Variability of 3 cytoplasmically encoded proteins in the *Triticum* genus

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The comparison of two-dimensional protein patterns from wheat alloplasmic lines (etiolated seedling stage) has allowed us to define three variable polypeptides with a cytoplasmic determination: the large subunit of ribulose biphosphate carboxylase/oxygenase and two other polypeptides that are likely to be two forms of the chloroplastic beta subunit of ATP synthase. Two cytoplasmic types are found among the *Triticum* species. Of the diploids studied only *T. speltoides* and *T. aucheri* had these same cytoplasmic markers as *T. aestivum*. *T. turgidum* and *T. timopheevi*.

INTRODUCTION

Many experiments have been done to study the cytoplasmic diversity in *Triticum* species (including the formerly called *Aegilops* species). Morphological and physiological studies on wheat alloplasmic lines (Kihara, 1951; Fukasawa, 1953; Hori and Tsunewaki, 1967; Maan and Lucken, 1970; Tsunewaki *et al.*, 1974; Kofoed and Maan, 1982) have led to the classification of 75 accessions (33 species) into 12 plasma types (Tsunewaki, 1980).

The large subunit (LS) of ribulose biphosphate carboxylase/oxygenase (Rubisco) has been used as a cytoplasmic marker. In the main study by Hirai and Tsunewaki (1981) two types were found among 39 *Triticum* cytoplasms. More recently, molecular data obtained by restriction fragment length polymorphism of organellar DNAs have revealed a great diversity, from which phylogenetic relationships have been deduced (Vedel *et al.*, 1978; Ogihara and Tsunewaki, 1982; Bowman *et al.*, 1983; Tsunewaki and Ogihara, 1983).

However the donor of the cytoplasm and B genome of the tetraploid *Triticum turgidum* (AABB) and hexaploid *T. aestivum* (AABBDD) cultivated wheats is still a subject of active controversy. Depending upon their experimental approaches, authors have proposed *T. bicorne* (Sears, 1956), *T. speltoides* (Sarkar and Stebbins,

1956; Riley *et al.*, 1958), *T. searsii* (Feldman and Kislev, 1977), *T. sharonense* (Kushnir and Halloran, 1981), all from the Sitopsis section, or *T. urartu* (Johnson, 1972).

On the other hand *T. speltoides* (Maan and Lucken, 1971) and *T. aucheri* (Tsunewaki, 1980), also from the Sitopsis section, have been suggested as the origin of the cytoplasm and G genome of the *timopheevi* group (AAGG, AAAAGG).

On the basis of the restriction patterns of chloroplast DNA (cpDNA) of alloplasmic Chinese Spring (CS) lines, Tsunewaki and Ogihara (1983) have proposed that an ancestor of *T. longissima* was the B genome donor and an ancestor of *T. aucheri* was the G genome donor.

The comparison of the proteins of wheat alloplasmic lines by high resolution two dimensional (2D) gel electrophoresis has led to the detection of variability in 3 cytoplasmically-encoded polypeptides (Bahrman and Thiellement, 1987). In this paper the variability observed for these 3 polypeptides in 50 *Triticum* genotypes is reported, with special emphasis on the Sitopsis section.

MATERIAL AND METHODS

Plant material

The various lines and accessions used in this study are listed in table 1. The alloplasmic lines of Chinese Spring (CS) were obtained from Prof. K. Tsunewaki (Kyoto, Japan) and the alloplasmic

Table 1 List of the plant material used and their 2D cytoplasmic type

Nuclear and cytoplasmic genomes	Plasmatype*	2D cytoplasmic type
Chinese Spring/ <i>tauschii</i> †	D	LS2, C3, C4
Selkirk/ <i>ventricosum</i> †	D	LS2, C3, C4
Selkirk/ <i>cylindricum</i> †	D	LS2, C3, C4
<i>T. tauschii</i> var. <i>typicum</i>	D	LS2, C3, C4
<i>T. tauschii</i> var. <i>meyere</i>	D	LS2, C3, C4
<i>T. tauschii</i> var. <i>Strangulatum</i>	D	LS2, C3, C4
Selkirk/ <i>juvenile</i> †	D ²	LS2, C3, C4
Selkirk/ <i>ovatum</i> †	M ⁰	LS2, C3, C4
Selkirk/ <i>muticum</i> †	M ^t	LS2, C3, C4
<i>T. monococcum</i> var. <i>monococcum</i>	A	LS2, C3, C4
<i>T. monococcum</i> var. <i>boeoticum</i>	A	LS2, C3, C4
<i>T. urartu</i> (5 accessions)	A	LS2, C3, C4
Selkirk/ <i>bicorne</i> †	S ^b	LS2, C3, C4
Selkirk/ <i>kostchii</i> †	S ^v	LS2, C3, C4
Selkirk/ <i>variabile</i> †	S ^v	LS2, C3, C4
<i>T. bicorne</i> (3 accessions)	S ^b	LS2, C3, C4
<i>T. sharonense</i> (3 accessions)	S ^l	LS2, C3, C4
<i>T. searsii</i> (2 accessions)	S ^{b,v}	LS2, C3, C4
<i>T. longissima</i> (4 accessions)	S ^{1,2} or S	LS2, C3, C4
<i>T. speltoïdes</i> var. <i>typicum</i> (2 accessions)	S or G	LS1, C1, C2
<i>T. speltoïdes</i> var. <i>polyatherum</i>	S or G	LS1, C1, C2
<i>T. speltoïdes</i> var. <i>compactum</i>	S or G	LS1, C1, C2
<i>T. aucheri</i>	G	LS1, C1, C2
Selkirk/ <i>timopheevi</i> †	G	LS1, C1, C2
Chinese Spring/ <i>turgidum</i> var. <i>dicoccoides</i> †	S	LS1, C1, C2
<i>T. turgidum</i> var. <i>dicoccum</i>	S	LS1, C1, C2
<i>T. turgidum</i> var. <i>durum</i>	S	LS1, C1, C2
<i>T. turgidum</i> var. <i>dicoccoides</i>	S	LS1, C1, C2
<i>T. turgidum</i> var. <i>carthlicum</i>	S	LS1, C1, C2
<i>T. aestivum</i> var. <i>aestivum</i> (8 lines)	S	LS1, C1, C2

* According to Tsunewaki 1980.

† Alloplasmic line.

Selkirk lines from Prof. S. S. Maan (Fargo, North Dakota, U.S.A.), the *Triticum turgidum*, *T. tauschii* and *T. monococcum* accessions were furnished by Dr Y. Cauderon (INRA Versailles, France) and Dr G. Doussinault (INRA le Rheu, France). The species of the Sitopsis section are represented by accessions obtained from Japan (Germ Plasm Institute of Kyoto), the United States (Prof. E. R. Sears, Columbia, Missouri) and France (INRA Le Rheu).

Protein extraction and electrophoresis

The proteins of 7-day-old dark germinated seedlings were extracted as previously described (Damerval *et al.* 1986) and the electrophoresis performed by two different methods: in 16×16 cm slab gels as described by Colas des Francs and Thiellement (1985) and in 20×24 cm slab gels as in Bahrman and Thiellement (1987). The analysis focuses on variation in 3 polypeptides easy to recognise using these two types of electrophoresis. In addition, data obtained in previous experiments (Zivy *et al.*, 1983, 1984) were included in the analysis.

RESULTS

As shown in fig. 1, six spots vary (presence/absence) according to the cytoplasm. Their behaviour suggests that they correspond to 3 pairs of cytoplasmic alleles: for each polypeptide the two allelic forms differ in isoelectric point and slightly in relative molecular mass. The LS1 and LS2 polypeptides are the two forms of the LS of Rubisco as demonstrated previously (Zivy *et al.*, 1984).

Figure 1 Sections of 2D gels of proteins obtained from alloplasmic Chinese Spring lines with *T. turgidum* (1) and *T. tauschii* (2) cytoplasm. Proteins with cytoplasmically determined variability are indicated. On the left the relative molecular mass of the proteins are given in kilodaltons.

The other polypeptides C1/C3 and C2/C4 are located in a chloroplastic membrane as shown by subcellular fractionation (Granier *et al.*, 1986). The two polypeptides found in one cytoplasm show the same pattern of partial enzymatic proteolysis (data not shown). The shift of the two protein spots on the gels from one to the other cytoplasmic type is exactly parallel. Thus it is tempting to

speculate that these two proteins are two products of the same gene. This may occur if one or both gene products undergo a post-translational modification such as phosphorylation or glycosylation which are known to induce changes in isoelectric point and even in relative molecular mass (Anderson and Anderson, 1979; Zannis and Breslow, 1981). These two proteins are cytoplasmically-encoded, as shown by comparing alloplasmic lines (table 1). Their isoelectric points, between pH 5 and 6, and their relative molecular mass, heavier than the LS one, circa 56–58 kilodaltons (see fig. 1), are in the range of the electrophoretic properties described for the beta subunit of the coupling factor CF1 of the chloroplastic ATP synthase complex, located in the chloroplastic membrane (Ellis, 1981; Howe *et al.*, 1982; Rémy and Ambard-Bretteville, 1983). It is thus hypothesised that C1/C2 and C3/C4 are two products of the *atpB* gene, which is located on the chloroplastic DNA (Howe *et al.*, 1982). These two spots have been described as cytoplasmic variants in previous reports from our laboratory with the spot numbers 1304 to 1307 (Zivy *et al.*, 1984). This clear and reproducible variation was considered partly as quantitative since the polypeptides were not completely separated with the method used at that time.

The observations for these 6 spots on the plant material examined are listed in table 1. Only 2 groups of cytoplasms can be discriminated using this criterion. One group consists of the representatives of the A, M and D genomes and many of the species from the Sitopsis section. *T. aestivum*, *T. turgidum*, *T. timopheevi* and only 2 diploid species, *T. speltoides* and *T. aucheri*, are found in the other group.

DISCUSSION

The results presented here are in accordance with the extensive study on alloplasmic CS lines by Hirai and Tsunewaki (1981) on the variation of the LS, except for *T. longissima*. The *T. longissima* LS was found by the Japanese workers to be identical to the *T. aestivum* LS. But, according to the data here reported (table 1), the *T. longissima* LS is different from the *T. aestivum* LS in the four accessions analyzed. As Tsunewaki and Ogiwara (1983) found no difference between the cpDNA of these same two alloplasmic lines, they concluded that *T. longissima* was the cytoplasmic donor of *T. aestivum*. However in no other comparison between cpDNA of closely related species did they find such identity. This fact, in addition

with the results reported above raises questions about the peculiar nature of the CS/*longissima* alloplasmic line used by Tsunewaki and his co-workers.

In every case examined the more acidic form of the LS of Rubisco is found associated with the more basic forms of C (table 1). Thus the appearance of mutated forms of the LS and C polypeptides must have occurred either simultaneously or in a relatively short period of time. In this connection, following the hypothesis that the C proteins are two forms of the beta subunit of ATP synthase, it is very remarkable that the two chloroplastic genes involved, *rbcL* and *atpB*, are very close to each other on the wheat cpDNA (Howe *et al.*, 1982).

The (LS1, C1, C2) type is found only among diploid species belonging to the Sitopsis section and in *T. turgidum*, *T. aestivum* and *T. timopheevi*. Therefore the mutation(s) likely occurred in an ancient form of Sitopsis, which transmitted its cytoplasm to *T. speltoides*, *T. aucheri*, to the B genome donor of *T. aestivum* and *T. turgidum* and to the G genome donor of *T. timopheevi*.

If we discard the hypothesis that the same mutational event(s) occurred independently in different cytoplasms, it can be concluded that *T. speltoides* and *T. aucheri* have cytoplasms that are more closely related to those of *T. turgidum*, *T. aestivum* and *T. timopheevi* than are the cytoplasms of any of the other diploids.

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Note added in proof: Preliminary immunoblotting experiments (with R. Rémy and F. Ambard-Bretteville, Laboratoire de Génétique Moléculaire des Plantes, Orsay) performed with anti-beta subunit from spinach chloroplasts confirmed the suggestion that the C proteins are indeed two forms of the beta subunit.

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