inoculation of seedlings with Agrobacterium rhizogenes

Sterilized seedlings (two weeks old) were pricked at 3 site/plant after removing the root group from it. Samples (50 seedlings) were inoculated with a sterile 0.25×9.5 mm Needle whose fine tip was submerged in the bacterial inoculum, as well as other 50 seedlings (control samples) were pricked by sterile needle without bacteria. The samples were then transferred to the surface of solidified MS medium and stored in the culture room at 25±2ºC with light intensity of 100 Lux.

D. Development of hairy root cultures

Hairy roots were developed at the inoculation sites after 10 days. Young hairy roots were enucleated 1.0-1.5 cm length and placed in 9.0 cm diam. plastic Petri-dishes containing 15 ml of agar solidified MS medium at a rate of 5 strands / plate, the plates were covered with lids and sealed with parafilm strips (Al- Mallah and Al- Nema, 2001). And the samples were kept under the same previous conditions.

E. Agropine test

Agropine test was performed according to the standard method (Tepfer and Tempé, 1981). 100 mg of hairy roots and normal roots were crushed separately in the presence of 0.1 N HCl, and the samples were centrifuged at 12.000 *g* / 10 min. 20 µl of each sample and standard agropine was spotted on Whatman No.3 mm paper (15 × 30 cm) and subjected to electrophoresis 300 V/cm, 60 min. Electophotogram was stained with silver nitrate ($AgNO_3$) for 15-30 min, then immerged in 2% methanolic NaOH, dried and submerged with 5% $Na_2S_2O_3$. Finally washed with tap water for 30 min and then allowed to air dry.

III. Results

A. Interaction between Agrobacterium rhizogenes and Capsicum annuum

The biological interaction process was succeeded between *A. rhizogenes* R1601 and *C. annuum* seedlings. Hairy roots were developed from injected sites on seedlings after 10 days (Figure 1, a). The number hairy roots formed on each seedling was 2-5 roots. After sequential transfer of these roots on solid MS medium they were obtained after two weeks (Figure 1, b). These adventitious hairy roots were negatively geotropism in their growth with dense of root hairs (Figure 1, c). Moreover, the responses of seedlings to inoculation by *A. rhizogenes* was 90% compared with the control samples that did not show any response (Table 1).

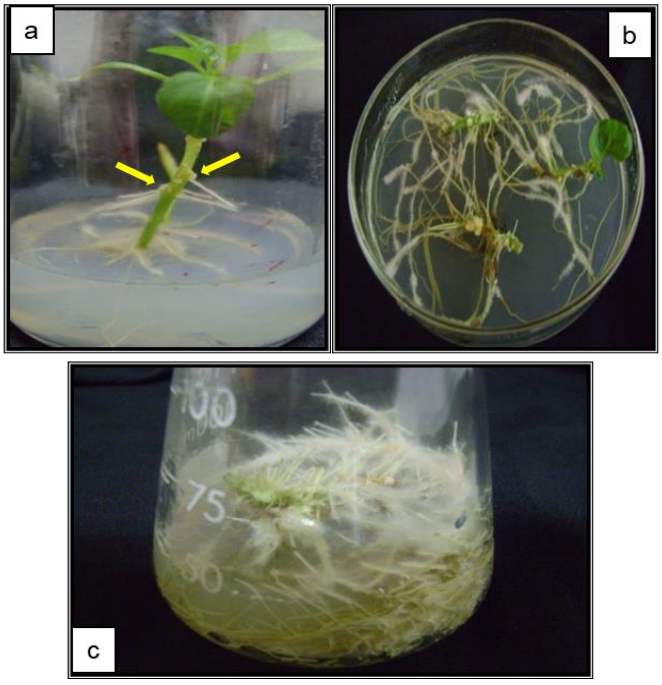

Figure 1. Transformed hairy roots formation on *Capsicum annuum* seedlings (sweet variety) inoculated via *Agrobacterium rhizogenes* 1601. a. Hairy roots formed on pepper seedlings at injection sites (10 days old) on solidified MS medium (arrowed). b. Hairy roots developed on seedlings (two weeks old). c. Culture of hairy roots having dense root hairs on solidified MS medium (6 weeks old).

Table 1. Transformed hairy roots production on paper *capsicum annuum* seedlings inoculated by *Agrobacterium rhizogenes*

Treatment	Number of seedlings inoculated	Number of seedlings forming roots	Average no. of hairy roots / seedling	Hairy root formation %
Seedlings	50	45	3.52	90
Control	50	0	0	0

B. Agropine test as evidence of genetic transformation.

The results of paper electrophoresis showed positive detection of agropine in the hairy root samples, while the normal root samples gave a negative detection of this test. A single spot was formed for the hairy root samples corresponding to its location to the standard agropine spot, while the normal root samples did not give any spot (Figure 2).

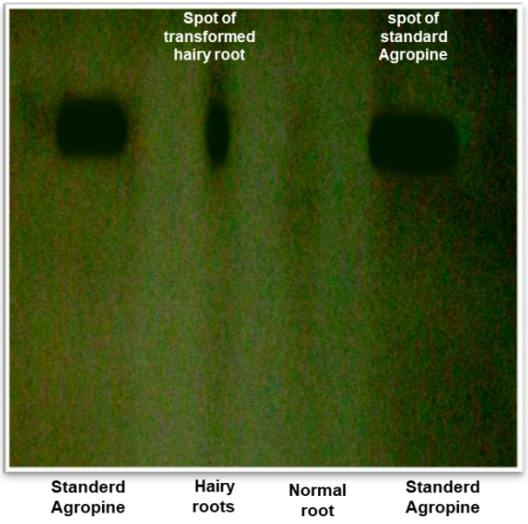

Figure 2. Chromatography sheet showing the spots of standerd agropine and agropine in transformed hairy roots induced on *capsicum annuum* seedlings by *Agrobacterium rhizogenes* 1601

IV. Discussion

The study included identifying the response of sweet pepper plants to a system of genetic transformation using strain R1601 of *A. rhizogenes*. The high response to pepper seedlings through their tolerance to direct injection of bacteria and their formation of hairy roots confirms the possibility of using this system and the response of plants to it (Hansen and Wright, 1999).

The success of the inoculation process by direct injection with this genetically modified bacterium containing Ri plasmids is due to the transfer of T-DNA segment containing the genes responsible for the formation of hairy roots and its random interference with the genotype of the plant cell (Vergunst and Hooykaas, 1999).

In this type of roots that is formed by the presence of genes responsible for making agropine (unusual amino acids) transferred on T-DNA segment into the genome of the transformed cells (AL-Mahdawe *et al.*, 2013). The rapid growth of transformed hairy roots and its dense content of root hairs, may due to the length of apical meristem for these roots compared with untransformed roots (Meyer, *et al*., 2000). The development of transformed hairy roots on various plant species are involving different time, like 15 days of inoculation in *Solanum nigrum* (Al-Mallah and Salih, 2006), and 4 weeks in *Beta vulgaris* (Al-Mallah and Al-Nema, 2012).

V. Conclusion

The current study concluded that the biological interaction between *Agrobacterium rhizogenes* strain R1601 and *Capsicum anuum* L. seedlings, was successful. Therefore, this study encourages conducting future research aimed at improving this economically important plant by continuing and applying modern technologies to obtain genetically modified plants.

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