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# HISTOLOGICAL CHANGES ACCOMPANYING ADVENTITOUS SHOOTS FORMATION OF FRAGRAIA X ANANASSA *IN VITRO* LEAVES

**[33]**

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## ABSTRACT

A tissue culture experiment was conducted to attain the adventitious buds initiation and growth from *Fragraia x ananassa* cv. Festival *in vitro* leaves. The leaves were excited from shoot propagated *in vitro* and cultured on Murashige and Skoog (MS) medium supplemented with 4.50, 6.75, and 9.00 mmol $l^{-1}$ thidiazuron (TDZ) for six weeks. Then explants were transferred to MS medium (salts and vitamins) free plant growth regulators for 8 weeks. This study included the histological and morphological responses of strawberry leaves with TDZ treatments in relation to some biochemical components. All TDZ treatments resulted in adventitious shoots development on leaf explants without callus formation. Growth in 6.75 mmol $l^{-1}$ TDZ led to an increment in the most morphological parameters compared to the rest treatments. The results showed that 6.75 mmol $l^{-1}$ of TDZ treatment significantly increases the number of shootlets per explant (5.0), total shoot (51.3), the number of leaves/explant (12) as compared with other treatments while the rest of parameters (the survival percentage, shoot length (cm), number of leaves/explant, root length (cm), and number of roots/explant) did not show any significant differences. Accumulation of chlorophylls, total sugars, and total soluble phenols was enhanced by 6.75 mmol $l^{-1}$ of TDZ. The histological observations showed that adventitious shoots initiated from some parenchyma of ground tissue in the midvein zone of the explant, whereas the parenchyma mesophyll and the epidermal cells did not involve in this process. The ontogenesis of adventitious shoots was described by successive stages with the following distinguishable anatomical structures: meristemoids, bud primordium, shoot apex with leaf primordial, branching of adventitious shoots. After four weeks of culture, cell dedifferentiation was recorded in the midvein parenchyma and forming small groups of divided cells called meistemoid centres. These centres forming new meristematic masses embedded in the ground tissue of the main vein. After six weeks, further development of these masses resulted in the formation of bud primordial with normal organized shoot apical meristems and leaf primordial and arising from the explant as small protrusions. After two weeks from subculture on MS free TDZ, the adventitious shoots continue to elongate with forming new lateral branches and clearly observed on the adaxial side of the explant. This procedure provides a simple and rapid approach to regenerate strawberry plants via direct organogenesis.

**Keywords:** Strawberry, *Fragraia x ananassa*, Adventitious shoots, Thiodiazuron (TDZ), Histological Changes.

## INTRODUCTION

Strawberry (*Fragraia x ananassa* cv. Festival) is a member of Rosaceae family. It considers one of the most profitable and important crops in Egypt and many countries, because of its delicious taste, low energy food and well-known source of vitamin C as antioxidants and phenolic compounds, mainly anthocyanin **(Sakila et al 2007)**. It also contains significant levels of anti-carcinogenic. In addition to fresh consumption, strawberry is widely used in the food industry and the strong demand existed in Europe during the months starting from late November until late March.

Conventional propagation techniques are laborious and expensive with numerous limitations and may not be suggested for effective and commercial multiplication **(Dhar 1998)**.

Plant tissue culture techniques have become a powerful tool for strawberry propagation on a large scale using the runner tips. However, these techniques have many problems fronted the production such as sterilization inefficiency and browning leads to explant death in the first stage of micropropagation **(Pirttila et al 2008).**

Adventitious shoot regeneration is influenced by some factors including plant genotype, explant type, components of basal medium and type, concentration of plant growth regulators **(Ćosić et al 2015; Haddadi et al 2013; San et al 2015)**. Previous reports recommended that cytokinins considered the most important regulatory factor for the meristem activities and morphogenesis process **(Werner et al 2003)**. Thidiazuron (TDZ) as a cytokinin was sufficient plant growth regulator in regenerate adventitious shoots from strawberry leaves and sepals **(Debnath 2005)**.

These are scarce reports recommended using the *in vitro* leaf explants as a source of strawberry regeneration **(Haddadi et al 2013).** Whereas it is extensively used in many other plants and produced normal plantlets **(Yepes and Aldwinckle 1994).**

There are few precise histological studies on the development stages during the initiation of direct strawberry organogenesis were reported **(Wang et al 2015; Bhandari and Roy, 2015).**

The purpose of the present study is to use the *in vitro* leaves of strawberry cv. Festival as explant to following the initiation and development of direct adventitious shoots histologically and provide a good understanding of this process.

## MATERIAL AND METHODS

### Culture establishment

The healthy runner's segments with runner tips of *Fragaria x ananassa*, cv. Festival were collected from Al Ahram Nurseries, El Manashi road, El Katatba, Qalubia, Egypt. The experiment was carried out in Plant Tissue Culture Lab of Agric. Botany Dept., Fac. of Agric. Ain Shams Univ., during the years of 2016–2018. The runner's segments were washed under tap water for 20 minutes, and then were sterilized by dipping in ethanol (70%) for 10 seconds, followed by surface sterilization with 0.1% $HgCl_2$ with detergent (Tween 20) 2-3 drops/ml for 6 minutes and rinsing at least 3 times with sterile distilled water. The shoot tips of the runner were directly inoculated to the tissue culture jar containing MS medium salts and vitamins **(Murashige and Skoog, 1962)** enhanced with 30 g $l^{-1}$ sucrose and 2.2 mmol $l^{-1}$ benzyl adenine (BA). The acidity of the medium was adjusted to pH 5.7 ± 0.1 prior to adding agar (7 g $l^{-1}$), then the medium was administered into glass jars (100 ml) containing 25 ml of the testing medium. The culture medium was autoclaved at 121°C and 1.1Kg/$cm^2$ for 20 min and cultures were incubated in a growth room 25 ± 2◦C under the light condition for 16 h/day photoperiods at an intensity of 2000 lux from white fluorescent lamps and subculture consistently at 4-week interims.

### Adventitious shoot organogenesis

Trifoliate leaves with the petiole (15 mm long) from one-month-old *in vitro* plantlets were used as explants. The edges of the leaves were cut, and wounds made on the surface and afterward cultured with the abaxial side down on the MS medium enhanced with 4.50, 6.75, and 9.00 mmol $l^{-1}$ thidiazuron (TDZ) for shoot induction. After 6 weeks, the explants were transferred to MS medium free growth regulator. The survival percentage, the number of shootlets per explant, total shoots, shoot length (cm), number of leaves/explant, root length (cm), and number of roots/explant were recorded after 14 weeks from culturing.

### Biochemical analyses

Total chlorophylls, sugars, and phenolic compounds were extracted by the method described by **Shahidi and Naczk (1995).** A half gram of fresh weight of produced shoots was submerged in ethanol (80%) and kept in a brown bottle for 24 h at 4◦C. The samples were re-extracted 3 times then the completed clarified extract rose to 30 ml with ethanol (80%).

Chlorophylls (Chl a & b) and carotenoids (Car) were performed in the previous ethanolic extract with UV-Vis spectrophotometer UV 9100 B, Lab-Tech at the following wavelengths: 664, 647 and 470 nm, for chlorophyll a, b and carotenoids, respectively **(Pompelli et al 2013)** and the concentration were calculated by equations as described by **Lichtenthaler (1987).**

Total soluble sugars (TSS) were measured according to the method recorded by **Dubois et al (1956)** and expressed as mg $g^{-1}$ fresh weight.

The total soluble phenols in the sample metabolic extracts were analyzed spectrophotometrically using a modification of the Folin–Ciocalteu colorimetric by the method of **Shahidi and Naczk (1995)** and expressed as µg of Gallic acid per g fresh weight of the sample.

**Histological studies**

To investigate the histological changes in *in vitro* leaves of *Fragaria* x *ananassa* cv. Festival, transverse and longitudinal sections were made in the leaves after 0, 2, 4 and 6 weeks from culturing. Samples were killed and fixed immediately in FAA solution (formalin-acetic acid and 50% ethanol 5:5:90) for 24 h at room temperature. Then, it dehydrated using ascending solutions of ethanol. Clearing by xylene and processed using the schedule of the paraffin method as described by **Abdelbar (2017).** Transverse sections (10µm) in thickness were made by LEICA rotary microtome model RM 2125 RTS. Sections were fixed on slides by means of Haupt's adhesive and stained with safranin-fast green **(Berlyn et al 1976).** Anatomical observations and measurements were attained using a Leica light Research Microscope model DM-500 provided with a digital camera LEICA ICC 50 HD with LAS E7 software version 2.1.0, 2012.

The experiment was arranged in a complete randomized design **(Gomez and Gomez, 1984)** with three replicates (jars), each replicate has three explants. The obtained results were subjected to statistical analysis of variance (ANOVA) in statistics (8th edition analytical software, USA) by **(Steel et al 1997)**. Differences between means were contracted by LSD method.

## RESULT AND DISCUSSION

### 1. Morphological and histological parameters

#### 1.1. Explant at culturing time

The young leaves which were excised from one-month-old *in vitro* plantlets have an oval shape with serrated edges, 4-5 mm in length and 6-7 mm in width. The petiole has 10-15 mm in length, cylindrical in shape, and green in colour **(Fig. 1-A)**. The leaflet cross-section shows its anatomical structure. The upper and lower epidermis consists of a single layer with unicellular trichomes. The mesophyll is differentiated into two layers of palisade tissue and loosely-packed cells of spongy parenchyma underneath **(Fig. 1-B)**. The vascular tissue is embedded in the ground tissue; it consists of eight strands of xylem outwardly and phloem tissue inwardly. The ground tissue of the midvein consists of large compact thin parenchyma **(Fig. 1-C)**.

#### 1.2. Initiation of the adventitious buds

After two weeks from culturing on MS medium contains various TDZ concentrations, observations of the transection revealed that several cell divisions initiated in the median vascular bundle. These mitotic divisions started in the phloem parenchyma followed by xylem parenchyma, which cause enlargement in the lamina and the mid vascular bundle area **(Fig. 1-D)**.

After four weeks from culturing, the morphogenesis process advanced, and the dedifferentiated cells gradually redifferentiated into two different cell types: the first redifferentiated into new xylem and phloem elements forming a nest-like shape embedded in the med vein **(Fig. 1-E)**. The second type has periclinal and anticlinal cell divisions which observed in some parenchyma of ground tissue in the midvein zone. Then, it gave rise to form small groups of meristematic cells called meristemoid centres **(Fig. 1- F, G &H)**.

After six weeks, high mitotic divisions were observed in the meristemoid centres forming new masses embedded in the ground tissue of the main vein area. These masses have small isodiametric cells, thin-walled, rich in the cytoplasm, and minimal vacuolated **(Fig. 2-A &B)**. These are the prominent features for the meristematic cells and considered bud primordia. Furthermore, the cells located in the outer peripheral zone of the apical meristem began to divide, subsequently, it gives rise to forming two leaf primordial **(Figs. 2-C,D)** then, the adventitious shoots can be visible on the adaxial side of the leaf in the form of a small protrusion **(Fig. 2-E)**.

#### 1.3. Development of adventitious shoots

After two weeks from transferring on free hormones MS medium, the adventitious shoots developed further (Fig.3-A), its main stem continued to elongate with forming new lateral branches which clearly observed on the adaxial side of the leaf **(Fig. 3-B)**.

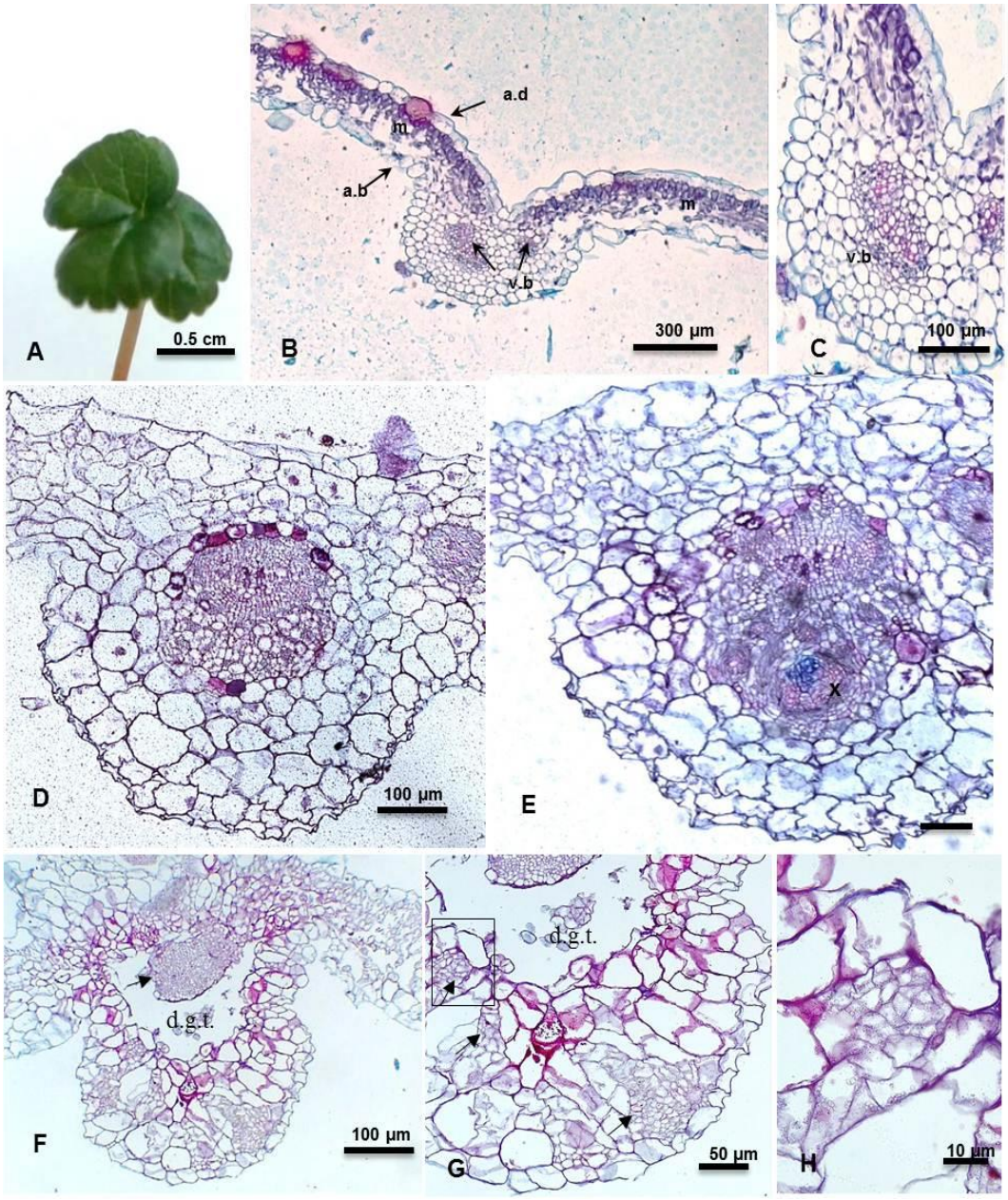


**Fig. 1.** (A) Morphology of *in vitro* strawberry cv. Festival leaf (explant) before culture. (B) Cross-section in the blade of the same explant shows the epidermal cells, the abaxial (ab), the adaxial (ad), mesophyll (m) and the vascular bundle(vb) of the mid vein. (C) Magnification of the midvein showing the structure of vascular bundle (v.b). (D) Two weeks after culturing on MS medium enhanced with various TDZ concentrations, note the cell divisions occurred in the phloem and xylem parenchyma of the vascular bunle (v.b). (E) Four weeks after culturing on the same medium, showing development of some newly vascular tissue forming a nest-like shape (xylem x). (F) Four weeks after culturing on the same medium, note the proliferation of newly vascular tissues (arrow) causing distortion for the med vein and degenrate the ground tissue around it. (G) Magnification of (F). Note the divisions in the ground tissue cells or the meristemoid centers (arrows) and the degenerated ground tissue (d.g.t.) of the midvein. (H) Magnification for the rectangle showed one meristemoid center.

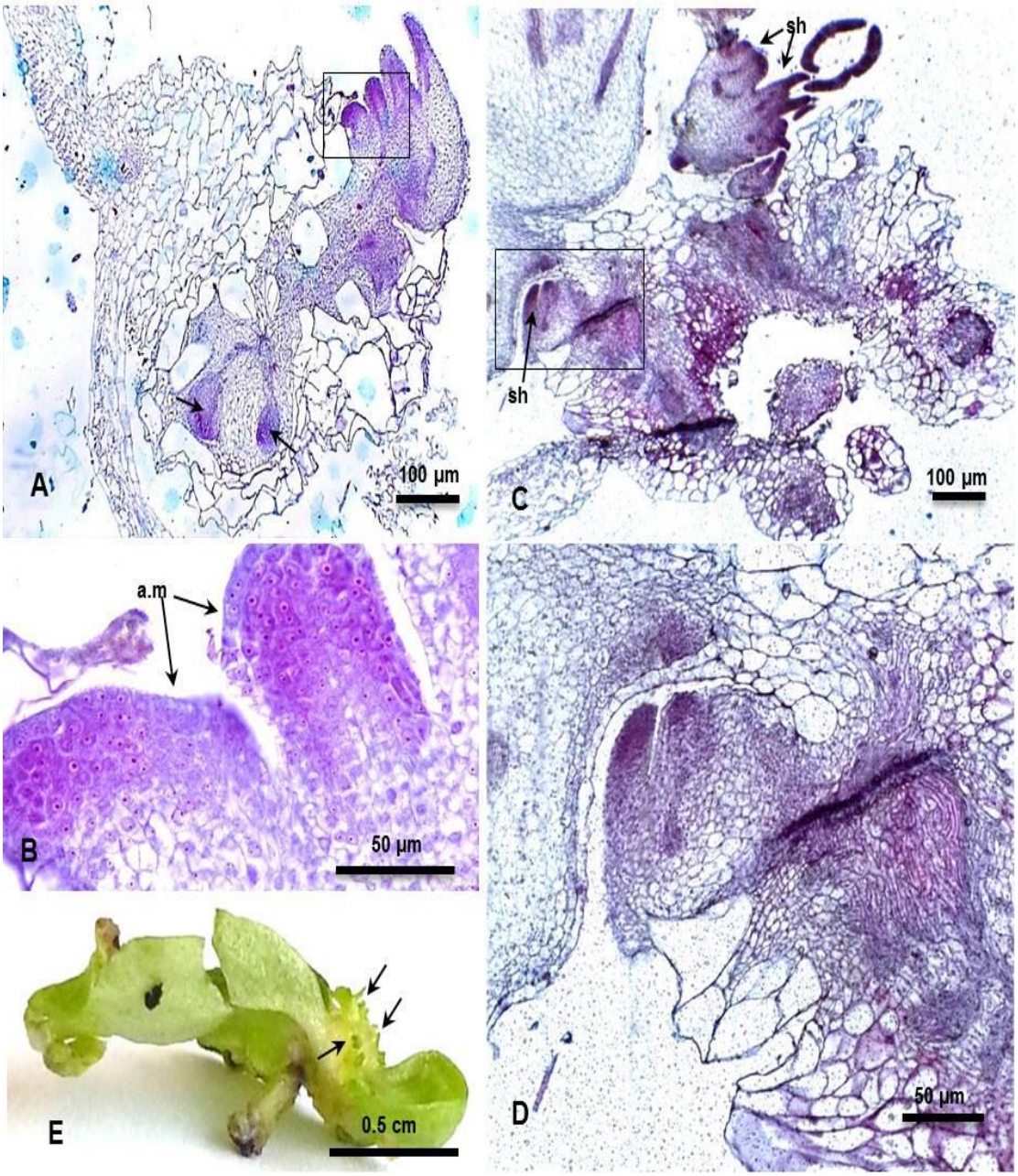


**Fig. 2.** (A) Six weeks after culture on the same medium, showing the meristematic masses embedding in the ground tissue of the midvein (Arrows). (B) Magnification of (A). Note a small isodiametric cells, thin-walled, rich in the cytoplasm and minimal vacuolated, forming the apical meristem (a.m.) of the bud primordia. (C) Six weeks of culturing in the same medium, the development of the adventitious shoots (sh) with two leaf primordial. Note the collapsed cells of the explant. (D) Magnification of (C) illustrates the adventitious shoots. (E) The adventitious shoots arising on the adaxial surface as small protrusions after six weeks of culture.

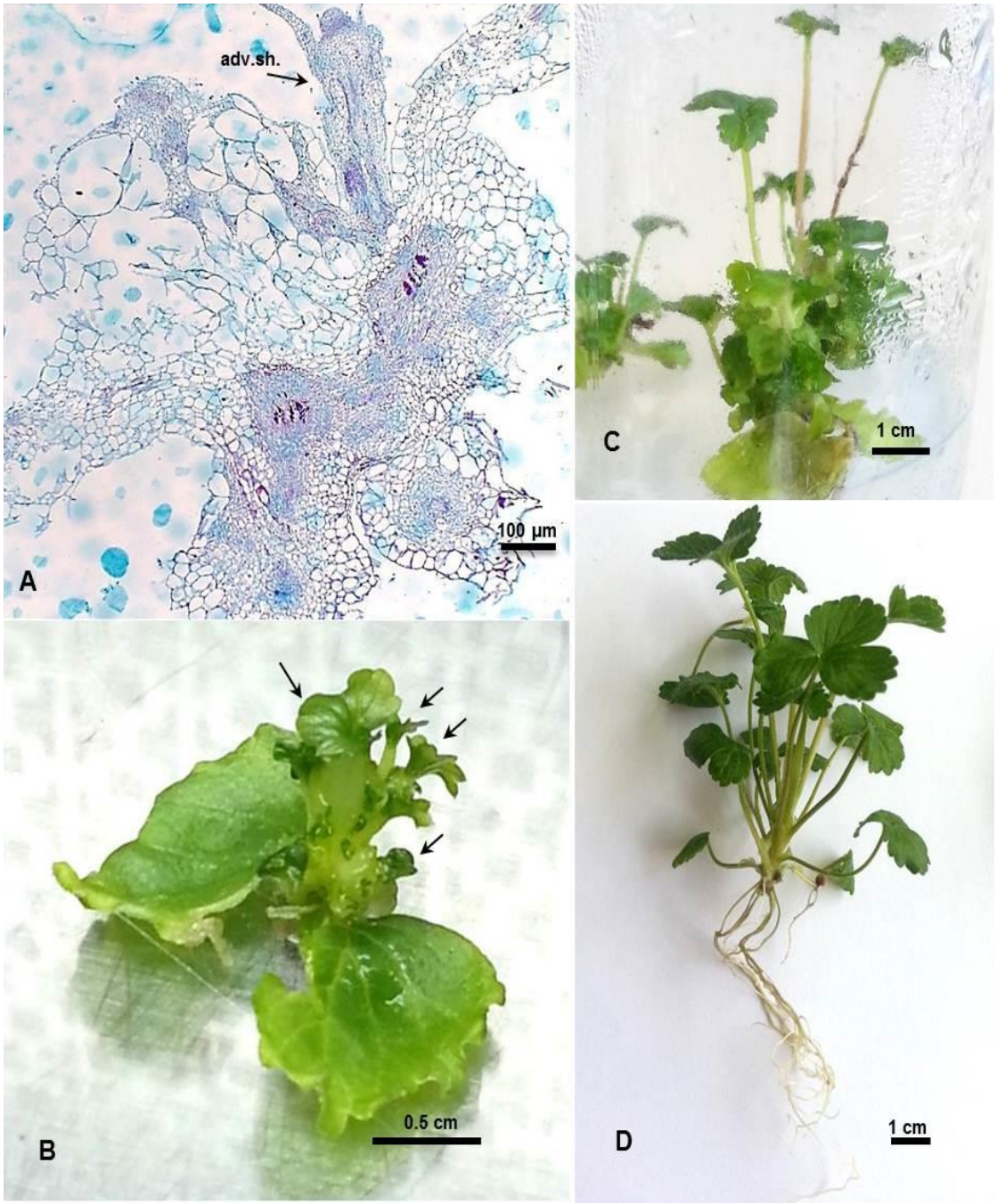


**Fig. 3.** (A) After two weeks of subculture on free-hormone medium, showing elongation of the adventitious shoots (adv.sh.). (B) After two weeks, morphology of the explant with newly elongated adventitious shoots (arrows). (C) After four week of subculture showed the elongated shoots and (D) showed the plantlet after eight week from subculture (2 subculture)

The present histological results indicated that the parenchymatous ground cells of the median vein of the *in vitro* leaves can form the adventitious shoots directly by TDZ. Whereas the epidermises and the mesophyll cells did not involve in this process. This finding matched with **(San José et al 2013).** Organogenesis is a difficult process that affected by many factors: tissue type, explant age, and hormonal balance in its tissues as well as the exogenous application of plant growth regulators in the culture medium. Many reports pointed that any normal living cells within the plant body, theoretically, acquired totipotency and has the capability to regenerate into an entire organism via formation *de novo* organs or somatic embryos. This process including cell dedifferentiation and redifferentiation is mainly organized by auxins and cytokinins **(Murthy et al 1998).** It is well-known that cytokinins assume an essential role in the developmental programs of the plant cells by inducing cell division and involved in plant morphogenesis **(Werner et al 2001; Srivastava, 2002).** In the present study, the adventitious shoots initiated directly from some meristemoid centres induced by TDZ. These centres formed through the dedifferentiation process took place in the ground tissue of the explant (no meristematic cells) to acquired meristematic activity. Thidiazuron one of the cytokinins which causes accumulation and/or synthesis of purine cytokinins. It able either alone or in combination with other growth-regulating substances to induce meristem formation, promotes shoots production *in vitro* cultures of many species **(Murthy et al 1998; Bunn et al 2005).** In strawberry, TDZ led to adventitious shoot regeneration induction in explants got from *in vitro* plantlets **(Cappelletti et al 2016; Debnath, 2005; Haddadi et al 2013).** Several reports indicated that synthesis or accumulation of endogenous cytokinins induction is promoted by TDZ **(Murthy et al 1998).** Thidiazuron can fulfil both the cytokinin and auxin requirements of various regeneration responses **(Jones et al 2007).** These responses may potentially occur through a variety of means including an increase in synthesis, a decrease in catabolism, or release of biologically active cytokinin molecules from non-active storage forms **(Kefford et al 1968).**

At the same time as, cellular dedifferentiation occurred in the ground tissue of *in vitro* leaf explant, the proliferation of the phloem and xylem parenchyma cells took place in the main vascular bundle. These proliferated cells redifferentiated into many treachery elements forming nest-like shapes and causes malformed and disorganized in the main vascular bundle shape. These results were matched with **(De Rybel et al 2014)**, they noted that the harmony among auxin and cytokinin in the vascular bundle acts as an organizer for the entire vascular bundle, and increasing the cytokinin activation rate in all vascular bundle cells increases periclinal cell division and generates a large disorganized vascular bundle. These newly treachery elements may establish vascular connections with the developing newly shoots.

## 2. Growth parameters

After 6 weeks from culturing, all the TDZ treatments showed buds formation from strawberry leaf explants without callus formation (Fig 2-E). In fact, it affirmed that by using TDZ at low concentration, callus arrangement could be utilized **(Haddadi et al 2013).** A regeneration technique with no callus stage may not be totally liberated from a somaclonal variation **(Husaini et al 2008).**

Data clarified in **Table (1)** showed the effect of various TDZ concentrations on the survival percentage, the number of shootlets per explant, total shoot, shoot length (cm), number of leaves/explant, root length (cm) and number of roots/explant after 14 weeks from culturing. The data revealed that TDZ treatments promoted and significantly increased the growth parameters except for survival percentage, shoot and root length, and number of roots/explant. The highest and significant parameters were recorded with 6.75 mmol $l^{-1}$ TDZ. **Cappelletti et al (2016)** reported that using medium supplemented with TDZ induced the best regeneration efficiency and highest shoots per explant from strawberry leaves. TDZ alone has additionally been utilized for strawberry shoot recovery **(Debnath, 2005). Murthy et al (1998)** demonstrated that TDZ has the unique property of emulating both auxin and cytokinin impact on differentiation and growth of cultured explant.

In the present study, following the investigation of **Debnath (2005),** root inception was done on MS medium without PGR, where plantlets rooted well **(Fig 3-D)**. The non-prerequisite for an auxin at the rooting stage shows that the plantlets may contain enough auxin **(Murti et al 2012).**

**Table 1.** Effect of thidiazuron (TDZ) treatments on strawberry leaves explant growth parameters after 14 weeks from inoculation.

| TDZ (mmol $l^{-1}$) | Survival % | No of shootlets/ explant | Total shoot | Shoot length (cm) | No of leaves/explant | Root length (cm) | No of roots/explant |
|---|---|---|---|---|---|---|---|
| | 55.00 | 2.7 $^{b}$ | 32.0 $^{b}$ | 5.2 | 11.0 $^{ab}$ | 4.00 | 9.0 |
| 6.75 | 88.67 | 5.0 $^{a}$ | 51.3 $^{a}$ | 5.5 | 12.0 $^{a}$ | 4.50 | 9.3 |
| 9.00 | 77.67 | 4.0 $^{ab}$ | 40.7 $^{b}$ | 5.8 | 08.7 $^{b}$ | 5.17 | 8.0 |
| LSD | N.S | 1.762 | 9.97 | N.S | 2.4 | N.S | N.S |

Means followed by different letters are significantly different

### 3. Biochemical composition

It is obvious from data of **Table (2)** that TDZ treatments significantly enhancing chlorophyll a, b and carotenoid concentrations, total soluble sugars, and phenolic content ratio. The highest content ratio of the biochemical component was observed with 6.75 mmol $l^{-1}$ TDZ. In this respect, **Genkov et al (1997)** indicated that TDZ enhanced chlorophyll substance and this corresponded with changes in chlorophyllase movement. They also mentioned that the use of phenylurea cytokinins as TDZ caused an increment in the chlorophyll a/chlorophyll b ratio. Also, **Oliveira et al (2008)** reported that *Annoma glabra* micropropagated plants showed increasing in carotenoids and total sugars when treated with TDZ.

**Khan et al (2016)** mentioned that the higher amounts of phenolic concentration distinguished in callus culture of *Fagonia indica* when contrasted with those in the control paralleled the inclusion of TDZ in the organogenesis of callus. TDZ may have activated stress on the plant cells during the development of callus; subsequently, the phenyl propanoid pathway may have turned on to deliver an adequate amount of phenolic acids and different antioxidants, to adapt to the pressure condition **(Bhargava et al 2013).**

**Table 2.** Effect of thidiazuron (TDZ) treatments on biochemical composition of strawberry plantlets after 14 weeks from starting

| TDZ (mmol $l^{-1}$) | Chl a | Chl b | Cart. | Total soluble sugars | Phenolic content |
|---|---|---|---|---|---|
| | mg $g^{-1}$ fw | | | | µg $g^{-1}$ fw |
| 4.50 | 0.937 $^{c}$ | 0.526 $^{b}$ | 0.185 $^{b}$ | 14.61 $^{b}$ | 0.103 $^{b}$ |
| 6.75 | 1.270 $^{a}$ | 0.696 $^{a}$ | 0.222 $^{a}$ | 18.38 $^{a}$ | 0.207 $^{a}$ |
| 9.00 | 1.168 $^{b}$ | 0.629 $^{ab}$ | 0.216 $^{ab}$ | 16.95 $^{a}$ | 0.152 $^{ab}$ |
| LSD | 0.099 | 0.154 | 0.035 | 1.442 | 0.056 |

Means followed by different letters are significantly different

## REFERENCES

**Abdelbar O.H. 2017.** Histological analysis of the developmental stages of direct somatic embryogenesis induced from *in vitro* leaf explants of date palm. In: Al Khayri, J.M., Mohan, J.S., Johnson, D.V. (Eds.), **Date Palm Biotechnology Protocols Tissue Culture Applications. I. Springer Press, pp. 145-162.**

**Berlyn G.P., Miksche J.P. and Sass J.E. 1976.** Botanical microtechnique and cytochemistry. Iowa State University Press, Ames, Iowa, USA, **pp. 24-100.**

**Bhandari M. and Roy S.K. 2015.** Standardization and establishment of an efficient protocol for in vitro multiplication of strawberry plant and its genetic stability testing. **Int. J. of Pharmaceutical Sci. Invention, 4(8), 7-12.**

**Bhargava A., Clabaugh I., To J.P., Maxwell B.B., Chiang Y.H., Schaller G.E., Loraine A. and Kieber J.J. 2013.** Identification of cytokinin-responsive genes using microarray meta-analysis and RNA-Seq in Arabidopsis. **Plant Physiology, 162, 272-294.**

**Bunn E., Senaratna T., Sivasithamparam K. and Dixon K.W. 2005.** *In vitro* propagation of *Eucalyptus phylacis* L. Johnson and K. Hill., a critically endangered relict from Western Australia. ***In Vitro* Cell Dev Biol Plant, 41, 812-815.**

**Cappelletti R., Sabbadini S. and Mezzetti B. 2016.** The use of TDZ for the efficient *in vitro* regeneration and organogenesis of strawberry and blueberry cultivars. **Sci. Hortic-Amsterdam, 207, 117-124.**

**Ćosić T., Motyka V., Raspor M., Savic´ J., Cingel A., Vinterhalter B., Vinterhalter D., Tra´vnı´c˘kova´ A., Dobrev P., Bohanec B. and Ninkovic´ S. 2015.** In vitro shoot organogenesis and comparative analysis of endogenous phytohormones in kohlrabi (Brassica oleracea var. gongylodes): effects of genotype, explant type and applied cytokinins. **Plant Cell Tissue Organ Cult 121(3), 741-760.** doi:10.1007/s11240-015-0742-2

**De Rybel B., Adibi M., Breda A.S., Wendrich J.R., Smit M.E., Novák O., Yamaguchi N., Yoshida S., Van Isterdael G., Palovaara J., Nijsse B., Boekschoten M.V., Hooiveld G., Beeckman T., Wagner D., Ljung K., Fleck C. and Weijers D. 2014.** Plant development. Integration of growth and patterning during vascular tissue formation in Arabidopsis. **Sci., B; 345, 1255215, 636-646.**

**Debnath S.C. 2005.** Strawberry Sepal: Another explant for thidiazuron induced adventitious shoot regeneration. ***In vitro* Cell. Dev. Biol. Plant, 41, 671-676.**

**Dhar M. 1998.** Techniques of vegetative and *in vitro* propagation of Jackfruit. **Ph.D. Thesis, Banggabandhu Shaikh Mujibar Rahman Agric. Univ., Salna, Gazipur, Bangladesh, 120 p.**

**Dubois M., Gilles K.A., Hamilton J.K., Rebers P. and Smith F. 1956.** Colorimetric method for determination of sugars and related substances**. Anal. Chem., 28(3), 350-356.**

**Genkov T., Tsoneva P. and Ivanova I. 1997.** Effect of cytokinins on photosynthetic pigments and chlorophyllase activity in *in vitro* culture of axillary buds of *Dianthus caryophyllus* L. **J. Plant Growth Regul., 16, 169-172.**

**Gomez K.A. and Gomez A.A. 1984.** Statistical procedures for agricultural research, 2nd ed. John Willey & Sons, New York, USA, **680 p**.

**Haddadi F., Aziz M.A., Kamaladini H. and Ravanfar S.A. 2013.** Thidiazuron-and zeatin-induced high-frequency shoot regeneration from leaf and shoot-tip explants of strawberry. **HortTechnology, 23(3), 276-281.**

**Husaini A.M., Aquil S., Bhat M., Qadri T., Kamaluddin T. and Abdin M. 2008.** A high-efficiency direct somatic embryogenesis system for strawberry (*Fragaria ananassa* Duch.) cultivar Chandler. **Crop Sci. Biotechnol., 11, 100-107.**

**Jones M.P.A., Yi Z., Murch S.J. and Saxena P.K. 2007.** Thidiazuron induced regeneration of *Echinacea purpurea* L.: micropropagation in solid and liquid culture systems. **Plant Cell Rep., 26, 13-19.**

**Kefford N.P., Zwar J.A. and Bruce M.I. 1986.** Antagonism of purine and urea cytokinin activities by derivatives of benzylurea. **Biochemistry and physiology plant growth substances. pp. 61-69.**

**Khan T., Abbasi B.H., Khan M.A. and Shinwari Z.K. 2016.** Differential effects of Thidiazuron on production of anti-cancer phenolic compounds in callus cultures of *Fagonia indica*. **Appl. Biochem Biotechnol., 179, 46-58.**

**Lichtenthaler H.K. 1987.** Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. **Methods Enzymol., 148, 350-382.**

**Murashige T. and Skoog F. 1962.** A revised medium for rapid growth and bioassays with tobacco tissue cultures. **Physiol. Plant., 15, 473-497.**

**Murthy B.N.S., Murch S.J. and Saxena P.K. 1998.** Thidiazuron: A potent regulator of *in vitro* plant morphogenesis. ***In Vitro* Cell. Dev. Biol. Plant, 34, 267-275.**

**Murti R.H., Debnath S.C. and Yeoury Y.R. 2012.** Effect of high concentration of thidiazuron (TDZ) combined with 1 H-indole-3- butanoic acid (IBA) on Albion strawberry (*Fragaria x ananassa*) cultivar plantlets induction. **African J. of Biotechnology, 11(81), 14696-14702.**

**Oliveira L.M., Paiva R., Santana J.R.F., Alves E., Nogueira R.C. and Pereira F.D. 2008.** Effect of cytokinins on *in vitro* development of autotropism and acclimatization of *Annona glabra* L. ***In vitro* Cellular & Development Biology Plant, Gaithersburg, 44, 128-135.**

**Pirttila A.M., Podolich O., Koskimaki J.J., Hohtola E. and Hohtola A. 2008.** Role of origin and endophyte infection in browning of bud-derived tissue cultures of Scots pine (Pinus sylvestris L.). **Plant Cell Tissue Organ Cult., 95, 47-55**

**Pompelli M.F., França S.C., Tigre S.C., Oliveira M.T., Sacilot M. and Pereira E.C. 2013.** Spectrophotometric determinations of chloroplastidic pigments in acetone, ethanol and dimethylsulphoxide. **Revista Brasileira de Biociências, 11(1), 52-58.**

**Sakila S., Ahmed M.B., Roy U.K., Biswas M.K., Karim R., Razvy M.A., Hossain M., Islam R. and Hoque A. 2007.** Micropropagation of strawberry (*Fragaria x ananassa* Duch.): A newly introduced crop in Bangladesh. **Amer-Eurasian J. Scientific Res., 2, 151-154.**

**San B., Li Z., Hu Q., Reighard G.L. and Luo H. 2015.** Adventitious shoot regeneration from in vitro cultured leaf explants of peach rootstock Guardian is significantly enhanced by silver thiosulfate. **Plant Cell Tissue Organ Cult 120(2), 757-765.** doi:10.1007/ s11240-014-0645-7

**San José M.C., Janeiro L.V. and Corredoira E. 2013.** Micropropagation of threatened black alder. **Silva Fenn, 47, 1-12.**

**Shahidi F. and Naczk M. 1995.** Food phenolics: sources, chemistry, effects, and applications. Technomic Publishing, Lancaster, Pennsylvania, USA. **pp. 481-482.**

**Srivastava L.M. 2002.** Plant growth and development. Academic Press, San Diego, California, USA. **pp. 303- 379.**

**Steel R.G.D., Torrie J.H. and Dicky D.A. 1997.** Principles and procedures of statistics: A biometrical approach, 3rd ed. **McGraw Hill, Inc. Book Co., New York, USA, pp. 352-358.**

**Wang H., Li M., Yang Y. and Jini W. 2015.** Histological and endogenous plant growth regulators changes associated with adventitious shoot regeneration from in vitro leaf explants of strawberry (*Fragaria x ananassa* cv. 'Honeoye'). **Plant Cell Tiss Organ Cult 123, 479-488.** DOI 10.1007/s11240-015-0851-y

**Werner T., Motyka V., Laucou V., Smets R., Van Onckelen H. and Schmu¨lling T. 2003.** Cytokinin-deficient transgenic Arabidopsis plants show multiple developmental alterations indicating opposite functions of cytokinins in the regulation of shoot and root meristem activity. **Plant Cell 15(11), 2532-2550.** doi:10. 1105/tpc.014928

**Werner T., Motyka V., Strnad M. and Schmulling T. 2001.** Regulation of plant growth by cytokinin. **Proc. Natl. Acad. Sci., 98, 10487-10492.**

**Yepes L.M. and Aldwinckle H.S. 1994.** Micropropagation of thirteen Malus cultivars and rootstocks, and effect of antibiotics on proliferation. **Plant Growth Regulation, 15, 55-67.**

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# التغيرات النسيجية المصاحبة لتكوين الافرع العرضية من الاوراق المنتجة معملياً لنبات الفراولة [33]

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**الموجـــــــز**

أجريت تجربة زراعة الأنسجة للتحقق من بدء وتطوير البراعم العرضية من أوراق الفراولة المنزرعة داخل المعمل. تم فصل الأوراق من البراعم المتجددة داخل المعمل، وزُرعت على بيئة موراشيج وسكوك مضاف اليها ثيديازورون 4.5 و 6.75 و 9.0 مليمول/ لتر لمدة ستة أسابيع. ثم نقلت المنفصلات إلى بيئة موراشيج وسكوك (املاح + فيتامينات) خالية من منظمات النمو لمدة 8 أسابيع. تضمنت هذه الدراسة الاستجابات التشريحية والمورفولوجية لأوراق الفراولة مع معاملات ثيديازورون وعلاقتها ببعض المكونات الكيموحيوية. أدت جميع معاملات ثيديازورون إلى ظهور براعم عرضية على الأوراق دون تكوين الكالس. أدي أستخدام ثيديازورون6.75 مليمول/لتر الي زيادة في معظم الخصائص المورفولوجية مقارنة مع بقية المعاملات. أظهرت النتائج أن معاملة ثيديازورون بتركيز 6.75 مليمول/لتر زادت معنوياً من عدد الأفرع الخضرية لكل منفصل نباتي (5.0)، إجمالي الأفرع الخضرية (51.3) ، عدد الأوراق/منفصل نباتي (12) مقارنة بالمعاملات الأخرى في حين إن بقية الخصائص المورفولوجية لم تظهر أي فروق معنوية (النسبة المئوية للبقاء – طول الأفرع الخضرية (سم) – عدد الأوراق/ منفصل نباتي – طول الجذر (سم) – عدد الجذور/منفصل نباتي). حثت معاملة ثيديازورون بتركيز 6.75 مليمول/لتر تراكم الكلوروفيل والسكريات الكلية والفينولات الكلية الذائبة.

أظهرت الملاحظات التشريحية أن البراعم العرضية بدأت من بعض بارانشيما النسيج الأساسي في منطقة العرق الوسطي من المنفصل النباتي، في حين أن بارانشيما النسيج المتوسط وخلايا البشرة لم تشارك في هذه العملية. تطورت البراعم العرضية من خلال مراحل متتالية مع وجود تراكيب تشريحية مميزة لكل مرحلة وهى: مراكز مرستيمية، بدائات براعم خضرية، قمة مرستيمية عادية مغطاة ببدائات أوراق، استطالة وتفرع براعم عرضية. بعد أربع أسابيع من الزراعة، لوحظت انقسامات لخلايا بارانشيما العرق الوسطي لتتكون مجموعات صغيرة من المراكز المرستيمية. هذه المراكز تكون كتلًا مرستيمية جديدة مدمجة في النسيج الأساسي لمنطقة العرق الوسطي. بعد ستة أسابيع، أدى المزيد من التطور لهذه الكتل إلى تكوين بدائات لبراعم خضرية ذات قمة مرستيمية عادية مغطاة ببدائات أوراق تنشأ من المنفصل النباتي على شكل نتوءات صغيرة. بعد أسبوعين من إعادة الزراعة على بيئة خالية من منظم النمو ثيديازورون، استمرت البراعم العرضية في الاستطالة لتشكل فروع جانبية جديدة ولاحظت بوضوح على الجانب القريب من المنفصل النباتي. يوفر هذا الإجراء طريقة بسيطة وسريعة لتجديد نباتات الفراولة من خلال التجديد المباشر للأعضاء الخضرية.

**الكلمات المفتاحية:** فراولة، فراجاريا أناناسا، ثيديازورون، أفرع عرضيه، التغيرات النسيجية

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