Theor Appl Genet (1990) 79:525–528

THEORETICAL AND APPLIED GENETICS

Production and meiotic analysis of autotriploid *Triticum speltoides* and *T. bicorne* *

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Received September 18, 1989; Accepted December 12, 1989
Communicated by K. Tsunewaki

Summary. Autotriploid *Triticum speltoides* and *T. bicorne* (2n = 3x = 21) were produced by pollinating autotetraploids with pollen from their respective diploids. The autotriploid plants were vigorous, male sterile, and morphologically resembled their diploid parents. At meiosis, *T. speltoides* (3x) averaged 2.52 univalents, 0.42 rod bivalents, 2.03 ring bivalents, 4.48 trivalents, and 0.03 chain quadrivalents per cell, and *T. bicorne* (3x) had 2.30 univalents, 0.20 rod bivalents, 2.10 ring bivalents, and 4.70 trivalents. Panhandle trivalents made up 27% of the total trivalents, and involved 18% of the total number of chromosomes observed in *T. bicorne* (3x), and 26% and 17% in *T. speltoides* (3x), respectively. The observed chromosome pairing in both triploids was predicted well from the expressions developed by Alonso and Kimber.

Key words: Wheat relatives – Meiosis – Chromosome pairing – Arm-pair switch – Autopolyploidy

Introduction

Induced autotetraploids have proved to be a useful tool in genome analysis (Kondo 1946, 1950; Kondo and Kamanoi 1958; Zennyozi 1960, 1965; Kimber and Yen 1988, 1989; Yen et al. 1988). Since autotetraploids have a tendency toward bivalentization (McCollum 1958; Timmis and Rees 1971; Avivi 1976; Yen and Kimber 1990), a study of whether bivalentization also occurs in autotriploids is important.

In this paper, artificial autotriploids of *T. speltoides* and *T. bicorne* are reported and their meiotic behavior is discussed.

* Contribution from the Missouri Agricultural Experiment Station. Journal Series No. 10932

Materials and methods

The materials used in this investigation are listed in Table 1. The meiotic behavior of both autotetraploids was reported in Yen and Kimber (1990). All the materials were from stocks maintained at the University of Missouri-Columbia.

Hybrids were made by hand-emasculation and -pollination. Embryos were dissected from developing seeds about 14–18 days after pollination and placed on Murashige and Skoog medium. Seedlings that developed were planted out into pots and grown in a glasshouse. Meiotic data were obtained from pollen mother cells (PMCs) fixed in 3:1 ethyl alcohol:glacial acetic acid for 24 h, and then stained by the Feulgen technique.

The meiotic data were analyzed by the numerical techniques described by Alonso and Kimber (1981). These techniques allow the calculation of two variables, c and x. The mean arm-pair frequency (c) is a measure of the number of arms bound by at least one chiasma divided by the maximum number of arms that can be bound. The range of c is from 0.0, when there is no pairing at all, to 1.0, when all the possible arms are paired. The range of values of x that is a measure of relative affinity between the two closer related genomes in a triploid hybrid is from 0.5, when all three genomes in a triploid are equally related, to 1.0, when two of the three genomes are so closely related that all the pairing involves only them. In the latter situation, two genomes are more closely related to each other and are equally distant from the third genome. The optimum value of x is calculated by minimum sum of squares of differences (SSD).

The Kimber-Alonso models assume that no arm-pair switches should occur. In fact, panhandle trivalents were fre-

Table 1. Materials used in this investigation

Species	Genomes	MU codes[a]	Diploid origin	Note
T. speltoides (4x)	SSSS	XX98	Iraq	
T. speltoides	SS	TS08	Israel	
T. bicorne (4x)	$S^bS^bS^bS^b$	XX105	Unknown	CI 47[b]
T. bicorne	S^bS^b	TB04	Israel	

[a] University of Missouri-Columbia accession codes
[b] CI is the U.S.D.A. plant introduction number

Table 2. The means, ranges, the values of c and x, and SSDs of autotriploids of *Triticum speltoides* and *T. bicorne*

Species	No. plants	No. cells		Univ.	Bivalent		Triv.	Quadriv.		c	x	SSD
					rod	ring		open	closed			
T. speltoides												
2n=20	1	20	Obs[a]	2.95 (1–7)	0.70 (0–2)	3.30 (1–7)	2.95 (0–5)	0.05 (0–1)	0.00 (0–0)	0.954		
2n=21	3	60	Obs	2.52 (0–8)	0.42 (0–2)	2.03 (0–6)	4.48 (1–7)	0.03 (0–1)	0.00 (0–0)	0.968		
			Opt	2.72	0.54	2.15	4.30	0.00	0.00		0.500	0.101
2n=22	1	20	Obs	3.15 (0–6)	0.65 (0–5)	4.00 (0–8)	2.65 (0–6)	0.30 (0–1)	0.10 (0–1)	0.953		
T. bicorne												
2n=21	2	40	Obs	2.30 (0–6)	0.20 (0–1)	2.10 (0–6)	4.70 (1–7)	0.00 (0–0)	0.00 (0–0)	0.986		
			Opt	2.47	0.19	2.27	4.54	0.00	0.00		0.500	0.084

[a] Obs – observed frequency; Opt – optimized frequency (Alonso and Kimber 1981)

quently observed in these autotriploids. Since a panhandle trivalent involves three chromosomes and at least three chiasmata, its occurrence will surely indicate homology. Panhandle trivalents are catalogued as chain trivalents to simplify optimization. Since the triploid model of Alonso and Kimber (1981) does not allow the optimization of meiotic data of aneuploids, no x values were calculated for the aneuploids of *T. speltoides* (Table 2).

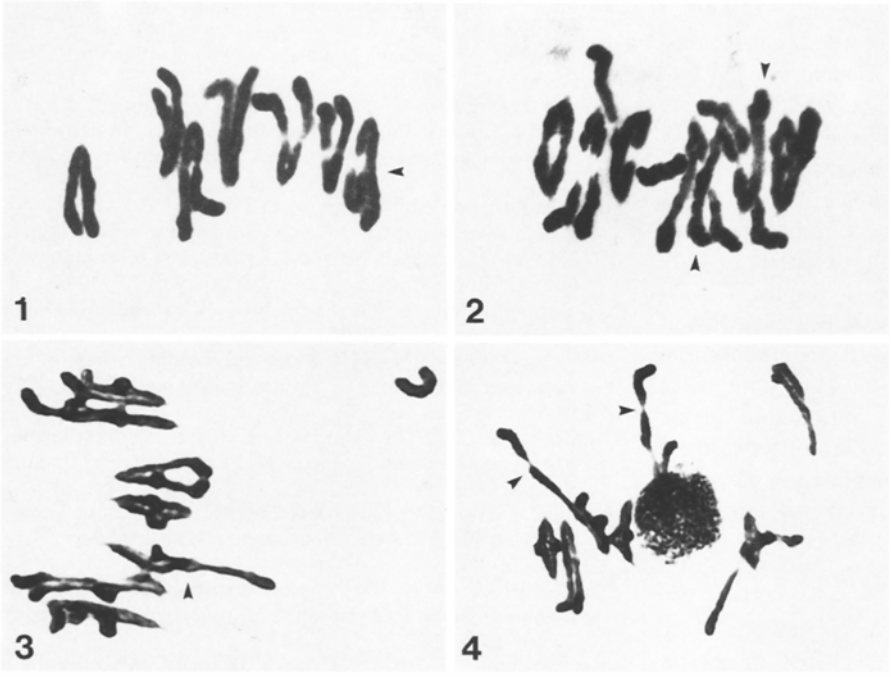
Results and discussion

This is the first report on the production and cytological study of autotriploids of *T. speltoides* and *T. bicorne*. These autotriploid plants were vigorous. Kuspira et al. (1986) reported that triploid *T. monococcum* was self-fertile, and it was evidently larger than the diploid *T. monococcum*. However, the triploids of *T. speltoides* and *T. bicorne* were both hard to distinguish morphologically from the diploids, unlike the tetraploids that were larger than the diploids. Apparently, the extra genome in these triploids does not cause a visible difference in gross plant size compared to the diploid. The triploid form of *T. speltoides*, an outcrossing species, was sterile. The self-fertility of the triploid *T. bicorne* was extremely low; only one selfed seed was obtained from 53 spikes.

Seven autotriploid *T. speltoides* plants were obtained. Of the five meiotically examined, three had a somatic chromosome number of 21 (Figs. 1 and 2), while the remaining two were 20 and 22, respectively. Five autotriploid *T. bicorne* plants were obtained; the one meiotically examined had 21 chromosomes (Fig. 3). At meiosis (Table 2), the open quadrivalents observed involved only 0.63% of the total number of chromosomes in the euploid *T. speltoides* plants and 0.95% in the 2n=20 plant. No closed quadrivalent was observed in these plants. However, the 2n=22 plant of *T. speltoides* had 6.67% of the total chromosomes involved in open quadrivalents and 0.95% in closed quadrivalents. The open quadrivalents in the euploid and 2n=20 plants probably resulted from the nonhomologous pairing. Apparent nonhomologous pairing was also observed in autotriploid *T. bicorne* (Fig. 4).

The x value of 0.500 demonstrated that the euploid triploids behaved as true autotriploids. There was little difference in pairing between triploids of the two species. The very low SSDs between the observed and calculated frequencies of meiotic configurations indicate that the model is a good fit. Therefore, pairing occurred randomly among the three homologues within each homologous group. Clearly, the bivalentization observed in the autotetraploid parents (Yen and Kimber 1990) did not take place in the autotriploids.

Mettin et al. (1984) reported 8.12 univalents, 1.98 rod bivalents, 2.74 ring bivalents, 1.12 trivalents, and 0.02 open quadrivalents in triploid *T. monococcum* var. *macedonicum*. Kuspira et al. (1986) observed 2.65 univalents, 0.09 rod bivalents, 2.41 ring bivalents, and 4.45 trivalents in triploid *T. monococcum* var. *hornemannaii*. Optimizing these meiotic data revealed x values of 0.945 and 0.621, respectively. It should be noted that no complex trivalents were observed by Mettin et al. (1984) and that the complex trivalents observed by Kuspira et al. (1986), which included some that were Y-shaped, comprised only 7% of the trivalents observed. It should also be noted that univalents were higher and rod bivalents and trivalents were lower than expected in both these cases, and that 2% of PMCs observed by Kuspira et al. (1986) contained three homologous univalents (indicated by three more univalents than the bivalents observed). These observations of Mettin et al. (1984) and Kuspira et al. (1986) strongly suggest that pairing level was reduced in



Figs. 1–4. 1 Chromosome associations at MI in PMCs of autotriploid *T. speltoides,* showing seven trivalents; *arrow head* indicates panhandle trivalent. **2** Chromosome associations at MI in PMCs of autotriploid *T. speltoides,* showing one univalent, one rod, and one ring bivalent, four trivalents, and one open quadrivalent. *Arrow heads* show the two ends of the open quadrivalent. **3** Chromosome associations at MI in PMCs of autotriploid *T. bicorne,* showing one univalent, one ring bivalent, and six trivalents; *arrow head* indicates panhandle trivalent. **4** Chromosome associations at MI of PMCs of autotriploid *T. bicorne,* showing two ring bivalents, three trivalents, and two open quadrivalents (marked with *arrow head*)

autotriploid *T. monococcum*. This would, in turn, reduce the number of trivalents, probably increase the number of ring bivalents, and thereby increase the value of *x*.

Panhandle trivalents were observed in all plants. They made up 27% of the total trivalents in *T. bicorne* and 26% in the euploid plants of *T. speltoides*. This means that 18% and 17% of the total chromosomes were involved in panhandle trivalents in the two autotriploids, respectively. In the 2n = 20 plant of *T. speltoides*, 25% of the trivalents or 11% of the total chromosomes were involved in panhandle trivalents but the percentages were only 13% and 5%, respectively, in the 2n = 22 plant. No Y-shaped trivalents were observed in any of these triploid plants. The occurrence of panhandle trivalents is most likely to result from arm-pair switch among homologues.

Yen and Kimber (1990) reported that arm-pair switch was not observed in autotetraploid *T. speltoides*, and involved 25% of the trivalents but only 6% of the total number of chromosomes of autotetraploid *T. bicorne*. Therefore, the high frequencies of panhandle trivalents observed in autotriploids of *T. speltoides* and *T. bicorne* suggest that arm-pair switch is promoted if a chromosome is present as a trisomic rather than a tetrasomic. Arm-pair switch can only occur if an arm has two or more chiasmata. When one chiasma has formed between two arms and a second is possible, whether the second chiasma is to occur between the same two arms or with a third homologue will surely depend on the availability of that third arm. If the chromosome concerned is trisomic, the third arm is fully available, for it has nothing else with which to pair. If, however, the chromosome is tetrasomic, the third and fourth homologous arms are likely to pair with each other, while the first and second homologous arms pair; this leaves little chance, if any, for any of the four homologous arms to allow its second chiasma to involve an arm that has not been involved in the first chiasma. The third homologous arm generally will only be available in a tetrasomic, if the third and fourth homologous arms fail to pair with each other. Therefore, more arm-pair switches are to be expected with trisomic than tetrasomic, and relatively more bivalents from the tetrasomic, as shown in Table 2. This argument is supported by the observation of others. Kuspira et al. (1986) found that complex trivalents involved 7% of the trivalents and 12% of the total chromosomes observed in their triploid *T. monococcum*. However, Kuspira et al. (1985) reported that complex trivalents represented 50% of the trivalents but only involved 1% of the total chromosomes in tetraploid *T. monococcum*. Consequently, arm-pair switch is evidently common in artificial autotriploids.

It should be noted that, in autotriploid *T. speltoides*, the arm-pair switch rate in the 2n = 20 plant differed little from the 2n = 21 plants, but the rate was much lower in the 2n = 22 plants. Since the only difference among these *T. speltoides* plants is presence or absence of one chromosome, it seems that arm-pair switch is more likely to occur in some homologous group than in others. This conclusion is supported by the data of Kuspira et al.

(1986), showing that only one of the seven *T. monococcum* chromosomes is responsible for the formation of complex trivalents in their triploid *T. monococcum*.

As shown in Table 2, both addition and loss of one chromosome in the female gamete of the autotetraploid parent resulted in reduction in trivalents and increase in ring bivalents in the aneuploid *T. speltoides*. It is expected that reduction in trivalents and increase in bivalents will occur when one group of homologues consists of only two chromosomes instead of three, as in the $2n=20$ *T. speltoides* plant. The large increase in ring bivalents in the tetrasomic autotriploid *T. speltoides*, together with a reduction in the number of trivalents, clearly indicates cytological bivalentization in that homologous group when four chromosomes are present. This observation is consistent with the cytological bivalentization described by Yen and Kimber (1990) in induced autotetraploid Triticeae.

References

Alonso LC, Kimber G (1981) The analysis of meiosis in hybrids. II. Triploid hybrids. Can J Genet Cytol 23:221–234

Avivi L (1976) The effect of genes controlling different degrees of homoeologous pairing on quadrivalent frequency in induced autotetraploid lines of *Triticum longissimum*. Can J Genet Cytol 18:357–364

Kimber G, Yen Y (1988) Analysis of pivotal-differential evolutionary patterns. Proc Natl Acad Sci USA 85:9106–9108

Kimber G, Yen Y (1989) Hybrids involving autotetraploid *Triticum umbellulatum*. Genome 31:1–5

Kondo N (1946) Conjugation of semi-homologous chromosomes between Cu and E genome in *Aegilops ovata*. Jpn J Genet 12:59–60

Kondo N (1950) Genome analysis of *Aegilops* using artificially induced autotetraploid *Aegilops squarrosa*, with special reference to D-genome. Oguma Commemoration Vol Cytol Genet 2:124–129

Kondo N, Kamanoi M (1958) Chromosome conjugation in the hybrids between Emmer wheat and induced autotetraploid *Aegilops squarrosa* or *Ae. bicornis*. Wheat Inf Service 7:19–20

Kuspira J, Bhambhani RN, Shimada T (1985) Genetic and cytogenetic analyses of the A genome of *Triticum monococcum*. I. Cytology, breeding behavior, fertility, and morphology of induced autotetraploids. Can J Genet Cytol 27:51–63

Kuspira J, Bhambhani RN, Sadasivaiah RS, Hayden D (1986) Genetic and cytogenetic analyses of the A genome of *Triticum monococcum*. III. Cytology, breeding behavior, fertility, and morphology of autotriploids. Can J Genet Cytol 28:867–887

McCollum GD (1958) Comparative studies of chromosome pairing in natural and induced tetraploid Dactylis. Chromosome 9:571–605

Mettin D, Schlegel R, Blüthner WD (1984) The meiotic behavior of a triploid *Triticum monococcum*. Wheat Inf Serv 59:1–2

Timmis JH, Rees H (1971) A pairing restriction at pachytene upon multivalent formation in autotetraploids. Heredity 26:269–275

Yen Y, Kimber G (1990) Meiotic behavior of induced autotetraploids in *Triticum* L. Genome (in press)

Yen Y, Kimber G, Metzger J (1988) Meiotic analysis of autotetraploid *Triticum umbellulatum*. Cereal Res Comm 16:101–104

Zennyozi A (1960) Chromosome conjugation on the hybrids between induced autotetraploid *Aegilops squarrosa* and *Triticum timopheevi*. Jpn J Genet 35:321–328

Zennyozi A (1965) Cytogenetical studies on the intergenomic hybrid between *Aegilops* and *Secale*. III. Chromosome pairing in the F_1 plant from 4x-*Ae. squarrosa* × *S. cereale* and the F_1 plants from the backcross, with special reference to the R and D intra-genome pairing. Jpn J Genet 40:325–335