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Influence of light on DNA content of *Helianthus annuus* Linnaeus

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ABSTRACT **Mean nuclear 2C DNA content (C equaling haploid DNA per nucleus) of the first leaf of the sunflower, *Helianthus annuus* L., is influenced by the quality and the quantity of light. Seedlings of two inbred lines, RHA 299 and RHA 271 were germinated and grown in controlled environmental conditions. Lighting was adjusted to provide different combinations of photon flux densities and red to far red (R:FR) ratios. At R:FR = 5.8 and photon flux densities of 170 μmol·m^{-2}·s^{-1}, 200 μmol·m^{-2}·s^{-1}, and 230 μmol·m^{-2}·s^{-1}, DNA content remained high and relatively constant ($\bar{x}$ = 6.97 pg for RHA 271 and $\bar{x}$ = 7.32 pg for RHA 299). When the photon flux density range (R:FR = 5.8) was elevated to 350 μmol·m^{-2}·s^{-1}, 410 μmol·m^{-2}·s^{-1}, and 470 μmol·m^{-2}·s^{-1}, mean DNA content was reduced to 6.23 pg (RHA 271) and 6.46 pg (RHA 299). At R:FR = 1.5, mean DNA content was consistently high (7.2–7.9 pg) only at the lowest photon flux density of 170 μmol·m^{-2}·s^{-1}. Significant decreases in DNA content (≤12%) were observed at photon flux densities of 200 μmol·m^{-2}·s^{-1} and 230 μmol·m^{-2}·s^{-1}. At the higher photon flux densities (350 μmol·m^{-2}·s^{-1}, 410 μmol·m^{-2}·s^{-1}, and 470 μmol·m^{-2}·s^{-1}) and R:RF = 1.5, the plants had extremely low DNA contents ($\bar{x}$ = 3.36 pg for RHA 271 and 3.41 pg for RHA 299) and high between-plant variance. The instability of DNA content, particularly for plants grown under light that is far red rich, suggests that phytochromes may be involved in regulating DNA content of the sunflower.**

Nuclear DNA content varies over several orders of magnitude among diploid eukaryotes (1). There is no overall correlation between DNA content and genetic complexity or evolutionary advancement (2), and differences in genome size exceeding 2- to 3-fold are commom among congeneric species (2). Furthermore, only a small amount of the nuclear DNA apparently has coding functions (3). These observations have collectively been called the C-value paradox (4, 5). Although extensive research over the last 3 decades has been directed toward resolving this paradox, no generally accepted solution has been found. Part of the difficulty in studying the evolution of genome size results from the fact that few plant and animal species are known in which the nuclear DNA content can be experimentally manipulated, independent of polyploidy, endopolyploidy, and aneuploidy.

The sunflower genus, *Helianthus*, is an ideal taxon in which to study genome organization and evolution of DNA content. Genome size varies more than 4-fold among diploid species, $2n = 34$ (6) and considerable variation in DNA content has been reported within the cultivated sunflower, *Helianthus annuus* (7–10). Nagl and Capesius (7) reported variation in nuclear DNA content exceeding 60% among cultigens of *H. annuus*. Michaelson *et al.* (10) and Cavallini *et al.* (9, 11) detected variation of 32% and 58%, respectively, among lines. Michaelson *et al.* (10) reported 48% differences in DNA amount among leaves of individual plants. Cavallini and Cremonini (12) initially proposed that aneusomaty (loss of chromosomes) occurring during mitotic divisions early in development resulted in tissues possessing a mixture of diploid and aneuploid cells and, therefore, variation in DNA content within *H. annuus* plants. However, Michaelson *et al.* (10) presented data indicating that if aneusomaty occurs in sunflowers it is rare. It is now evident that the variation in DNA content in *H. annuus* results from quantitative differences in the DNA of chromosomes, apparently due to differences in the copy number of repetitive sequences (11, 13).

Because abundant variation in DNA content exists among and within sunflower plants and the DNA amount displays instability, we initiated a study of *H. annuus* to detect parameters that might influence the stability of DNA content (14). Although increasing levels of nitrogen fertilizer resulted in higher DNA content, other environmental factors tested such as heat stress, water deficit, and phosphorous fertilizer level had little or no significant effect on nuclear 2C DNA content or its stability. The one constant that emerged from these studies was that plants growing in one of two growth chambers displayed instability in DNA content, whereas plants growing in the second growth chamber had relatively stable DNA contents. Because the major differences between the two growth chambers were irradiance and the ratio of red to far red light (R:FR), it was suggested that either of these factors may induce instability in DNA content (14). Herein, we report experiments indicating that both irradiance and R:FR ratio influence the quantity of nuclear DNA content in the first leaf pair of two inbred strains of *H. annuus*.

MATERIALS AND METHODS

Plant Material. Two U.S. Department of Agriculture inbred oilseed lines of *H. annuus*, RHA 271 and RHA 299, originally obtained from C. C. Jan (U.S. Department of Agriculture, Agricultural Research Service, Fargo, ND), were used in this study because both had been previously documented as showing DNA content instability (10, 14). *Hordeum vulgare* cv Sultan (2C DNA content = 11.12 pg; C equals haploid content per nucleus) was used as a DNA content standard.

The sunflowers were grown in medium texture vermiculite in one gallon pots and saturated every other day with a solution containing trace elements, 45 ppm nitrogen, 48 ppm phosphorous, and 51 ppm potassium prepared from Peters Pete-Lite Special, 15–16-17. The pots were placed in a 3.6-m^2 walk-in growth chamber with a 16 hr/28°C day and an 8 hr/20°C night. The newly expanded first leaf pair was used to determine 2C nuclear DNA content. All measurements of seedlings for an individual experimental set were done within a 4-day interval. Plants used were phenotypically healthy and no yellowing or signs of senescence were apparent.

Photon Flux Density and R:FR Ratios. Light intensity was measured as the integrated irradiance between 400 nm and 800 nm using a LiCOR LI-1800 portable spectroradiometer. Red light was measured as the integrated irradiance between 654 nm and 666 nm. Far red was measured as the integrated irradiance between 724 nm and 736 nm. Different lighting

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Abbreviations: C, haploid DNA content per nucleus; R:FR, red to far red ratio.

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Table 1. Mean 2C DNA content (pg DNA) of the first leaf pair for control plants grown at three photon flux densities with R:FR = 5.8

	RHA 271				RHA 299			
Photon flux density	*N*	pg DNA	Duncan's groupings*	SE	*N*	pg DNA	Duncan's groupings*	SE
170 μmol·m^{-2}·s^{-1}	10	6.898	A	0.096	11	7.367	A	0.082
200 μmol·m^{-2}·s^{-1}	10	6.984	A	0.109	11	7.253	A	0.656
230 μmol·m^{-2}·s^{-1}	10	7.019	A	0.049	10	7.327	A	0.054
	$\bar{x}$ = 6.967				$\bar{x}$ = 7.316			

Total number of plants (*N*), mean 2C nuclear DNA content (pg DNA), and standard error of the mean (SE).
*Multiple range test groupings calculated after an F-test, significance at the 1% level.

levels within an experiment were produced by placing pots at different distances from the light bank. The distances were also adjusted between experiments so that the irradiance for the fluorescent lighting used in the control experiments (R:FR = 5.8) was the same as that in the experiments using combined fluorescent and incandescent lights (R:FR = 1.5).

Determination of Nuclear DNA Content. Nuclei were prepared for flow cytometry by procedures modified from Galbraith *et al.* (15) and Michaelson *et al.* (10) as detailed in Johnston *et al.* (14) and Price and Johnston (16). The samples of nuclei were stained with propidium iodide and analyzed with a Coulter "Epics" Elite flow cytometer adjusted to 0.5 W of laser power at 514 nm. The mean DNA content of sunflower samples was determined by dividing the mean channel number fluorescence of the sunflower by the mean channel number fluorescence of the barley internal standard and multiplying by the 11.12 pg of the barley. The data analyzed had a full coefficient of variation about the 2C mean no larger than 5.0 and 2C to 4C mean ratios between 1.96 and 2.13.

Statistical Analysis. Statistics were conducted using the General Linear Model Procedure (Proc GLM) and Duncan's test of significance between means (SAS statistical package, SAS Institute, Cary, NC, USA Release 6.08). To compare between experiments with different means, the overall results were analyzed using zero means adjusted data in which the data were weighted to correct for unequal sample size and adjusted so that the data summed to zero within each experiment. The results are presented as pg DNA per nucleus above (+) or below (−) the mean for each experiment.

RESULTS

Controls. Two sets of controls were run. The first set consisted of sunflower plants germinated under three photon flux densities (170 μmol·m^{-2}·s^{-1}, 200 μmol·m^{-2}·s^{-1}, 230 μmol·m^{-2}·s^{-1}) with relatively high R:FR ratio (R:FR = 5.8). No significant differences in DNA content were observed among plants of either strain (Table 1). This demonstrates that at a high R:FR ratio under relatively low irradiance, DNA content is not influenced by photon flux density. The second set of controls consisted of plants germinated under conditions of increased photon flux densities (350 μmol·m^{-2}·s^{-1}, 410 μmol·m^{-2}·s^{-1}, 470 μmol·m^{-2}·s^{-1}, R:FR = 5.8). The results (Table 2) show that the mean DNA content was at least 11% lower for these plants and an effect of photon flux density was apparent only at the highest irradiance level in RHA 271.

Experimental Results. Two sets of experiments were done to determine the effects of R:FR and irradiance on mean DNA content of the two sunflower strains. The first one was done at relatively higher photon flux densities, 350 μmol·m^{-2}·s^{-1}, 410 μmol·m^{-2}·s^{-1}, 470 μmol·m^{-2}·s^{-1} (Table 2). The first leaf pairs of sunflowers differentiated at high photon flux densities and a relatively low R:FR ratio, which was FR rich (R:FR = 1.5) were characterized both by extremely low DNA content ($\bar{x}$ = ca 3.4 pg for both RHA 271 and RHA 299), and high between-plant variation (Table 2). The means were 54% and 53% of the control mean values for RHA 271 and RHA 299 plants grown under the same photon flux levels but with R:FR = 5.8 (Table 2). An effect of increased photon flux density on DNA content at these higher photon flux densities was observed in both strain RHA 271 and RHA 299.

The second set of experiments involved three replicates of plants germinated at lower photon flux densities (170 μmol·m^{-2}·s^{-1}, 200 μmol·m^{-2}·s^{-1}, 230 μmol·m^{-2}·s^{-1}) and R:FR = 1.5 (Table 3). Replicate 1 shows that the DNA content of strain RHA 299 decreased from 7.87 pg to 6.93 pg, or 12%, with increase photon flux densities. Strain RHA 271 did not respond in this experiment. In replicate 2, DNA content decreased about 3–5% with increased photon flux density in RHA 271 (7.20 pg to 6.99 pg) and RHA 299 (7.21 pg to 6.86 pg). In the third replicate, 6 of 15 incandescent bulbs burned out during the experiment and were replaced. This resulted in a change in the R:FR ratio during the course of the experiment. A higher R:FR ratio apparently occurred early in the experiment when the plants at higher irradiance are growing faster. Even though the results of replicate 3 are not totally comparable to replicates 1 and 2, we chose to leave these data in the analysis because they provide another measure of the sensitivity of the plants to light quality. Under these conditions the mean DNA content of RHA 271 decreased with increased photon flux densities from 7.33 pg to 6.45 pg, or ≈12%; the plants of strain RHA 299 did not respond. The results in Table 3, compared with those of the controls in Table 1, indicate DNA content of plants germinated under these relatively low photon flux levels varies only when the irradiance is rich in the far red light spectrum.

Table 2. Mean 2C nuclear DNA content (pg DNA) of the first leaf pair from sunflower seedlings of inbred strains RHA 271 and RHA 299 grown under R:FR = 5.8 and R:FR = 1.5 at photon flux densities of 350 μmol·m^{-2}·s^{-1}, 410 μmol·m^{-2}·s^{-1}, and 470 μmol·m^{-2}·s^{-1}

	R:FR = 5.8								R:FR = 1.5							
Photon flux density	pg DNA RHA 271	Duncan's grouping*	*N*	SE	pg DNA RHA 299	Duncan's grouping*	*N*	SE	pg DNA RHA 271	Duncan's grouping*	*N*	SE	pg DNA RHA 299	Duncan's grouping*	*N*	SE
350 μmol·m^{-2}·s^{-1}	6.320	A	7	0.297	6.366	A	6	0.350	4.286	C	3	0.386	3.458	C	3	0.337
410 μmol·m^{-2}·s^{-1}	6.482	A	8	0.351	6.464	A	7	0.360	2.760	D	3	0.287	3.951	C	3	0.530
470 μmol·m^{-2}·s^{-1}	5.883	B	8	0.382	6.552	A	7	0.169	3.042	D	15	0.077	2.820	D	16	0.064
	$\bar{x}$ = 6.225				$\bar{x}$ = 6.460				$\bar{x}$ = 3.363				$\bar{x}$ = 3.410			

*Multiple range test groupings calculated after an F-test, significance at the 1% level.

Table 3. Mean DNA content (pg DNA) of the first leaf pair for experimental plants grown at three photon flux densities (170 μmol·m^{-2}·s^{-1}, 200 μmol·m^{-2}·s^{-1}, 230 μmol·m^{-2}·s^{-1}) with R:FR = 1.5

	RHA 271				RHA299			
Photon flux density	*N*	pg DNA	Duncan's groupings*	SE	*N*	pg DNA	Duncan's groupings*	SE
Replicate 1								
170 μmol·m^{-2}·s^{-1}	9	7.471	A	0.187	8	7.869	A	0.106
200 μmol·m^{-2}·s^{-1}	8	7.662	A	0.079	11	7.498	B	0.140
230 μmol·m^{-2}·s^{-1}	7	7.652	A	0.155	11	6.929	B	0.140
Replicate 2								
170 μmol·m^{-2}·s^{-1}	17	7.197	A	0.037	12	7.208	A	0.044
200 μmol·m^{-2}·s^{-1}	6	7.235	A	0.078	5	6.963	B	0.064
230 μmol·m^{-2}·s^{-1}	8	6.994	B	0.102	9	6.856	B	0.073
Replicate 3								
170 μmol·m^{-2}·s^{-1}	9	7.328	A	0.368	11	7.268	A	0.291
200 μmol·m^{-2}·s^{-1}	8	6.882	AB	0.225	4	6.429	A	0.327
230 μmol·m^{-2}·s^{-1}	9	6.446	B	0.223	12	7.280	A	0.183

Total number of plants (*N*), mean 2C nuclear DNA content (pg DNA), and standard error of the mean (SE).
*Multiple range test groupings calculated after an F-test. Different letters within a strain of a replicate are significantly different at the 1% level.

Table 4 shows the pooled DNA values for the experimental and control plants (Tables 1–3) using zero means adjusted data. The results show a significant decrease in mean DNA content with increasing photon flux densities for plants grown under R:FR = 1.5, and no significant differences in DNA content with increasing photon flux densities for plants germinated under R:FR = 5.8.

DISCUSSION

DNA content varies several hundred-fold among presumed diploid angiosperms, up to 9-fold among diploid congeneric plant species (17–19) and to a smaller but nonetheless extensive degree among (17, 19, 20) and within (10, 21) plants of a species. Although considerable correlation has been detected between DNA content and environmental factors (2, 18–20) and between DNA amount and cellular and growth parameters (22), the evolutionary significance of variation in DNA content is not well understood.

The plant genome is considered to be fluid with repetitive sequence amplification, divergence, and deletion occurring over time (3, 23). It has been proposed that the plant genome may be unstable and respond to physical, chemical, or genetic stresses by amplifying or deleting DNA sequences and hence altering DNA content (24, 25). The ability of the genome to respond to environmental factors may be a strategy for adaptation to a changing environment (24, 25). The processes responsible for turnover of DNA sequences in the genome remain to be elucidated; however, the activity of transposable elements, retrotransposons, unequal crossingover, and saltatory amplifications or deletions are generally considered to be likely mechanisms for alteration of 2C DNA content. There is experimental evidence indicating that at least some of the mechanisms are sensitive to genetic and environmental parameters, e.g., nitrogen and phosphorous fertilizer-induced DNA content changes in flax (26), nitrogen fertilizer-induced changes in sunflowers (14), and the destabilization of DNA content in *Microseris* (27) and *Nicotiana* (28, 29) hybrids. Waters and Schaal (30) reported that in *Brassica nigra* heat shock induces a statistically significant reduction in the number of rRNA cistrons. The average rDNA copy number was reduced 37%, relative to nonstressed control plants, and was heritable to the next generation. Dhillon (31) reported DNA content of nuclei of the eastern cottonwood *Populas deltoides* to be 27% higher in plants grown under long compared with short days.

Table 4. Zero adjusted means (Mean) and standard errors (SE) for 2C nuclear DNA contents from Tables 1–3

R:FR	Strain	Irradiance	*N*	Mean	Duncan's grouping*	SE
5.8	RHA 271	Low	17	−0.073	A	0.095
		Mid	18	0.046	A	0.104
		High	18	−0.151	A	0.115
	RHA 299	Low	17	0.099	A	0.079
		Mid	18	0.005	A	0.086
		High	17	0.084	A	0.109
1.5	RHA 271	Low	38	0.286	A	0.080
		Mid	25	0.040	AB	0.071
		High	39	−0.180	B	0.062
	RHA 299	Low	34	0.142	A	0.073
		Mid	23	0.010	AB	0.086
		High	48	−0.352	B	0.095

The values shown are the deviations from the mean of each control or experiment.
*Multiple range test groupings calculated after an F-test. Different letters within a strain of a replicate are significantly different at the 1% level.

The DNA content of the plants grown under stable conditions in the current study are between 6.9 pg and 7.4 pg. However, plants grown at relatively high photon flux levels and a R:FR = 1.5 have less than 50% of the mean 2C DNA content of those grown at lower photon flux densities at R:FR = 5.8 (Tables 1 and 2). Johnston *et al.* (14) reviewed the evidence that leaf nuclei of sunflower differentiate at the 2C level and that the differences in DNA content among sunflower leaves are apparently not the result of endoreduplication of genomes or arrest of DNA replication between the 2C and 4C level. This evidence included: (*i*) the nuclei of composites typically differentiate at G_1 (2C) (32) and endopolyploidy is rare (33); (*ii*) in sunflower, endopolyploid nuclei do not exist (34, 35) or are infrequent (7); (*iii*) a Feulgen microspectrophotometric analysis indicated that leaf interphase nuclei have one-half the absorbancy of late prophase 4C root tip nuclei; (*iv*) cytograms of nuclei from differentiated sunflower leaves show a major 2C peak and a minor 4C peak and when the 2C peak is low there is a comparable drop in the 4C peak (14); and (*v*) variation in DNA content in the sunflower is accompanied by changes in the copy number of repetitive DNA sequences (11, 13).

The data herein show that environmental components influencing DNA content instability in *H. annuus* include both light quality and quantity. Higher photon flux densities produced lowered overall mean DNA contents, but the maximum effect of photon flux density was dependent upon the R:FR ratio. We propose that the induction of DNA content instability requires a quantitative amount of FR light, most readily

provided by a spectrum with a low R:FR ratio. The DNA content of the plants of Table 2 lead us to suggest that even at high R:FR ratios, if irradiance is high, sufficient FR light can be provided to reduce DNA amounts, but the maximum reduction is obtained only for plants grown under the low R:FR ratio (Table 2).

The differential response to R:FR ratios suggest that phytochromes may be involved in the instability of DNA content phenomenon in sunflowers. However, the amount and duration of far red light and the critical stage of meristem or leaf development where instability can be induced remain to be determined. Phytochromes exist in multiple molecular forms and are a regulatory photoreceptor shown to function as a switch in influencing many diverse physiological phenomena such as germination, stem elongation, floral induction, and the induction of some enzymes (36, 37). If the observed DNA instability is phytochrome regulated, exposure to red light (660 nm) would convert P_r to P_{fr} (38); the P_{fr} form would be necessary for DNA stability. The exposure to far red light (725 nm) would change P_{fr} back to P_r leading to instability of DNA content. Although the mode of action of phytochromes in regulating DNA content is not understood, P_r may trigger induction of enzymes that can recognize and remove noncoding DNA sequences, perhaps repetitive DNA regions that are methylated. Regardless of the mechanisms generating the DNA content variation, considerable amount of DNA is involved. The DNA content of plants of the current study varied over a 5 pg range from 2.8 pg (Table 2) to 7.9 pg (Table 3). This variation represents 15 times more DNA than present in *Arabidopsis thaliana* nuclei containing 2C = 0.34 pg (39).

The adaptive significance, if any, of the light effects on DNA content of the sunflower remains to be determined. Because reflected light has a lower R:FR ratio to its spectrum than does unobstructed light (40), the far red light-induced instability and reduction of nuclear DNA content may represent an adaptation for shade-avoidance in competition with neighboring plants.

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1. Sparrow, A. H., Price, H. J. & Underbrink, A. G. (1972) *Brookhaven Symp. Biol.* **23,** 451–494.
2. Price, H. J. (1976) *Bot. Rev.* **42,** 27–52.
3. Flavell, R. B. (1980) *Annu. Rev. Plant Physiol.* **31,** 569–596.
4. Thomas, C. A. (1971) *Annu. Rev. Genet.* **5,** 237–256.
5. Raff, R. A. & Kaufman, T. C. (1983) *Embryos, Genes, and Evolution* (Macmillan, New York).
6. Sims, L. & Price, H. J. (1985) *Am. J. Bot.* **72,** 1213–1219.
7. Nagl, W. & Capesius, I. (1976) *Plant Syst. Evol.* **126,** 221–237.
8. Olszewska, M. J. & Osiecka, R. (1983) *Biochem. Physiol. Pflanz.* **78,** 581–599.
9. Cavallini, A., Zolfino, C., Natali, L., Cionini, G. & Cionini, P. G. (1989) *Theor. Appl. Genet.* **77,** 12–16.
10. Michaelson, M. J., Price, H. J., Johnston, J. S. & Ellison, J. R. (1991) *Am. J. Bot.* **78,** 1238–1243.
11. Cavallini, A., Zolfino, C., Cionini, G., Cremonini, R., Natali, L., Sassoli, O. & Cionini, P. G. (1986) *Theor. Appl. Genet.* **73,** 20–26.
12. Cavallini, A. & Cremonini, R. (1985) *Z. Pflanzenzuecht.* **95,** 118–124.
13. Natali, L., Cavallini, A., Cionini, G., Sassoli, O., Cionini, P. G. & Durante, M. (1993) *Theor. Appl. Genet.* **85,** 506–512.
14. Johnston, J. S., Jenson, A., Czeschin, D. G., Jr., & Price, H. J. (1996) *Am. J. Bot.* **83,** in press.
15. Galbraith, D. W., Harkins, K. R., Maddox, J. M., Ayres, N. M., Sharma, D. P. & Firoozabady, E. (1983) *Science* **220,** 1049–1051.
16. Price, H. J. & Johnston, J. S. (1996) in *Methods of Genome Analysis in Plants: Their Merits and Pitfalls*, ed. Jauhar, P. P. (CRC, Boca Raton, FL), pp. 115–132.
17. Bennett, M. D. (1985) in *Plant Genetics*, ed. Freeling, M. (Liss, New York), pp. 283–302.
18. Bennett, M. D. & Smith, J. B. (1976) *Philos. Trans. R. Soc. London Biol. Sci.* **274,** 227–274.
19. Price, H. J. (1988) *Ann. Mo. Bot. Gard.* **75,** 1248–1257.
20. Price, H. J. (1988) *Evol. Trends Plants* **2,** 53–60.
21. Jackson, R. C., Crane, C. F., Johnston, J. S., Ellison, J. R. & Price, H. J. (1993) *Chromosome Res.* **1,** 53–61.
22. Bennett, M. D. (1974) *Brookhaven Symp. Biol.* **25,** 344–366.
23. Flavell, R. B. (1982) in *Genome Evolution,* eds. Dover, G. A. & Flavell R. B. (Academic, New York), pp. 301–323.
24. Walbot, V. & Cullis, C. A. (1983) *Plant Mol. Biol. Rep.* **1,** 3–11.
25. Walbot, V. & Cullis, C. A. (1985) *Annu. Rev. Plant Physiol.* **36,** 367–396.
26. Cullis, C. A. & Cleary, W. (1986) *Can. J. Genet. Cytol.* **28,** 252–259.
27. Price, H. J., Chambers, K. L., Bachmann, K. & Riggs, J. (1983) *Am. J. Bot.* **70,** 1133–1138.
28. Gerstel, D. U. & Burns, J. A. (1966) in *Chromosomes Today*, eds. Darlington, C. A. & Lewis, K. R. (Plenum, New York), Vol. 1, pp. 41–56.
29. Moav, J., Moav, R. & Zohary, D. (1968) *Genetics* **59,** 57–63.
30. Waters, E. R. & Schaal, B. A. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 1449–1452.
31. Dhillon, S. S. (1988) in *Genetic Manipulation of Woody Plants,* eds. Hanover, J. W. & Keithley, D. E. (Plenum, New York), pp. 265–274.
32. Brossard, D. (1977) *New Phytol.* **79,** 423–429.
33. Nagl, W. (1978) *Endopolyploidy and Polynemy in Differentiation and Evolution* (North-Holland, New York).
34. Van't Hof, J. (1965) *Exp. Cell Res.* **39,** 48–58.
35. Evans, L. S. & Van't Hof, J. (1975) *Am. J. Bot.* **62,** 1060–1064.
36. Quail, P. H. (1991) *Annu. Rev. Genet.* **25,** 389–409.
37. Furuya, M. (1993) *Annu. Rev. Plant Physiol.* **44,** 617–645.
38. Salisbury, F. B. & Ross, C. W. (1992) *Plant Physiology* (Wadsworth, Belmont, CA), pp. 438–463.
39. Galbraith, D. W., Harkins, K. R. & Knapp, S. (1991) *Plant Physiol.* **96,** 985–989.
40. McNellis, T. W. & Deng, X.-W. (1995) *Plant Cell* **7,** 1749–1761.