**Chloroplast and mitochondrial DNAs of alloplasmic common
wheat with cytoplasms of *Agropyron glaucum*,
Ag. trichophorum and *Haynaldia villosa***

Tohru SUZUKI¹, Naoki MORI², Chiharu NAKAMURA^{2*},
Ivan PANAYOTOV³ and Chukichi KANEDA²

¹*Division of Science of Biological Resources, Graduate
School of Science and Technology, and*

²*Laboratory of Plant Genetics, Faculty of Agriculture, Kobe
University, 1 Rokkodai-cho, Nada-ku, Kobe 657, Japan*

³*Institute for Wheat and Sunflower, Dobroudja,
near General Toshevo, Bulgaria*

(Received 5 June 1992)

ABSTRACT

Cytoplasms of *Agropyron glaucum*, *Ag. trichophorum* and *Haynaldia villosa* are functionally different as revealed by several physiological characteristics of the alloplasmic lines having these cytoplasms and the same wheat nucleus. To find genetic differences among the cytoplasms of these species at molecular level, we studied chloroplast (ct) and mitochondrial (mt) DNAs using the alloplasmic lines. Cytoplasms of the two *Agropyron* species had closely related ctDNAs, whereas their mtDNAs differed greatly in their structure. As compared to the ct and mtDNAs of common wheat, those of the *Agropyron* species and *Hy. villosa* were found to have been equally diverged.

1. INTRODUCTION

Alloplasmic lines of wheat which have been produced through recurrent backcrosses using wheat as pollen parents have provided useful experimental materials in studying cytoplasmic diversity and interaction between nuclear and cytoplasmic, organellar genomes in *Triticum* and its related genera (Tsunewaki, 1989).

Cytoplasms of two wheatgrass species, *Agropyron glaucum* and *Ag. trichophorum*, are both incompatible with nuclei of common wheat, *Triticum aestivum*, causing a gross reduction of plant vigor and male sterility of the alloplasmic lines that have nuclei of common wheat and cytoplasms of the two *Agropyron* species (Tsujimoto et al., 1987; Nakamura et al., 1991; Suzuki et al., 1992). This nucleus-cytoplasmic incompatibility, however, is cross-compensated for by the presence of specific chromosome arm (short-arm telocentric, nucleolus-organizing chromosome) derived from either *Agropyron* species, indicating that the mechan-

* Corresponding author

ism(s) determining the compatibility/incompatibility is the same for the cytoplasms of both *Agropyron* species (Suzuki et al., 1992). On the other hand, interactions between the common wheat nuclei and/or the telocentric *Agropyron* chromosomes with *Agropyron* cytoplasms differed depending on the *Agropyron* species, suggesting some genetic differences between the two cytoplasms with respect to the compatible/incompatible reactions against common wheat nuclei (Nakamura et al., 1991; Suzuki et al., 1992).

The genus *Agropyron* is complex and comprises well over 100 species (Dewey, 1984). However, no information is yet available on their chloroplast and mitochondrial DNAs. Toward elucidating the genetic similarity and dissimilarity between *Ag. glaucum* and *Ag. trichophorum*, we studied structures of their chloroplast and mitochondrial DNAs. In this study we included an alloplasmic common wheat with cytoplasm of *Haynaldia villosa* which shows compatibility with nuclei of common wheat (Panayotov, 1983).

2. MATERIALS AND METHODS

Three alloplasmic lines of common wheat, *Triticum aestivum* L., with cytoplasms of *Agropyron glaucum* (Desf. ex DC) Roemer & Schltes., *Ag. trichophorum* (Link.) Richt. or *Haynaldia villosa* Schur. (Panayotov, 1983) were used as sources of cytoplasms from which organellar DNAs were isolated. The alloplasmic lines with *Agropyron* cytoplasms included two sublines each either with or without telocentric *Agropyron* chromosomes. As controls, two euplasmic cultivars of common wheat, "Chinese Spring (CS)" and "Siete Cerros 66 (SC)", were used, the latter of which was a nucleus donor common for all the alloplasmic lines.

Chloroplasts were isolated from 9-day-old seedlings according to the method of Ogihara and Tsunewaki (1988), and ctDNAs were extracted by the phenol/chloroform extraction method. After digestion by seven restriction endonucleases (*Bam*HI, *Bgl*II, *Eco*RI, *Hind*III, *Pst*I, *Sma*I and *Xho*I), ctDNAs were analyzed by 0.8% agarose gel electrophoresis.

Mitochondria were isolated from 7-day-old, dark-grown seedlings. Etiolated leaves were cut into pieces and ground in buffer containing 50 mM Tris-HCl (pH 8.0), 3 mM Na₂-EDTA, 0.44 M mannitol, 0.1% (w/v) BSA and 1 mM 2-mercaptoethanol using a pre-cooled mortar with pestle. The homogenate was passed through four layers of gauze followed by two layers of Miracloth (Calbiochem). The filtrate was centrifugated at 1,900×g for 10 min, and the supernatant was recentrifugated at 19,000×g for 20 min to collect mitochondrial pellets. After treatment of the pellets by DNase I (40 µg/ml), mtDNAs were extracted by the phenol/chloroform extraction method and digested by four restriction endonucleases (*Bam*HI, *Eco*RI, *Hind*III and *Xho*I). After electrophoresis using 0.8% agarose gel, mtDNAs were transferred to nylon membranes (Hybond N, Amersham) and hybridized with digoxigenin-labeled probes.

Probes used were 18S-5S ribosomal RNA gene from common wheat (Falconet et al., 1984), ATPase subunit a gene (*atpA*) from pea (Morikami and Nakamura, 1987), and ATPase subunit 9 gene (*atp9*) from *Oenothera* (Schuster and Brennicke, 1989) and cytochrome c oxidase subunit 1 gene (*cox1*) from *Oenothera* (Hiesel et al., 1987). Signal detection was made by dig-ELISA method (Boehringer Mannheim).

The genetic relatedness of ct and mtDNAs among cytoplasms of the two common wheat cultivars and the three alloplasmic lines was estimated by the percentages of restriction fragments and hybridized fragments shared among them.

3. RESULTS

Structures of ct and mtDNAs of the two common wheat cultivars, CS and SC, and the alloplasmic lines of SC with cytoplasms of *Ag. glaucum*, *Ag. trichophorum* and *Hy. villosa* were compared. In case of the alloplasmic lines with *Agropyron* cytoplasms, the sublines carrying a pair of the corresponding telocentric *Agropyron* chromosomes were used as main sources of the organellar DNAs.

The two common wheat cultivars showed identical restriction fragment patterns of ctDNA for all the enzymes used (Table 1). The percentages of restriction fragments of ctDNAs shared between common wheat and *Ag. glaucum*, *Ag. trichophorum* and *Hy. villosa* were 83.9, 84.4 and 86.8%, respectively. The restriction fragment patterns of *Ag. glaucum* and *Ag. trichophorum* were identical except when digested by *EcoRI* and *HindIII*, thus 98.2% of their ctDNA fragments were common between them (Table 1 and Fig. 1). The restriction fragment pattern of ctDNA of *Hy. villosa* differed from those of common wheat

Table 1. Frequency (% , above the diagonal) and number^a (below the diagonal) of chloroplast DNA restriction fragments shared among common wheat, *Ag. glaucum*, *Ag. trichophorum* and *Hy. villosa* as revealed by seven restriction enzymes

Cytoplasm donor ^b	CS	SC	Ag. glc	Ag. trc	Hy. vll
CS	—	100	83.9	84.4	86.8
SC	226/226	—	83.9	84.4	86.8
Ag. glc	188/224	188/224	—	98.2	78.7
Ag. trc	189/224	189/224	218/222	—	77.8
Hy. vll	197/227	197/227	177/225	175/225	—

^a Numerators and denominators represent the number of shared fragments and the total number of fragments observed in each comparison, respectively.

^b Ag. glc, Ag. trc and Hy. vll represent the alloplasmic lines of common wheat cv. SC with cytoplasms of *Ag. glaucum*, *Ag. trichophorum* and *Hy. villosa*, respectively.

Fig. 1. Restriction fragment patterns of ctDNAs of common wheat cv. SC and the alloplasmic lines of SC with cytoplasms of *Ag. glaucum* (glc), *Ag. trichophorum* (tre) and *Hy. villosa* (vll) after digestion by *Bam*HI, *Bgl*II, *Eco*RI and *Hin*dIII. Arrowheads indicate fragments which differed between glc and tre.

and the two *Agropyron* species for all the enzymes used except *Pst*I. The percentages of fragments shared between *Hy. villosa* and *Ag. glaucum* and between *Hy. villosa* and *Ag. trichophorum* were 78.7% and 77.8%, respectively (Table 1). Average total genome sizes of the ctDNAs analyzed in this study were 127.7, 125.9, 125.8 and 126.0 kb for common wheat, *Ag. glaucum*, *Ag. trichophorum* and *Hy. villosa*, respectively. These accounted for about 94% of the ct genome size of the gramineous plants (Bowman et al., 1981; Ogihara and Tsunewaki, 1982; Hirai et al., 1985; Kataoka et al., 1987; Murai and Tsunewaki, 1987; Ogihara and Tsunewaki, 1988; Murai et al., 1989).

The restriction fragment patterns of mtDNAs differed among all the cytoplasms for all the enzymes studied (Fig. 2). The Southern hybridization patterns of

Fig. 2. Restriction fragment patterns of mtDNAs of common wheat cv. SC and the alloplasmic lines of SC with cytoplasms of *Ag. glaucum* (gle), *Ag. trichophorum* (trc) and *Hy. villosa* (vll) after digestion by *Eco*RI and *Hind*III.

mtDNAs differed in all or among three cytoplasms depending on combinations of the enzymes and probes used (Fig.3). The two common wheat cultivars were identical for both the restriction fragment patterns of all the enzyme digests and the hybridization patterns of all combinations of the enzymes and probes. The percentages of restriction fragments of mtDNAs shared between common wheat and *Ag. glaucum*, *Ag. trichophorum* and *Hy. villosa* were 45.4, 45.7 and 38.5%, respectively (Table 2), and those of hybridized fragments were 41.6, 44.4 and 40.0% (Table 3). mtDNAs of *Ag. glaucum* shared 46.0% of the restriction

Fig. 3. Southern hybridization patterns of mtDNAs of common wheat cv. SC and the alloplasmic lines of SC with cytoplasms of *Ag. glaucum* (gle), *Ag. trichophorum* (tre) and *Hy. villosa* (vll) as revealed by probing *Bam*HI and *Xho*I digests with 18&5S rDNA and *atpA*.

fragments with that of *Hy. villosa*, while *Ag. trichophorum* did 54.7% (Table 2). Similar values were obtained between them based on the hybridized fragments (Table 3). In contrast to ctDNAs, mtDNAs of *Ag. glaucum* and *Ag. trichophorum* differed in about 40% of the restriction fragments as well as in the hybridized fragments (Tables 2 and 3). The diversity of mtDNAs among the cytoplasms, therefore, was much greater than that of ctDNAs.

In the alloplasmic lines with *Agropyron* cytoplasms, we compared restriction

Table 2. Frequency (% , above the diagonal) and number^a (below the diagonal) of mitochondrial DNA restriction fragments shared among common wheat, *Ag. glaucum*, *Ag. trichophorum* and *Hy. villosa* as revealed by four restriction enzymes

Cytoplasm donor ^b	CS	SC	Ag. glc	Ag. trc	Hy. vll
CS	—	100	45.4	45.7	38.5
SC	190/190	—	45.4	45.7	38.5
Ag. glc	84/185	84/185	—	60.1	46.0
Ag. trc	86/188	86/188	110/183	—	54.7
Hy. vll	74/192	74/192	86/187	104/190	—

a and b See legends to Table 1.

Table 3. Frequency (% , above the diagonal) and number^a (below the diagonal) of mitochondrial DNA restriction fragments shared among common wheat, *Ag. glaucum*, *Ag. trichophorum* and *Hy. villosa* as revealed by the combinations of four restriction enzymes and four mtDNA clones as probes

Cytoplasm donor ^b	CS	SC	Ag. glc	Ag. trc	Hy. vll
CS	—	100	41.6	44.4	40.0
SC	80/80	—	41.6	44.4	40.0
Ag. glc	32/77	32/77	—	61.5	41.1
Ag. trc	36/81	36/81	48/78	—	51.9
Hy. vll	30/75	30/75	30/73	40/77	—

a and b See legends to Table 1.

fragment patterns of mtDNAs of the two sublines either with or without telocentric *Agropyron* chromosomes. No differences were found between the two sublines when the mtDNAs were digested by *Bam*HI, *Eco*RI and *Xho*I (Fig. 4). Although our study was limited, the result suggested that the presence or absence of the telocentric chromosomes did not cause any detectable structural changes in the mtDNAs of these alloplasmic lines.

4. DISCUSSION

Our results obtained by the analyses of ct and mtDNAs of the alloplasmic lines of common wheat with cytoplasms of *Ag. glaucum* and *Ag. trichophorum* showed that the cytoplasms of the two *Agropyron* species are different at molecular level (Tables 1, 2 and 3). This is in accordance with the functional differences of these cytoplasms as revealed by growth vigor, fertility and photosynthetic and respiratory characteristics of the alloplasmic lines having these cytoplasms and the same

Fig. 4. Comparison of restriction fragment patterns of mtDNAs between the alloplasmic sublines of SC having cytoplasm of *Ag. trichophorum* (trc-SC) with (+t) and without telocentric chromosomes derived from *Ag. trichophorum*.

wheat nucleus (Nakamura et al., 1991; Suzuki et al., 1992). The observed structural differences were far greater in the mtDNAs than in the ctDNAs, between the cytoplasms of common wheat and the two *Agropyron* species. The result might indicate that the incompatibility of *Agropyron* cytoplasms with wheat nucleus is related to their mtDNAs.

Agropyron is a complex genus (Dewey, 1984). According to Tsvelev (1983),

Ag. glaucum and *Ag. trichophorum* are classified in *Elytrigia intermedia* subsp. *intermedia* and *trichophora*, respectively. Dewey (1984) further classified perennial grasses of Poaceae, tribe Triticeae, based on their nuclear genomic relationships. According to this classification, *Ag. intermedium* (Host) Beauvois, *Ag. glaucum* (Desf. ex DC) Roemer & Schltzes, *Ag. trichophorum* (Link.) Richt. and *Elymus hispidus* (Opiz) Melderis are all included in one species, i.e. *Thinopyrum intermedium* (Host) Barkworth & DR Dewey, because of their same nuclear genome constitution. The presence of different cytoplasm types in a particular species has been reported, e.g. in *Aegilops triuncialis*, which is known to have originated after amphidiploidization between *Ae. caudata* and *Ae. umbellulata*, three types of chloroplast genome exist, one from *Ae. caudata*, one from *Ae. umbellulata* and the other one as a derivative of that of *Ae. caudata* (Murai and Tsunewaki, 1986). Nasuda et al. (1991) confirmed the presence of the two chloroplast genome types among accessions of *Ae. caudata*, thus further suggesting a polyphyletic origin of *Ae. triuncialis*. To clarify the cytoplasmic lineage of the two *Agropyron* species, a further study on the inter- or intraspecific variations of ct and mtDNAs in the genus *Agropyron* is necessary.

It has been generally accepted that restriction fragment length polymorphism analysis of ctDNAs provides a reliable means in studying phylogenetic relationship among species and genera, because of their genomic stability (Palmer, 1985). We showed that the ctDNAs of *Ag. glaucum*, *Ag. trichophorum* and *Hy. villosa* were equally diverged from that of common wheat and their estimated genetic relatedness was larger than that between the ctDNAs of *Hy. villosa* and the two *Agropyron* species (Table 1). Although the magnitude of genetic relatedness was much smaller, a similar relation was observed among the mtDNAs of these species (Tables 2 and 3). Our results agree with the phylogenetic relationships proposed by Baum (1978) among these three genera, *Triticum*, *Agropyron* (*Elytrigia*) and *Haynaldia* (*Dasyphyrum*).

T. Suzuki is awarded a Fellowship of the Japan Society for the Promotion of Science (JSPS) for Japanese Junior Scientists. This work was supported in part by a Grant-in-Aid for Encouragement of Japanese Junior Scientists from the Ministry of Education, Science and Culture, Japan (MESJCJ). The work was also supported by a Grant-in-Aid for Scientific Research (No. 0156005) from MESJCJ and a grant for International Cooperative Research from JSPS.

REFERENCES

- Baum, B. R. (1978). Generic relationships in Triticeae based on computations of Jardine and Sibon Bk clusters. *Can. J. Bot.* **56**, 2948–2954.
- Bowman, C. M., Koller, B., Delius, H. and Dyer, T. A. (1981). A physical map of wheat chloroplast DNA showing the location of the structural genes for the ribosomal RNAs and the large subunit of ribulose 1, 5-bisphosphate carboxylase. *Mol. Gen. Genet.* **183**, 93–101.
- Dewey, D. R. (1984). The genomic system of classification as a guide to intergeneric hybridization in the perennial Triticeae. In: *Gene manipulation in plant breeding* (ed: J. P. Gustafson), pp. 209–

279. Plenum, New York.
- Falconet, D., Lejeune, B., Quetier, F. and Gray, M. W. (1984). Evidence for homologous recombination between repeated sequences containing 18S and 5S ribosomal RNA genes in wheat mitochondrial DNA. *EMBO J.* **3**, 297–302.
- Hiesel, R., Schobel, W., Schuster, W. and Brennicke, A. (1987). The cytochrome oxidase subunit I and III genes in *Oenothera* mitochondria are transcribed from identical promoter sequences. *EMBO J.* **6**, 29–34.
- Hirai, A., Ishibashi, T., Morikawa, A., Iwatsuki, N., Shinozaki, K. and Sugiura, M. (1985). Rice chloroplast DNA: a physical map and the location of the genes for the large subunit of ribulose 1,5-bisphosphate carboxylase and the 32 kD photosystem II reaction center protein. *Theor. Appl. Genet.* **70**, 117–122.
- Kataoka, J., Ogiwara, Y. and Tsunewaki, K. (1987). Chloroplast DNA variation observed among *Hordeum* species. *Barley Genetics* **5**, 515–524.
- Morikami, A. and Nakamura, K. (1987). Structure and expression of pea mitochondrial F1 ATPase α -subunit gene and its pseudogene involved in homologous recombination. *J. Biochem.* **101**, 967–976.
- Murai, K. and Tsunewaki, K. (1986). Molecular basis of genetic diversity among cytoplasms of *Triticum* and *Aegilops* species. IV. CtDNA variation in *Ae. triuncialis*. *Heredity* **57**, 335–339.
- Murai, K. and Tsunewaki, K. (1987). Chloroplast genome evolution in the genus *Avena*. *Genetics* **116**, 613–621.
- Murai, K., Naiyu, X. and Tsunewaki, K. (1989). Studies on the origin of crop species by restriction endonuclease analysis of organellar DNA. III. Chloroplast DNA variation and interspecific relationships in the genus *Secale*. *Jpn. J. Genet.* **64**, 35–47.
- Nakamura, C., Yamakawa, S. and Suzuki, T. (1991). Recovery of normal photosynthesis and respiration in common wheat with *Agropyron* cytoplasms by telocentric *Agropyron* chromosomes. *Theor. Appl. Genet.* **81**, 514–518.
- Nasuda, S., Terachi, T. and Tsunewaki, K. (1991). Chloroplast DNA variation in *Aegilops caudata* L. *Jpn. J. Genet.* **66**, 752 (Abstract).
- Ogiwara, Y. and Tsunewaki, K. (1982). Molecular basis of the genetic diversity of the cytoplasm in *Triticum* and *Aegilops*. I. Diversity of the chloroplast genome and its lineage revealed by the restriction pattern of ct-DNAs. *Jpn. J. Genet.* **57**, 371–396.
- Ogiwara, Y. and Tsunewaki, K. (1988). Diversity and evolution of chloroplast DNA in *Triticum* and *Aegilops* as revealed by restriction fragment analysis. *Theor. Appl. Genet.* **76**, 321–332.
- Palmer, J. D. (1985). Comparative organization of chloroplast genomes. *Ann. Rev. Genet.* **19**, 325–354.
- Panayotov, I. (1983). The cytoplasm in Triticeae. *Proc. 6th Int. Wheat Genet. Symp.*, pp. 481–497.
- Schuster, W. and Brennick, A. (1989). Conserved sequence elements at putative processing sites in plant mitochondria. *Curr. Genet.* **15**, 187–192.
- Suzuki, T., Nakamura, C., Sakagami, H., Mori, N., Panayotov, I. and Kaneda, C. (1992). Interaction between telocentric nucleolus-organizing chromosomes of two *Agropyron* species and their cytoplasms in alloplasmic lines of common wheat. *Jpn. J. Genet.* **67**, 133–145.
- Tsujimoto, H., Panayotov, I. and Tsunewaki, K. (1987). Behavior of an extra chromosome carried by alloplasmic common wheat lines having *Agropyron trichophorum* cytoplasm. *Jpn. J. Genet.* **62**, 291–299.
- Tsunewaki, K. (1989). Plasmon diversity in *Triticum* and *Aegilops* and its implication in wheat evolution. *Genome* **31**, 143–154.
- Tsvelev, N. N. (1983). *Grasses of the Soviet Union. Part 1*. Oxonian Press Pvt. Ltd., India.