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Response of *Syngonium podophyllum* L. 'White Butterfly' shoot cultures to alternative media additives and gelling agents, and flow cytometric analysis of regenerants

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Abstract. *Teixeira da Silva JA. 2015. Response of* Syngonium podophyllum *L. 'White Butterfly' shoot cultures to alternative media additives and gelling agents, and flow cytometric analysis of regenerants. Nusantara Bioscience 7: 26-32. Syngonium podophyllum* L. (arrowhead vine) is a popular leafy indoor pot plant whose tissue culture has been established, primarily through *in vitro* shoot culture, but several interesting aspects have not yet been explored. In this study, cv. 'White Butterfly' was used to investigate the response of shoot formation to alternative gelling agents and media additives. Gellan gum (Gelrite®) at 2 g/L resulted in greater leaf production, plantlet fresh weight and higher chlorophyll content (SPAD value) than all other gelling agents tested, including agar, Bacto agar, phytagel, oatmeal agar, potato dextrose agar, barley starch and corn starch, when on a basal Hyponex® (NPK = 6.5: 6: 19; 3 g/L) medium. Several alternative liquid medium additives tested (low and full-fat milk, Coca-Cola®, coffee, Japanese green, Oolong, and Darjeeling teas) negatively impacted plant growth, stunted roots and decreased chlorophyll content (SPAD value) of leaves. Plant growth on medium with refined sucrose or table sugar responded similarly. Poor growth was observed when crude extract from a high rebaudioside-containing stevia (*Stevia rebaudiana* Bertoni) line - an artificial sweetener - was used. Leaf tissue from the control did not show any endopolyploidy but low levels of endopolyploidy (8C) were detected in some treatments.

Keywords: agar, alternative gelling agents, milk, oatmeal agar, table sugar.

INTRODUCTION

Driving down the cost of plant tissue culture is a constant objective of companies and research centers in developing countries (Purohit et al. 2011). Prakash et al. (2004) estimated that chemicals in micropropagation media cost slightly less than 15% of the total cost while the cost of gelling agent per unit media is 73.53%, although that estimate was made in India and is likely to vary from country to country. Alternative low-cost gelling agents have been tested, including sago powder, isabgol husk, guar gum, cassava flour, xantham gum (Maliro and Lameck 2004; Jain and Babbar 2005, 2006; Gour and Kant 2011) and several other media alternatives tested on a hybrid orchid, *Cymbidium* (Teixeira da Silva and Tanaka 2009; Van et al. 2012), papaya and chrysanthemum (Teixeira da Silva 2014a) and also tested on *Syngonium podophyllum* L. (arrowhead vine) in this study. The choice of gelling agent is an important factor determining the success of a plant tissue culture protocol (reviewed by Cameron 2008). The rheological and diffusion properties of gelling agents have been shown to affect shoot development in apple and black locust (Dobránszki et al. 2011) and *Amelanchier canadensis* (Fira et al. 2013), and also influence the incidence of hyperhydricity in *Aloe* (Ivanova and van Staden 2011).

Despite the established tissue culture of *S. podophyllum*, either through somatic embryogenesis (Zhang et al. 2006) or protocorm-like bodies (PLBs; Cui et al. 2008), most commercial tissue culture of arrowhead vine involves the use of shoot tips as clonal propagation units. The use of table sugar or alternative medium additives and gelling agents in the tissue culture of this ornamental and is the fundamental objective of this study. As a practical objective, the ability to identify suitable and effective alternative media additives and gelling agents could potentially allow costs to be reduced.

The objective of this study was thus to test a range of medium additives, some traditional, others not, that might improve shoot and plantlet growth of this ornamental in vitro. These additives formed three groups: (i) gelling agents; (ii) liquids serving as alternatives to water; (iii) table sugar versus regular sucrose. There are two reasons to test these substances: (i) as a purely scientific exploration of substances that have never been tested before in the plant science literature; (ii) to find alternatives that could serve as low-cost options for developing countries.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals and reagents, which were of tissue culture grade or the highest grade available, were purchased from Nacalai-Tesque (Kyoto, Japan), Wako Pure Chemical Industries (Osaka, Japan) or Sigma-Aldrich (St.

Louis, MI, USA), whichever offered the best price at the same grade. Gellan gum (Gelrite®) was purchased from Merck (USA). The following were bought from local supermarkets in Takamatsu (Japan) (brand name indicated in parentheses): low and full fat milk (Meiji), Coca-Cola®, instant, granulated coffee (Nescafé, Nestlé), Japanese green tea (Itoh), Oolong tea (Suntory), Darjeeling tea (Tetley), table sugar (Mitsui Sugar Co. Ltd.), corn starch (A-Price). Stevia (*Stevia rebaudiana* Bertoni) was a Chinese cultivar (high level of rebaudioside) grown under field conditions.

The rationale for the selection of these alternatives is explained next. Milk is a daily commodity around the world that is often discarded in a bid to manipulate milk prices (Food and Water Watch 2010). Milk can thus be a suitable substrate if water is not available. Tea and coffee are well-known antioxidants (e.g., Anissi et al. 2014), and thus by removing reactive oxygen species (ROS) (Korir et al. 2014) from in vitro cultures, their growth may be theoretically improved. The exogenous addition of antioxidants has already been shown to improve the growth of hybrid *Cymbidium* (Teixeira da Silva 2013) and *Brassica napus* (Hoseini et al. 2014) cultures. Stevia is an alternative sweetener to sucrose, being as much as 300-fold sweeter (Meireles et al. 2006). To date, no study has yet explored the extract of this plant as an alternative to sucrose in plant in vitro culture. A cheap and popular drink available globally (every country except for Cuba and North Korea) is Coca-Cola® (http: //en.wikipedia.org/wiki/Coca-Cola), which could serve, in a degassed form, as an alternative to sucrose in plant in vitro cultures.

Plant cultures, explants, and general media preparation

Original in vitro plant cultures and mother (donor) plants (*S. podophyllum* cv. 'White Butterfly') were obtained courtesy of Mr. Kobayashi of the Kobayashi Botanical Gardens (Kan-onji, Kagawa Prefecture, Japan). Plants were established and amassed over several months and maintained as for *Spathiphyllum*, explained in detail in Teixeira da Silva et al. (2006). Initial stock shoots (rootless, 5 cm in length) were transferred to 80 mL of Hyponex® (6.5: 6: 19; 3 g/L; Hyponex, Osaka, Japan) medium solidified with 2 g/L of Gellan gum, at 5 shoots/bottle, with 3% (w/v) sucrose in an air-tight Magenta glass bottle (75 mm wide × 250 mm tall) to multiple stocks. Apical shoots 2 cm long with two fully open/developed leaves were harvested from actively growing clonal cultures and used as the explants for all tissue culture experiments. All media were adjusted to pH 5.8 with 1N NaOH or 1 N HCl prior to autoclaving at 121°C, 100 KPa for 15 min. No plant growth regulators were used and additives (gelling agents or medium additives), which were added prior to autoclaving, were not filter sterilized. Shoots from all controls and treatments were cultured at 25 ± 1°C under a 16-h photoperiod with a light intensity of 45 µmol m^{-2} s^{-1} provided by plant growth fluorescent lamps (Plant Lux, Toshiba Co., Japan). All cultures were initiated simultaneously (within 3 days) and tracked for 90 days.

Substrate, medium additive, and photoautotrophic treatments

Three sets of experiments were established to test the effect of substrate and/or gelling agent and medium additives on shoot growth and plant development. All experiments were repeated in triplicate. In all experiments, 5 shoots were placed in each 500 mL Magenta bottle and grown on 100 mL of Gellan gum (2 g/L)-solidified Hyponex®. In set 1, there was no "standard" control (i.e., treatment against which all other treatments were compared) since no industry standard exists yet; thus treatments were compared against each other. In set 2, distilled water-based medium served as the "standard" or control. In set 3, the "standard" (control) was commercial sucrose.

Set 1 (alternative medium gelling agents). Oatmeal agar (30 g/L), agar, bacto agar, potato dextrose agar and phytagel (all four at 8 g/L), barley starch and corn starch (both at 4 g/L), and Gellan gum (Gelrite®) (2 g/L). Pre-experimental trials were conducted to test which concentration was firm, not rock-hard, nor jelly-like so as to support the growth of shoots and plantlets.

Set 2 (innovative additives). Full and low-fat milk (100%, v/v), Oolong tea (50%, v/v), Coca-Cola®, coffee, stevia extract, Japanese green, Oolong, and Darjeeling teas (2%, v/v). Coca-Cola® was shaken for 24 h prior to use in medium to de-gas the soft drink. A stock solution of teas, stevia extract and coffee was prepared. For coffee and stevia extract, 10 g/L (coffee powder or fresh stevia leaves) was added to boiling water, stirred well for 5 min and then added to culture medium until a 2% (v/v) final concentration was obtained. Similarly, Japanese green tea and Darjeeling tea stocks were prepared by infusing 5 teaspoons of dry leaves or a tea-bag (approx. 5 g), respectively for 3 min in boiling water and then added to culture medium until 2% (v/v) final concentration was obtained. Oolong tea was purchased as a ready-made bottled tea and was used as such in media.

Set 3 (tissue culture grade sucrose vs table sugar). Table sugar was added to medium at the same concentration as sucrose, i.e., 2% (v/v). Sucrose costs 2200 JPN Yen/kg while refined table sugar (sugar-cane-derived) costs 158 JPN Yen/kg (118 JPN Yen = 1 US$; January 2015), i.e., ~14 times higher cost.

Morphological and physiological analyses

The growth and development of plants were evaluated after 90 days following culture initiation, i.e., plating shoot tips (Figure 1A). Survival percentage, total number of newly formed leaves (i.e., excluding the initial two leaves), and total plantlet net fresh weight (FW) were determined. Plantlet survival was calculated based on the percentage of plants being totally green and with root formation. Plantlet height was not measured because of vertical and horizontal expansion of shoot clusters. Chlorophyll content of three random leaves of plantlets from each flask, while still attached to the plant, was measured by a chlorophyll meter (SPAD-502, Minolta Co., Japan) and reported as the SPAD value. The SPAD value is highly correlated with chlorophyll content ($R^2 = 0.89$) and thus serves as a simple and effective way, through SPAD units, to reflect chlorophyll content (Coste et al. 2010).

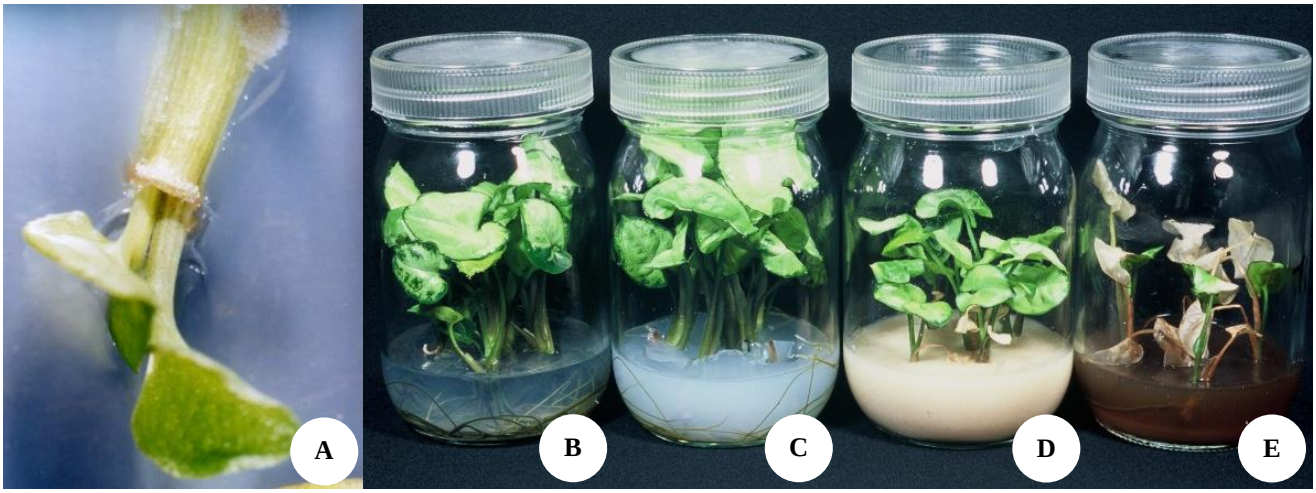

Figure 1. In vitro micropropagation of *Syngonium podophyllum* L. cv. 'White Butterfly'. Initial explants approximately 2 cm long and with two small, fully-developed leaves (A) were grown on several media, including, 2 g/L Gellan Gum-gelled medium (B), 8 g/L agar-gelled medium (C), 100% (v/v) full fat milk, which curdles and hardens after autoclaving (D), and de-gassed Coca-Cola® on 2 g/L Gellan Gum-gelled medium (E), all supplemented with 3 g/L Hyponex® (6.5: 6: 19) and 3% (w/v) sucrose, demonstrating the capacity to grow (or not) on media with different gelling agents/bases and additives, albeit with different growth responses.

Flow cytometry

The protocol follows that used by Teixeira da Silva and Tanaka (2006), but was adjusted for arrowhead vine. Nuclei were isolated from 0.5 cm^2 of leaf material (leaf stalk, lamina and mid-vein, pooled) from all treatments in Table 1 by chopping in a few drops (5-10) of nucleic acid extraction buffer (PartecCystain UV Precise P, Germany), and left to digest on ice for 5 min. Three separate leaves (youngest, developing leaves) from three different plants for each treatment were used. The nuclear suspension was then filtered through a 30 µm mesh size nylon filter (CellTrics®) and five times of Partec Buffer A (2 µg/mL 4,6-diamidino-2-phenylindole (DAPI), 2 mM $MgCl_2$, 10 mM Tris, 50 mM sodium citrate, 1% PVP K-30, 0.1% Triton-X, pH 7.5; Mishiba and Mii 2000) was added at room temperature for 5 min. Nuclear fluorescence was immediately measured using a Partec® Ploidy Analyser and relative fluorescence intensity of the nuclei was analyzed when the coefficient of variation was < 3% by counting a minimum of 5000 nuclei for each sample. Diploid barley (*Hordeum vulgare* L.) cv. 'Ryufu', courtesy of Prof. Shin Taketa (Faculty of Agriculture, Kagawa University), served as the internal control.

Statistical analyses

Experiments were organized according to a randomized complete block design (RCBD, n = 60 per treatment). Each experiment was repeated three times and each experiment had three replicates. For all parameters tested, data analyses were carried out using IRRISTAT version 3.0. Following one-way analysis of variance (ANOVA), Duncan's multiple range test (DMRT) at $P = 0.05$ and the student's *t*-distribution (standard error, Excel 2010) were used to test for differences between means. Percentage values were arc-sin transformed prior to analysis.

RESULTS AND DISCUSSION

Only two studies exist on the tissue culture of *Syngonium podophyllum* (Zhang et al. 2006; Cui et al. 2008). Those studies are important because they indicate protocols for callus formation from somatic embryos and plantlet regeneration from PLBs, respectively, although the terminology pertaining to PLBs in the latter study is being questioned (Teixeira da Silva 2014b). In clonal commercial production, shoot tips are used. This implies that there is a large gap in our understanding of the response of this ornamental in vitro. Thus, by understanding the response of 'White Butterfly', a popular cultivar, to different gelling agents and media additives would narrow this information gap. This study examined the impact of a wide range of substrates and medium additives using the rationale outlined above.

Syngonium shoots grew best on Gellan gum-based medium (Figure 1B), followed by agar-based medium (Figure 1C) but performed poorly on all other agar types. Plantlets performed poorly on medium when supplemented with milk (full-fat or low-fat) (Figure 1D), Coca-Cola® (Figure 1E), coffee or tea (Japanese green, Oolong and Darjeeling teas). The exact reason for this poor performance is not known. However, these additives are not naturally occurring and are man-made, thus one possibility is that enzymes required for their degradation and subsequent uses simply do not exist in plants. For example, in humans, enzymes such as lactase, amylase or catalase (non-exhaustive list) are produced that result in the breakdown of lactose, amylose or hydrogen peroxide in milk. However, while plants produce catalase, lactase and amylase are not, or are rare (e.g., Stano et al. 2011), thus growth in milk might thus be inhibited simply because the plant cannot degrade the substrate to a physiologically

useful form. One way of being able to use milk by plants would be to genetically modify the plant with lactase or catalase gene. Observing trends across treatments for gelling agent (set 1) in Table 1, the following can be concluded: (i) plantlet survival and SPAD value were highest when agar or Gellan gum were used; (ii) most number of new leaves and greatest plantlet FW were obtained when Gellan gum served as the gelling agent; (iii) endopolyploidy was detected in cultures with barley or corn starch or potato dextrose agar. Generally, for the clonal production of plants, changes to morphology, genetics or other performance-related indices are undesired. Therefore, for clonal production, the incidence of endopolyploidy may result in undesirable phenotypes. However, in contrast, in ornamentals, where mutations or induced variations are desired to create novel phenotypes, endopolyploidy may serve a useful function. A wider discussion of endopolyploidy may be found in a recent review by Scholes and Paige (2015). Observing trends across treatments for medium additives (set 2) in Table 1, the following can be concluded: (i) all parameters (plantlet survival, plantlet FW, SPAD value, most new leaves formed) were highest when distilled water served as the basal medium liquid; (ii) endopolyploidy, which is correlated to systematics, organ, life strategy and genome size (Barow and Meister 2003), was detected in cultures grown in the presence of Coca-Cola® or coffee. Finally, in set 3, table sugar could be used as efficiently as tissue culture grade sucrose, resulting in significantly equivalent performance for all parameters observed.

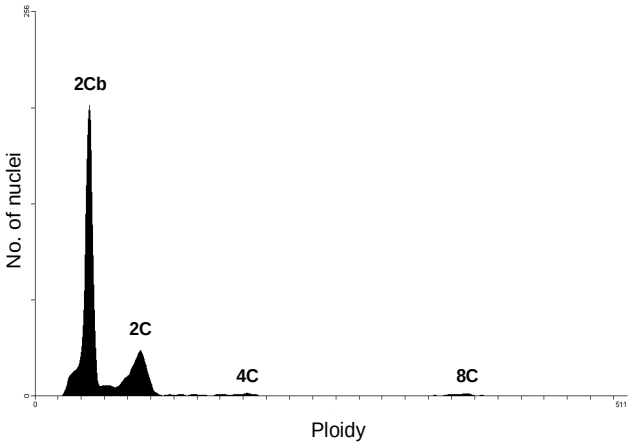

Figure 2. Flow cytometric analysis showing low levels of endopolyploidy (8C) in leaves of *Syngonium podophyllum* L. cv. 'White Butterfly' exposed to treatments marked with * in "Leaf ploidy" column of Table 1. 2Cb = 2C peak for diploid barley (*Hordeum vulgare* L.) cv. 'Ryufu' (internal standard).

Table 1. Effect of alternative gelling agents, medium additives and carbohydrate sources on *Syngonium podophyllum* L.cv. 'White Butterfly' plantlet growth and development, measured 90 days after culture of shoot tips.

Treatments	Plantlet survival (%)	SPAD value	No. new leaves	Plantlet net FW (mg)	Leaf ploidy (2C: 4C: 8C)
Gelling agent[1]					
Agar	91 a	41.6 a	5.2ab	462 b	90 : 10 : 0
Bacto agar	24 cd	36.2 b	1.3 cd	117 d	92 : 8 : 0
Barley starch	31 c	34.6 b	1.1 cd	186 c	90 : 9 : 1*
Corn starch	26 cd	28.0 bc	2.4c	133 d	89 : 8 : 3*
Gellan gum (Gelrite®)	100 a	43.8 a	6.3 a	581 a	88 : 12 : 0
Phytagel	18 d	29.6 bc	4.1 b	126 d	92 : 8 : 0
Potato dextrose agar	0 e	0 d	0.1d	Neg.	93 : 6 : 1*
Oatmeal agar	46 b	24.0 c	0.2d	23 e	91 : 9 : 0
Alternative liquid-based medium additives[1]					
Distilled water-based	100 a	44.2 a	6.4 a	572 a	89 : 11 : 0
Coca-Cola®	0 e	0 d	0 d	Neg.	93 : 6 : 1*
Coffee	0 e	0 d	0 d	Neg.	94 : 5 : 1*
Milk Full-fat	62 c	16.1 c	1.4 a	253 d	95 : 5 : 0
Low-fat	58 d	18.2 c	2.1 bc	286 d	95 : 5 : 0
Stevia extract	64 c	31.6 b	3.4 b	351 c	91 : 9 : 0
Tea Darjeeling	71 bc	33.2 b	3.2 b	401 b	94 : 6 : 0
Japanese green	77 b	31.6 b	2.4 bc	427 b	93 : 7 : 0
Oolong	70 bc	29.8 b	2.2 bc	416 b	94 : 6 : 0
Carbohydrate source[1]					
TC grade sucrose	100 a	44.4 a	5.6 a	546 a	91 : 9 : 0
Table sugar	98 a	43.6 a	5.1 a	532 a	90 : 10 : 0

Note: [1] Exact concentration and methods of preparation are explained in detail in the materials and methods section. Means within a column within each set of experiments (A, B, C) and for each plant character followed by the same letters are not significantly different at P=0.05 by Duncan's multiple range test, n= 60 per treatment. In all cases, 5 shoot tips were plated per culture vessel. Percentage values arc-sin transformed prior to analysis. FW = fresh weight that exceeds the fresh weight of a single "starting" explant (Figure 1A). Neg. = negligible (< 5 mg). Leaf ploidy levels involved the average of three samples (different leaves) per treatment. * indicates the presence of endopolyploidy (see Figure 2). TC = tissue culture.

Most of the factors assessed in this study have been shown to affect the growth of plantlets in vitro of other species, but never all for a single plant. The choice of gelling agent affected organogenesis in hybrid *Cymbidium* plantlet cultures in which Gellan gum resulted, in general, in better plant growth parameters than Bacto agar and oatmeal agar while the number of roots was highest on Gellan gum as was the fresh and dry mass of shoots and roots although more leaves were produced on Bacto agar (Van et al. 2012). The difference in performance, as indicated above for milk, may be related to the lack of suitable enzymes to degrade the substrates into utilizable carbohydrate sources. Gellan gum is a highly pure gelling agent, thus the existence of other impurities in other gelling agents may also be an influential factor. It is for this reason that Gellan gum tends to be more expensive than regular agar, although prices differ widely between manufacturers. In that study, the chlorophyll content of *Cymbidium* plantlets grown in oatmeal agar was lowest among all basal medium treatments; finally, oatmeal agar-based medium strongly inhibited the initiation of new leaves and roots compared to other gelling agents. As observed in this study (Table 1), Gellan gum formed more PLBs that oat meal agar and potato dextrose agar in another hybrid *Cymbidium* (Teixeira da Silva and Tanaka 2009). Thus, in this study, none of the alternative gelling agents tested could support the growth or arrowhead vine as effectively as Gellan gum. However, in countries where only such gelling agents exist, growth is possible, but may account for poor visual aspect or low productivity. Visual aspect is important for this ornamental, which is prized for its visually attractive leaves, but the visual aspect of poor in vitro growth might not be an important issue if such media were to be used for medicinal plants, horticultural crops or agronomic species.

The choice of gelling agent affected adventitious shoot regeneration capacity and water content (i.e., the state of hyperhydricity) of French marigold (*Tagetes minuta*) shoots (Modi et al. 2009) or *Aloe* (Ivanova and van Staden 2011). The higher the agar concentration, the lower the hyperhydricity of *Dianthus caryophyllus* shoots (Casanova et al. 2008). When phytagel was used as the gelling agent, shoots of *Malus x domestica* (apple) (Turner and Singha 1990), *Pyrus communis* (pear) (Kadoka and Niimi 2003) and *Scrophularia yoshimurae* (Tsay et al. 2006) became hyperhydric, but the physiological status of apple and black locust shoots was attributed to the rheological and diffusion properties of the gelling agents (Dobránszki et al. 2011). Van et al. (2012) did not observe hyperhydricity in *Cymbidium* plantlets in any gel-based media. In this study, hyperhydricity was not observed but leaf margins tended to appear "burnt", or dehydrated, possibly due to a lack of moisture in the medium. Seven commercial agar brands caused different organogenic responses in rose (*Rosa hybrid* L. cv. 'Motrea'), lily (*Lilium longiflorum* cv. 'Enchantment') and cactus (*Sulcorebutia alba*) (Scholten and Pierik 1998). *Phalaenopsis* leaf segments obtained from shoots derived from flower-stalk cuttings cultured in vitro on a Gelrite®-solidified medium resulted in the formation of more callus-derived PLBs than when agar was used as the medium solidifying agent (Ishii et al. 1998). Three-fold higher dry weight was observed with tobacco and wild carrot cultures grown on medium gelled with corn starch than on medium gelled with agar (Henderson and Kinnersley 1988). A mixture of corn starch and Gelrite® was a suitable substitute for agar in the in vitro cultivation of apple and red raspberry (Zimmerman et al. 1995). Unlike for arrowhead vine (Table 1), starches from barley, corn, potato, rice, and wheat were all suitable substitutes for agar in the culture of barley (*Hordeum vulgare* L.) seeds, the most effective being that from barley (Sorvari 1986). 'Isubgol', derived from the mucilaginous husk derived from the seeds of *Plantago ovata*, is used in the tissue culture and seed germination of *Syzygium cumini*, *Datura inoxia* (Babbar and Jain 1998), and *Dendrobium chrysotoxum* (Jain and Babbar 2005). The choice of gelling agent affected the regeneration efficiency on selective medium in tulip (*Tulipa* sp.), gladiolus (*Gladiolus* sp.) and tobacco transformation experiments (Chauvin et al. 1999). The level of impurities within a gelling agent might contribute to the outcome of an organogenic pathway, as was demonstrated for *Ranunculus asiaticus* shoots grown in basal medium containing one of three commercial agars (Beruto and Curir 2006). Agar was better than sago powder, guar gum orisabgol husk as a media-solidifying agent for *Balanites aegyptiaca* and *Phyllanthus emblica* (Gour and Kant 2011).

Similarly, where pure water might not be available, or where water may be more expensive than milk, or Coca-Cola®, or rare, such as in countries with desert conditions or limited water supply, low-fat milk can support the growth of arrowhead vine, but not as effectively as a water-based medium. The prices of water - and expensive but essential commodity - due to its link to so many sectors, will undoubtedly increase (Campbell and Tilley 2014), and this may signal the need to find an alternative to water for plant tissue culture systems in the future.

There is always interest in finding alternatives to sucrose as a carbohydrate source for the heterotrophic culture of plants in vitro. In this study, the use of stevia extract or Coca-Cola® as an alternative sugar (carbohydrate) source resulted in poor plantlet growth but table sugar performed as effectively as commercial sucrose (Table 1). In chrysanthemum, other carbohydrates can influence rhizogenesis, caulogenesis (shoots) and somatic embryogenesis (reviewed in Teixeira da Silva et al. 2013), callus and PLB formation in *Cymbidium* hybrids (Teixeira da Silva et al. 2007) and root induction in tree peony (*Paeonia suffruticosa* Andr.) (Wang et al. 2012). There is a possible weakness of this study related to the level at which these liquid additives were added to basal medium and to their osmotic nature. It is possible that tea, at lower concentrations (or higher dilutions), the use of other commercial sodas, or the use of a more dilute concentration of stevia leaf extract may serve as useful forms of carbohydrate and antioxidants in low concentrations for the improved in vitro culture of plants. Evidently, this will require more extensive testing and a wider selection of plants to ascertain the optimal concentrations for practical use in plant tissue culture. If the new substrate has too much of an osmotic gradient, the plant may be stressed as

water flows out from the plant into the medium, thus these substrates and alternatives to plant tissue culture may also serve as new forms of stressors for the study of plant stress physiology. Also, as suggested above, especially for additives like milk, there may be interest in generating transgenic plants carrying the lactase gene to be able to degrade lactose, thus allowing the plant to use this carbohydrate source for growth and development.

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DISCLAIMER AND CONFLICTS OF INTEREST

The author does not specifically endorse any of the brands used in this study. These were used with the pure intention of scientific exploration in mind. The author declares no conflicts of interest, financial or other.

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