theoretical value of 45.3. This total of 11 means exceeds the two expected (5% level) and constitutes statistical argument for the presence in maize of primary non-homologue association.

In the presence of agents which disrupt microtubules, all the homologue ADV's were increased and the number of significant means of comparisons among non-homologues was reduced from 11 to one; i.e., the distributions became random.

J.D. Horn

Effects of cycloheximide on the frequency of somatic polar metaphase observed — We have found that a short pulse of cycloheximide resulted in a marked increase in the frequency of observed polar metaphase. The sensitivity of the cell to this treatment was cell-cycle time specific. A 15-minute cycloheximide treatment (75 ug/ml) at the beginning of prophase resulted in a marked increase in polar metaphases at 45-60 minutes post-treatment at 27°C and at 135 minutes post-treatment at 18°C (Table 1). We interpreted these results as being indicative of a cell-cycle time specific event.

Table 1. Dividing nuclei (%) following cycloheximide (15'; 75 ug/ml) treatment.

Control	Stage	Minutes following treatment							
		27°C				18°C			
		0	30	60	90	0	30	90	135
60	Prophase	42	61	55	68	57	68	76	63
21	Metaphase	36	18	15	5	24	18	18	6
6	Polar metaphase	6	15	25	27	7	6	6	31
7	Anaphase	8	4	3	0	6	4	0	0
6	Telophase	8	2	2	0	8	4	0	0

Cycloheximide is an inhibitor of protein synthesis. The proteins necessary for coiling of the somatic chromosomes are presumably already synthesized by the time of the onset of prophase since normal-appearing metaphase chromosome morphology is evident in cycloheximide-induced polar metaphase nuclei. Proteins necessary to uncouple the chromosomes from the nuclear membrane, for the breakdown of the nuclear membrane and for spindle fiber synthesis are not produced after the challenge with cycloheximide.

J.D. Horn

Description of chlorophyll mutants by in vivo spectrophotometry — Virescent chlorophyll mutants have long been recognized for their potential not only as genetic tools but also as vehicles for the study of development of the photo-

synthetic apparatus. Little of this potential has been realized, however, due in part to the absence of an adequate method for quantification of the greening process. Not only does the coloration of virescent mutants change with time, but both the rate and pattern of color development are subject to environmental control. We have found that there are limitations to the traditional methods by which the status of greening is determined by extractable chlorophyll. The purpose of this report is to describe a rapid and convenient method for description of virescent phenotypes, based on absorbance characteristics of chlorophyll in the tissue.

The method involves determination of an absorbance index (AI) calculated from an *in vivo* absorption spectrum and takes advantage of the increasing availability of spectrophotometers designed to accommodate dense, light-scattering samples. In our system a small disk (5 to 7 mm diameter) is punched from the leaf with a cork borer. The absorption spectrum of the disk is recorded over the range 650 nm to 750 nm, and the difference in absorbance is calculated between the chlorophyll peak (675 nm) and the long wave-length minimum (735 nm). The absorbance difference (read to 2 decimal places) is multiplied by 100 to remove the decimal and the resulting value is taken as an index of chlorophyll content.

In order to obtain valid absorbance measurements with dense, light-scattering tissue, it is necessary that the instrument have a high efficiency of light collection and a low-noise photomultiplier circuit (see Butler, W.L., Ann. Rev. Plant Physiol. 15:451-470, 1964). An increasing number of spectrophotometers which will accommodate light-scattering samples are commercially available. We use a Biospect 61 scanning spectrophotometer (Agricultural Specialties Co.) fitted with a Hewlett-Packard 7035B X-Y recorder. Stray light is limited by sample holder design which, while allowing the measuring beam to pass through the disk, restricts light leakage around the edge of the disk. Light collection is enhanced in that the sample is closely juxtaposed to the surface of a large (ca. 2-inch diameter) end-window photomultiplier tube. The Biospect is a single-beam instrument which employs an electronic compensation network for baseline adjustment. We use filter paper, which has approximately the same density and scatter properties as the corn leaf, as a reference for setting a flat baseline. Spectra are usually recorded over full scale ranges of 1.0 or 2.0 A. Total absorbance at the 675 nm peak frequently exceeds 3.0 A so that a zero offset must be used to bring the sample on scale. Thus, the absolute absorbance values at 675 nm and 735 nm include system response and have little meaning. However, subtraction of one from the other (A_{675} - A_{735}) cancels out the system response

and the resulting difference is an accurate and valid absorbance measurement.

Among the major advantages of this method is its speed. One minute or less is required for each sample, far less time than is required for traditional chlorophyll extractions.

There is, however, a significant correlation between the *in vivo* AI and extractable chlorophyll levels. In the experiment presented in Table 1 an AI was obtained for each of four disks harvested as a group from various leaf sources. The four disks were then pooled and chlorophyll extracted and quantified by Arnon's method, using absorbance at 652 nm (Arnon, D.I., Plant Physiol. 24:1-5, 1949). In Table 1 the extractable chlorophyll values are compared with the mean absorbance index (AI). A departure from linearity is observed above an AI of approximately 100 to 120, a deviation which is common with dense, light-scattering samples.

Table 1. Comparison of AI with extractable chlorophyll.

AI	60	74	83	107	123	138	148	196
Chl (mg/gfw)	0.75	0.90	0.98	1.36	1.63	2.00	2.20	3.93

The use of an *in vivo* AI to describe the status of a greening system provides an essentially non-destructive assay. For example, the very same tissue sample from which the AI has been obtained may itself be used for further analysis of interest to the investigator, such as in the experiment described above. Furthermore, small disks may be taken from leaves without injury to the plant. The same leaf or plant may then continue its development and be available for sampling again at a later date.

Finally, using the *in vivo* AI, it is possible to quickly and accurately construct a profile of a leaf or whole plant. We have done this with Oh 43 and with the virescent mutants *v*, *v16* and *v18* in the Oh 43 background. The results show quite clearly that the pattern of greening in leaves of both *v16* and *v18* is essentially like that of the wild type, in which the old cells near the tip accumulate the highest levels of chlorophyll with a gradual decrease in chlorophyll content toward the base of the leaf. In the *v* leaf, however, the pattern is essentially the opposite, indicating a significantly different developmental program. Thus, with this *in vivo* AI method we can now describe, with far greater simplicity and precision, the patterns of development in virescent and other

chlorophyll mutants and determine the influence of environmental parameters on the expression of the virescent (or chlorophyll deficient) phenotypes.

W.G. Hopkins, D.B. Hayden and D.B. Walden

Requests for seed — 1. We are interested in observing the 'ABPHYL' syndrome (AJB 59:466-472, 1972) in several different leaf size and leaf arrangement backgrounds. We would appreciate receiving a few seeds of such isolates, whether the isolates be specific mutant stocks or unique inbreds.

2. As indicated in the preceding report, we have techniques at hand which permit quantification of the greening processes in plants. Mutants such as virescents are now more amenable to analysis. We are interested in examining any virescent mutant and shall be prepared to perform tests of allelism with known mutants. I shall be grateful to colleagues if they would make available to us some seed of any virescent line unless they obtained the stock earlier from the Coop.

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The effect of K10 on chiasmata — It has been shown that K10 promotes crossing over in proximal regions of certain chromosomes, but the data are not yet inclusive enough to say that all chromosomes are affected similarly. A study of the effect of K10 on the number and distribution of chiasmata was undertaken to investigate the influence of this accessory chromatin on the total genome.

Sporocytes were taken from a line segregating k10 k10 and K10 k10, and chiasmata were studied at metaphase I. Data were collected from ten cells in nine plants each of k10 k10 and K10 k10. A chart was constructed with a schematic representation of tetrads having various numbers of proximal and distal exchanges, and a tally was made of the number of each of these tetrad types. For each genotype an average was obtained for the number of distal exchanges and proximal exchanges and the total number of chiasmata (Table 1). Statistical analysis was done by means of a t test.

Table 1. Effect of K10 on chiasmata.

	Average chiasmata per cell			Total number of chiasmata		
	distal	proximal	total	distal	proximal	total
k10 k10	7.73	11.06	18.78	696	995	1691
K10 k10	5.54	13.87	19.42	499	1249	1748
P	<.001	<.001	<.01			