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Henry R. Owen, *Eastern Illinois University*
R. E. Veilleux
F. L. Haynes, *North Carolina State University at Raleigh*
K. G. Haynes, *North Carolina State University at Raleigh*

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Abstract

Flow-cytometric analysis of pollen samples obtained from Solanum phureja, clone PP5, grown under 10, 14, and 18 hour photoperiods at the Southeastern Plant Environment Laboratories of North Carolina State University yielded two populations of pollen based on size parameters. These populations corresponded to pollen separation based on propidium iodide staining of DNA. Anther culture response from plants grown under 14 and 18 hour photo periods for 8, I 0, 12, and 14 weeks was greatest from 8 week old plants grown under a 14 hour photoperiod. Net photosynthesis was significantly higher for plants grown under a 10 hour photoperiod than for plants grown under either a 14, or 18 hour photoperiod. A significant positive correlation was found between net photosynthesis and tuber yield. Results suggest that vegetative growth is increased under short photoperiods, floral development is favored under long photoperiods, and androgenesis is greatest from young plants grown under an intermediate photoperiod.

Introduction

Interploid hybridization between tetraploid S. tuberosum cultivars and diploid potato species is currently being exploited to introduce new germ plasm into potato cultivars (17, 18, 19). This technique has relied primarily upon 2n pollen formation in a diploid species and subsequent crossing to a tetraploid cultivar, producing a 4x=2x hybrid. Tuber yield of such hybrid has approached the tetraploid parent (21). Unfortunately, seed set in 4x=2x crosses is highly variable and generally inferior to intraploid crosses (10). Frequency of2n pollen formation has been shown to be affected by physiological (6) and morphological factors (21), as well as genotypic predispositions (14, 18).

Estimations of 2n pollen frequency have commonly been based on microscopic examination and visual scoring of large pollen grains (9). Flow-cytometric analysis of pollen samples labeled with a fluorescent DNA stain, on the other hand, is able to offer simultaneous measurement of size and DNA content of a large number of pollen grains with great rapidity and accuracy (15). In addition, it is able to display DNA distributions of subpopulations within a sample based on size parameters alone, thus allowing for separate DNA analysis of two size classes, such as large and small pollen grains.

Extraction of monoploid genotypes from diploid species has been envisioned as a useful first step in germplasm development, because it allows for direct phenotypic selection prior to genomic reconstruction (7, 23). Reconstruction utilizing

monoploid, anther-derived genotypes could be accomplished via somatic hybridization (cell fusion) between two unrelated genotypes which have been selected for superior characteristics, or by sexual hybridization via a 4x=2x cross after chromosome doubling and restoration of fertility. As with 2n pollen formation, response to anther culture has been shown to vary with environment (12) and genotype (2).

Selection for increased photosynthetic capacity has been examined as a method for improving yield of existing crop species (5, 8, 11, 24). In addition to genotype, photosynthetic capacity is strongly influenced by environmental factors (1, 11), and thus selection efforts require strict environmental control to reduce variability within genotypes. Photoperiod has been shown to influence several developmental and physiological processes in Solanum species, most notably tuberization (4, 13) and flowering (3, 6), which generally are favored under short and long day cycles, respectively. Detection of genotypes which demonstrate greater net photosynthesis may depend upon whether vegetative or floral development is favored.

The objectives of this study were to examine the effect of photoperiod on the three aforementioned characteristics of interest to current breeding efforts, i.e., 2n pollen production, anther culture response, and net photosynthesis utilizing a diplandrous (2n pollen-producing) clone of Solanum plzureja. This clone has been selected for enhanced response to anther culture, but has not previously been examined for net photosynthetic rate.

Materials and Methods

The following studies were conducted on plants grown at the Southeastern Plant Environment (Phytotron) Laboratories of North Carolina State University. Tubers from a diploid, diplandrous clone of Solanum phureja, P.I. 225669, (hereafter, PP5), were planted in flats containing **1**13 Peat-lite (Redi Earth, W.R. Grace Co.) and~ gravel (standard phytotron substrate), and placed in 3 photoperiod rooms (chamber size B). The chambers were set for 3 photoperiods, 10, 14 and 18 hrs. After the initial l 0 hr day length (650 µmol m-2 s-1 photosynthetic photon flux density, or PPFD), low intensity incandescent lights (50 µmol m-2 s-l PPFD) were used to extend the photoperiods in the remaining 2 chambers to 14 and 18 hrs. All chambers were maintained at 18 C for the minimum I 0 hr light period and 14 C for the remaining 14 hr period. Plants were watered 3 times weekly throughout the experiment with a nutrient solution containing 106 ppm N, 10 ppm P, and Ill ppm K. Sprouted tubers were transplanted into individual 15 cm pots 6 wks after planting (6 plants per chamber).

Flow cytometnc analyses - Pollen samples from each photoperiod treatment were collected 10 wks after experiment initiation and placed in a desiccator at 4 C. Pollen samples from the 3 photoperiods were placed in I ml of buffer solution [882 mg sodium citrate, 419 mg morpholinopropanesulfonic acid (MOPS), 915 mg MgCl2, 0.25 ml Triton X-100/250 ml]. To each sample, 0.5 ml of RNAase solution (80 mg

ribonuclease-A in 100 ml buffer solution) was added and the samples were incubated at 23 C for 30 min. Subsequently, 0.25 ml of propidium iodide (PI) solution (40 mg PI in 100 ml buffer solution) was added to each sample, incubated on ice for an additional 30 min, and analyzed within 3 hrs. Stained samples were filtered through a 37 μm nylon mesh and analyzed using an Epics V, Model 752 laser flow cytometer and cell sorter (Coulter Electronics, Hialeah, FL). Laser excitation was 300 mW, 488 nm from a 5 W Innova 90 Argon Laser (Coherent Inc., Palo Alto, CA). Three parameters were recorded: forward angle light scatter (FALS) and 90 degree light scatter (90LS, 488 nm dichroic filter) for size measurements, red fluorescence (RFL, 590 nm dichroic, 610 nm long pass filter) for DNA measurements. Multi parameter Data Acquisition and Display System (MDADS) and Easy 88 microcomputer analysis (Coulter Electronics) were used for data collection and analysis. FALS was collected linear integral, 90LS log integral, and RFL linear peak red. Histograms of number of nuclei per fluorescence channel contained 256 channels and were gated on FALS and 90LS dual parameter 64 X 64 channels resolution histograms defining the populations of interest. The fluorescence signal from PI-stained preparations is proportional to the DNA content of particles (pollen grains) passing through the flow sheath. Chicken red blood cells and a microsphere standard (Coulter Electronics) were used as calibration standards. Pollen grain counts were set at 5 ,000/sample.

Anther cultures - Pre-anthesis floral buds from plants in the 14 and 18 hr photoperiod chambers were collected at 8, 10, 12, and 14 wks, wrapped in moist paper, and kept at 4 C for 3 days prior to anther culture. Flowering in the 10 hr chamber was insufficient for anther culture. Floral buds were disinfested by a 30 sec dip in 70% ethanol, followed by immersion in a 5.25% sodium hypochlorite solution for 20 min and 3 rinses in sterile, distilled water. Anthers were dissected from the buds and plated on a solid/liquid bilayer medium [7 ml bottom layer containing MS salts and vitamins (16), 60 g/l sucrose, 5 g/l activated charcoal, 7 g/l agar, and 1 mg/l N6-benzylaminopurine (BAP), pH 5.8; 1 ml top layer containing same as bottom, but lacking activated charcoal and agar] as described by Veilleux, et al (20). Ten anthers were placed in each petri plate and incubated at 25 C under a 16 hr photoperiod for 4 wks. Embryos arising from within the cultured anthers were transferred to 25 X 150 mm culture tubes containing 20 ml of filter-sterilized embryo medium (MS salts, 100 mg/l inositol, 0.4 mg/l thiamine, 0.1 mg/l gibberellic acid (GA3), 20 g/l sucrose, 7 g/l agar, pH 5.8) and incubated as above (22). After 6 wks, plant lets were transferred to MS basal medium for rooting.

Photosynthesis and yield measurements - After 8 wks of growth, measurements of net photosynthesis [carbon dioxide exchange rate (CER) expressed as the volume of C02 depleted by the plant leaf surface per unit time] were taken for all plants during the middle of the light period using an infrared gas analyzer (Anarad, Model AR-500R) equipped with an external reference (ambient C02). CER measurements were taken 3 times on fully expanded leaflets from nodes 4, 5, and 6. Measurements were repeated at 10 and 12 wks after experiment initiation. Total tuber weight and tuber number (greater than I cm diam) were recorded for each plant at harvest (16 wks

after planting).

Results

2n pollen formation- Pollen size was found to correspond reasonably well with DNA content in each of the 3 photoperiods (Figure I). As the photoperiod increased, the pollen population shifted to a more obvious bimodal distribution, indicating increasing 2n pollen frequency based on size parameters (Figure la-c) and DNA content (Figure ld-f). The major and minor peaks occurred at channels 60 and 90 (out of 256 channels total) and corresponded to small (Figure lg-1) and large (Figure lj-/) pollen subpopulations, respectively.

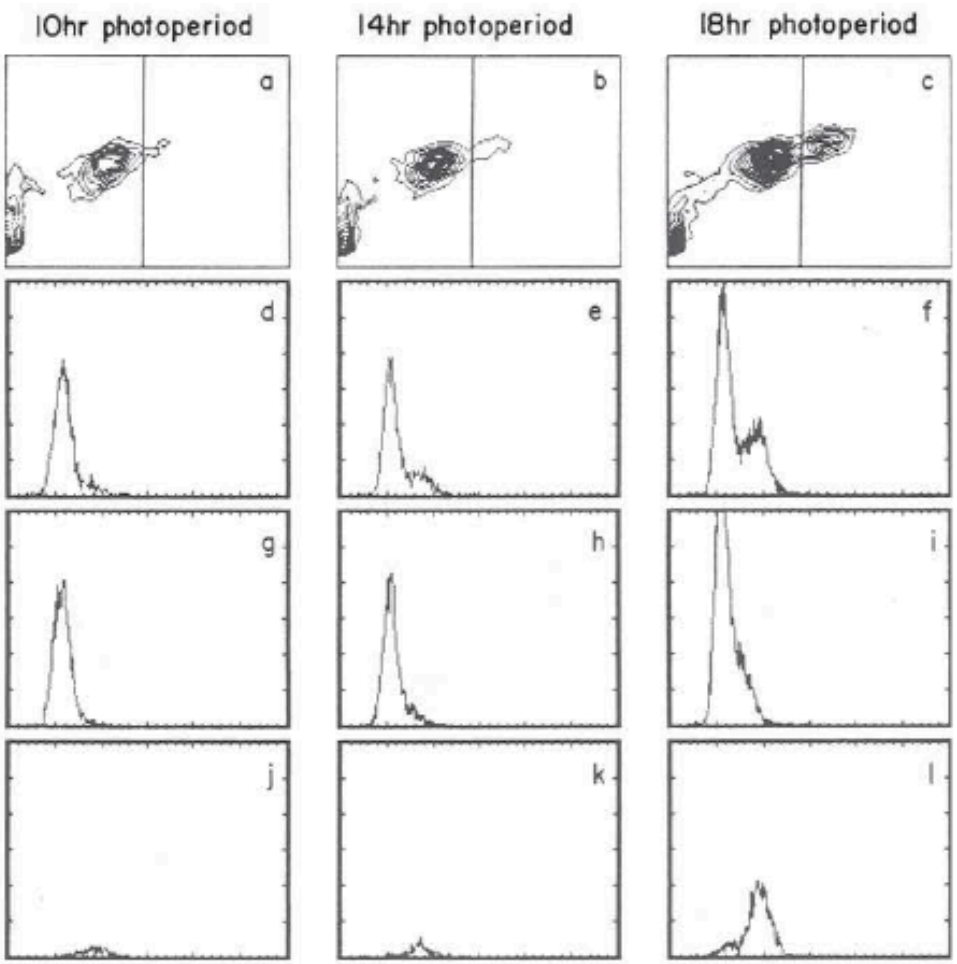

FIG. 1. Flow-cytometric analyses of pollen size [measured by forward angle (x-axis) and 90° (y-axis) light scatter] and DNA distribution (measured by fluorescence of propidium iodide) of a 2*n* pollen-producing clone of *Solanum phureja* grown under three photoperiods (vertical columns). After elimination of debris appearing in the lower left of figures *a-c*, the pollen populations were analyzed for DNA content (*d-f*). X-axis equals channel number, y-axis equals frequency of nuclei (of 5,000 analyzed) per channel. The pollen populations in figures *a-c* were then divided (vertical line) to determine subpopulation percentages and to reanalyze subpopulations for DNA content (*g-i*, major population of mostly 1*n* pollen and *j-l*, minor population of mostly 2*n* pollen).

In the sample from the 10 hr photoperiod, 62% of the particles which recorded a positive fluorescent event were contained within the major population. Similarly, samples from the 14 and 18 hr photoperiods recorded major populations corresponding to 59 and 62% of their total counts (Figure la-c). By contrast, minor populations, corresponding to the larger-sized pollen grains, were 7, 8 and 16% of the total counts from the 10, 14, and 18 hr photoperiods, respectively. Remaining counts fell outside of the populations of interest and consisted mainly of particles (debris, aborted pollen) recording very low size values in the scattergrams. This increased frequency of particles recording large size and DNA values in the 18 hr sample indicates a twofold increase in 2n pollen in the sample taken from plants grown under an 18 hr photoperiod. However, the subpopulation of larger pollen did include a small frequency of In pollen, as determined by DNA content (Figure 1l).

Response to anther culture - The highest response to anther culture was observed on anthers taken from the first flowers on plants in the 14 hr chamber (Table 1). The number of pre-anthesis floral buds formed varied weekly and by photoperiod. Both flowering frequency and duration reached a maximum in the 18 hr chamber. Anthers taken from plants grown under an 18 hr photoperiod produced fewer embryoids and plantlets, even though a greater number of anthers was cultured from this photoperiod. Frequency of embryoids and plantlets was superior from plants grown under a 14 hr photoperiod, as demonstrated by mean embryoids/anther. This increased response, however, was almost entirely the result of anthers cultured at 8 wks. With plants grown under an 18 hr photoperiod, maximum response to anther culture, as indicated by mean plantlets per anther, occurred at 12 wks.

Net photosynthetic rate - CER did not vary significantly among the 8, 10, and 12 wk measurements. In addition, no significant interaction was found (5% level) between week and photoperiod treatments (data not shown); thus, weekly measurements of CER were combined for further data analysis. Mean separation of CER by Student-Newman-Keuls' test at the 1% level was significant between photoperiods. Mean CERs were 2.7, 1.3, and 0.8 μl CO_2 dm-2s-1 for the 10, 14, and 18 hr photoperiods, respectively (Table 2). In addition, significant correlations (5% level; N= 54) were found between CER and both final tuber weight (0.54) and tuber number (0.74).

Discussion

Pollen size was found to be a fairly accurate gauge of DNA content, and allows for estimation of 2n pollen frequency based upon visual scoring of pollen preparations. In addition, it makes possible the separation of2n from ln pollen based solely on size parameters. Because flow sorting of pollen samples based upon size does not require fluorescent staining, the potential exists for recovery of viable pollen grains within size classes.

TABLE 1. — *Anther culture response of* Solanum phureja, *genotype PP5, taken from plants grown under 14 and 18 hr photoperiods for 8, 10, 12, and 14 wks.*

Photoperiod (hrs)	Age of plant (wks)	Anthers plated	Embryoids formed	Plantlets regenerated	Embryoids per anther	Plantlets per anther
14	8	40	43	22	1.08	0.55
14	10	300	6	0	0.2	0
14	12	80	0	0	0	0
14	14	0	0	0	0	0
Total		420	49	22	0.12	0.05
18	8	20	0	0	0	0
18	10	340	16	3	0.05	0.01
18	12	200	7	7	0.04	0.04
18	14	200	2	0	0.01	0
Total		760	25	10	0.03	0.01

TABLE 2. — *Mean CO_2 exchange rate (CER, µl CO_2 depleted per square decimeter leaf surface per second) measured after 8 wks of growth, mean tuber number per plant, and mean tuber wt (g per plant) 16 wks after planting for* S. phureja *clone PP5 at 3 photoperiods.*

Photoperiod hrs light	CER µl CO_2 dm^{-2} s^{-1}	Tuber number per plant	Tuber wt. (g) per plant
10	2.7	7.3	106.0
14	1.3	5.7	95.0
18	0.8	2.5	33.4

For S. phureja clone PP5, 2n pollen frequency was greatest under an 18 hr day length. Thus, screening of several genotypes for 2n pollen frequency may not give an accurate measurement of maximum frequency unless the genotypes being tested behave similarly for the given photoperiod under which the plants are grown.

For PPS, net photosynthesis and tuberization reached their maximum under short photoperiods. Conversely, flowering and 2n pollen formation peaked under long daylengths. Thus, vegetative development was favored under short day lengths, whereas long day lengths stimulated floral development. Response to anther culture in this genotype was greatest from 8 wk old plants grown under a 14 hr photoperiod. Anther culture itself involves a redirection from floral development (pollen formation) to vegetative development (embryo formation); therefore, the

best physiological and environmental conditions for embryoid formation and plantlet regeneration may be from young plants grown under a moderate photoperiod.

This work demonstrates that vegetative and floral development in vivo and plant regeneration from anther culture each impose their own unique set of environmental conditions for maximum response, including photoperiod exposure. This dichotomization of developmental processes caused by photoperiod may be useful, for example, when determining environmental conditions to maximize either harvest index, seed set, or in vitro response.

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