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Induction of hairy roots producing –agropine on hypocotyls explant of sunflower and tomato by *Agrobacterium rhizogenes* 1601 harbouring Ri- plasmid

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ABSTRACT

Transformed hairy roots were induced on Helianthus annuus and Lycopersicon esculentum by inoculation of hypocotyl explants with agropine wild type Agrobacterium rhizogenes 1601 harbouring Ri-plasmids. Susceptibility assessment of these plants to this strain of A. rhizogenes produced 90 and 60% transformation efficiencies respectively.. These roots were rapid negative-geotropism in their growth on agar-solidified MS0 medium and having dense of root hairs. Interestingly, they spontaneously produce callus in sunflower. Paper electrophoresis of extract for both types of hairy roots demonstrated the separation of black spots of atropine.

KEYWORDS

Hairy root;
Sunflower;
Tomato *Agrobacterium rhizogenes*;
Ri- plasmid.

INTRODUCTION

Sunflower, *Helainthus annuus* L. (Compositae) is a popular oil crop. Tomato, *Lycopersicon esculentum* L. (Solanaceae) is an important vegetable plants. Both of these plant species are of considerable value for human nutrition. Hairy roots are genetically stable and contain different cells. These hairy roots pathological syndrome of dicotyledonous plants following wounding and infection with *Agrobacterium rhizogenes*[1]. This strain *Agrobacterium rhizogenes* R1601 was classified according to the opine produced[2]. In many plant species infected with this strain induced several gentotypes of hairy roots such as in sugarbeet[3] Taget[4], carrot[5] and rubber[6]. Due to the difficulties of sunflower regeneration and transformation,and two pointed out the shortcut for improving tomato plants away from breeding program this work was proposed, aimed to produce hairy root cultures using the virulence strain R1601 of *A. rhizogenes*.

MATERIALS AND METHODS

Bacterial strain

Agropine-type *Agrobacterium rhizogenes* R1601 was supplied by professor E. Nester, Univ. of Washington, USA. Liquid APM[7] medium supplemented with 100 mgL^{-1} of each kanamycin and carbencillin was used to maintain this bacteria and suspension preparation.

Plant materials

Seeds of both sunflower (*Helainthus annuus* L.) and tomato (*Lycopersicon esculentum* L.) were

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obtained from local market. Each type of seeds were surface - sterilized by soaking in a 6 % solution of sodium hypochloride (NaOcl) for 15min. And thoroughly rinsed with sterile water[8]. The surface sterilized seeds were placed separately on the surface of 20 ml aliquots of agar-solidified MS[9] in 9.0 cm diam. Plastic Petri dishes (Sterilin, UK). They were kept in dark conditions in the culture room at 25 ± 2°C. The produced axenic hypocptyls were used in inoculation experiments.

Bacterial suspension and inoculation

Inoculum was prepared by inoculating 20 ml aliquots of liquid APM, containing 100 mgL^{-1} of each kanamycin and carbencillin with a loopful of agrobacterial growth and incubated for 48 hours. Bacterial suspension was centrifuged at 600 rpm/5.0 min. The precipitated bacteria was resuspended by the addition of 1.0 ml of freshly prepared liquid APM medium[10].

The optical density of this suspension was measured spectrophotometerically (Cecil,1100). Stem segments 3.0 cm long were exiled from four weeks old sunflower and tomato seedlings. Each type of these explants were inoculated by direct injection method[11] using fine needle dipped in bacterial inoculum and directly injected in selected sites of stems. The inoculated specimens were plunged vertically in agar-solidified MS0 medium and kept in culture room condition previously mentioned.

Establishment of agrobacterial -free hairy roots culture

Single root of two cm length were excised separately from infected hypocotyls of both plant species. They were placed on agar-solidfied MS0 media provided with gradual conc. 150,200,250 mgL^{-1} of cefotatime. Each type was trasferred sequentially and stay for two weeks on each conc. later they were cultivated on hormone -free MS medium.

Paper electrophoresis

Two hundreds milligrams of freshly developed hairy roots were excised and minced separately by pestle and mortar in the presence of 1N HCL. The supernant was loaded on whatman filter paper No 3 MM of 20 × 20 cm dimension and fixed in the apparatus (Esselte studiums s-11285 stockholm Sweden). The buffer solution consisted of Formic acid: Acetic acid: Water (5:15:80 v:v:v). The used voltage was 300 volt which allow to pass through for one hour[12].

RESULTS

Axenic seedlings production

Healthy and axenic sunflower and tomato seedlings were produced from surface sterilized seeds cultruted on the surface of agar-solidfied MS0 medium. They were used as source of stem explants to be used in inculation experiments.

Inoculation of explants and hairy root induction

The obtained results indicates that both type of plants respond positively to inoculation with *A. rhizogenes* (TABLE 1)

TABLE 1 : Induction of hairy roots on stem explants of sunflower and tomato by *A. rhizogenese* R1601 agropine type.

Stem explant of	No. of explants Inoculated	Responsed	Timeneed (day)
Helianthus annuus L.	50	45	7
(Control)	25	0	0
Lycopersicon esculentum	50	30	10
(Control)	25	0	0

The observations that all seedlings tolerate the inoculation process. Moreover, ninety percent of sunflower stem explants inoculated with *A. rhizogenes* produced hairy root in both inoculated and non-inoculated positions (Figure 1A) whereas, sixty percent of agrobacteral inoculated tomato explants stimulated this type of hairy roots (Figure 1B).

Production of bacterial - free hairy root culture

The results indicate that cultivation of hairy root on solid MS medium supplied with gradual conc. of cefotoxime was efficient to remove *Agrobacterium rhizogenes* from those culture. After that they were periodically subcultured on MS medium without addition of growth regulators.

Characterization of hairy roots

Hairy roots produced on sunflower were thick, elongated, white in color and have dense of root hairs (Figure 1C) whereas those produced on tomato were slim, yellow in color with dens of root hairs (Figure1 .D). Both genotypes of hairy root were negatively geotropism in their growth.

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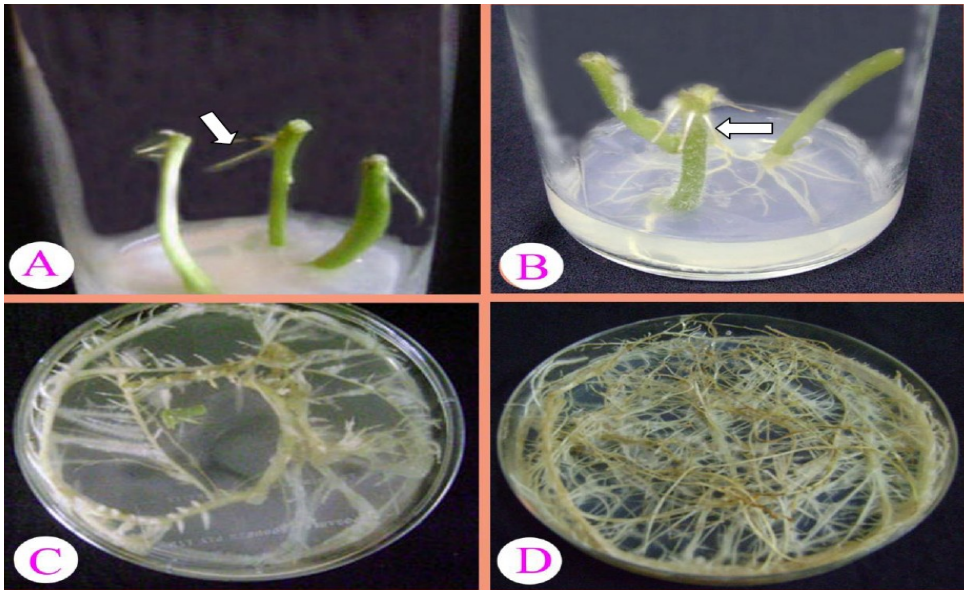

Figure 1 : Hairy roots induction on sunflower and tomato by agropine-type *Agrobacterium rhizogenes* 1601. (A) Initiation of hairy roots produced on hypocotyl explants of sunflower 7 days of inoculation. (B) Induction of hairy roots on hypocotyl explants of tomato 10 days of inoculation. (C) Culture of hairy roots in (A) grown on agar-solidified MS0 medium (D) Culture of bacterial free hairy roots in (B) grown on agar -solidified MS0 medium

Growth of hairy roots

The growth of hairy roots produced on sunflower stem explants were more active and longer than those produced on tomato stem explant (Figure 2).

Root samples of 2.0cm length excised from normal seedlings of sunflower and tomato failed to continue their grow on surface of agar-solidifed MS0 medium.

Data of agropine

Data obtained from electrophoretogram revealed the separation of black spotsfrom extraction of hairy roots induced on sunflower and tomato corresponded in their position to the standard agropine (Figure 3). These results proved that these tissue were genetically transformed by *A. rhizogenes*.

DISCUSSION

The differences in hairy root induction on (sunflower) *H. annuus* and(tomato) *L. esculeutum* may be due to two reasons including that the natural exudates released from the wounded tissues may not be sufficient to dicit the activities of the vir-genes in the *A. rhizogenes* R1601. Also, these exudates may have an inhibitory

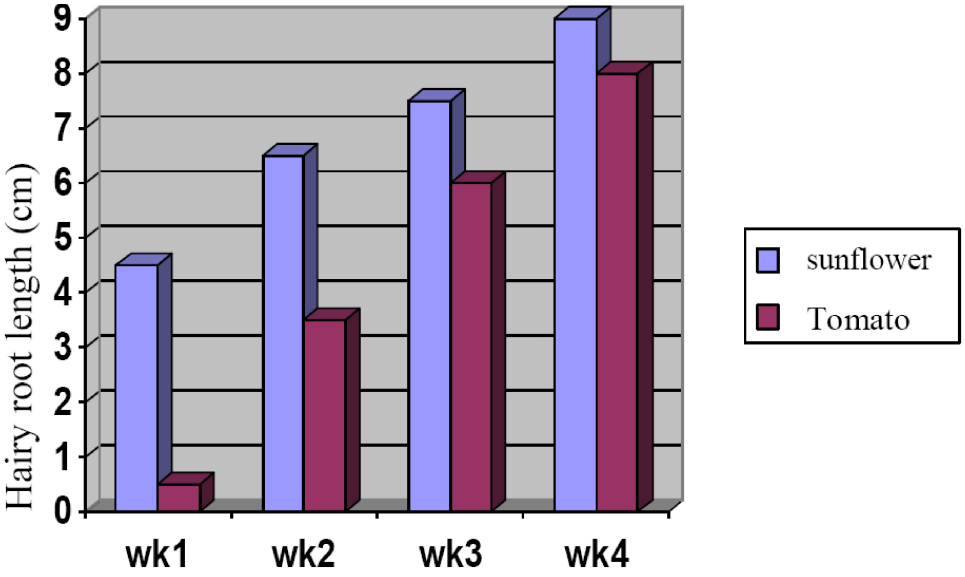

Figure 2 : Growth of hairy roots induced on hypcotyl explants of sunflower and tomato

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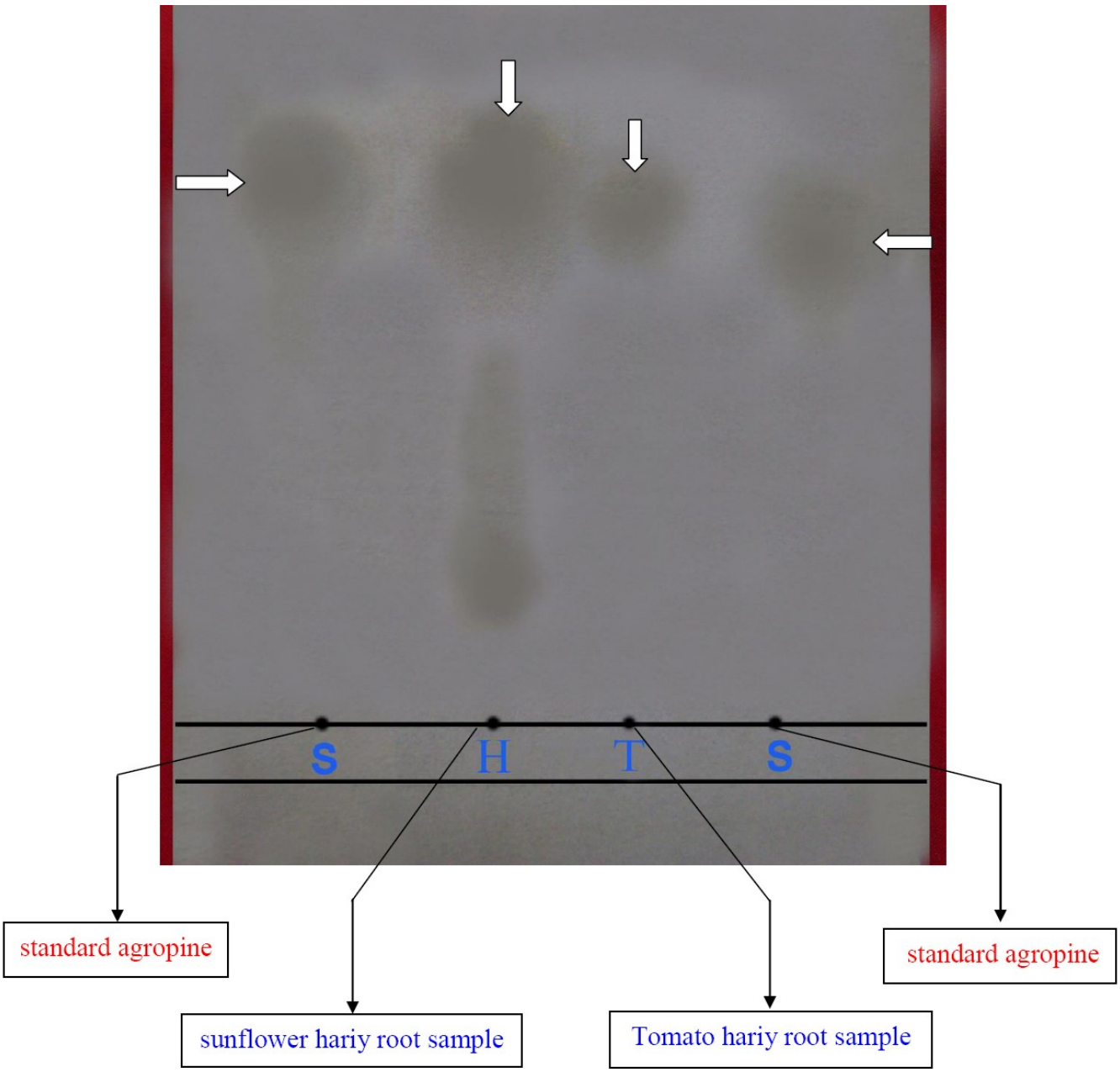

Figure 3 : Electrophoretogram of hairy roots induced on hypocotyl explants of sunflower and tomato by agropin-type *A. rhizogenes* 1601

effect on the *A. rhizogenes* strain used[13].

In this study the genotypes of hairy roots induced on both type of plants by the same *A. rhizogenes* on hormone-free MS basal medium were similar to genotypes of hairy roots induced on *L. esculentum*[14] and *Ammi mojus*[15].

The type of opine produced by this strain of *Agrobacterium rhizogenes* is agropine. This is perhaps due to agropin strain contain both TL and TR regions in its Ri plasmid which make it more virulent as mentioned in other investigation[16,17]. The hairy roots induction occurred at the cut region as well as at other region- in this study was similar with the observation noted in other plant species. The diverse susceptibilities of in stem explants to *A. rhizogenes* may be due to their physical conditions and to the physiological characteristics of these tissues in response to culture. In conclusion the production of callus from those hairy roots spontanously represents a short cut to produce plants especially in plants difficult in culture[18].

REFERENCES

[1] J.Draper, R.Scotl, D.Armitage, J.Hamil;

Transformation of dicotyledonous plant cells using Ti plasmid of Agrobacterium tumefaciens and the Ri plasmid of A. rhizogenes. In: J.Draper, R.Scott P.Armitagc, R.Walden, (Eds); Plant Genetic Transformation and Gene Expression A Laboratory Monual - Black Scientific Publications. Oxford, 69.160 **(1988)**.

[2] D.J.Hamill, J.A.Lidgget; Hairy root cultures opportunities and key protocols for studies in metabolic engineering In: M.P.Doran, (Ed); Hairy Roots Cultures and Applications. Harwood Academic Publishers, Netherlands, 1-29 **(1997)**.

[3] M.K.Al-Mallah, Q.S.Al-Nema; Putative genetically modified callus derived from transformed hairy roots induced on sugarbeet (Beta vulgaris L.) explants by Agrobacterium rhizogenes 1601 harbouring Ri-plasmid. Iraqi J.Biotechn., **11**, 455-463 **(2012)**.

[4] K.M.Al-Hamdani, M.K.Al-Mallah, A.O.Al-Atrakchi; Isolation and detection thiophene from Tagetes patula-plants produced from tissue cultures and transformed hairy roots. Mesopotamia J.Agric., **36**, 22-30 **(2008)**.

[5] M.K.Al-Mallah, A.A.Mohammed; Transfer of Ri T-DNA genes of Agrobacterium rhizogenes 1601 via direct microinjection and co-cultivation to carrot, Daucus carota L.Tissue and production of transformed hairy root cultures. Iraq J.Biotechn., **11**, 227-239 **(2012)**.

[6] R.M.Abdullah, M.K.Al-Mallah; Transfer of T-DNA genes from Agrobacterium rhizogenes 1601 to indian rubber Ficus elastica plant. Proceeding of the 2nd Scientific Conference for Roxb Biol.Sci. College of Science, Nov.Univ of Mosul, IRAQ, 16-17 **(2011)**.

[7] A.J.Morgan, P.N.Cox, D.A.Turner, E.Peel, M.R.Davey, K.M.Gartland, B.J.Mulligan; Transformation of tomato using an Ri-plasmid vector. Plant Sci., **49**, 37–49 **(1987)**.

[8] P.Suraninpog, S.Chanprame, H.J.Cho, J.M.Widholm, A.Waranyuwat; Agrobacterium-mediated transformation of mungbean Vigna radiata L.Wilczek.Walailak J.Sci.&Tech., **1**, 38-48 **(2004)**.

[9] T.Murashige, F.Skoog; A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol.Planta., **15**, 473-497 **(1962)**.

[10] M.K.Al-Mallah, M.R.Davey, E.C.Cocking; Enzymatic treatment of clover root hairs removes a barrier to Rhizobium-host specificity. Biotechnology, **5**, 1319-1322 **(1987)**.

[11] M.R.Davey, P.Anthony; Plant Cell Culture, Essential Methods. Wiley-Blackwell, **(2010)**.

[12] D.A.Tepfer, J.Tempe; Production of di-agropine par des vaccines formees sous I·action Agrobacterium rhizogenes, Acad.Sci.Paris.Ser. III, **292**, 212-218 **(1981)**.

[13] K.M.A.Gartland, M.R.Davey; Agrobacterium Protocols. Method in Molecular Biology.Humans Press, New Jersey, U.S.A., **(1995)**.

[14] R.Y.Hashimoto, C.F.M.Menck, M.A.Van Sluys; Negative selection driven by cytosine deaminase gene in lycopersicon esculentum hairy roots. Plant Sci., **141**, 175-181 **(1999)**.

[15] A.Krolicka, I.Staniszewska, K.Bielawski, E.Malinski, J.Szotranek E.Lojkowska; Establishment of hairy root cultures of Ammi majus. Plant Sci., **160**, 259-264 **(2001)**.

[16] M.Otani, M.Mii, T.Handa, H.Kamada, T.Shimada; Transformation of sweet potato (Ipomoea batatas L. Lom.) plants by Agrobacterium rhizogenes. Plant Sci., **94**, 151-159 **(1993)**.

[17] K.M.Oksman-Caldentey, R.Hiltunen; Transgenic crops for improved pharmaceutical products. Field Crop Res., **45**, 57-69 **(1996)**.

[18] W.Y.Mei, J.B.Wang, D.Luo, J.F.Jia; Regeneration of plants from callus cultures of roots induced by Agrobacterium rhizogenes on Alhagi pseudoalhagi. Cell Res., **11**, 279-284 **(2001)**.