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Recovery of normal photosynthesis and respiration in common wheat with *Agropyron* cytoplasms by telocentric *Agropyron* chromosomes

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Summary. Alloplasmic common wheat (*Triticum aestivum* L. cultivars 'Penjamo 62' and 'Siete Cerros 66') with cytoplasms of wheatgrass (*Agropyron trichophorum* and *Ag. glaucum*) showed two aberrant phenotypes, i.e., gross reduction in plant vigor and male sterility. Plant vigor and male fertility were restored by cytoplasm-specific telocentric chromosomes (telosomes). Studies on carbon assimilation and consumption and on oxygen evolution and uptake showed that maximum rates of apparent photosynthesis were significantly lower in the alloplasmic lines than in their corresponding euplasmic lines and that the telosomes restored a normal level of photosynthesis. The decreased apparent photosynthetic rates in the alloplasmic lines were shown to be not due to decreased rates of true photosynthesis but to increased rates of dark respiration in the green leaves. In contrast, dark respiration in the roots was significantly low in the alloplasmic lines. The alloplasmic lines also showed decreased rates of respiratory consumption of new photosynthates. These results suggest that growth depression and male sterility in the alloplasmic lines are related to aberrant mitochondrial function, which is compensated for by cytoplasm-specific telosomes.

Key words: *Agropyron* cytoplasms – Common wheat – Growth depression and male sterility – Photosynthesis and respiration – Telocentric *Agropyron* chromosomes

Introduction

Since 1951, when Kihara first introduced *Aegilops caudata* cytoplasm into common wheat *Triticum aestivum* (Kihara 1951), many alloplasmic lines of common and durum wheat with cytoplasms of *Triticum* and related wild species have been produced (for a review, see Tsunewaki 1980). These alloplasmic lines were established through recurrent backcrosses using common and durum wheat as pollen parents. However, certain alloplasmic lines have retained an extra chromosome derived from cytoplasmic parents throughout the substitution backcrosses. One mechanism of such chromosome retention in the alloplasmic lines is the necessity of the cytoplasm-specific chromosomes for the restoration of plant vigor and male fertility (Maan 1976).

In an alloplasmic common wheat line (cv 'Penjamo 62') with a cytoplasm of wheatgrass (*Agropyron trichophorum*), growth depression and male sterility occurred, and a cytoplasm-specific telosome restored normal vigor and male fertility (Tsujimoto et al. 1987). These lesions observed in the alloplasmic line are likely due to functional disharmony, which results from imbalance between cytoplasmic and nuclear genomes.

In this study, we analyzed photosynthesis and respiration in four alloplasmic lines with two common wheat nuclei (cultivars 'Penjamo 62' and 'Siete Cerros 66') and two *Agropyron* cytoplasms (*Ag. glaucum* and *Ag. trichophorum*). We will present data which suggest that growth depression and male sterility commonly occurring in these alloplasmic lines are related to aberrant mitochondrial function.

Materials and methods

Plant materials

Euplasmic common wheat, *Triticum aestivum* (L.) Thell. cultivars 'Penjamo 62' and 'Siete Cerros 66', and alloplasmic lines having these common wheat nuclei and cytoplasms of *Agropyron trichophorum* (Link.) Richt. and *Ag. glaucum* Dest. were

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Table 1. The euplasmic lines and the alloplasmic common wheat lines with *Agropyron* cytoplasms used in the study

Cytoplasmic donor	Nuclear donor	Telosome[a]	Abbreviation
Ag. glaucum	*T. aestivum*		
	cv 'Penjamo 62'	+	(gl)-Pj+t
		–	(gl)-Pj
	cv 'Siete Cerros 66'	+	(gl)-SC+t
		–	(gl)-SC
Ag. trichophorum	*T. aestivum*		
	cv 'Penjamo 62'	+	(tri)-Pj+t
		–	(tri)-Pj
	cv 'Siete Cerros 66'	+	(tri)-SC-t
		–	(tri)-SC

[a] Symbols + and – indicate the presence and absence of one or two cytoplasm-specific telosomes, respectively

used (Table 1; Panayotov 1983). Plants with and without one or two *Agropyron*-derived telosomes were selected in the selfed progenies of the alloplasmic lines based on seed plumpness and shriveling, respectively, and on numbers of roots emerging at germination (Tsujimoto et al. 1987; see 'Results and discussion'). The presence of telosomes was further confirmed by counting chromosome numbers in root tips. All lines were planted using artificial soil (aquaball, AQA Plants, Tokyo) in glass vials, watered periodically with nutrient solution, and grown for 3 weeks at day-night temperatures of 20–15 °C under natural light. After 3 weeks, plants were transferred to soil in pots and grown for an additional 7 weeks under the same conditions.

Determination of photosynthesis and respiration by far-red spectrophotometry

At 3 and 10 weeks after imbibition, six plants were selected randomly from each line and placed in a CO_2 assimilation chamber. Gaseous CO_2 containing stable, isotopic $^{13}CO_2$ (65 and 70% of the total CO_2 at a concentration of ca. 500 ppm in two separate experiments) was introduced into the chamber, and plants were kept for 6 h at 25 °C under a saturating intensity of light (350–600 $\mu E \cdot m^{-2} \cdot s^{-1}$) provided by cool white fluorescent lamps. After this CO_2 assimilation period, half of the plants from each line were removed, treated immediately with liquid nitrogen, and dried at 105 °C for 30 min followed by 80 °C for 8 h. The remaining half of the plants were kept in the dark at 25 °C under natural CO_2 conditions for the following 6 or 12 h. After this respiration period, plants were dried in the same way.

After measuring dry matter weights, the whole plants were ground to powder by Sample Mill (Heiko, Tokyo). The powdered samples (0.2–0.5 mg) were burned at 900 °C and the gaseous total CO_2 was led into a far-red spectrophotometer (Nippon Bunko, Tokyo). The amounts of ^{13}C and ^{12}C in the total CO_2 were determined based on the spectrum differences in far-red absorption between ^{13}C and ^{12}C. The amount of total carbon assimilated during the period of photosynthesis under the constant percent $^{13}CO_2$ in the total CO_2 was calculated as the product of RSA and sample dry matter weight applied, where RSA represents the relative specific activity defined as the ratio between percent ^{13}C atom excess in the samples and percent ^{13}C atom excess in the assimilation chamber. The RSA thus stands for the relative amount of newly assimilated carbon in the total carbon assimilated before and during the photosynthesis period.

The amount of new photosynthates consumed during the subsequent respiration period in the dark was measured as the difference between the amount of new photosynthates immediately after the photosynthesis period and that remaining after the respiration period.

Determination of photosynthesis and respiration by polarography using an oxygen electrode

At 3 weeks after imbibition, more than three plants were selected randomly from each line and green leaves and roots were harvested. The leaves and roots were cut into pieces and subjected to polarography using a Klark-type oxygen electrode (Rank Brothers, Cambridge). The rates of oxygen uptake and evolution were measured in 3 ml of buffer containing 100 m*M* Tricine-HCl (pH 7.5) and CPW salts (Frearson et al. 1973) at 25 °C. After obtaining a steady state of dark respiration, the system was illuminated by a saturating intensity of light at ca. 500 $\mu E \cdot m^{-2} \cdot s^{-1}$ provided by Fiber Optic Light Source (Nikon, Tokyo). When another steady state resulted, a solution of $NaHCO_3$ was added to a final concentration of 3 m*M* and the rate of O_2 evolution (the photosynthetic capacity) was recorded.

Results and discussion

Growth depression and male sterility in the alloplasmic lines

We studied four alloplasmic lines with two common wheat nuclei and two *Agropyron* cytoplasms to examine the following two aspects: (i) whether or not growth depression and male sterility are general phenomena in the alloplasmic common wheat with cytoplasms of *Agropyron* species, and (ii) whether or not the recovery of normal growth and male fertility occurs in the alloplasmic lines by the presence of cytoplasm-specific chromosomes.

All of the alloplasmic lines with one *Agropyron* telosome produced plump and shriveled seeds. Chromosome counting in root tips confirmed that all of the plump seeds were with one or two *Agropyron* telosomes and most of the shriveled seeds were without the telosomes. Growth depression occurred in the alloplasmic lines without telosomes from the early stages of germination throughout their development. In the alloplasmic lines without telosomes, germination was delayed until 4–6 days after imbibition, while in the euplasmic lines and the alloplasmic lines with telosomes, germination occurred within 1–2 days after imbibition. The euplasmic lines and the alloplasmic lines with telosomes had three roots emerging from each germinating seed, while the alloplasmic lines without telosomes had only one or, rarely, two roots per seed even at 7 days after imbibition. Growth depression was apparent in both the root tissues and the above-ground tissues (data not shown). Table 2 shows dry matter weights of the 3-week-old plants in the euplasmic lines and the alloplasmic lines with and without telosomes. Severe growth depression was observed in the alloplasmic lines without telosomes, but almost normal growth and vigor were recovered by the presence of one or two cytoplasm-specific telosomes. At 10 weeks, the

Table 2. Dry mattern weights and the amounts of total carbon in the euplasmic lines and the alloplasmic lines with and without telosomes. Entries with the same letters are not significantly different at the 5% level, according to Duncan's test

Line[a]	Dry matter (mg)		Total C (mg/g dry wt.)	
	3 weeks	10 weeks	3 weeks	10 weeks
Pj, SC	110a	378a	456a	444a
(gl)-Pj, SC+t	85b	254b	459a	445a
(gl)-Pj, SC	24c	72d	435a	430a
(tri)-Pj, SC+t	107a	234b	435a	425a
(tri)-Pj, SC	26c	133c	465a	426a

[a] The alloplasmic lines with +t represent those having one or two cytoplasm-specific telosomes

Table 3. Selfed seed sets and seed weights in the euplasmic lines and the alloplasmic lines with and without telosomes. Selfed seed sets were based on numbers of seeds per spike and seed weights on 100 seeds

Line[a]	Selfed seed set[b]	Seed weight (g)
Pj, SC	75.7	5.0
(gl)-Pj, SC+2t	58.9	4.8
(gl)-Pj, SC	0.6	1.2
(tri)-Pj, SC+2t	42.6	5.5
(tri)-Pj, SC	3.1	1.9

[a] The alloplasmic lines with +2t represent those having two cytoplasm-specific telosomes
[b] (gl)-Pj and (tri)-Pj were completely sterile

alloplasmic lines with *Ag. trichophorum* cytoplasm showed a certain degree of growth recovery, although growth depression in this line was still significant as compared to the corresponding euplasmic lines and the alloplasmic lines with telosomes. The interaction between cytoplasmic and nuclear genomes on growth was thus expressed differently at different developmental stages between the alloplasmic lines with different cytoplasms.

The alloplasmic lines without telosomes showed limited levels of male fertility as revealed by selfed seed sets (Table 3). The selfed seed sets in the alloplasmic lines without telosomes differed, depending on combinations of common wheat nuclei and *Agropyron* cytoplasms: the lines with nuclei of 'Penjamo 62' set no seeds, irrespective of *Agropyron* cytoplasms, but those with nuclei of 'Siete Cerros 66' set a few seeds per spike, the seed set in the line with *Ag. trichophorum* cytoplasm being higher than in the line with *Ag. glaucum* cytoplasm. The seeds set upon selfing in the alloplasmic lines with nuclei of 'Siete Cerros 66' were shriveled and seed weights were much lighter than the corresponding euplasmic lines and the alloplasmic lines with telosomes (Table 3). The alloplasmic lines with telosomes not only showed high degrees of recovery

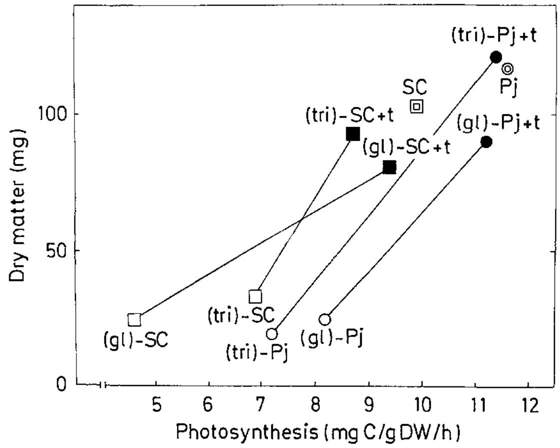

Fig. 1. Dry matter weights and the maximum photosynthetic rates in 3-week-old plants of the euplasmic lines and the alloplasmic lines with and without telosomes. The alloplasmic lines with telosomes showed photosynthetic rates higher than the corresponding alloplasmic lines without telosomes (significant at the 1% level, according to Duncan's test). No significant differences were found in the photosynthetic rates between the alloplasmic lines with telosomes and the corresponding euplasmic lines. For the line designation, see legend to Table 1

in the selfed seed sets but also seeds became plump and seed weights were as high as those in the euplasmic lines.

Photosynthesis and respiration in the alloplasmic lines

Cytoplasmic genomes code for enzymes necessary in two essential cytoplasmic functions, i.e., photosynthesis and respiration. The fact that growth depression occurs in the nonphotosynthetic tissues of the alloplasmic lines without telosomes indicated that the lesion might be related to mitochondrial function. To examine this, we studied photosynthesis and respiration in the alloplasmic lines with and without specific telosomes in comparison with those in the euplasmic lines.

No significant differences were found in the amount of total carbon per unit dry matter weight among the lines (Table 2). The amount of total carbon did not differ at different developmental stages studied. The values of RSA determined by far-red spectrophotometry, however, differed significantly among the lines to give significantly different, maximum apparent photosynthetic rates (the apparent photosynthetic capacity): all the alloplasmic lines without telosomes showed lower apparent photosynthetic capacity than the corresponding euplasmic lines, and the cytoplasm-specific telosomes restored normal levels of photosynthetic capacity in the 3-week-old plants (Fig. 1). In the 10-week-old plants, the alloplasmic lines with *Ag. trichophorum* cytoplasm showed no significant differences in the apparent photosynthetic capacity compared to the corresponding euplasmic lines and the alloplasmic lines with telosomes (Fig. 2). The normal levels of apparent photosynthetic capacity observed in the

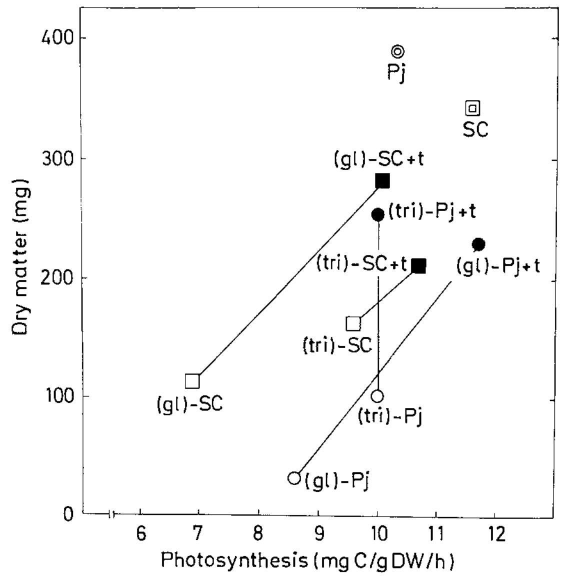

Fig. 2. Dry matter weights and the maximum photosynthetic rates in 10-week-old plants of the euplasmic lines and the alloplasmic lines with and without telosomes. The alloplasmic lines wiht *Ag. glaucum* cytoplasm and telosomes showed photosynthetic rates higher than the corresponding alloplasmic lines without telosomes (significant at the 1% level, according to Duncan's test). No significant differences were found in the photosynthetic rates between the alloplasmic lines with telosomes and the corresponding euplasmic lines. For the line designation, see legend to Table 1

10-week-old alloplasmic lines with *Ag. trichophorum* cytoplasm appeared to correlate with the growth recovery of these lines at this stage (Fig. 2 and Table 2). On the other hand, the alloplasmic lines with *Ag. glaucum* cytoplasm that showed severe growth depression had significantly low, apparent photosynthetic capacities.

Since the true photosynthetic capacity and the dark respiration could not be determined by far-red spectrophotometry, determinations of these two parameters were made by the polarographic method using an oxygen electrode. No significant differences were found in the maximum rates of true photosynthesis among the euplasmic lines and the alloplasmic lines with and without telosomes in the green leaves of the 3-week-old plants, except for the alloplasmic lines with a cytoplasm and telosomes of *Ag. glaucum,* which showed true photosynthetic rates significantly higher than the other lines (Table 4). In contrast, the rates of dark respiration in the green leaves were significantly higher in the alloplasmic lines without telosomes than in the euplasmic lines and the alloplasmic lines with telosomes. Accordingly, the rates of maximum apparent photosynthesis became lower in the alloplasmic lines without telosomes (Table 4), which agreed with the observation using far-red spectrophotometry (Fig. 1).

It was thus suggested that growth depression in the alloplasmic lines was related to their abnormally high dark respiration, thus to their lower apparent photosynthesis in the green leaves. Interestingly, however, respiration in the roots was significantly lower in the alloplasmic lines without telosomes than in the euplasmic lines and the alloplasmic lines with telosomes (Table 4). In the euplasmic lines and the alloplasmic lines with telosomes, root respiration was higher than leaf respiration, whereas in the alloplasmic lines without telosomes, root respiration was lower than leaf respiration. The decreased root respiration was correlated with the decreased root growth in the alloplasmic lines, and the telosomes restored normal root respiration and growth.

Table 4. Photosynthesis and respiration in the euplasmic lines and the alloplasmic lines with and without telosomes. The measurements were made using green leaves and roots of 3-week-old plants. Entries with the same letters are not significantly different at the 5% level, according to Duncan's test

Line[a]	Photosynthesis ($nmol\ O_2/min/g$ fresh wt)		Dark respiration ($nmol\ O_2/min/g$ fresh wt)	
	Apparent	True	Leaf	Root
Pj, SC	369ab	471b	102bc	173bc
(gl)-Pj, SC+t	442a	552a	110bc	227a
(gl)-Pj, SC	283c	443b	160a	107d
(tri)-Pj, SC+t	352ab	453b	100c	182b
(tri)-Pj, SC	302bc	434b	132ab	116cd

[a] The alloplasmic lines with +t represent those having one or two cytoplasm-specific telosomes

The spectrophotometric analysis of respiration showed that the rates of new photosynthates consumed by the leaves of the 3-week-old (data not shown) and 10-week-old plants (Fig. 3) during the respiration period in the dark were lower in the alloplasmic lines without telosomes than in the euplasmic lines and the alloplasmic lines with telosomes. Since the alloplasmic lines without telosomes showed significantly increased leaf respiration (Table 4), the results indicated that leaf respiration in the alloplasmic lines depended more on the stored carbohydrates than on the new photosynthates in the absence of telosomes. The lines with *Ag. glaucum* cytoplasm, which showed severe growth depression throughout the developmental stages (Table 2), consumed fewer new photosynthates than the alloplasmic lines with *Ag. trichophorum* cytoplasm (Fig. 3), which showed some degrees of growth recovery at the late developmental stages (Table 2). The decreased root respiration and the increased leaf respiration together with the limited ability of the alloplasmic lines to use new photosynthates effectively as respiratory substrates might likely cause, at the least, growth depression in the alloplasmic lines.

It has been well documented that male sterility is related to mitochondrial function (Levings and Pring 1979; Leaver and Gray 1982; Hanson and Conde 1985; Siculella and Palmer 1988). The alloplasmic lines with

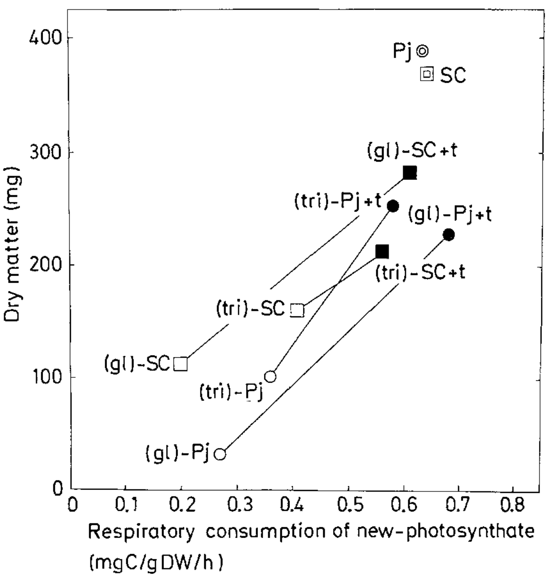

Fig. 3. Dry matter weights and the rates of consumption of new photosynthates in 10-week-old plants of the euplasmic lines and the alloplasmic lines with and without telosomes. For the line designation, see legent to Table 1

nuclei of 'Ciete Cerros 66', especially with a cytoplasm of *Ag. trichophorum,* showed some degrees of male fertility as revealed by the selfed seed sets (Table 3). In this line, the rate of consumption of new photosynthates was highest among the alloplasmic lines without telosomes (Fig. 3). Although in order to find a definitive answer, respiratory characterization must be made using the tissues where male sterility actually is being expressed, e.g., pollen grains or tapetum cells, the results suggest that the aberrant mitochondrial function observed in the alloplasmic lines might be related not only to growth depression but also to male sterility.

In the alloplasmic common wheat with *Agropyron* cytoplasms, both growth depression and male sterility are cytoplasm specific (Tsujimoto et al. 1987). The preferential retention of *Agropyron* telosomes occurs because the telosomes are necessary for the restoration of plant vigor and male fertility. A similar mechanism of preferential chromosome retention has been reported in alloplasmic durum wheat with cytoplasms of *Aegilops squarrosa* and *Ae. cylindrica* (Maan 1976; Ohtsuka 1980) and in alloplasmic common wheat with rye cytoplasm (Maan and Lucken 1971; Nakata et al. 1986). However, genetic control of male sterility in the alloplasmic common wheat with *Agropyron* cytoplasms differs from that in the alloplasmic durum wheat with *Aegilops* cytoplasms: in the former, male sterility is sporophytically controlled as in alloplasmic common wheat with rye cytoplasm, while in the latter, male sterility is gametophytically controlled. In both cases, the cytoplasm-specific chromosomes should carry genes whose products play essential role(s) in the restoration of normal plant vigor and male fertility. The alloplasmic lines with defective organella whose function is restored by the cytoplasm-specific chromosomes should provide a good experimental system for dissecting functional interaction between cytoplasmic and nuclear genomes.

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