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Haploid production in durum wheat by the interaction of *Aegilops kotschyi* cytoplasm and 1BL/1RS chromosomal interchange

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Abstract. The present study describes the development of an alloplasmic haploid-inducer in *durum* wheat cv 'Cando'. This cultivar possesses the homozygous wheat-rye translocation 1BL/1RS from the 6x-wheat cv 'Veery'. The nucleus of 4x-'Cando-Veery 1BL/1RS' was introduced into *Aegilops kotschyi* cytoplasm by initially using (*kotschyi*)-Salmon as the maternal parent. In the cross of this alloplasmic *durum* line with 'Cando-Veery 1BL/1RS', which was used as the recurrent pollen parent, haploids (n = 14) were produced. The frequency of haploids increased from 5.7% in the F_1 generation to 14% in the BC_1 generation. The presence of rye chromosome arm 1RS and the concomitant loss of 1BS in '(*kotschyi*)-Cando-Veery 1BL/1RS' are necessary for haploid induction. Proposals are made which may enable the use of haploids produced by nucleo-cytoplasmic interactions in future wheat breeding programs.

Key words: Durum wheat-rye translocation 1BL/1RS – *Aegilops kotschyi* cytoplasm – Haploid induction – Alloplasmic 4x-wheat

Introduction

The induction of haploidy in higher plants has received considerable attention in recent years as haploids have proven valuable in genetic studies, studies on genome relationships, and particularly in accelerating the process of breeding for pure lines. In *Triticum aestivum* L. there are well-established methods for the production of haploid plants from anther culture (Henry and De Buyser 1990; Lashermes 1992) and from the pollination of wheat by *Hordeum bulbosum* (Barclay 1975), maize (Laurie and Reymondie 1991), and pearl millet (Ahmad and Comeau 1990).

Another procedure that does not require embryo rescue is the production of haploids by nucleo-cytoplasmic interactions (Kihara and Tsunewaki 1962; Mukai 1981; Tsunewaki and Mukai 1990). Kihara and Tsunewaki (1962) first reported that *T. aestivum* cv 'Salmon' induced haploids at a frequency of about 30% when its cytoplasm was substituted for by that of *Aegilops caudata*. Later studies revealed that all of the cytoplasms belonging to the C, C^u, M^u, Mt, S^1, and S^v plasma types produced haploids at frequencies varying from 10% to 20% when transferred to cv 'Salmon' (Tsunewaki et al. 1976; Mukai 1981; Tsunewaki and Mukai 1990). Among these haploid-inducing cytoplasms the plasma of *Aegilops uniaristata* (M^u type), *A. kotschyi* Boiss., and *A. variabilis* (both S^v type) produced little or no deleterious effects on wheat characters, with *A. kotschyi* cytoplasm being considered the most promising among all alien cytoplasms for widening the genetic variability of common wheat (Tsunewaki et al. 1985). Haploid induction in '(*kotschyi*)-Salmon' ('kSlm') has been associated with the interaction of the 1BL/1RS translocation in 'Salmon' and the cytoplasm of *A. kotschyi* (Kobayashi and Tsunewaki 1980).

The study described here examined the production of haploids in durum wheat (2n = 4x = 28) that resulted from the interaction of *A. kotschyi* cytoplasm and the 1BL/1RS chromosomal interchange.

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Table 1. Frequencies of haploids in *Triticum turgidum* ssp. *durum* cv 'Cando-Veery 1BL/1RS' with *A. kotschyi* cytoplasm

Year	Generation	Number of plants	Halploid (H)	Twins[a] (T)	% (H + T)
1991	F_1	35	2	0	5.7
1992	BC_1	157	20	2	14.0

[a] Haplo-diplo type (n–2n)

Material and methods

'(*kotschyi*)-Salmon' ('kSlm'), kindly provided by K. Tsunewaki, Kyoto, Japan, was first used as the female parent in a cross performed in 1981 at Weihenstephan, Germany, with common wheat cv 'Disponent', which carries a 1BL/1RS translocation. 'kSlm' is a cytoplasm substitution line of cv 'Salmon' with *A. kotschyi* cytoplasm. The pedigree is *A. kotschyi*/'Chinese Spring^3'/'Salmon^6' and in 1980 reached the BC_5 generation (Kobayashi and Tsunewaki 1980).

The backcrossing of 'kSlm' with cv 'Disponent' for two generations resulted in a haploid production frequency of 17% (F. J. Zeller, unpublished). These plants were further crossed with 4x-'Creso × Cando-Veery 1BL/1RS', and 35-chromosome plants were cytologically selected. 'Creso' is an Italian 4x-durum wheat, 'Cando' a 4x-durum wheat grown in the USA, and 'Veery' is a hexaploid CIMMYT-spring wheat carrying the 1BL/1RS wheat-rye translocation (Merker 1982). The production of 4x-'Cando-Veery' possessing the 1BL/1RS translocated chromosome pair was described by Friebe et al. (1987). The hybrids between '(*kotschyi*)-Disponent'/'Creso × Cando-Veery' were twice backcrossed to 4x-'Cando-Veery 1BL/1RS', and one 28-chromosome plant with two satellited chromosomes (6B) was obtained. This plant is presently designated '(*kotschyi*)-Cando-Veery 1BL/1RS'. The progeny of this plant having *A. kotschyi* cytoplasm was used as the female parent in further crosses with 4x-'Cando-Veery 1BL/1RS'. The somatic chromosome number was scored using the standard Feulgen staining method.

Results

Two haploid plants (n = 14) were obtained from 35 F_1 seedlings of the cross '(*kotschyi*)-Cando-Veery 1BL/1RS' × 'Cando-Veery 1BL/1RS'. The tetraploid F_1 plants possessing *A. kotschyi* cytoplasm were backcrossed to 'Cando-Veery 1BL/1RS' using the latter as the recurrent pollen parent. A seed-set frequency of about 28% was obtained from a total of 846 florets pollinated. There was no seed-set in the isolated spikes. The majority of the BC_1 seeds were normally plump; however, about one-third of the BC_1 seeds was shrivelled, and 20% did not germinate.

In the population of BC_1 seedlings the frequency of haploid plants was approximately 14%. The frequency of haploid production was 2–3 times higher than the frequency (5.7%) observed in the previous F_1 generation (Table 1). Cytological analysis revealed that the mitotic metaphase chromosome number of all of the haploid plants was n = 14 and that each haploid complement possessed only one satellited chromosome (Fig. 1), presumably chromosome 6B. Two twin (n–2n) plants were obtained.

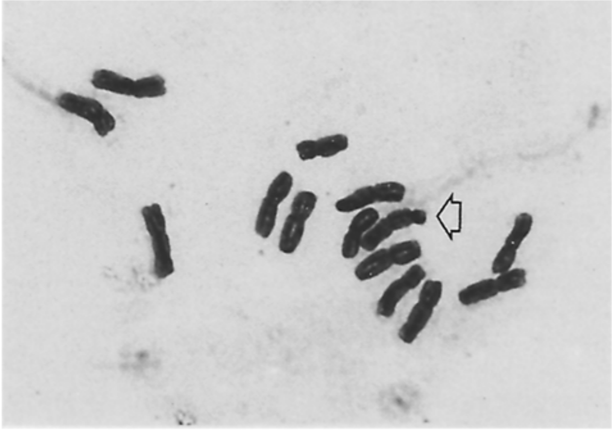

Fig. 1. Mitotic metaphase plate of a '(*kotschyi*)-Cando-Veery 1BL/1RS' haploid plant showing n = 14 chromosomes; only a single SAT-chromosome is visible (*arrow*)

Discussion

Results of the study described here reveal that haploid plants can be induced in alloplasmic tetraploid wheat as a result of the interaction of *A. kotschyi* cytoplasm and the 1BL/1RS chromosomal translocation. Kobayashi and Tsunewaki (1980) showed that haploid induction in hexaploid wheat cv 'Salmon' with *A. kotschyi* cytoplasm was due to the presence of the wheat-rye translocation in the egg cell. 'Salmon', a common wheat, had been shown to possess a 1BL/1RS translocation (Zeller 1973). Additional studies on the genetic mechanism of haploid induction in 'kSlm' elucidated that haploid production depends on the presence of a parthenogenesis-inducing cytoplasm and a nuclear gene, designated *Ptg* (parthenogenesis), located on the short arm of rye chromosome 1R (1RS) and the absence of another nuclear gene, *Spg* (suppression of parthenogenesis), on the short arm of wheat chromosome 1B (Tsunewaki and Mukai 1990).

At the hexaploid level, other available evidence also indicates that haploid production is governed by the concurrent absence of 1BS and presence of 1RS. The wheat cv 'Amigo' possessing the complete 1B wheat chromosome pair and a 1AL/1RS chromosome translocation (Zeller and Fuchs 1983) and another wheat line with complete chromosome pair 1B and translocated chromosome 1DL/1RS (Shepherd 1973) were crossed and backcrossed to 'kSlm', but haploid plants were not produced (F. J. Zeller, unpublished). This demonstrated that haploid induction is not possible in the presence of both 1BS and 1RS. Similarly, in a study with another haploid-inducer plasma from *A. caudata*, no haploids were induced in '(*caudata*)-nulli 1B' of cv 'Chinese Spring' when 1RS was absent (F. J. Zeller, unpublished).

In the present study the frequency of haploid durum plants in the BC_1 generation (14%) is slightly higher than the frequency of 'kSlm' (12.5%), but a lower frequency of twins was observed (1.3%) in a comparable BC_1 generation reported by Tsunewaki et al. (1974). Haploid frequency in 'kSlm' in the BC_2 generation later increased to 17% (Tsunewaki et al. 1976). Mukai (1981) also reported that with further increase in the substitution backcrosses from the BC_4 to BC_{11} generations the frequency of haploids in *A. columnaris*/*T. macha*$^{5-12}$ alloplasmic wheat lines increased from 18% to 32%. Thus, it may be anticipated that the frequency of haploids in durum wheat will also increase upon further substitution backcrosses.

One prominent effect of the *A. kotschyi* cytoplasm in alloplasmic durum wheat is male sterility. Isolated spikes in the BC_1 generation did not produce any seeds. In common wheat, Mukai and Tsunewaki (1979) reported that sterility is due to the absence of a fertility-restoring gene (*Rfv1*) against *A. kotschyi* cytoplasm that is located on the satellited short arm of chromosome 1B in cv 'Chinese Spring'. The genetic distance of the *Rfv1* gene from the centromere based on telocentric mapping has been estimated to be 34 cM (Hamawaki and Mukai 1980). Recently, using the Giemsa staining technique Mukai and Endo (1992) showed that the *Rfv1* gene is located close to the interstitial C-band in the satellited chromosome of 1B.

Haploid induction to be of use in practical wheat breeding necessitates the production of a self-fertile, haploid-inducing strain. Studies in alloplasmic common wheat reveal difficulties in producing these strains due to the position of the nuclear genes involved in the haploid-induction mechanism (Mukai and Tsunewaki 1979; Tsunewaki and Mukai 1990). It is therefore important that a search for a translocation line carrying only the fertility-restoration gene (*Rfv1*), which is located on the satellite without the *Spg* gene, should be undertaken. At present, deletion stocks having various deficiencies in the 1BS wheat chromosome that have been derived from the backcrossed progeny of a monosomic *A. cylindrica* chromosome addition line of cv 'Chinese Spring' are available (Endo 1990; Mukai and Endo 1992). Attempts have also been made in tetraploid wheat to induce and classify translocations involving the A and B genome chromosomes of wheat (Sendino and Perera 1984; Nishikawa et al. 1986). A study using these materials may produce a translocation involving only the fertility-restoration gene, *Rfv1*. Concurrent fertility restoration studies and C-banding screening will enhance the process of obtaining the desired genetic line.

Another approach is to find in existing wheat accessions or artificially induced lines a recessive *spg* allele on a normal 1B chromosome. Tsunewaki and Mukai (1990) have assumed that heterozygotes (*spg Rfv1*/*Ptg*) can be produced that could give rise to a high haploid frequency line (40%) like the one obtained by Mukai and Nakanishi (1982). Moreover, half of the progeny from the heterozygotes will produce fertile diploids after chromosome doubling.

The results of our study indicates that the haploid induction mechanism operates at the tetraploid level as in hexaploid wheat. In addition, haploid production through the interaction of alloplasmic wheat and the 1BL/1RS translocation, free from in vitro culture, has an advantage over other means that need artificial media. Moreover, alloplasmic haploid-inducers in wheat can be used to convert any non-haploid-producing cultivars into a haploid producer (Tsunewaki and Mukai 1990). The production of haploids by introducing *A. kotschyi* cytoplasm into common wheats possessing the 1BL/1RS translocated chromosome pair, which are widely distributed in the world (Rajaram et al. 1990), and alloplasmic durum wheats having the 1BL/1RS translocation can be a valuable tool in future wheat breeding programs.

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