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Responses of alloplasmic (cytoplasm = *Triticum timopheevii*) and euplasmic wheats (*Triticum aestivum*) to photoperiod and vernalization*

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Summary. Studies were conducted to determine the influence of the male sterility-inducing cytoplasm of *Triticum timopheevii* (Zhuk.) Zhuk. on response of several common winter wheat (*T. aestivum* L.) nuclear genotypes to photoperiod and vernalization. Comparative studies of cytoplasmic substitution lines provide information on the role of the cytoplasmic genetic mechanism in growth and development. In the case of cytoplasmic male sterility-based hybrid production systems, ubiquity of sterility-inducing cytoplasm in derived hybrids warrants thorough characterization of its influence on plant phenotype. Factorial combinations of cytoplasm (*T. timopheevii* and *T. aestivum*), nuclear genotype, and photoperiod or vernalization treatments were evaluated under hydroponic conditions in controlled environment chambers. Interaction of cytoplasm, photoperiod, and nuclear genotype was significant in one or more experiments for days to anthesis and potential spikelet number, and interaction of cytoplasm, vernalization, and nuclear genotype was significant for days to spike emergence. Long day length was associated with increased percentage seed set in one study, but interactions of photoperiod and cytoplasm were not detected for percentage seed set. Interactions involving cytoplasm and photoperiod or vernalization were interpreted as evidence of the existence of genetic factors in cytoplsam of *T. timopheevii* which alter photoperiod or vernalization responses of alloplasmic plants relative to responses exhibited by euplasmic plants. Since photoperiod and vernalization responses are critical to adaptation, *T. timopheevii* cytoplasm can alter adaptability of *T. aestivum*. The specific effect would be nuclear genotype dependent, and does not appear to be of a magnitude greater than that induced by nuclear genetic variability at loci conditioning photoperiod or vernalization responses or other adaptation-determining characteristics. Normal multilocation/year testing of alloplasmic hybrids should therefore adequately identify zones of adaptation.

Key words: *Triticum aestivum* – *T. timopheevii* – Wheat – Photoperiod – Vernalization – Male sterility – Alloplasmic hybrids

Introduction

Substituting common wheat (*Triticum aestivum* L.) nuclear genotypes into cytoplasm of *T. timopheevii* (Zhuk.) Zhuk. via nucleus substitution backcrossing usually resulted in male sterile phenotypes (Fujigaki and Tsunewaki 1976). The main effect of *T. timopheevii* cytoplasm was on male fertility, although effects on leaf and spike characteristics, maturity, sprouting resistance, and grain protein content are known (Fujigaki and Tsunewaki 1976; Jost et al. 1976). Hybrid wheat breeding programs frequently employ *T. timopheevii* cytoplasmic male sterility (CMS) to produce hybrid seed (Hayward 1975). Studies of cytoplasmic substitution lines provide information on the role of the cytoplasmic genetic mechanism in growth and development. Ubiquity of sterility-inducing cytoplasm in hybrids derived from CMS-based systems warrants thorough characterization of its influence on plant phenotype, a point dramatized by the 1970 Southern corn leaf blight

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epidemic on maize hybrids carrying Texas cytoplasm (National Academy of Sciences 1972).

Responses to vernalization and photoperiod condition developmental timing and, consequently, adaptation in wheat. The primary characteristic that distinguished winter wheats from spring wheats was the large response of the former and the small response of the latter to vernalization (Klaimi and Qualset 1974). The major effect of vernalization on responsive wheats was acceleration of development leading to flowering (Grant 1964), although related morphological effects, such as reduced leaf and spikelet numbers, were common (Berry et al. 1980; Wall and Cartwright 1974). Photoperiod insensitivity was a key factor in wide adaptation of the spring-type semi-dwarf wheats developed at the International Maize and Wheat Improvement Center (CIMMYT) (Borlaug et al. 1966). Common wheat, when responsive, was a quantitative long-day plant and flowered rapidly under long day (short night) conditions (Major 1980).

Nuclear genes on at least 11 chromosomes determined vernalization response (Grant 1964), but most variation among genotypes was caused by three to five major genes, with low response partially dominant and epistatic to high response (Klaimi and Qualset 1974; Law et al. 1976). At least two major genes with multiple alleles governed photoperiod response (Klaimi and Qualset 1973). Cytoplasm affected vernalization response as evidenced by enhanced responsiveness of the cultivar 'Chinese Spring' when cytoplasm of *T. aestivum* was replaced by that of *T. ovatum* (L.) Raspil (Cahalan and Law 1979). The photoperiod response of *T. turgidum* L. (selection 56-1) was altered by cytoplasms of *T. umbellulatum* (Zhuk.) Bowden and *T. monococcum* L. (Sasakuma 1976). *Triticum timopheevii* cytoplasm had no effect on the photoperiod response of selection 56-1 in that study.

We report here results of controlled environment studies to determine the influence of *T. timopheevii* cytoplasm on response of several common wheat nuclear genotypes to vernalization and photoperiod treatments. The studies involved factorial combinations of nuclear genotypes, cytoplasms, and controlled environments. Information concerning the influence of photoperiod on the expressivity of male fertility restorer factors was also obtained.

Materials and methods

Three studies, two on photoperiod effects and one on vernalization effects, were conducted in controlled environment chambers. Radiant energy was provided by eight 1.8-m-long fluorescent tubes (G.E. F72T12/CW/SHO) and three 300-watt incandescent bulbs, except for the vernalization study, where only fluorescent tubes were used. Photosynthetic photon flux at canopy height was about 350,000 nE s^{-1} cm^{-2} in the photoperiod studies and 275,000 nE s^{-1} cm^{-2} in the vernalization study.

Genotypes used were highly homozygous components of the *T. timopheevii*-based CMS hybrid wheat system. Information regarding the wheat genotypes used is given in Table 1. Plants were cultured hydroponically with Hoagland solution (Hoagland and Arnon 1950) in 2-liter containers. Randomization was restricted so that each container held six plants of a single nuclear genotype. Three plants in each container had *T. timopheevii* cytoplasm (alloplasmic) and three plants had *T. aestivum* cytoplasm (euplasmic).

In the first photoperiod experiment (Experiment I), eight genotypes were evaluated in 14-h/10-h (day-night) and 16-h/8-h regimes. The genotypes were F_1's derived from crosses among lines listed in Table 1. The crosses and cytoplasm types of the F_1's were I24/R100 (*aestivum*) and AI24/R100 (*timopheevii*); I24/R108 (*aestivum*) and AI24/R108 (*timopheevii*); B123/R100 (*aestivum*) and AB123/R100 (*timopheevii*); and B123/R108 (*aestivum*) and AB123/R108 (*timopheevii*). The eight genotypes represent a factorial combination of four nuclear genotypes and two cytoplasms. B-line/R-line cross designations refer to nuclear genotypes; e.g., the designation I24/R100 represents the nuclear genotype of both I24/R100 and AI24/R100.

Table 1. Designation, type, cytoplasm, and pedigree of winter genotypes of *Triticum aestivum* used in photoperiod and vernalization studies

Designation	Type[a]	Cytoplasm	Pedigree
R100	R-line	*timopheevii*	*T. timo.*/2*Iohardi//Wichita/3/Kaw
R108	R-line	*timopheevii*	*T. timo.*/2*Iowin//2*Quivira/3/ *T. timo.*/3*Itana
I24	B-line	*aestivum*	Bezostaya
AI24	A-line	*timopheevii*	CMS (*timopheevii*)I24 (10 + backcrosses)
B123	B-line	*aestivum*	Palo Dura/Parker
AB123	A-line	*timopheevii*	CMS (*timopheevii*) B123 (10 + backcrosses)
B116	B-line	*aestivum*	CI 12406/Palo Dura
AB116	A-line	*timopheevii*	CMS (*timopheevii*) B116 (10 + backcrosses)
62-1	B-line	*aestivum*	Sturdy (CI 13864) selection
A62-1	A-line	*timopheevii*	CMS (*timopheevii*)62-1 (10 + backcrosses)

[a] R-line = male fertility restorer; A-line = alloplasmic male sterile; B-line = euplasmic male fertile maintainer of A-line

Seedlings were grown in vermiculite and vernalized in a 12/12-h photoperiod regime at 2.5 °C for nine weeks before they were transplanted to the hydroponic system. Temperature at plant canopy height was 21 °C for the remainder of the study. There were four observations (containers) per treatment combination. Observations within a photoperiod were arranged at random. All plants received 13 days of a 12/12-h photoperiod after which photoperiod treatments of 14/10-h and 16/8-h were imposed. The two additional hours of light in the 16/8-h versus the 14/10-h treatments were provided by three 300-watt incandescent bulbs.

Characters measured on the main culm of each plant were days to anthesis; potential spikelet number (PSN) expressed as number of nodes in the rachis; plant height at the spike base; flag leaf width; flag leaf length; and percentage seed set (unbagged) calculated as $\frac{\text{seed set}}{(\text{PSN} - \text{UND}) \times 2} \times 100$, where UND = undeveloped basal spikelets.

The second photoperiod experiment (Experiment II) evaluated the four F_1's (2 alloplasmic, 2 euplasmic) from the first study that had either B123/R100 or B123/R108 as their nuclear genotype. The four genotypes represent a factorial combination of two nuclear genotypes and two cytoplasms. Increased replication and 17/7-h and 20/4-h photoperiod regimes were used. Seedlings grown in Petri dishes were vernalized for six weeks in a 9/15-h photoperiod at 5 °C prior to transplanting to the hydroponic system. Temperature at canopy height was 25 °C for the remainder of the experiment. There were five observations (containers) per treatment combination and observations within a photoperiod were arranged at random. Initial photoperiod treatments were 14/10-h and 17/7-h. After ten days, day lengths were extended 3 h giving photoperiods of 17/7-h and 20/4-h. The 3 additional hours of light in the 20/4-h versus the 17/7-h treatment were provided by three 300-watt incandescent bulbs. Plant characters measured were the same as those for the first experiment.

The vernalization study (Experiment III) used genotypes 62-1, A62-1, B116, and AB116 (Table 1) in factorial combination with vernalization treatments of four, six, and eight weeks duration. The four genotypes represent a factorial combination of two nuclear genotypes and two cytoplasms. B-line designations refer to nuclear genotypes; e.g., the designation 62-1 represents the nuclear genotype of both 62-1 and A62-1. Seedlings from seeds germinated in Petri dishes were transplanted to vermiculite and subsequently transferred at the two-leaf stage to a vernalization chamber with a 9/15-h photoperiod and a temperature of 9 °C (± 1 °C). Treatments were timed so that all vernalized seedlings were simultaneously transplanted to the hydroponic system. Observations were assigned at random to positions within a single chamber bench. There were three observations (containers) per treatment combination. A 16/8-h photoperiod and a constant temperature at canopy height of 18 °C (± 2 °C) were maintained throughout the balance of the study. Characters measured for the main culm of each plant were days to spike emergence, potential spikelet number, and final leaf number.

All studies were analyzed as factorial combinations of cytoplasm, nuclear genotype, and either photoperiod or vernalization treatments. The mean performance of all plants of a single cytoplasm within a container was used as a datum in the statistical analysis. Plants which failed to head were disregarded. The restricted randomization procedures necessitated use of two error terms in the analysis of variance, since within-container error variation was expected to be less than among-container error variation.

Results

F-ratio probabilities and error mean squares for the analyses of variance for the photoperiod and vernalization experiments are presented in Tables 2 and 3, respectively. The 4-week vernalization treatment was disregarded in the analysis of the third experiment because few plants had headed when the study was terminated after 120 days. Except for flag leaf width in the first experiment, error b was always smaller than error a, reflecting the decreased error variation of within-container comparisons relative to among-container comparisons.

Experiment I

Interactions involving cytoplasm, photoperiod, and nuclear genotype were detected for both days to anthesis and potential spikelet number (Table 2). Details of the three-way interactions for days to anthesis and potential spikelet number are presented in Figs. 1 and 2, respectively.

The mean potential spikelet number was 0.32 nodes greater for plants with *T. timopheevii* cytoplasm than for euplasmic plants.

Increased day length reduced mean days to anthesis and potential spikelet number 3.5 days and 0.50 nodes, respectively. Mean reductions associated with increased day length were also found for flag leaf length (3.4 cm), flag leaf width (0.13 cm), and plant height (5.8 cm). Nuclear genotype main effects were found for all of the above traits except flag leaf length.

The overall mean percentage seed set was 62.13%. Mean percentage seed set in the 16/8-h photoperiod

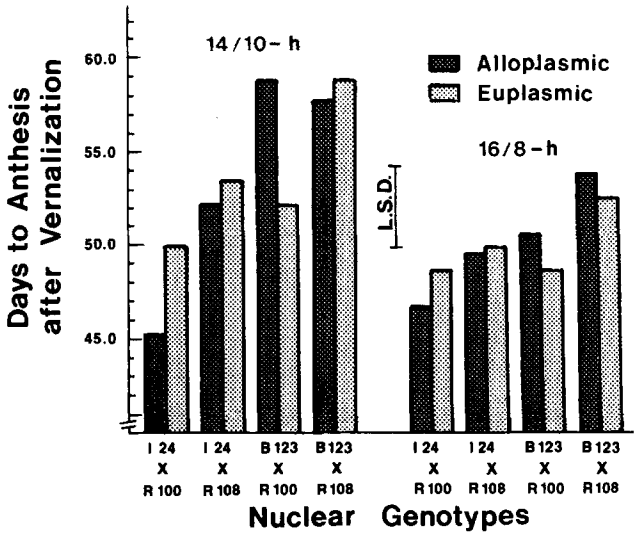

Fig. 1. Days to anthesis after vernalization for main spikes of alloplasmic (cytoplasm = *Triticum timopheevii*) and euplasmic lines of four F_1 nuclear genotypes of *T. aestivum* grown in 14/10-h (day/night) and 16/8-h photoperiod regimes (Experiment I)

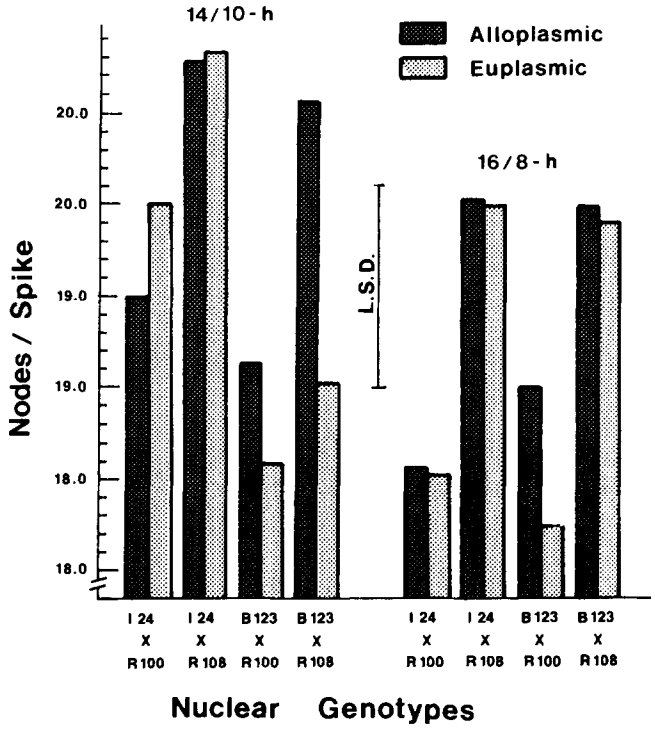

Fig. 2. Potential spikelet number for main spikes of alloplasmic (cytoplasm = *Triticum timopheevii*) and euplasmic lines of four F_1 nuclear genotypes of *T. aestivum* grown in 14/10-h (day/night) and 16/8-h photoperiod regimes (Experiment I)

exceeded the mean for the 14/10-h photoperiod by 6.1%. Mean percentage seed set was greatest for nuclear genotype B123/R100 (77.4%) and, within that nuclear genotype, the mean for euplasmic plants significantly exceeded the mean for alloplasmic plants by 13 percentage points. Interactions involving cytoplasm and photoperiod were not significant.

Experiment II

Twenty-five of the 120 plants failed to reach spike emergence by the 65th day when the study was terminated. Plants which headed performed as relatively homogeneous groups, giving a coefficient of variation for days to anthesis (calculated using error b) of 6.3%. The distribution of non-headed plants was not associated with any photoperiod or genotype treatment.

Interaction of cytoplasm, photoperiod, and nuclear genotype was significant for potential spikelet number (Table 2). Details of that three-way interaction are presented in Fig. 3.

The mean days to anthesis for plants with *T. timopheevii* cytoplasm was 3.5 days greater than that for euplasmic plants. Increased day length reduced time to anthesis and potential spikelet number 4.7 days and 1.94 nodes, respectively. A nuclear genotype main effect was detected for days to anthesis only; no significant treatment effects were found for flag leaf width, flag leaf length, or plant height. The overall mean for percentage seed set was 67.6%. Insufficient data were available for percentage seed set to analyze them with a balanced design. The GLM procedure of the Statistical Analysis System (SAS) was consequently employed; it revealed no significant treatment effects for percentage seed set.

Experiment III

Interaction of cytoplasm, vernalization, and nuclear genotype was significant for days to spike emergence (Table 3). Details of that interaction are shown in Fig. 4.

T. timopheevii cytoplasm increased mean days to spike emergence 1.72 days. Increased duration of

Table 2. F-ratio probabilities and error mean squares for studies of alloplasmic (cytoplasm = *Triticum timopheevii*) and euplasmic lines of F_1 nuclear genotypes of *T. aestivum* grown in 14/10-h (day/night) and 16/8-h photoperiod regimes (Experiment I) and 17/7-h and 20/4-h photoperiod regimes (Experiment II)

Source	Experiment I							Experiment II		
	df	DA[a]	PSN	% SS	FLW	FLL	HT	df	DA	PSN
Photoperiod (P)	1	***[b]	*	*	***	***	*	1	**	*
Nuclear genotype (NG)	3	***	***	***	***	NS	*	1	*	NS
P×NG	3	*	NS	NS	NS	NS	NS	1	NS	*
Error a	24	7.56	0.59	119.66	0.01	7.03	105.76	16	22.14	5.26
Cytoplasm (C)	1	NS	*	NS	NS	NS	NS	1	**	NS
C×P	1	NS	NS	NS	NS	NS	NS	1	NS	NS
C×NG	3	***	*	*	NS	NS	NS	1	NS	NS
C×P×NG	3	*	*	NS	NS	NS	NS	1	NS	*
Error b	24	3.9	0.25	101.20	0.01	5.27	15.46	16	10.25	1.27

[a] DA = days to anthesis; PSN = potential spikelet number; UND = number of nodes at the spike base with underdeveloped spikelets; % SS = percentage seed set; FLW = flag leaf width; FLL = flag leaf length

[b] *, **, *** and NS = probability levels of 0.05, 0.01, 0.001, and not significant, respectively

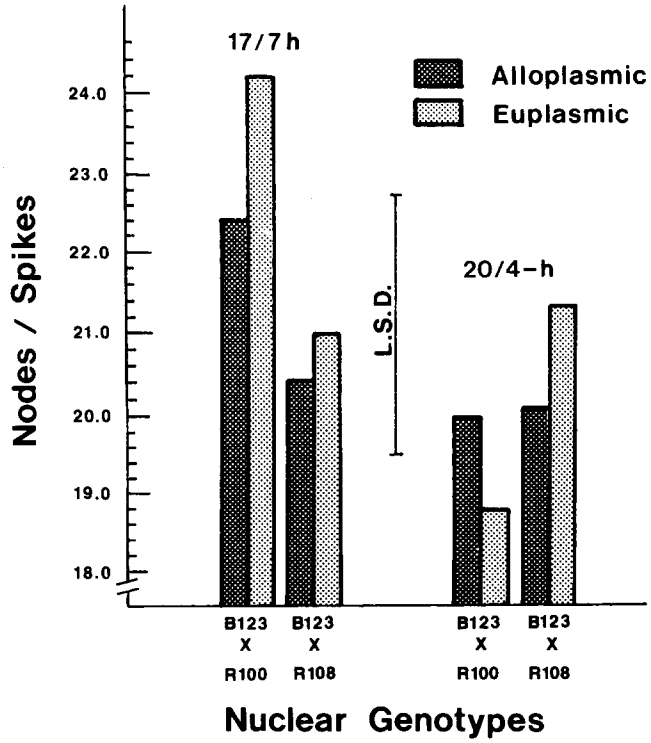

Fig. 3. Potential spikelet number for main spikes of alloplasmic (cytoplasm = *Triticum timopheevii*) and euplasmic lines of two F_1 nuclear genotypes of *T. aestivum* grown in 17/7-h (day/night) and 20/4-h photoperiod regimes (Experiment II)

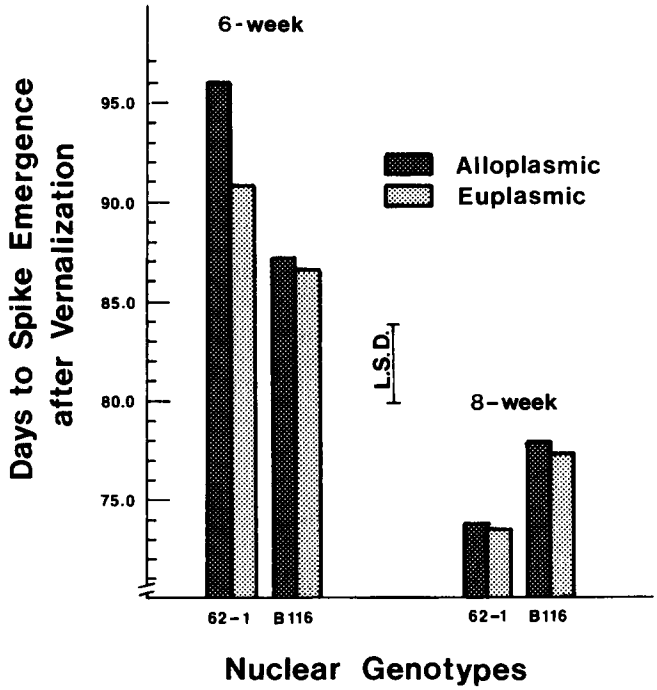

Fig. 4. Days to spike emergence after vernalization for main spikes of alloplasmic (cytoplasm = *Triticum timopheevii*) and euplasmic lines of *T. aestivum* vernalized for six and eight weeks (Experiment III)

Table 3. F-ratio probabilities and error mean squares for study of alloplasmic (cytoplasm = *Triticum timopheevii*) and euplasmic lines of *T. aestivum* vernalized for six and eight weeks (Experiment III)

Source	df	DSE[a]	PSN
Vernalization (V)	1	***[b]	***
Nuclear genotype (NG)	1	NS	NS
V×NG	1	**	*
Error a	8	10.07	1.13
Cytoplasm (C)	1	*	NS
C×V	1	NS	NS
C×NG	1	NS	NS
C×V×NG	1	*	NS
Error b	8	1.71	0.44

[a] DSE = days to spike emergence; PSN = potential spikelet number

[b] *, **, ***, and NS = probability levels of 0.05, 0.01, 0.001, and not significant, respectively

vernalization reduced mean days to anthesis and potential spikelet number 14.6 days and 2.43 nodes, respectively. For both days to spike emergence and potential spikelet number, nuclear genotype 62-1 with either cytoplasm was markedly more sensitive to vernalization than was nuclear genotype B116. That differential sensitivity to vernalization reversed days to spike emergence rank of the two nuclear genotype means in the two vernalization treatments. The overall mean for final leaf number was 10.4. No treatment effects were found for that trait or for plant height.

Discussion

The influence of nuclear genotype on response of *T. aestivum* to photoperiod and vernalization conditions is well documented (Sasakuma 1976). The interactions reported here involving cytoplasm and photoperiod or vernalization treatment indicate that factors in cytoplasm of *T. timopheevii* modify responses of *Triticum aestivum* to photoperiod or vernalization conditions. Similar results from studies using other cytoplasms and/or nuclear genotypes from the Triticinea were reported for photoperiod (Sasakuma 1976) and vernalization (Cahalan and Law 1979) response. Those studies indicated that the cytoplasmic genetic mechanism plays an integral role in plant response to photoperiod and vernalization conditions. Our studies of interactions involving cytoplasm and photoperiod or vernalization also involved nuclear genotype and demonstrated that the effects of the nuclear and cytoplasmic genetic mechanisms are interdependent.

Environmentally induced variability in the expressivity of male fertility restorer genes is a major problem

in applying the *T. timopheevii* based-CMS hybrid wheat system (Schmidt et al. 1970). We used A-line/B-line × R-line hybrids in the photoperiod studies to study the influence of photoperiod on fertility restoration. However, percentage seed set on unbagged heads, our measure of male fertility, was low in both photoperiod studies. The lack of a significant cytoplasm effect and/or interaction involving cytoplasm and photoperiod for percentage seed set in all except one instance suggested that fertility restoration was complete in all photoperiods or that other factors reduced mean fertility below a threshold level above which differences in the alloplasmic and euplasmic hybrids would be expressed.

The significant main effect of photoperiod in the first study, where increased percentage seed set was associated with longer day length, contrasted with predictions based on observations of CMS-derived wheat hybrids grown at different latitudes (Schmidt et al. 1970). However, in the first two years of field trials of the International Wheat Restorer Germplasm Screening Nursery no singe environmental factor was significantly correlated with male fertility (Jost 1980).

The results suggested that adaptability of *T. aestivum* to environments differing in photoperiod and/or vernalization conditions was altered by substituting normal cytoplasm with *T. timopheevii* cytoplasm. The direction and magnitude of the effect of *T. timopheevii* cytoplasm was nuclear genotype dependent, but its effect on adaptability was probably no more significant than the effects of the existing nuclear genetic variability at loci conditioning photoperiod and vernalization responses (Halloran and Boydell 1967; Klaimi and Qualset 1973), maturity (Major 1980), or cold resistance (Cahalan and Law 1979). Normal multilocation/year testing procedures would identify the range of adaptation of a given alloplasmic hybrid.

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