FINAL TECHNICAL REPORT FOR NASA PROJECT NCC 2-726, entitled:

"CULTIVATION OF TOMATO TISSUES CAPABLE OF FORMING FLOWERS AND FRUITS IN VITRO"

(The final extension of this grant terminated on October 31, 1995)

The final phase of this research project was designed to develop a practical method for producing a steady supply of fresh cherry tomato fruits over a period of several months, for possible use as a fresh vegetable supplement to a standard diet of astronauts on extended missions. This effort was successful.

We were able to excise immature flowers from Pixie tomato plants grown in a controlled ccondition room, implant them on arftificial media under aseptic conditions, and get them to develop into edible fruits in a little over a month. The medium (Murashige-Skoog) was purchased from Sigma, supplemented with sugar plus a synthetic analog of the plant hormone cytokinin, and adjusted to pH 5.8. A temperature of at least 25 degrees centigrade and visible light helped to produce ripe red fruits within 7 weeks.

To ensure a steady supply of such tomatoes, we found it possible to store the explanted flower buds in MS medium at 5 degrees centigrade for at least 6 weeks without significant loss of ability to develop into fruits. This means that many containers could be prepared before launch and put into a refrigerator; a convenient number could then be removed periodically to guarantee a succession of harvests during the life of an extended mission. Details are found in the attached reprints. Subsequent applications for funds for flight or continued research were denied, and the project was terminated.

Arthur W. Galston
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Formation *in vitro* of ripe tomato fruits from thin layer explants of flower pedicels

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Abstract

Thin longitudinal sections cut from pedicels of fifteen cultivars of tomato (*Lycopersicon esculentum*) were grown *in vitro* on Murashige-Skoog medium supplemented with various concentrations of different auxins and cytokinins. Isatin (an auxin precursor slowly converted to an active auxin) was the most effective source of auxin for the formation of buds without prior root formation, while zeatin was the most effective cytokinin for growth and development of the buds. Flower buds and ripe fruits developed consistently from explants of the cultivar Pixie Hybrid II treated with 10 μM isatin plus 3 μM zeatin as the cytokinin. Fruits developed parthenocarpically, grew to a diameter of about 15 mm, ripened promptly, and possessed normal color and flavor.

Abbreviations: BAP = benzylaminopurine; IAA = indole-3-acetic acid; IBA = indole-3-butyric acid; IPA = isopentyladenosine; NAA = α-napthaleneacetic acid

1. Introduction

The thin-layer culture technique, first described by Tran Thanh Van and coworkers [24, 26] and reviewed recently by Compton and Veilleux [9] was at first used primarily as a tool for the study of morphogenesis in *Nicotiana*. Explants from pedicels or peduncles, generally containing 3–10 layers of epidermal and subepidermal cells, produced calli, roots and ultimately vegetative and reproductive buds, depending primarily on the auxin:cytokinin ratio in the medium, and also on polyamine titer [15, 23] of the tissue. Later studies extended the use of this technique to other plants, including *Cichorium intybus* [4], *Nautilocalyx lynchei* [25], *Petunia hybrida* [17], *Torenia fournieri* [7] and *Lycopersicon esculentum* [2, 8], all of which formed flower buds. In *Nicotiana*, pollination of flowers formed *in vitro* resulted in the formation of mature fruits with viable seeds [27].

The present work, describing the application of the tobacco thin-layer culture technique to the production of edible fruit in another solanaceous plant, the patio size tomato (*Lycopersicon esculentum* cv. Pixie Hybrid II), arose from an appreciation of the needs of our space program for readily-available fresh vegetables to supplement the diets of astronauts during prolonged spaceflight. Over the past few years, techniques have been developed for the growth of such major food crops as dwarf wheat [5], rice [14] and potato [29] in NASA life support units designated as Controlled Ecological Life Support Systems (CELSS) [13]. Such units utilize photosynthetically active green plants to produce food, oxygen and potable water while absorbing carbon dioxide and light quanta, and recycling minerals from vegetable and human waste produced by the crew. In theory, CELSS units could function indefinitely, satisfying the caloric needs of astronauts subjected to prolonged space travel. Recently, emphasis has also been placed on the production of fresh vegetables because of their great nutritional and psychological value to members of the crew during prolonged space flight [1].

This contribution describes the rapid production of cherry tomato sized fruits *in vitro* through use of a thin-layer explant culture technique that could easily be adapted for use for the continuous produc-

tion of edible fresh plant material during prolonged spaceflight. The use of *in vitro* techniques for food production in a CELSS has at least two advantages: 1) Because there are no roots, stems or even leaves on most of these cultures, they can be packaged conveniently and economically in light plastic containers to conserve space and mass, both of which are critical for extended spaceflight missions. 2) The maintenance of asepticity guarantees that at least part of the plant material carried in a CELSS will be protected against infection by microorganisms [10] that may develop to critical levels during prolonged recirculation of liquid media in CELSS-supported missions.

2. Materials and methods

2.1 *Plant material*

Fifteen cultivars of *Lycopersicon esculentum* were grown from seeds in a controlled growth room on a 16L:8D photoperiod with a 9:1 watt:watt energy mixture of fluorescent and incandescent light, 20 W m^{-2} at 27 °C. From WA Burpee and Co, Warminster, PA 18974, we obtained seeds of the cultivars Gardeners's Delight, Golden Tomboy, Pixie Hybrid II, Small Fry, Sundrop and Sweet 100; from Geo W Park Seed Co, Greenwood, SC 29647 we obtained Better Bush and Early Girl; from Stokes Seeds Ltd, Buffalo, NY 14240 we obtained Cherry Gold, Red Robin, Stakeless, Tiny Tim and Yellow Canary; from Thompson and Morgan, Jackson, NJ 08527 we obtained Red Alert and a mutant strain designated LA 2705. Plants were grown in plastic pots containing washed vermiculite and were subirrigated twice daily with 1.2 g L^{-1} solution of 'Hyponex' (Hydroponics Chemicals Co, Copley, OH). Explants, harvested 6–12 weeks after seed planting, were taken from pedicels taken from below the abscission layer of flowers at the pre-anthesis, anthesis, post-anthesis, and green fruit stages. In addition, pedicels from above the abscission layer, and occasionally anthesis stage peduncles were also selected from the same aged plants.

The explants were first washed several times with tap water, then sterilized for 10 minutes in a solution containing 10% Clorox (0.5% sodium hypochlorite), 30 USP units of Nystatin mL^{-1} and one drop of Tween-20 per 100 mL. After four rinses with sterilized water, longitudinal sections, about 2 mm wide and 10 mm long, consisting of approximately 15 surface cell layers (somewhat thicker than the usual thin layer explant) together with cortical and vascular tissue, were cut from the pedicels and peduncles, and placed, cut side down, on the surface of agar medium in capped, but unsealed test tubes (25 × 100 mm Pyrex, 1 explant each in 10 mL medium) or Phytatrays (Sigma, 114 × 86 × 65 mm polystyrene, 10 explants each in at least 50 mL medium). Both types of culture containers produced acceptable and equivalent results. For most experiments a modified Murashige and Skoog (MS) [19] medium (Sigma, MS 5519) plus 3% sucrose with various combinations of auxins and cytokinins was used (Table 1). The pH of the medium was generally adjusted to 5.8 with KOH after the addition of 0.6% agar. The effects of pH (4.8 to 7.4), increasing the vitamins (nicotinic acid, 1 mg L^{-1}; pyridoxine, 1 mg L^{-1} and thiamine, 10 mg L^{-1}), substituting glucose (1.5 or 4%), changing sucrose (1–5%) and adding activated charcoal (0.03 or 0.3%) on *de novo* flower bud formation were also studied.

The cultures were grown in growth cabinets at 27 °C under a 16 h photoperiod (cool white fluorescent tubes, 10 W m^{-2}). Changes in the light intensity (5 W m^{-2}), duration (12 h) and quality (Sylvania GRO Lux) were also studied to examine possible effects on the induction and development of flower buds. Twice-weekly observations were made on *in vitro* morphogenesis of flower buds and fruit formation in 15 tomato cultivars. Each experiment was repeated 2 to 4 times, with at least 20 explants per treatment. Data represent means ± SE.

2.2 *Chemicals*

All chemicals, including nutrient solutions and hormones were purchased from Sigma Chemical Co.

3. Results

3.1 *Optimization of medium*

As reported earlier [2] and confirmed in the present experiments, thin layer explants from pedicels or peduncles produced callus tissue best when grown on modified MS medium with 3% sucrose, 0.6% agar and pH adjusted to 5.8. Depending somewhat on the auxin and cytokinin concentrations, *de novo* root and shoot formation were frequent [2], while flower bud formation occurred sporadically, depending not only on the hormone concentrations and environmental conditions, but also on the cultivar used. Substituting

Table 1. Competence of tomato (Pixie Hybrid II) explant source to form flower buds when cultured in 3 μM zeatin and 10 μM isatin. For each treatment at least 30 explants were used

Source of explant	Percent of total explants with flower buds		
	Maximum	Mean	S.E.
Pre anthesis pedicel below abscission zone	45	24	7
Anthesis pedicel below abscission zone	50	28	6
Post anthesis pedicel below abscission zone	40	23	8
Green fruit pedicel below abscission zone	0	0	0
Anthesis pedicel above abscission zone	7	4	2
Peduncle	20	6	6

glucose, changing the sucrose or vitamin concentrations, or adding activated charcoal to the culture medium did not increase the number of flower buds (data not shown). Flower bud formation was inhibited at the lowest pH (4.8) tested, but remained stable within the pH range of 5.8 to 7.4.

3.2 *Competence of explants from various sources*

As Table 1 indicates, explants taken from the pedicel below the abscission zone were more competent to form flower buds than explants taken from the peduncle, while explants taken from the pedicel above the abscission zone seldom produced floral buds. Competence for induction of flower buds from below-abscission-zone pedicel explants remained constant from pre-anthesis to post-anthesis stages, but similar pedicel explants taken from the green fruit stage were incapable of producing flower buds. The age of the flower cluster on any plant had no effect on the competence of the explant to produce flower buds. Paired longitudinal sections of the same pedicel often did not produce the same number of flower buds or shoots when treated similarly, presumably because of differences in tissue sampling or wounding of members of the pairs.

3.3 *Effects of auxins and cytokinins*

Flower bud formation from anthesis stage pedicel explants occurred on culture media containing several combinations of auxins and cytokinins (Table 2). The mean frequency of explants producing floral buds ranged from 5–26% depending on the growth regulators used, and was highest with 10 μM isatin (indole 2,3-dione; [6]) and 3 μM zeatin (Table 2). While the variability is high, up to 50% of the explants produced floral buds. Earliest floral bud formation was achieved at 9 days with 3 μM isatin and 1 μM zeatin but only about a maximum of 10% of the explants formed flowers at these concentrations (Table 2). *De novo* flower bud formation with similar maximum efficiency occurred with most of the other hormone concentrations. Initiation of flower buds was not observed when 2,4-dichlorophenoxy acetic acid (0.3 to 10 μM) or gibberellic acid (1 to 100 μM) were used in combination with zeatin at 3 μM (data not shown). Zeatin appeared to be the best cytokinin because it produced the maximum frequency of buds (50%) and also in combination with many auxins, formed buds in at least 10% of the cultures (Table 2).

3.4 *Origin and development of flower buds to ripe fruits*

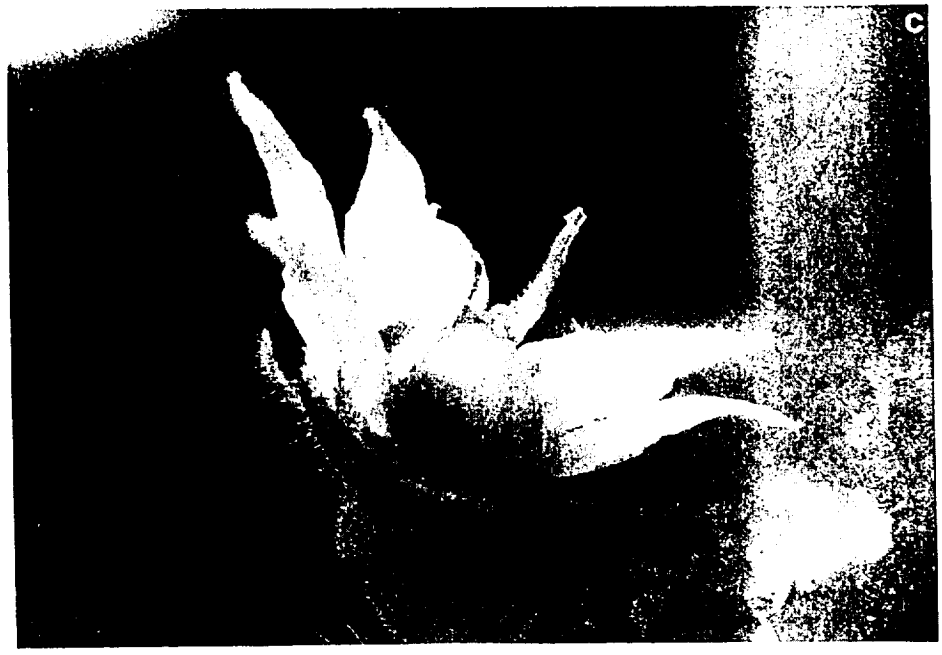
Floral buds appeared to arise in clusters directly from the thin layer explant (Fig. 1a), as seen by sections of the callus, or accompanied by shoots (Fig. 1b) or from shoots with leaves. In some cultures, the floral buds were small, grew in clusters mainly from shoots and did not develop further. Usually only the larger buds without shoots developed to the anthesis (Fig. 1c) and green fruit stage (Fig. 2a) and some eventually formed ripe fruits (Fig. 2b). However when individual small buds were excised and grown on medium containing 1 μM BAP, about 66% buds developed to flowers which grew to form ripe fruits.

Ripe fruits (Fig. 2b) were obtained with a frequency of about 1% in experiments with pedicel explants from the cultivar 'Pixie Hybrid II' cultured on a medium containing 10 μM isatin and 3 μM zeatin. The ripe fruits were parthenocarpic, and attained a maximum diameter of about 15 mm. With these grown regulators, flower buds were usually induced after about 30 days in culture and ripe fruit developed about 45 days later.

Fig. 1. Initiation and development of flower buds on tomato ('Pixie Hybrid II') pedicel explants grown on MS medium containing 10 μM isatin and 3 μM zeatin (for details see Materials and methods). (a) and (b) Development of flower buds in clusters without and with leaves respectively. (c) An induced flower bud in bloom.

3.5 *Cultivar specificity*

Since the maximum flower bud formation occurred with 10 μM isatin and 3 μM zeatin in 'Pixie Hybrid II', this combination of growth substances was tested on the other cultivars. Besides 'Pixie Hybrid II', flower buds formed on pedicel explants from only two other cultivars, Yellow Canary and LA 2705 (mutant), but



not from the explants of the other 12 cultivars. In these two cultivars the maximum frequency of bud formation never exceeded 10%, and the flower buds did not develop into ripe fruits. Clearly there is considerable variation in behavior between cultivars.

4. Discussion

In an earlier investigation [2] we reported the regeneration of complete plants from *in vitro* culture of thin layer explants of tomato pedicel. We now report

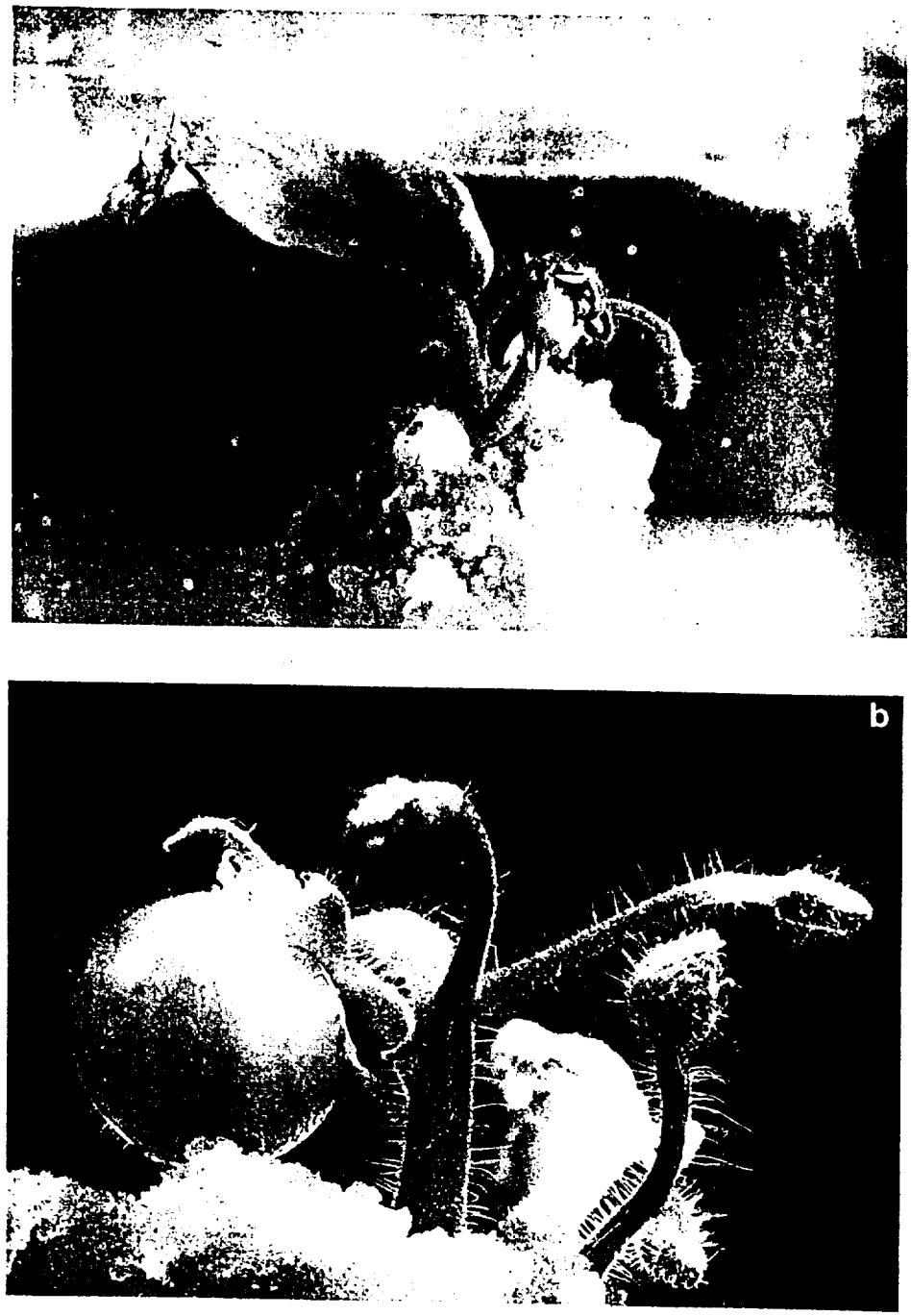


Fig. 2. Stages in the development of ripe fruit on tomato ('Pixie Hybrid II') pedicel explants. Culture conditions as in Figure 1. **(a)** Formation of floral buds one of which developed to the green fruit stage. (b) Formation of a ripe fruit about 75 days from start of culture.

Table 2. Floral bud formation from thin layer pedicel explants of tomato ('Pixie Hybrid II'), cultured with auxin and cytokinin concentrations yielding at least 10% frequency of flower production from the total number of explants. For each treatment at least 20 explants were used

Auxin(μM)	Cytokinin (μM)	Day of first floral bud appearance	Percent of total explants developing flower buds		
			Maximum	Mean	S.E.
None	Zeatin (3)	18	12	10	4
Isatin (3)	Zeatin (1)	9	10	5	3
Isatin (10)	Zeatin (3)	28	50	26	6
Isatin (100)	Zeatin (3)	35	25	25	0
IBA (3)	None	32	10	5	4
IBA (3)	IPA (1)	32	20	20	0
IBA (1)	Zeatin (1)	27	10	10	0
IBA (1)	Zeatin (3)	18	14	13	6
IBA (3)	Zeatin (3)	25	12	8	4
IAA (10)	Kinetin (10)	41	10	10	0
IAA (0.3)	Zeatin (3)	40	10	5	4
IAA (1)	Zeatin (3)	31	10	5	4
IAA (3)	Zeatin (3)	40	10	10	0
NAA (0.1)	BAP (1)	37	10	10	0
NAA (0.1)	Zeatin (3)	31	10	5	4
NAA (0.3)	Zeatin (3)	31	10	7	7

the direct formation of ripe tomato fruit from flowers induced on similar thin layer explants. *De novo* floral bud formation from thin layer explants is difficult to achieve in comparison with shoot and root formation and has been thus far successfully achieved in only a few genera, described in the Introduction. Mature fruits and seeds have been reported thus far only from tobacco thin layer cultures [27]. Ripe fruit development from flower buds induced *de novo* on thin layer explants is apparently even more difficult to achieve, and has to our knowledge not yet been reported for any edible fruits. In this study we report the formation of flower buds from pedicel explants of 'Pixie Hybrid II' in cultures containing several combinations of growth substances. Some of these flowers developed to green fruits which eventually ripened. Recently, Compton and Veilleux [8] reported development of green fruit on elongated shoots with several developed leaves starting with pedicel explants of the tomato cultivar 'Red Alert'. In view of the variation within a cultivar, it is not surprising that we were not able to get flower buds from pedicel explants with this cultivar, but did observe shoot formation.

Isatin, indole 2,3-dione, used in combination with the cytokinin zeatin, was most effective in supporting *de novo* floral bud formation, some of these flowers developing into ripe fruits. In general, no root initiation occurred in cultures in which isatin was the sole auxin. Isatin has previously been shown to act as an auxin after a time lag, based on its promotion of the straight growth of oat coleoptile and etiolated and green pea stem sections, and its facilitation of cell division in excised *Pelargonium* pith callus cultures [6, 12]. During studies of the metabolism of ^{14}C-labeled isatin in pea stem sections, it was found [16] that isatin is converted to isatic acid (an active auxin) as well as tryptophan, itself a probable precursor of IAA. Thus, the unique efficacy of isatin in the present study is probably due to its action in providing a steady source of a low concentration of auxin, rather than a sudden high concentration followed by a decline as a culture is initiated and allowed to age.

Several other auxins, when used at 0.1 to 10 μM in combination with cytokinins, were effective in flower bud formation with tomato pedicel explants. In tobacco, by contrast, auxin levels higher than 1 μM inhibited flower bud development on inflorescence stem thin layer sections [15, 23]. We [15, 23] and others [24, 26] found that changing the concentration of auxins from 1 μM to 10 μM inhibited flower bud formation in favor of vegetative shoot formation on thin layer explants of inflorescence stalks of tobacco.

In contrast, high auxin levels were required for flower formation in tomato pedicel explants in the experiments of Compton and Veilleux [8].

Flower initiation and morphogenesis are complex phenomena known to be regulated by genetic [18], hormonal, nutritional and environmental factors [3, 11, 22, 28]. In our experiments, only a few of the flower buds induced to form on the explants were able to enlarge and form ripe fruits. Furthermore, many other auxin-cytokinin combinations tested failed to produce any flower buds. We hypothesize that a population of morphogenetically competent cells with the potential to differentiate must be present in the cut section, in direct contact with the culture medium, for flowers to develop. There is no clear evidence yet of the differentiation of callus cells formed from the thin layer explant. Further improvement in the rate of conversion of flowers to fruits now seems possible since the transfer of induced flower buds from the thin layer explants to cultures containing 1 μM BAP overcomes any block in the isatin and zeatin culture medium and allows further development of the flower buds to ripe fruits.

It appears likely that the efficiency of the development of flower buds to the fruit stage is low because the type or concentrations of growth substances needed for ripening of the ovary into a fruit differ from those required for floral bud initiation. One would not expect that one fixed concentration or set of hormones would carry the thin layer explants through callus growth and floral initiation all the way to ripe fruit formation. For example, while studying the development of excised floral buds of tomato cultured *in vitro*, Rastogi and Sawhney [20, 21] reported that BAP was the most effective and zeatin the least effective cytokinin for development of flower buds to maturity, yet we have consistently found zeatin best for floral initiation *in vitro*. Auxins also differ in their effects on the *in vitro* growth and development of floral buds; they have been sometimes reported as essential for initiation and growth of some floral organs but in other cases as either ineffective or inhibitory. Thus, although the frequency of the development of ripe fruit from thin layer explants is low at present, there is a potential for substantial improvement by manipulation of the hormonal, nutritional and environmental conditions.

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Isatin as an auxin source favoring floral and vegetative shoot regeneration from calli produced by thin layer explants of tomato pedicel

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Abstract

Thin layer explants taken from the pedicels and peduncles of flowering tomato plants yielded calli with great organogenetic potential. Of the 15 cultivars tested, 7 regenerated roots, shoots and eventually entire fruit-bearing plants. Calli grown on modified Murashige-Skoog medium responded to varied auxins and cytokinins with different morphogenetic patterns. Thus, naphthaleneacetic acid yielded root-producing calli, while the auxin precursor isatin (indole 2,3–dione) caused the production of calli with vegetative and floral shoots, rarely yielding roots. This may be related to isatin's slow, steady conversion to an active auxin (Plant Physiol 41:1485–1488, 1966) in contrast with naphthaleneacetic acid's immediate presentation of a high level of active auxin. The highest incidence of vegetative shoot (100%) and flower (50%) formation was obtained with 10 μM isatin and 3 μM zeatin. A few of the flowers developed into ripe fruits. The high frequency of induction of vegetative shoots and flowers before roots with isatin suggests its utility in micropropagation from plant tissue cultures.

Abbreviations: BAP = benzylaminopurine; 2, 4-D = 2, 4-dichlorophenoxyacetic acid; GA_3 = gibberellic acid; IAA = indole-3-acetic acid; IBA = indole-3-butyric acid; IPA = isopentyladenosine; KN = kinetin; NAA = naphthaleneacetic acid.

Introduction

The study of direct *in vitro* organogenesis has been advanced by the development of techniques in which cultures are started from thin layer explants, generally of flower stalks [17]. The *de novo* production of shoots, roots and flowers from calli produced by cultures from such thin layer explants is generally regulated by manipulating the auxin-cytokinin ratio [18], although the addition of spermidine may also lead to flower production under some circumstances [9]. In cultures derived from thin layer explants of inflorescence stalks of tobacco cv Wisconsin 38 [9, 16], equimolar NAA and KN in the micromolar range produce floral buds exclusively, while raising the concentration of KN tenfold leads to shoot development. These results agree with several other reports on tobacco thin layer tissue culture [17, 18, 20]. Direct organogenesis from thin cell layer explants has also been reported in many other plants including *Begonia rex* [4], *Beta vulgaris* [6], *Brassica napus* [10], *Bryophyllum daigremontianum* [1], *Psophocarpus tetragonolobus* [19] and more recently *Petunia hybrida* [12] and *Lycopersicon esculentum* [5].

Since *de novo* flower formation from thin layer explants of tomato might be useful in elucidating details of its floral initiation and morphogenesis, and since regeneration of plants through root and shoot organogenesis *via* thin layer explants could facilitate propagation of new genomes produced by genetic transformation, we undertook this study. Our aim was to develop techniques for efficient *in vitro* formation of roots, shoots, and flowers and regeneration of complete

tomato plants from thin layer explants of inflorescence tissue.

Materials and methods

Plant material

The following fifteen cultivars of *Lycopersicon esculentum* were studied: Baxter's Bush, Cherry Gold, Gardeners Delight, Golden Tomboy, Kodine, LA2705, Pixie Hybrid II, Red Alert, Red Robin, Small Fry, Stakeless, Sundrop, Sweet 100, Tiny Tim and Yellow Canary. They were grown in a controlled growth room under a 16L/8D photoperiod with a 9:1 (watt:watt) energy mixture of fluorescent and incandescent light at a fluence rate of 17.6 Wm^{-2} at 27 °C. All cultivars flowered continuously at maturity. Plants were grown in plastic pots containing washed vermiculite and were subirrigated twice daily with 1.2g/L solution of 'Hyponex' (Hydroponics Chemicals Co, Copley, OH). Plants with one to many inflorescences, some containing young green fruits, were selected for explantation. The peduncles and pedicels from flowers at various stages of anthesis or fruit formation were excised, rinsed several times with tap water and sterilized for 10 min in a solution containing 7% Clorox (0.4% sodium hypochlorite), Nystatin (30 USP units/ml, Sigma) and one drop of Tween 20 per 100 ml, then rinsed four times in sterile distilled water. Longitudinal strips (about 2 mm wide × 10 mm long) of pedicel or peduncle tissue, containing epidermal, cortical and occasionally vascular tissue (approximately 15 cell layers) were cut and placed into either Pyrex culture tubes (20 × 100 mm, 1 explant in 10 ml of medium) or polycarbonate Phytatrays (Sigma, 6–10 explants in 50 ml medium). The culture medium was a modified MS 9274 (Murashige and Skoog, 13, Sigma) including 3% sucrose, 0.8% agar and various combinations of auxins and cytokinins (Tables 1 and 2). The pH of the medium was adjusted to 5.8 with KOH or NaOH. Cultures were grown in controlled growth cabinets under a 16h photoperiod of white fluorescent light, 50 Wm^{-2} at 27 °C. Changing the light quality, intensity or duration did not affect the growth of the cultures, which were examined weekly under a dissection microscope for evidence of roots, shoots or floral bud formation. The regenerated seedlings from these cultures were transfered to vermiculite and grown in the controlled growth room to the ripe fruit stage. Each experiment was repeated at least twice. Competence for flower, shoot and root formation and plant regeneration was tested in 15 tomato cultivars selected mainly because of early ripening of cherry-type tomatoes. Of all the varieties, Pixie Hybrid II gave the best results; accordingly, the data presented below deal with this variety.

Chemicals

All chemicals, including nutrient solutions, were purchased from Sigma.

Results

Root organogenesis

De novo root formation was observed from both pedicel and peduncle inflorescence explants grown on MS basal medium supplemented with various auxin : cytokinin ratios or with gibberellic acid. NAA in combination with different cytokinins (Kn, zeatin, IPA and BAP) was most effective (Table 1) when compared with IAA, while 2, 4-D and GA_3 were least effective. Early and vigorous root formation occurred with low to medium (0.1 – 3 μM) levels of auxins and cytokinins while higher concentrations of cytokinins delayed or inhibited rooting (data not shown). In contrast, root formation in the presence of isatin and zeatin was always either totally inhibited or greatly delayed, occurring after the appearance of vegetative and floral buds. IBA, when used alone (3 μM to 10 μM) to induce rooting, caused the earliest appearance of roots (4 days, data not shown), but was ineffective in combination with the cytokinins. There were no significant differences in the effects of these growth substances on the ability of any cultivar explant to produce roots. In several instances abundant aerial roots developed directly from the explants.

Shoot organogenesis

In general, medium to high concentrations of cytokinins (3 μM to 10 μM) with low to medium concentrations (0.1 μM to 3 μM) of all auxins, were most effective in causing direct shoot formation (Table 2). Isatin, an auxin precursor rather than an immediately active auxin [3], was required in higher concentrations. Vigorous and early *de novo* shoot formation occurred consistently after about 13d (Table 2) when isatin was used as an auxin source in combination with zeatin as

Table 1. Root organogenesis on cultures derived from Pixie Hybrid II tomato pedicel explants with different concentrations of NAA and cytokinins. All combinations of hormone concentrations listed below produced roots on more than 50% of the calli derived from pedicel explants. For each treatment 10 to 12 explants were used

Auxin		Cytokinin	
Type	μM	Type	μM
NAA	0.3	BAP	0.3
	0.3	BAP	1.0
	0.3	IPA	1.0
	0.3	IPA	3.0
	0.3	IPA	10.0
	0.3	Kn	0.3
	0.3	Kn	10.0
	1.0	Kn	0.3
	1.0	Kn	10.0
	3.0	Kn	0.3
	3.0	Kn	10.0
	10.0	Kn	3.0
	10.0	Kn	10.0
NAA	0.1	Zeatin	0.1
	0.1	Zeatin	3.0
	1.0	Zeatin	0.1
	1.0	Zeatin	3.0
	1.0	Zeatin	10.0
	3.0	Zeatin	0.1
	3.0	Zeatin	1.0
	3.0	Zeatin	3.0

Fig. 1. Formation of floral buds on tomato (Pixie Hybrid II) callus developed from thin layer pedicel explants grown on MS (9274 Sigma) medium containing 3 μM zeatin and 10 μM isatin. Flower buds occurred on 6 week old cultures.

a cytokinin; abundant shoot formation also occurred when BAP was substituted for zeatin. The frequency of shoot induction with isatin ranged from 70 to 100%, with several shoots per explant. NAA in combination with zeatin was more effective than IBA or zeatin alone, while IAA in combination with Kn also yielded shoots with an efficiency ranging from 70 to 90%. Neither GA_3 nor 2, 4-D in combination with various cytokinins was effective in producing shoots. As with root organogenesis, explants from all 15 of the selected tomato cultivars were capable of producing shoots when cultured in various concentrations of auxin and cytokinin.

Floral organogenesis

Flower formation from inflorescence explants occurred in about 50% of the cultures when isatin (10 – 100 μM) was combined with zeatin (3 μM). The combination of zeatin with IAA, IBA and NAA also produced flowers on about 10 – 20% of the cultures. As with vegetative shoot formation, zeatin was the most effective cytokinin. The number of floral buds per explant ranged from 1 to 8 (Fig. 1). In a few instances, the floral buds opened and the pistils developed into ripe, parthenocarpic fruits, about 15mm in diameter, but in the majority of cultures, the flower buds were small and did not develop further, or yielded abnormal flowers that did not develop into fruits. No flower formation

Table 2. Shoot organogenesis on calli derived from Pixie Hybrid II tomato pedicel explants, with auxin: cytokinin ratios yielding at least 50% frequency of shoot production. For each treatment 10 to 12 explants were used

Auxin(μM)	Cytokinin (μM)	Shoot appearance day	% efficiency
IAA, 3.0	BAP, 10	29	62
Isatin, 30.0	BAP, 10	27	90
Isatin, 100.0	BAP, 10	27	70
NAA, 0.1	BAP, 10	18	50
IAA, 0.3	Kinetin, 10	28	70
IAA, 1.0	Kinetin, 10	23	78
IAA, 3.0	Kinetin, 10	23	80
IAA, 10.0	Kinetin, 10	23	90
IBA, 3.0	Kinetin, 10	24	50
	Zeatin, 3	18	67
IBA, 1.0	Zeatin, 3	21	75
Isatin, 10.0	Zeatin, 3	13	100
Isatin, 100.0	Zeatin, 3	13	100
NAA, 0.1	Zeatin, 3	16	80
NAA, 0.3	Zeatin, 3	16	90

was observed at all when either 2, 4-D or GA_3 was used in combination with any cytokinin. Thus far, *de novo* floral bud formation has occurred only on explants obtained from the cultivars Pixie Hybrid II, Yellow Canary and LA 2705; in all three, the pedicel was more effective in flower formation than the peduncle. Details of floral organogenesis from pedicel explants will be published elsewhere.

Plant regeneration

Explants showing competence for root and shoot organogenesis developed into seedlings *in vitro* and regenerated into complete plants when transferred to vermiculite and grown in the controlled growth room. These plants showed vigorous growth and produced abundant fruits of 25–50 mm diameter, with viable seeds. Successful plant regeneration from inflorescence explants of tomato was achieved from 7 cultivars: Kodine, Golden Tomboy, Pixie Hybrid II, Red Alert, Tiny Tim, Small Fry and Sundrop. Most of the experiments were done with Pixie Hybrid II. The time required for attainment of various developmental stages was as follows: callus, about 10d; shoot and root differentiation, 2 to 3 weeks depending on the hormone ratio; seedlings, about 6 weeks, and complete plants, about 4 months.

Discussion

In general *in vitro* flower development is more difficult to accomplish in culture than other types of morphogenesis, even when inflorescence explants are used to start the cultures. This study extends the range of plants from which inflorescence explants can be used to regenerate plants [6, 10, 14]. In agreement with other reports [2, 5, 9, 17], we found that direct organogenesis, especially formation of flower buds from pedicel explants, strongly depends on the genotype and the nature and concentration of the growth regulator. Of the various auxins tested in the presence of cytokinins, NAA was most effective for root formation and isatin for vegetative shoot organogenesis, the latter of which occurred in most of the cultivars. Flower formation was more restricted, occurring most frequently with isatin and zeatin in the cultivar Pixie Hybrid II. In contrast to root organogenesis, shoot and flower formation never occurred when 2, 4-D or GA_3 were used as growth regulators. As in this study, an influence of the type of explant and genotype specificity for flower formation has been reported in *Brassica* [7] tomato [5], and sunflower [14]. Increased plant vigor in the regenerated plants obtained by *in vitro* propagation of inflorescence explants observed here has also been reported in *Saintpaulia ionantha* Wendl [2].

In the present study, the auxin precursor isatin was most effective in shoot and flower bud formation, especially when used in combination with zeatin. A frequency of almost 100% for *de novo* formation of shoots and about 50% for formation of flower buds in cultures developed from explants grown with the isatin-zeatin combination suggests that isatin is acting as a steady source of auxin supply, rather than as an immediate source of high auxin concentration. Earlier studies [3, 8] reported that isatin acts like an auxin in promoting elongation of etiolated and green pea stem sections and in facilitating division of excised *Pelargonium* pith callus cultures. Isatin (indole 2, 3-dione) lacks several features generally considered essential to an auxin molecule [15], such as a carboxyl group at the end of a carbon chain of certain minimum length. However, studies of the metabolism of ^{14}C-labeled isatin in pea stem sections [11] showed that it is readily converted to the auxin isatic acid, and also serves as a precursor of trytophan, which can then yield IAA. Its action in promoting shoot rather than root initiation may well be related to this role as auxin precursor, rather than as an auxin itself.

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