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Photoperiod affects the growth and development of yam plantlets obtained by *in vitro* propagation

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Abstract

The effects of photoperiod on the development of *in vitro* grown plantlets of yam (*Dioscorea alata* L.), were investigated. Plantlets were transplanted into pots, acclimatizated until they reached vegetative stages V_1 (3 leaves) or V_2 (8 leaves), and then grown under 12-h or 16-h photoperiod. The formation and development of underground tubers was only induced under 12-h photoperiod. Tuber initiation was not related to the initial vegetative stage of plants, and the tubers were visible at about 18 - 24 d. On the contrary, a 16-h photoperiod inhibited tuber formation and stimulated vine and leaf growth. The total dry matter production and the number of leaves per plant of V_1 stage plants were 50 and 30 % lower respectively, after 44 d under 12-h compared to 16-h photoperiod. These parameters were not influenced by photoperiod in V_2 stage plants. Consequently, the effect of 12-h photoperiod on dry matter of V_1 plants was attributed to a source limitation related to the early initiation of tuberization. The transfer of plants grown under 12-h to 16-h photoperiod stopped tuber growth and starch accumulation. On the other hand, it stimulated the shoots and the roots to grow.

Additional key words: *Dioscorea alata*, short days, saccharides, tuber induction.

Introduction

Yam represents a main source of sugar for human nutrition in tropical countries. However, yams are vegetatively propagated and most of their diseases, particularly those caused by fungi, viruses and nematodes are transmitted through planting materials. Understanding tuber growth and bulking, as well as controlling various pathogens could be achieved with the use of plants obtained from *in vitro* culture.

Tuberization is under the influence of a number of interactive variables, such as environment, mother tuber, genetics, partitioning of assimilate and other factors (Orkwor and Ekanayake 1998). The production of yam tuber is under the control of photoperiodic conditions (Lacointe and Zinsou 1987, Mantell and Hugo 1989, Hayashi and Ishihata 1991, Shiwachi *et al*. 2002). It is probable that warm temperature may retard tuber growth (Onwuene 1978). Variation in tuber initiation is caused by differences in *Dioscorea* species and cultivars within species. It occurs 12 weeks after sprouting in *D. alata* (Chapman 1965) and takes as long as 17 weeks in *D. trifida* (Ferguson *et al*. 1980). However, discrimination has not been well established between individual factors controlling the initiation and bulking of tubers.

Our work aims to study the influence of day length on the growth of plants obtained from in *vitro* propagation instead of seed tubers. This method permits, not only the use of healthy material, but also avoids the influence of the mother tuber on plant development. Furthermore, the response of *in vitro* grown plantlets is more homogeneous and faster compared to vegetatively-propagated plants.

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Abbreviations: LD - long day (16 h); SH - short day (12 h).

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Materials and methods

Plants growth conditions: Cuttings from nodal explants of *Dioscorea alata* L. cv. Belep were subcultured every 2 months from a bank of plantlets (INRA, C.I.V. Laboratory, Petit-Bourg, Guadeloupe). These were indexed for potyvirus group with monoclonal antibodies (*Agdia Inc.*, Elkart, Indiana, USA). Single nodes were inoculated in 25 × 150 mm glass tubes on 10 cm^3 of 6 g dm^{-3} agar-gelled Murashige and Skoog (1962; MS) medium supplemented with 10 µg dm^{-3} biotin, 1 mg dm^{-3} each of, nicotinic acid, pyridoxine-HCl, thiamine-HCl, calcium pantothenate, cysteine chlorhydrate and 3 mg dm^{-3} benzyladenine, containing 30 g dm^{-3} sucrose under a 16-h photoperiod and irradiance of 50 µmol m^{-2} s^{-1}. Plants were acclimatized on peat pastilles (*Jiffy 7*, *Puteaux*, Les Clayes sous Bois, France) during 15 d, then transplanted into pots containing a mixture of sterilised vegetable mould and soil until vegetative stage V_1 (3 ± 1 leaves) or V_2 (8 ± 1 leaves). They were allowed to grow in a bioclimatic chamber under controlled temperature (28/25 °C day/night) and 12-h photoperiod (SD) or 16-h photoperiod (LD). Irradiance provided by fluorescent tubes (3 *Sylvania Gro-lux*, Raunheim, Germany, and 5 *Cool white*, *Osram*, Munich, Germany) was about 50 µmol m^{-2} s^{-1}. Leaf area was measured using a leaf area meter (*3100 Li-COR*, Lincoln, NE, USA). For each trial, eight plants were harvested, and samples taken from the diverse parts of the plant. The above samples of roots and tubers were stored at -20 °C in 95 % (v/v) ethanol for sugar analysis. Dry matter of every plant part was evaluated after air drying at 60 °C in an oven.

Extraction and quantification of sugar: Starch was extracted from the ethanol-insoluble material by solubilization in DMSO/HCl (8 M; 20:5). The homogenate was incubated at 60 °C for 30 min, then centrifuged at 5 000 *g* (*Rotor 57311000*, Jouan, France) for 15 min. After recovery of supernatant, the pellet was extracted twice, in the same conditions as described above. The supernatant as a whole, was brought to pH 4.5 with NaOH and the volume adjusted to 50 cm^3 with distilled water. Diluted samples of starch (1:50) were hydrolysed to glucose in the presence of amylo-glucosidase (EC 3.2.1.3) in 100 mM Na-acetate pH 4.5 at 60 °C. The D-glucose formed was determined by enzymatic method (Bergmeyer *et al*. 1974).

Statistical analysis: All calculations were carried out with *SYSTAT* (*SPSS Inc.*, Chicago, USA) software. The separate variance Student's *t*-test (Mead and Curnow 1983) was applied, in order to compare two samples (one obtained from plants incubated under SD and the other from plants incubated under LD). Mean and standard error (SE) of the mean were calculated for each sample. The observed difference between the two means was significant at $P \leq 0.05$.

Results

Total dry matter of plantlets increased linearly during three months. The number of leaves per plant under LD, increased to 9.1 ± 0.9 and 10.4 ± 0.6, respectively, after 44 d in V_1 and V_2 plants. Under SD, it was 6.4 ± 0.9 and 11.6 ± 0.8 in V_1 and V_2 plants. At the same time, the total area of leaves of V_1 plants increased to 162 and 343 cm^2 under SD and LD compared to 314 and 354 cm^2 for V_2 plants (Table 1,2). Under LD, the distribution of fresh matter (FM) into the different plant parts was similar in V_1 and V_2 plants (Fig. 1*A*,*C*). About 62 % of the FM was found in leaves. Under SD, the FM of leaves, roots and stems decreased by 54, 81, and 66 %, respectively, in V_1 plants, and 17, 38, and 30 %, respectively, in V_2 plants (Fig. 1*B*,*D*). The tubers were visible from 24 - 28 d under SD.

Table 1. Effects of photoperiod at V_1 stage on the growth of *D. alata.* Each value is the mean ± SE of 8 replicates (* - means significantly different at $P < 0.05$).

Time [d]	Leaf area [cm^2 $plant^{-1}$]		Dry matter [g $plant^{-1}$]	
	LD	SD	LD	SD
4	34 ± 5	33 ± 5	0.11 ± 0.01	0.11 ± 0.01
14	86 ± 9	108 ± 14	0.34 ± 0.04	0.31 ± 0.04
24	138 ± 27	128 ± 16	0.61 ± 0.13	0.46 ± 0.06
34	190 ± 32	152 ± 28	0.84 ± 0.14	0.64 ± 0.12
44	343 ± 23	162 ± 29*	1.88 ± 0.12	0.91 ± 0.12*

Table 2. Effects of photoperiod at V_2 stage on the growth of *D. alata.* Each value is the mean ± SE of 8 replicates (means were not significantly different at $P < 0.05$).

Time [d]	Leaf area [cm^2 $plant^{-1}$]		Dry matter [g $plant^{-1}$]	
	LD	SD	LD	SD
4	52 ± 6	52 ± 6	0.25 ± 0.03	0.25 ± 0.03
11	100 ± 12	103 ± 25	0.38 ± 0.04	0.39 ± 0.09
18	176 ± 24	179 ± 25	0.77 ± 0.12	0.66 ± 0.10
28	249 ± 40	250 ± 49	1.27 ± 0.20	1.35 ± 0.29
38	354 ± 35	314 ± 63	1.92 ± 0.25	1.92 ± 0.39

After 44 d, the total dry matter of the V_1 stage plants was 50 % less under SD compared to LD (Table 1). Also the number of leaves per plant and total leaf area were 30 and 50 % lower under SD. None of these parameters were influenced by photoperiod in V_2 stage (Table 2). On the other hand, the distribution of DM in the different plant parts was similar in V_1 and V_2 plants. About 56, 10, 11 and 22 % of the plant DM was found in leaves, stem, roots and tubers respectively. Under SD, 50 % of V_2 stage plants produced aerial tubers (bulbils) after 3 months. This phenomenon correlated with a reduction of the underground tuber mass by a factor of 3.5, but was not observed in V_1 plants.

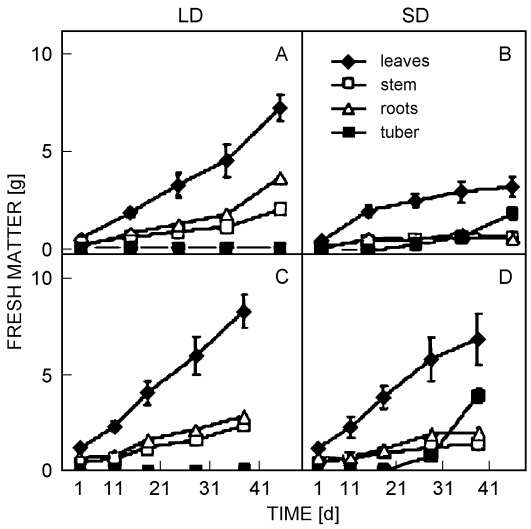

Fig. 1. Effects of photoperiod on the partitioning of FM in the diverse plant parts. Plantlets of *D. alata* were transplanted into pots and incubated under natural long day conditions until stage V1 (*A, B*) or V2 (*C, D*), then transferred to bioclimatic chambers and incubated under LD or SD. Each point represents the mean of 8 replicates; error bars represent SE.

In order to examine the reversibility of the effects of SD, plants were allowed to grow for 2 months under SD, and then half of them transferred to LD. After 44 d, DM of plants attained, was 44 % under SD+SD, compared to SD+LD (Table 3). Under SD+SD the FM of leaves and stems decreased, whereas the FM of tubers doubled (Fig. 2). Under SD+LD the FM of leaves, stems and roots was multiplied by approximately 7, after 44 d. At the beginning of the transfer, the FM of the tubers represented 56 % of the plants, but after 44 d under LD and SD, it reached 28 and 78 % respectively. The starch concentration in tubers (expressed on a FM basis) remained unchanged under LD (38 ± 8 mg g^{-1}), but increased to 105 ± 9 mg g^{-1} under SD.

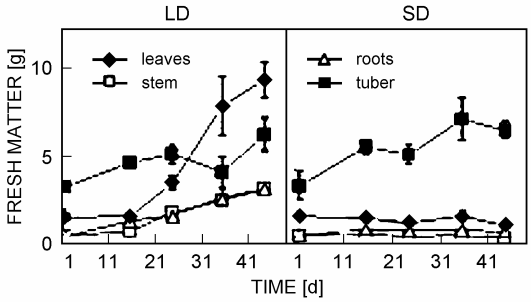

Fig. 2. Effects of LD and SD after 2 months acclimatization of plantlets under SD. *D. alata* plantlets were transplanted into pots and incubated under SD during 2 months, then transferred to bioclimatic chambers and incubated under LD or SD. Each point represents the mean of 6 replicates. *Error bars* represent SE.

Table 3. Effects of the transfer from SD to LD, on the growth of *D. alata.* V_1 plants were as a preliminary incubated under SD for 60 d, then incubated under LD or SD. Each value is the mean ± SE of 8 replicates (* - means significantly different at $P < 0.05$).

Time [d]	Dry matter [g plant^{-1}] SD+LD	SD+SD
1	0.92 ± 0.17	0.92 ± 0.17
15	1.18 ± 0.06	1.05 ± 0.10
24	1.39 ± 0.16	0.98 ± 0.09
35	2.06 ± 0.16	1.47 ± 0.25
44	2.81 ± 0.23	1.24 ± 0.11*

Discussion

Short days (12 h or less), as found in most of the humid and subhumid tropical yam zones, favours tuber initiation of various tuberous plants (Alvarenga and Valio 1989, Sladký and Bartošová 1990, Okubo *et al.* 1992). Furthermore, the initiation of tuberization and tuber bulking may be regulated differently. In yam bean, tuber bulking only takes place during SD, whereas the initiation of tuberization always occurs 6 - 8 weeks after sowing (Vaillant and Desfontaines 1995). The response to photoperiod was similar to that in potato cuttings (Ewing and Wareing 1978).

In *D. alata* cv. Belep, the formation and development of underground and aerial (bulbils) tubers was only induced by SD. Tuber initiation was not related to the

initial vegetative stage of plants. Tubers were visible as soon as 18 - 24 d under SD. This was quite early compared to the field conditions, where the tuber initiation occurred 12 weeks after sprouting (Chapman 1965). In fact, during the first phase of growth (sprouting to the 6^{th} week), the growth of plantlet depended entirely on the stored food in the planted tuber. The plant became autotrophic from the 12^{th} week after sprouting (Orkwor and Ekanayake 1998) and the tuber initiation occurred. Bigger tuber size in *D. alata* has also been found to result in longer leaf expansion phase and to hasten tuber initiation (Enyi 1972). Under SD, 50 % of V_2 stage plants produced aerial tubers (bulbils) after 3 months probably been due to a limitation of underground tuber growth because of the size of the pots. Rodriguez (1997) obtained similar results, after physical limitation of the underground tuber sink. The data did not agree with the fact that aerial tubers could be secondary sinks, developed only after the primary sink has passed its maximum period of bulking, as suggested by Ferguson
et al. (1980). On the other hand, bulblet formation from *Lilium* was also found to be effective in 16-h photoperiod (Lian *et al*. 2003).

SD affected the number of leaves and the total plant dry matter of V_1 plants. Owing to the simultaneous formation of stems and leaves (source) and tubers (sink) in V_1 and V_2 plants, the fact that these parameters were decreased by SD only in V_1 plants, implies that there could be an optimum source/sink balance. Consequently, V_1 plants produced less dry matter, probably in relation with source limitation due to early initiation of tuberization. These results indicated that the induction of tuberization affected the export of assimilate towards tubers but could have little influence on CO_2 fixation. The same results were obtained on yam bean (Vaillant and Desfontaines 1995) where the amount of assimilates was similar under SD compared to LD.

In yam, the effects of SD were reversed by LD, which stopped tuber growth but stimulated shoot and root growth. These results were similar to those obtained by Yoshida and Kanahama (1999) in Chinese yam, where the SD behaviour may be interrupted at any stage and replaced by LD behaviour. Short period of LD can also temporarily delay or inhibit tuber formation in potato (Struik *et al*. 1988).

Little is known of the factors that determine either the rate of synthesis or the remobilization of starch, in the starch-storing organs of higher plants such as tubers. The fact that LD decreased starch accumulation in tubers, indicated that the main sinks for carbon changed from tubers under SD to apex and roots under LD. The need to identify controlling factors in assimilate partitioning and use in tuber remains an important priority. It remains also to be seen, whether the photoperiodic response is influenced by other factors like temperature, as was described in potato (Snyder and Ewing 1989).

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