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Research article

IN VITRO FLOWERING AND PLANTLETS ELONGATION IN "SUNDEW" *DROSERA CAPILLARIS*

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ABSTRACT: Experimental data pertaining to the possible participation of mineral nutrients, plant growth regulators, particularly gibberellic acid, carbohydrates and vernalization, in flowers or inflorescence initiation and plantlets elongation in "sundew" *Drosera capillaris* (a long-day plant) were obtained. The plantlets were cultivated in MS medium supplemented with 0.01 mg/L NAA, 0.01 mg/L GA_3, 2% sucrose and 0.18% Phytagel under 12/12, 16/8 and 20/4 h (light/dark) photoperiodic conditions at 24-26°C for 90 days. Under very long day conditions (20/4 h) the flowering process was significantly increased (40-60%) when plantlets of *D. capillaries* were cultured on medium MS 1/10 and with addition of gibberellic acid (0.1 and 1.0 mg/L) and sucrose (4-6%). These factors not replace the effect of long days to induce flowering. Both foliar development and root formation was much greater on MS media supplemented with 0.5 mg/L IAA in all photoperiodic conditions.
Key words: Assimilates, Floral initiation, Gibberellic acid, Morphogenesis, Salicylic acid.

INTRODUCTION

Carnivorous or insectivorous plants have attracted considerable attention from many biologists for several hundred years, not only because of their special nutritional requirements and ecological adaptations, but also because of their value as medicinal herbs [1]. This botany group is formed by the Droseraceae, Sarraceniaceae, Nepenthaceae, Byblidaceae and Cephalotaceae families. The genus *Drosera*, which consists of about 125 species [2], represents a really good example of plant evolution and functional adaptation. Numerous *Drosera* species are distributed worldwide across tropical to tundra regions, except for deserts and Antartica, specifically are concentrated in Australia and New Zealand, while others can be found only in America, South Africa or in Europe.

Various *Drosera* species are medicinal herbs containing valuable secondary metabolites. The most important are naphthoquinones, especially plumbagin and 7-methyljuglone and flavonoids. Importantly, naphthoquinones, are thought to be responsible for therapeutic effects against respiratory diseases in humans, including asthma, bronchial infections, whooping cough and tuberculosis. The naphthoquinones, in special plumbagin, also inhibits development of parasitic nematodes and insects. It was also shown that naphtoquinones have antiviral, antibacterial, antifungal, aphrodisiac, antispasmodic, antileprosy, antisclerotic and anticancer properties [3]. Recently *Drosera capensis*, a medicinal plant of South Africa was screened for their antimycobacterial activity, and the MIC was ranged from 0.781 to 6.25 mg/mL against *Mycobacterium smegmatis* [4], and the inhibitory activity of *in vitro* plants from *Drosera capillaris*, collected in the Cauca valley (Cali, Colombia), against *M. tuberculosis* was observed; *M. tuberculosis* growth was inhibited in 40 and 93.1% range and 2.5 and 5 mg/mL treatments, respectively [5].

In addition, the genus *Drosera*, represents an ideal model for developmental studies of plant evolution, including developmental and physiological experiments that could address specific roles for phytohormones in macromorphological evolution [6]. Particularly, is interesting for studies of functional and structural adaptations (including insectivory nutrition and gemmae production) to various environments [3]. Furthermore, this genus with pollen grains permanently organized in tetrads may exhibit an intermediate state in an evolutionary shift in pollination systems from cross-pollination to self-pollination [7].

In vitro flowering has been reported in several plant species. Flowering of *Kalanchöe blossfeldiana* was achieved in response to short-day inductive cycles; nodal explants were cultured *in vitro* on a low nutrient hormone-free medium, and was observed that nitrogen in the form of NH_4NO_3 and KNO_3 promoted flowering and vegetative growth in different ways [8]. Flower buds were induced in zygotic embryos of ginseng (*Panax ginseng*) cultured on MS medium supplemented with all combinations of 1.0 mg/L BA, 1.7 mg/L GA_3 and 1.3 mg/L ABA, and in all cases flower buds were formed on elongated axillary branches from the cotyledonary node, while the apices remained vegetative [9]. In *Perilla frutescens*, shoots regenerated from cotyledonary and hypocotyl explants were cultured on MS medium containing sucrose, ammonium nitrate and 1.0 mg/L of BA, and after 40 d of culture, 86.2% of shoots flowered and most of which self-fertilized *in vitro* and produced mature fruits with viable seeds [10]. High frequency early *in vitro* flowering of the self-pollinated seedlings of a tropical orchid hybrid, *Dendrobium* 'Madame Thong-In', was developed; coconut water was required to trigger the transitional shoot apical meristem and BA enhanced inflorescence stalk initiation and flower bud formation [11]. Recently, using aseptic plantlets obtained from stem node explants of hybrid red miniature rose (*Rosa hybrid* cv. Fairy Dance), the effects of shoot physiological status, medium ingredients, and culture thermoperiod on *in vitro* flowering were evaluated [12], however, in the *Drosera* genus exist a limited number of reports [3].

The induction of *in vitro* flowering could be an option for *in vitro* improvement through selective breeding method as well as in the study of reproductive biology of *Drosera*. In this paper we report the effect of photoperiod and others physiological factors on *in vitro* flowering and plantlets elongation in "sundew" *Drosera capillaris* Poiret, an important ornamental and medicinal plant, widely distributed in North, Central and South America (Venezuela, Colombia and Brazil).

MATERIAL AND METHODS

Drosera capillaris Poiret, a long-day plant with 14-16 h of approximate critical day in natural conditions, was collected in the Cauca Valley (Cali, Colombia) and was introduced, in tissue culture form in 1988, in the Germplasm Bank of the Plant Genetic Resources and Tissue Culture Laboratory (Universidad Nacional Pedro Ruiz Gallo, Lambayeque, Peru), by Dr. G.E. Delgado-Paredes.

Whole plantlets, with 1 cm length and 3-5 leaves were excised from actively growing plants and inserted up-right into 25x150 mm test tubes containing 15 mL propagation medium and closed with snap cap closures. The medium consisted of MS mineral salts [13], 100 mg/L myo-inositol, 1.0 mg/L thiamine HCl, 0.01 mg/L naphtaleneacetic acid (NAA), 0.01 mg/L gibberellic acid (GA_3), 2% (w/v) sucrose, and 0.18% (w/v) Phytagel (Sigma Chemical Co.). The medium was adjusted to pH 5.8 ± 0.1 with 0.5 N KOH or 0.5 N HCl before autoclaving at 120°C for 15 min. The cultures were maintained under 12/12 (short day), 16/8 (long day/control) and 20/4 (very long day) h (light/dark) photoperiod (76 mmol m^{-2} s^{-1} supplied by Gro-lux tubes) at 24-26°C for 90 days. In order to verify the effect of different parameters in interaction with the photoperiod on *in vitro* flowering process, various treatments were established: MS concentration (1 MS, 1/5 MS and 1/10 MS concentration), growth regulators [indoleacetic acid (IAA), benzylaminopurine (BAP), GA_3, abscisic acid (ABA) and salicylic acid (SA)], carbohydrates (sucrose, glucose and maltose) and osmoregulatory compounds (sucrose, mannitol and sorbitol). Likewise, to verify the influence of low temperature (vernalization) the whole plantlets were grown in refrigeration (8-10°C) for 30 days, with 2-h photoperiod, before of itself establishment under the same photoperiodic conditions as in general experiment. For calculate the percentage of flowering response of the *in vitro* plantlets, twenty-five explants were used per treatment in each test and each treatment was repeated twice. Results were analyzed comparing the variance and the means using Duncan's multiple range test at $P < 0.05$. Data of both number and length of roots not is showed in table.

RESULTS AND DISCUSSION

Flowering is considered as a complex process regulated by a combination of environmental and genetic factors and its induction under *in vitro* culture is extensively rare; however, several explants as hypocotyl, petiole, floral bud, leaf discs, petal and superficial tissue, has been used for *in vitro* flowering mainly in *Nicotiana tabacum*, *Cichorium intybus* and *Streptocarpus nobilis* [14].

Effect of mineral substances in culture medium

MS/10 culture medium concentration [with 6 mM N (NO_3^-, NH_4^+) and 2 mM K^+] enhanced both the number of cultures with floral buds (42%) (Fig.1a) and the number of flower buds per culture, but only under very long day conditions (Fig. 1b); the plantlets elongation (Fig.1c) was very similar than control in all photoperiodic conditions tested (Table 1). As additional information, both number and length of roots were slightly decreased.

The effect of mineral nutrients on flowering in most plants is poorly known. In *Pharbites nil*, was studied the flowering response in relation to C and N contents of plants cultured in nitrogen poor media and in *Lemna paucicosta* was studied the flower induction by nitrogen deficiency [15]; in that study the *in vitro* flowering was induced by lower levels of nitrogen supplemented in the culture medium. On the contrary, in SDP *Streptocarpus nobilis*, the higher levels of KNO_3 were markedly inhibitory of *in vitro* flowering [16]. Recently was reported the induction of *in vitro* flowering in *Capsicum frutescens* under the influence of $AgNO_3$ (40 µM) and $CoCl_2$ 30 (µM); both Ag^{++} and Co^{++} ions to inhibit ethylene action and ethylene production respectively [17]. In *Anthemis xylopoda*, a critically endangered Turkish endemic, the maximum flowering percentage was obtained when the shoots were cultured on ½MS medium (~30 mM NH_4NO_3 and 10 mM KNO_3) containing 1.0 mg/L IBA [18], and in hybrid red miniature rose, increasing the potassium nitrate to ammonium nitrate ratio or increasing the phosphate concentration in MS medium had a positive effect on *in vitro* flowering [12].

Table - 1 Effect of the photoperiod (12/12, 16/8 and 20/4 h) on *in vitro* flower formation and plantlets elongation of *D. capillaris* in several MS culture media concentrations after 90 days of culture.

Concentration	Flowering (%)/Plantlets elongation (cm)		
	12/12	16/8	20/4
MS	0.0/2.0 ± 0.3 c	0.0/2.3 ± 0.5 c	8.5/2.0 ± 0.4 b
MS/5	0.0/1.8 ± 0.3 c	0.0/2.0 ± 0.2 c	0.0/1.7 ± 0.5 c
MS/10	0.0/1.9 ± 0.4 c	10.0/2.2 ± 0.6 a	42.0/1.9 ± 0.4 a

Data shown are mean ± SE of two experiments; each experiment consisted of 25 replicates.
For *in vitro* flowering means within a column followed by the same letter are not significantly different at 0.05 level according Duncan's multiple range test.

Table - 2 Effect of the photoperiod (12/12, 16/8 and 20/4 h) on *in vitro* flower formation and plantlets elongation of *D. capillaris* in several plant hormones concentration after 90 days of culturing on MS medium.

IAA	BAP	GA_3	Flowering (%)/Plantlets elongation (cm)		
	(mg/L)		12/12	16/8	20/4
0.0	0.0	0.0	0.0/2.0 ± 0.3 c	0.0/2.3 ± 0.5 c	8.5/2.0 ± 0.4 b
0.1			0.0/2.3 ± 0.4 c	0.0/2.6 ± 0.1 c	10.0/2.4 ± 0.4 b
0.5			0.0/2.8 ± 0.4 c	10.0/3.2 ± 0.5 b	0.0/3.1 ± 0.4 c
	0.5		0.0/1.0 ± 0.2 c	0.0/1.3 ± 0.3 c	0.0/1.1 ± 0.2 c
	1.0		0.0/1.0 ± 0.1 c	0.0/1.2 ± 0.3 c	0.0/1.0 ± 0.2 c
		0.1	0.0/2.1 ± 0.3 c	0.0/3.2 ± 0.4 c	50.0/2.2 ± 0.4 a
		1.0	0.0/2.2 ± 0.3 c	0.0/3.3 ± 0.4 c	42.0/2.2 ± 0.3 a

Data shown are mean ± SE of two experiments; each experiment consisted of 25 replicates.
For *in vitro* flowering means within a column followed by the same letter are not significantly different at 0.05 level according Duncan's multiple range test.

Table - 3 Effect of the photoperiod (12/12, 16/8 and 20/4 h) on *in vitro* flower formation and plantlets elongation of *D. capillaris* in ABA and SA interaction after 90 days of culturing on MS medium.

ABA	SA	Flowering (%)/Plantlets elongation (cm)		
(mg/L)		12/12	16/8	20/4
0.0	0.0	0.0/2.0 ± 0.3 c	0.0/2.3 ± 0.5 c	8.5/2.0 ± 0.4 ab
0.1		0.0/2.0 ± 0.6 c	0.0/2.2 ± 0.3 c	0.0/2.1 ± 0.4 c
0.5		10.0/2.0 ± 0.4 a	0.0/1.9 ± 0.3 c	10.0/1.6 ± 0.4 a
	0.1	0.0/2.1 ± 0.3 c	12.0/2.5 ± 0.5 a	12.0/2.4 ± 0.4 a
	1.0	0.0/2.3 ± 0.3 c	0.0/2.6 ± 0.4 c	0.0/2.7 ± 0.4 c

Data shown are mean ± SE of two experiments; each experiment consisted of 25 replicates.
For *in vitro* flowering means within a column followed by the same letter are not significantly different at 0.05 level according Duncan's multiple range test.

In addition, in *Rosa indica*, the 3-week intervals for two consecutive subcultures on MS medium supplemented with 0.5 mg/L IAA, 1.0 mg/L BA and 50 mg/L silver nitrate under photoperiod 16/8 (light/dark cycle) were efficient for flower induction [19].

In natural conditions, *Drosera* grows mostly on acidic substrates which are often deficient in nitrogen and phosphorus [20]. Insects as prey were proposed to be a valuable additional source of nitrogen. For example, an optimal growth of *D. binata* was achieved after application of insects to plants, while addition of inorganic nitrogen resulted in slower growth and flower inhibition [21]. In our work, very low levels of both inorganic nitrogen and phosphorus, supplemented to culture medium, was markedly stimulatory on *in vitro* flowering

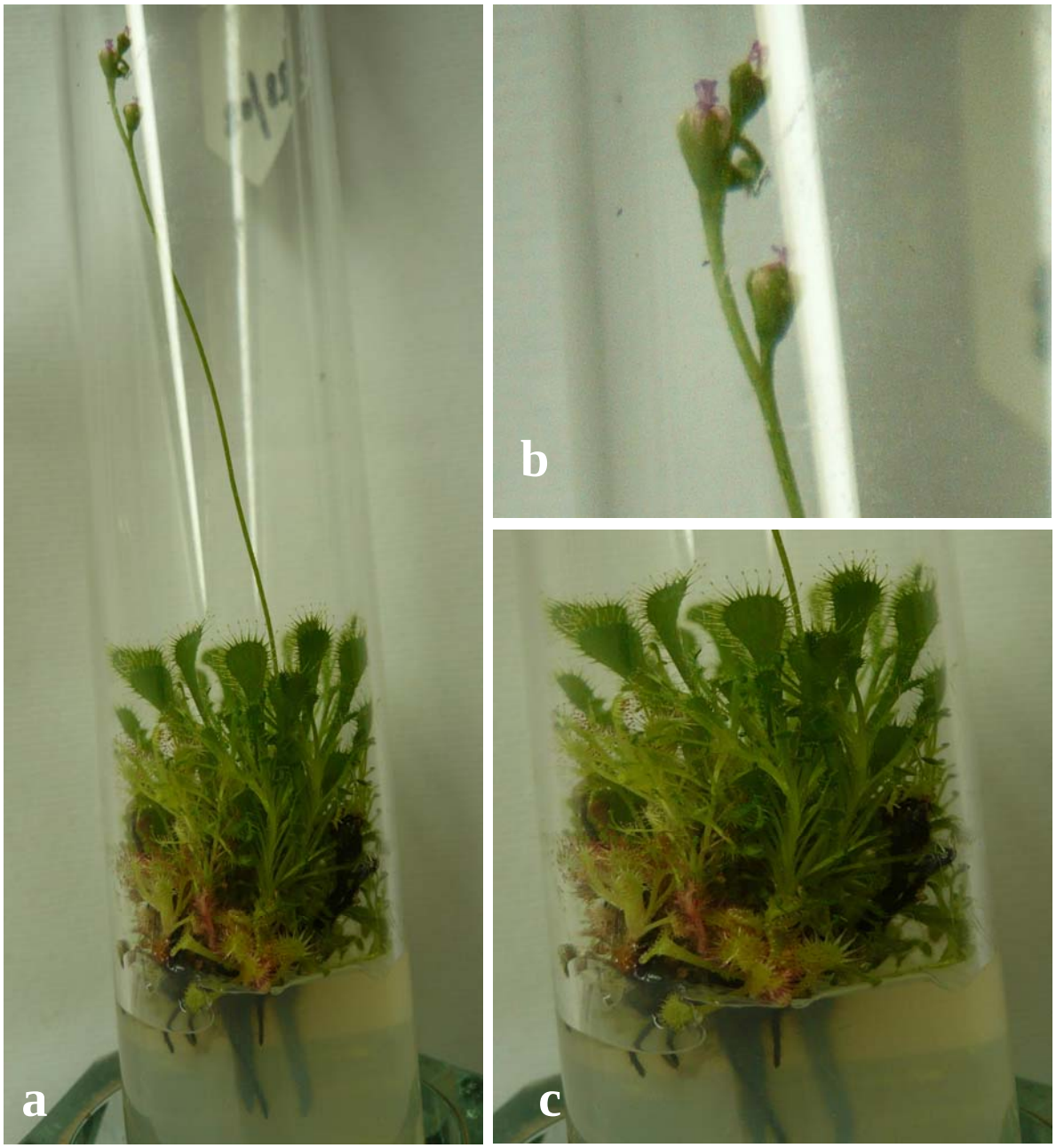

Fig-1. a. Plantlets of *Drosera capillaris* with inflorescence after 90 days of culture under very long day (20-4 h) conditions in MS medium with 0.01 mg/L NAA and 0.01 mg/L GA_3 b. Inflorescence and c. Plantlets elongation

Table-4 Effect of the photoperiod (12/12, 16/8 and 20/4 h) on *in vitro* flower formation and plantlets elongation of *D. capillaris* in several carbohydrates concentration after 90 days of culturing on MS medium.

Sucrose	Glucose	Maltose	Flowering (%)/Plantlets elongation (cm)		
	(%)		12/12	16/8	20/4
2.0			0.0/2.0 ± 0.3 c	0.0/2.3 ± 0.5 c	8.5/2.0 ± 0.4 b
4.0			0.0/1.7 ± 0.5 c	0.0/2.3 ± 0.3 c	58.0/2.3 ± 0.5 a
6.0			0.0/2.2 ± 0.5 c	10.0/1.9 ± 0.2 b	42.0/1.9 ± 0.6 ab
	2.0		0.0/1.5 ± 0.5 c	0.0/1.6 ± 0.5 c	0.0/1.6 ± 0.4 c
	4.0		0.0/1.6 ± 0.4 c	0.0/2.3 ± 0.5 c	0.0/1.7 ± 0.5 c
		2.0	0.0/1.6 ± 0.4 c	0.0/1.7 ± 0.4 c	0.0/1.2 ± 0.5 c
		4.0	0.0/1.8 ± 0.5 c	0.0/1.7 ± 0.4 c	0.0/0.8 ± 0.3 c

Data shown are mean ± SE of two experiments; each experiment consisted of 25 replicates.
For *in vitro* flowering means within a column followed by the same letter are not significantly different at 0.05 level according Duncan's multiple range test.

Table - 5 Effect of the photoperiod (12/12, 16/8 and 20/4 h) on *in vitro* flower formation and plantlets elongation of *D. capillaris* in low temperature (vernalization) conditions after 90 days of culturing on MS medium.

Temperature (°C)	Flowering (%)/Plantlets elongation (cm)		
	12/12	16/8	20/4
Control (24-28)	0.0/2.0 ± 0.3 c	0.0/2.3 ± 0.5 c	10.0/2.0 ± 0.4 b
Pre-treatment 8-10	17.0/1.9 ± 0.3 ab	0.0/2.0 ± 0.3 c	25.0/2.0 ± 0.4 a

Data shown are mean ± SE of two experiments; each experiment consisted of 25 replicates.
For *in vitro* flowering means within a column followed by the same letter are not significantly different at 0.05 level according Duncan's multiple range test.

Effect of photoperiod

Only under very long day (20/4 h) conditions, 0.1 mg/L IAA treatment was scarcely stimulatory for flower buds induction (10%); likewise, both 0.1 and 1,0 mg/L GA_3 treatments, but only under very long day conditions, were significantly stimulatory for flower buds initiation (about 50%). In the case of cytokinin BAP no effects on flower buds induction were observed; the plantlets elongation was markedly higher than control, whereas in the case of BAP the plantlets elongation was markedly inhibits in all photoperiodic conditions tested (Table 2). As additional information, both number and length of roots were also significantly increased with addition of both IAA and GA_3 but with addition of BAP did not, where the roots formation was completely inhibits.
In saffron plant (*Crocus sativus*), used as a source of food additives and component of traditional medicine, the optimal condition for *in vitro* flowering induced (37.5-40%) was in the dark at 20°C [22]; however, in embryogenic cultures of Kinnow mandarin (*Citrus nobilis* x *C. deliciosa*), the inductive photoperiod for *in vitro* flowering was 12/12 h [23]. In *Capsicum frutescens* [17], *Eulophia graminea* [24], and *Boerhaavia diffusa* [25] the inductive photoperiod required was 16/8 h. In *Lycopersicon esculentum* flowering was not observed in dark [26]. In other study, the effects of different light qualities and growth regulators on *in vitro* flowering of *Phyllanthus tenellus*, commonly known as "quebra-pedra" in Brazil, was realized; the plantlets grown under white and blue lights showed the highest *in vitro* flowering percentage (70% and 56%, respectively), and a reduction of flowering was observed under red, yellow and green light exposure [27]. Plausible candidates for genes that are implicated in the transition to flowering are those that encode photoreceptors; the phytochromes, which perceive red and far-red light, and the cryptochromes, which perceive UV-A and blue light, are two major classes of photoreceptors found in most higher plants and that are involved in flowering [28]. In *Arabidopsis*, the phytochromes A and B, along with the cryptochromes 1 and 2, are involved in day length response [29]. In addition, genetic studies in *Arabidopsis* have discovered one of the components of a potential 'light quality pathway', the *Phytochrome and Flowering Time 1* (*PFT1*) gene [30].

Effect of plant growth regulators

Exogenous auxins (either indole acetic acid or naphthalene acetic acid) can both inhibit and promote flower initiation in SDP (*Pharbites nil* and *Chenopodium rubrum*) and LDP (*Lolium temulentum* and *Sinapis alba*), and inhibition is far more widespread than promotion [31].

In *Sinapis*, as in several other plants, auxin is promotive at low doses and inhibitory at high doses; this inhibition at high doses might simply be related to a general growth inhibition or also an auxin-induced ethylene biosynthesis [31]. In fact, the significance of these results are uncertain; nevertheless, Metzger (1995) [32] believe that it is possible that auxins play a role in some of the processes associated with evocation such as a loss in apical dominance and alteration in phyllotaxis, but this remains to be shown conclusively. Likewise, not only IAA or NAA, but also other auxins, should be considered. Recently was reported the induction of *in vitro* flowering in *Withania somnifera* in culture medium with 0.1 mg/L IAA - kinetin (KIN) (0.5-4.0 mg/L) [33], in *Brassica campestris* with 1.0 IAA - 0.5 mg/L KIN [34], in *Crocus sativus* with 5.0 mg/L NAA and 7.0 mg/L BAP [22], in *Pentanema indicum* with 2.0 mg/L IBA [35], and also in *Anthemis xylopoda* 1.0 mg/L IBA was found more efficient than IAA in the induction of flower buds [18]. In our work, IAA to result highly inhibitory in flower buds formation.

Cytokinins have been observed to promote flower formation in a few species; however, have been also observed that exogenous cytokinins cause promotion and inhibition of flower initiation in a variety of species, although the effects are much more frequent than inhibitory ones [36]. In many of the cases, the promotive effect of cytokinins is seen only in induced or marginally induced plants. In *Chenopodium*, *Anagallis* and *Sinapis*, inhibition or promotion has been observed depending on the amount of cytokinin applied and/or timing of treatment; thus there is apparently an optimal dose and time of sensitivity [31]. In addition, in the LDP *Sinapis alba*, an increase in the endogenous cytokinin levels in both leaves and phloem exudate was observed 16 h after the beginning of LD [37].

There is not convincing evidence linking cytokinins and flower induction in a cause and effect relationship [32]; however, was reported that thidiazuron (TDZ) 0.5 mg/L and 2.0 mg/L BAP induced *in vitro* flowering in *Dendrocalamus strictus* [38] and *Lycopersicon esculentum* [26], respectively. Additionally, in embryogenic cultures of Kinnow mandarin (*Citrus nobilis* x *C. deliciosa*) the maximum percentage (31.9%) of cultures producing flowers and maximum number (5.6) of flowers per culture was observed on MS medium supplemented with 2.0 mg/L KIN and 4% sucrose at 12-h photoperiod [23], in *Boerhaavia diffusa*, an Indian medicinal plant, the highest percentage of flowering was obtained on medium supplemented with BAP 2.0 mg/L [25], in *Scoparia dulcis* simultaneous regeneration of shoots and roots and *in vitro* flowering were achieved from the nodal explants on MS medium supplemented with 0.5 mg/L KN and 2.0 mg/L IAA [39], in *Spilanthes acmella*, other Indian ornamental and medicinal plant, the maximum percentage (50%) of flower induction occurred when regenerated shoots were cultured on MS medium supplemented with 2.0 mg/L BAP and 0.5 mg/L IAA under photoperiod of 16/8 h [40], and in red miniature rose the more suitable plant growth regulators for *in vitro* flowering was a combination of 3.0 mg/L BA and 0.1 mg/L NAA [12]. In our work, BAP to result highly inhibitory in both flower buds formation and plantlets elongation.

Of all the plant hormones that have been applied to plants under strictly noninductive conditions, only GAs have been shown to effectively cause flower formation in a wide variety of species. In general, LDP (*Hieracium aurantiacum* and *Blitum virgatum*) and plants with a cold requirement (*Gerum urbanum* and *Lunaria annua*) are responsive to exogenous GAs while SDP and DNP are not [32]. Likewise, shoot multiplication and *in vitro* flowering was induced from nodal explants of *Ceropegia bulbosa* in culture medium with 1.0 mg/L GA_3 - 0.5 mg/L BA [41]. In view of the large number of species in which exogenous GAs cause vegetative plants to flower under noninductive conditions, it is logical to conclude that GAs have a critical role in the regulation of flower formation. On the other hand, in some rosette LDP, such as *Samolus*, *Rudbeckia*, and *Arabidopsis*, growth retardants suppress both bolting and flowering in LD, and GA overcomes these effects, suggesting that in these species GAs are the primary controlling factors of flowering; in other rosette LDP, however -e.g. spinach, *Silene*, and *Agrostemma*- growth retardants suppress bolting, reduce considerably GA levels, but leave flowering unaffected [31]. Summarizing, it was shown that GAs have a variety of roles in reproductive development depending on the species and the response type; in view of all of this, the role of GAs in flower initiation needs reexamination [31, 32]. GA_3, in media containing three concentrations (1.0, 2.0 and 10.0 mg/L) was used in order to induce the reversion of olive tree vegetative buds towards a floral ones *in vitro*; however, few cases of reversion were observed mainly on the medium containing 10.0 mg/L GA_3 [42]. In our work, GA_3 to result highly stimulatory in both flower buds formation and plantlets elongation.

GA molecular targets were first described in barley, with the *GAMYB* gene being an integral part of GA signal transduction, and over-expression of GAMYB protein mimics the effects of GA application [43]; likewise, GA affects two floral transition repressors, *GIBBERELLIC ACID INSENSITIVE* (*GAI*) and *RGA* (*Repressor of ga1-3*), both of which are *DELLA* class proteins [44]; these DELLA proteins may act as the target for GA-directed ubiquitination followed by subsequent proteasome-mediated destruction [28].

In both ABA and SA the effects on flower bud induction were few evident and erratic; only about 10% of plantlets developed flowers at 0.5 mg/L ABA and 0.1 SA in almost all photoperiodic conditions tested; in the case of ABA the plantlets elongation decreased as ABA level increased and were markedly lower than control, whereas in the case of SA the plantlets elongation increased as SA level increased and were markedly higher than control (Table 3). As additional information, both number and length of roots were also significantly increased with addition of SA but additioning ABA did not.

In vitro flowering and fruiting were induced in plants regenerated from calli of leaf explants of *Lycopersicon esculentum*; MS medium supplemented with 2.0 mg/L BAP displayed the best response followed by MS with 2.0 mg/L BAP, 1.0 mg/L ABA and 0.5 mg/L IAA [26]; likewise, flower buds of ginseng (*Panax ginseng*) were induced on the medium with either BA, BA + GA_3, or BA + GA_3 + ABA, and in all cases flower buds were formed on elongated axillary branches from the cotyledonary node, while the apices remained vegetative [9]. The main effect of ABA has been fully described in several reviews. ABA induces stomatal closure, inhibits shoot growth, induces storage protein synthesis in seeds, counteracts the effect of gibberellin on α-amylase synthesis in cereal grains, affects the induction and maintenance of some aspects of dormancy in seeds and induces gene transcription in response to wounding [45]. With the discovery of ABA as a potent, naturally occurring growth inhibitor that was possible involved in the control of bud dormancy, it was reasonable to suspect that ABA might be the flower inhibitor [32]. Indeed, previous observations showed that exogenous ABA is inhibitory in several SDP and LDP (for example *Spinacea oleracea* and *Lolium temulentum*) grown in inductive conditions, and endogenous ABA levels in several photoperiodic species do not bear consistent relationships with day length [36]. However, when certain SDPs (for example, Japanese morning glory and *Chenopodium rubrum*) are already slightly induced and then treated with ABA, flowering is promoted; but no conclusions can be drawn because ABA inhibits flowering in other SD and LD species and is completely innocuous in still others [46]. On the other hand, no causal relationship was observed between endogenous ABA levels and the ability to flower. Thus, ABA does not appear as a major determinant in the floral transition, except perhaps in some species [31]. In the case of *Spinacea*, when ABA levels were in fact higher under LD than SD, the apparent inhibition of flower formation might have been the result of a delay in inflorescence development rather than inhibition of flower initiation, indicating that ABA does not have a role in the regulation of flower formation in LDP as a graft-transmissible inhibitor [32].

Recent reviews have demonstrated that SA has a relevant role in the control of physiological and biochemical processes in plants. Among these morphogenetic processes affected by SA are flowering and tuberization; however, the most typical biological response is a systemic signal for induction of disease resistance [47], even if the physiological mechanisms of SA action in growth and morphogenetic processes remain unknown. Results obtained in two differents materials, *Streptocarpus nobilis* -a short day plant- and *Ullucus tuberosos* -which form tubers under short days-, was showed a common aspect, the stimulatory effect of SA on stem elongation; however, only in *S. nobilis* the flowering process was induced and the percentage of cultures with floral buds and the number of flower buds per culture was significantly enhanced in all concentrations tested (0.05 - 0.25 mg/L) [48].

Effect of carbohydrates and osmoregulatory compounds

The higher sucrose concentration (4 and 6%) enhanced the flower buds formation about 40 to 60% approximately, but only under very long day (20/4 h) conditions; however, in both glucose and maltose treatments (2 and 4%) no effects were observed; the plantlets elongation was very similar with addition of high sucrose concentration, whereas in the case of both glucose and maltose the plantlets elongation was markedly lower than control (Table 4). As additional information, both number and length of roots were also significantly increased only with addition of both sucrose and glucose but not with addition of maltose, showed in this case a negative response in all photoperiodic conditions tested. Available evidence suggests that an early change of the assimilates concentration, particularly carbohydrates, at the apical bud is critical for flower initiation, but that this modification is not sufficient alone to trigger initiation; they are only part of a complex controlling system in which other regulating agents also operate, such as various plant growth substances and substrates [36, 49]. For many physiologists, assimilates have only a supportive role in flower initiation, simply providing energy for the biosynthetic reactions associated with flowering.

A counter "nutrient diversion" hypotesis postulates that induction, whatever the nature of the involved factors, is a means to modify the source/sink relationships within the plant in such a way the shoot apex receives a higher concentration of assimilates than under noninductive conditions [50, 51]. A number of studies using material grown *in vitro* have emphasized the role of carbohydrates (sucrose and glucose) for flower initiation and have pointed out that sucrose concentration optimal for flower initiation is higher than that required for vegetative growth [36]. A list of LDP and SDP for which carbohydrates applications stimulate the floral response is relatively numerous, sometimes even in noninductive conditions [49].

For example, in *Brassica campestris*, a quantitative LDP, sucrose feeding has recently been shown to cause flower initiation in 8-h SD [52]. This effect, which is not related to the osmotic properties of the carbohydrates solution, is not mediated by a general stimulation of growth and is thus specific for floral transition. On the other hand, inhibition of floral initiation by sucrose applications as reported for *Lemna paucicostata* [53] may result from a supraoptimal sucrose concentration in the plant or from an inadequate timing application. In the case of maltose application using material grown *in vitro* is does not known; however, no genetic evidence for the nutrient diversion model exists to date, but analysis of carbohydrate to nitrogen ratios prior to and during floral transition show that under vegetative conditions, the ratio is consistent but at time of flowering nitrogen transport becomes proportionally greater to the apex [54].

In the treatments with the osmoregulatory compounds sucrose-sorbitol and sucrose-mannitol only detrimental effects, in flowering and plantlets elongation, were observed. In this case application using material grown *in vitro* is does not known.

Effect of temperature

The pre-treatment with vernalization (8-10°C) was slightly stimulated for flower buds under short day (12/12 h) and very long day (20/4 h) conditions with 17 and 25%, respectively; the plantlets elongation was very similar with the control treatments in all photoperiodic conditions tested (Table 5); however, in red miniature rose, the percentage of *in vitro* flowering was significantly higher at day/night temperature of 28/20^{o}C than at other constant temperatures [12]. As additional information, both number and length of roots were also very similar with the control treatment.

Vernalization is a checkpoint through which many plants must pass, where flowering is facilitated by a prolonged exposure to cold temperature and subsequently induced by other inductive pathways; is a somatically heritable state since once vernalization has occurred, daughter cells derived from induced SAM cells retain the state induced by extended cold exposure [55]. Vernalization also promotes flowering in species not normally considered cold requires. For instance, the LDP *Spinacea oleracea* responds to thermoinductive temperatures with a shortening of the critical daylength requirement. In other LDP as well, low night temperatures can compensate for a long dark period (i.e., SD) resulting in flowering under normally noninductive conditions [32].

Vernalization in *Arabidopsis* involves several steps that culminate in the stable repression of *FLOWERING LOCUS C* (*FLC*) gene, and stable repression of *FLC* occurs through the activity of two other genes, *VERNALIZATION 1* and *2* (*VRN1* and *VRN2*) [28].

CONCLUSION

A micropropagation system for *Drosera capillaris* has been developed using whole plantlets, with 1 cm length and 3-5 leaves. In the current study, the role of different factors such as photoperiod, mineral nutrition, carbohydrates, plant hormones and cold pre-treatment in the regulation of flowering was verified. Although *in vitro* flowering was observed, more reliable culture regimes need to be elucidated; however, this protocol represents an easy way to produce plantlets of *D. capillaris* in a short period of time covering the whole life cycle.

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REFERENCES

[1] Lecoufle M., 1990. Carnivorous plants: care and cultivation. Blandford, London.

[2] Culham A., Gornall, R.J. 1994. The taxonomic significance of naphtoquinones in the Droseraceae. Biochem. System. Ecol. 22:507-515.

[3] Samaj J., Blehová, A., Repcák, M., Ovecka, M., Bobák, M. 1999. *Drosera* species (sundew): *In vitro* culture and the production of plumbagin and other secondary metabolites. In: Biotechnology in Agricultural and Forestry, Vol. 43 Medicinal and Aromatic Plants XI. Eds. Y.P.S. Bajaj, Springer-Verlag, Berlin Heidelberg, pp. 105-135

[4] Mativandlela S.P., Meyer, J.J., Hussein, A.A., Houghton, P.J., Hamilton, C.J., Lall, N. 2008. Activity against *Mycobacterium smegmatis* and *M. tuberculosis* by extract of South African medicinal plants. Phytother. Res. 22:841-845.

[5] Alvarado J., Vásquez, H., Delgado, G.E., Trevisan, D., Horna, O., Pereira, J. Rojas, C. 2010. Actividad inhibitoria de plantas *in vitro* de *Drosera capillaris* sobre *Mycobacterium tuberculosis*. Rev. Peru. Biol. 17:353-358
[6] Albert V.A., Williams S.E., Chase M.W. 1992. Carnivorous plants: phylogeny and structural evolution. Science 257:1491-1495.
[7] Takahashi H. 1988. Ontogenic development of pollen tetrads of *Drosera capensis*. L. Bot. Gaz. 149:275-282.
[8] Dickens C.W.S., Van Staden J. 1987. The *in vitro* flowering of *Kalanchöe bloosfeldiana* Poellniz. I. Role of culture conditions and nutrients. J. Exp. Bot. 39:461-471.
[9] Lee H.S., Lee K.-W., Yang S.G., Liu J.R. 1991. *In vitro* flowering of ginseng *(Panax ginseng* C. A. Meyer) zygotic embryos induced by growth regulators. Plant & Cell Physiol. 32:1111-1113.
[10] Zhang T. 2007. *In vitro* flowering of *Perilla frutescens.* 2007. In Vitro Cell. Dev. Biol. Plant. 43:91-94,
[11] Sim G.E., Loh C.S., Goh C.J. 2007. High frequency early *in vitro* flowering of *Dendrobium* Madame Thong-In (Orchidaceae). Plant Cell Rep. 26:382-393.
[12] Zeng S., Liang S., Zhang Y.Y., Wu K.L., Teixeira da Silva J.A., Duan J. 2013. *In vitro* flowering red miniature rose. Biol. Plantarum 57:401-409.
[13] Murashige T., Skoog F. 1962. A revised medium for rapid growth and bio-assays with tobacco tissue cultures. Physiol. Plant. 15:473-497.
[14] Floh E.I.S., Handro W. 1999. Floração *in vitro*. In: Técnicas e Aplicações da Cultura de Tecidos de Plantas. Eds. A.C. Torres & L.S. Caldas. ABCTP/EMBRAPA-CNPH, Brasilia, pp. 213-226.
[15] Tanaka D. 1986. Flower induction by nitrogen deficiency in *Lemna paucicostata* 6746. Plant Cell Physiol. 27:875-880.
[16] Simmonds J.A. 1982. *In vitro* flowering on leaf explants of *Streptocarpus nobilis*. The influence of culture medium components on vegetative and reproductive development. Can. J. Bot. 60:1461-1468.
[17] Sharma A., Kumar V., Giridhar P., Ravishankar G.A. 2008. Induction of *in vitro* flowering in *Capsicum frutescens* under the influence of silver nitrate and cobalt and pollen transformation. Plant Biotech. 11(2).
[18] Erdağ B.B., Emek Y.C. 2009. Adventitious shoot regeneration and *in vitro* flowering of *Anthemis xylopoda* O.Schwarz, a critically endangered Turkish endemic. Turk. J. Biol. 33:319-326.
[19] Pratheesh P.T., Kumar M.A. 2012. *In vitro* flowering in *Rosa indi*ca L. Int. J. Pharm. Biol. Sci. 2:196-200.
[20] Stewart C.N., Nilsen E.T. 1992. *Drosera rotundifolia* growth and nutrient in a natural population with special reference to the significance of insectivory. Can. J. Bot. 70:1409-1416.
[21] Chandler G.E., Anderson J.W. 1976. Studies on the nutrition and growth of *Drosera* species with reference to the carnivorous habit. New Phytol. 76:129-141.
[22] Jun Z., Xiaobin C., Fang C. 2007. Factors influencing *in vitro* flowering from styles of saffron. Acta Hortic. 739:313-320.
[23] Singh B., Sharma, S., Rani, G., Virk G.S., Zaidi A.A., Nagpal A. 2006. *In vitro* flowering in embryogenic cultures of Kinnow mandarin (*Citrus nobilis* Lour x *C. deliciosa* Tenora). Afr. J. Biotech. 5:1470-1474.
[24] Chang C., Hu W-H., Chen Y-C., Su Y-L., Chiu Y.T. 2010. *In vitro* flowering and mating system of *Eulophia graminea* Lindl. Bot. Stud. 51:357-362.
[25] Sudarshana, I.M.S., Niranjan M.H., Girisha S.T. 2008. *In vitro* flowering, somatic embryogenesis and regeneration in *Boerhaavia diffusa Linn*. - A medicinal plant. Global J. Biotech. & Biochem. 3:83-86.
[26] Sheeja T.E., Mandal A.B. 2003. *In vitro* flowering and fruiting in tomato (*Lycopersicon esculentum* Mill.). Asia Pacific Jour. Molec. Biol. & Biotech. 11:37-42.
[27] Victorio C.P., Lage C.L.S. 2009. *In vitro* flowering of *Phyllanthus tenellus* Roxb. cultured under different light qualities and growth regulators. Gen. Appl. Plant Physiol. 35:44–50.
[28] Tremblay R., Colasanti J. 2006. Floral induction. In: Annual Plant Reviews: Flowering and its Manipulations. Ed. C. Ainsworth. Blackwell Publishing Ltd, Oxford, UK, pp. 28-48.
[29] Mouradov A., Cremer F., Coupland G. 2002. Control of flowering time: Interacting pathways as a basis for diversity. Plant Cell 14 (suppl.), S111–S130.
[30] Cerdan P.D., Chory J. 2003. Regulation of flowering time by light quality. Nature 423:881-885.
[31] Bernier G. 1988. The control of floral evocation and morphogenesis. Ann. Rev. Plant Physiol. Plant Mol. Biol. 39:175-219.
[32] Metzger J.D. 1995. Hormones and reproductive development. In: Plant Hormones. Physiology, Biochemistry and Molecular Biology. Ed. P.J. Davies. 2nd Edition. Kluwer Academic Publishers, The Netherlands, pp. 617-648.

[33] Saritha K.V., Naidu C.V. 2007. *In vitro* flowering of *Withania somnifera* Dunal. -An important antitumor medicinal plant-. Plant Sci. 172:847-851.
[34] Verma R., Singh R.R. 2007. Regeneration and *in vitro* flowering in *Brassica campestris* (L.) var. *Bhavani*. Our Nature 5:21-24.
[35] Sivanesan I., Jeong B.R. 2007. Micropropagation and *in vitro* flowering in *Pantanema indicum* Ling. Plant Biotech. 24:527-532.
[36] Bernier G., Kinet J.-M., Sachs R.M. 1981. The Physiology of Flowering. Vol. I. Boca Raton:CRC.
[37] Lejeune P., Kinet J.-M., Bernier G. 1988. Cytokinin fluxes during floral induction in the LDP *Sinapis alba* L. Plant Physiol. 86:1095-1098
[38] Singh M., Jaiswal U., Jaiswal V.S. 2000. Thidiazuron-induced *in vitro* flowering in *Dendrocalamus strictus* Ness. Curr. Sci. 79:1529-1530.
[39] Karthikeyan S., Prasad R., Mahendran T.S., Rajagopal K., Ravendran V. 2009. Direct regeneration and *in vitro* flowering of *Scoparia dulcis* L. Indian J. Sci. Technol. 2:55-57.
[40] Yadav K., Singh N. 2011. *In vitro* flowering of shoots regenerated from cultured nodal explants of *Spilanthes acmella* murr. - an ornamental cum medicinal herb. Analele Universității din Oradea - Fascicula Biologie Tom. XVIII 1:66-70.
[41] Britto S.J., Natarajan E., Arockiasamy D.I. 2003. *In vitro* flowering and shoot multiplication from nodal explants of *Ceropegia bulbosa* Roxb. var. *Bulbosa*. Taiwania 48:106-111.
[42] Chaari-Rkhis A., Maalej M., Messaoud S.O., Drira N. 2006. *In vitro* vegetative growth and flowering of olive tree in response to GA_3 treatment. Afr. J. Biotechnol. 5:2097-2302.
[43] Cercos M., Gomez-Cadenas A., Ho T.H.D. 1999. Hormonal regulation of a cysteine proteinase gene, EPB1, in the aleurone cells of germinating barley seeds: cis- and trans-acting elements involved in the gibberellin induct ion. Plant J. 19:107-118.
[44] Cheng H., Qin L., Lee S., Fu X., Richards D., Cao D., Luo D., Harberd N., Peng J. 2004. Gibberellin regulates *Arabidopsis* floral development via suppression of DELLA protein function. Development 131:1055-1064.
[45] Davies P.J. 1995. The plant hormones: their nature, occurrence, and functions. In: Plant Hormones. Physiology, Biochemistry and Molecular Biology. Ed. P.J. Davies. 2nd Edition. Kluwer Academic Publishers, The Netherlands. pp. 1-12
[46] Salisbury F., Ross C.W. 1992. Plant Physiology. Fourth Edition. Wadsworth Publishing Company. Belmont, California. 682 p.
[47] Raskin I. 1995. Salicylic acid. In: Plant Hormones. Physiology, Biochemistry and Molecular Biology. Ed. P.J. Davies. 2nd Edition. Kluwer Academic Publishers, The Netherlands. pp. 188-205.
[48] Handro W., Mello C.M., Manzano M.A., Floh E.I.S. 1997. Enhancement of stem elongation and flower bud regeneration by salicylic acid. R. Bras. Fisiol. Veg. 9:139-142.
[49] Bodson M., Bernier G. 1985. Is flowering controlled by the assimilate level?. Physiol. Vég. 23:491-501.
[50] Sachs R.M., Hackett W.P. 1983. Source-sink relation-ships and flowering. In: Strategies of Plant Reproduction. Ed. W.J. Meudt). Allanheld, Osmun, Totowa. pp. 263-272.
[51] Lejeune P., Bernier G., Requier M.-C., Kinet J.-M. 1993. Sucrose increase during floral induction in the phloem sap collected at the apical part of the shoot of the long-day plant *Sinapis alba* L. Planta 190:71-74.
[52] Friend D.J.C., Bodson M., Bernier G. 1984. Promotion of flowering in *Brassica campestris* L. cv. Ceres by sucrose. Plant Physiol. 75:1085-1089.
[53] Sotta B. 1978. Interaction du photopériodisme et des effects de la zéatine, du saccharose et de l'eau dans la floraison du *Chenopodium polyspermum*. Physiol. Plant. 43:337-342.
[54] Rideout J.W., Raper C.D.J. Miner G.S. 1992. Changes in ratio of soluble sugars and free amino nitrogen in the apical meristem during floral transition of tobacco. Int. J. Plant Sci. 153:78-88.
[55] Sung S.B., Amasino R.M. 2004. Vernalization and epigenetics: how plants remember winter. Curr. Opin. Plant Biol. 7:4-10.