Theor Appl Genet (1988) 75:912–916

Synthesis and evaluation of *Triticum durum* – *T. monococcum* amphiploids

R. S. Gill, H. S. Dhaliwal and D. S. Multani
Punjab Agricultural University, Regional Research Station, Gurdaspur-143521, Punjab, India

Received June 25, 1987; Accepted November 30, 1987
Communicated by G. S. Khush

Summary. Nine *Triticum durum – T. monococcum* amphiploids ($AABBA^mA^m$) were synthesized by chromosome doubling of sterile triploid F_1 hybrids involving nine *T. durum* (AABB) cultivars and a *T. monococcum* (A^mA^m) line. The triploid F_1 hybrids had a range of 4–7 bivalents and 7–13 univalents per PMC. The synthetic amphiploids, however, showed a high degree of preferential pairing of chromosomes of the A genomes of diploid and tetraploid wheats. The amphiploids were meiotically stable and fully fertile. Superiority of four amphiploids for tiller number per plant, 100-grain weight, protein content and resistance to Karnal bunt demonstrated that these could either be commercially exploited as such after overcoming certain inherent defects or used to introgress desirable genes into durum and bread wheat cultivars. Methods for improvement of these amphiploids are discussed.

Key words: *Triticum durum – Triticum monococcum* – Triploids – Amphiploids – × *Durococcum* – Evaluation

Introduction

The new manmade cereal, triticale (× *Triticosecale* Witmack), has become popular among farmers in certain areas of the world. Several amphiploids involving *Triticum* and different species of *Aegilops*, *Agropyron* and *Hordeum* have been synthesized. *Triticum zhukovskyi* ($A^tA^tB^tB^tA^mA^m$), the natural amphiploid between *T. timopheevi* and *T. monococcum*, has also been artificially synthesized (Watanabe et al. 1956; Upadhya and Swaminathan 1965; Dhaliwal and Johnson 1976; Driscoll et al. 1979) and was found to have perfect diploid chromosome pairing and high seed set.

The cultivated diploid wheat *T. monococcum* (A^mA^m) is an important reservoir of useful genes for resistance to various diseases and high protein content (Vallega 1978; Sharma et al. 1981; Dhaliwal and Gill 1982; Gill et al. 1983). The useful genetic variation of *T. monococcum* could be combined with high productivity of *T. durum* in *T. durum – T. monococcum* amphiploids. Furthermore, as *T. durum* was subjected to more rigorous improvement through various breeding programmes than *T. timopheevi*, the *durum-monococcum* amphiploids are likely to be superior to *T. zhukovskyi*. The amphiploids of *T. durum* and *T. monococcum* involving well adapted, high-yielding durum varieties have not been studied extensively.

In the present investigation, nine amphiploids have been synthesized involving nine genetically diverse durum varieties and one accession of *T. monococcum*. Four of these amphiploids were evaluated for their meiotic behaviour, fertility, disease resistance and for different agronomic and quality characteristics. The importance of the amphiploids and their further improvement is discussed.

Materials and methods

The materials for this investigation comprised ten different cultivars of *T. durum* viz. PCD 57, WC 6001, DWL 5031, Boy$^{\text{'S'}}$, Mexi.C75, Produra, Cocorit, Yavaros 79, PBW 34 and PBW 117 and an accession of *T. monococcum* with spring habit. The durum cultivars PCD 57, WC 6001, Mexi.C75, Yavaros 79 and Boy$^{\text{'S'}}$ were received from C IMMYT, Mexico; Cocorit, Produra and *T. monococcum* were received from the University of California, Riverside (USA); and DWL 5031, PBW 34 and PBW 117 are double dwarf and rust resistant (yellow and brown) durum varieties from Ludhiana, India. To synthesize the amphiploids, triploid hybrids were made between *T. durum*, cultivars PCD 57, DWL 5031, WC 6001, Boy'S', Mexi.C75, Produra, Cocorit, Yavaros 79, PBW 34, PBW 117 and *T. monococcum* in 1982–1983 and 1983–1984. Reciprocal crosses were also attempted. In order to induce polyploidy the seedlings of *durum* × *monococcum* or reciprocal triploid hybrids were treated by keeping their coleoptiles immersed in 0.25% colchicine solution (in 5% DMSO solution) for 3.5 h. Synthetic amphiploids were selected and cytologically confirmed. For meiotic studies, freshly emerging spikes of triploid and amphiploids were fixed in Carnoy's 6:3:1 fixative for 24 h and transferred to 70% alcohol until used. Anther squashes were made in 1% acetocarmine to study their chromosome associations at metaphase I and chromosome distribution at early anaphase I. A minimum of 50 metaphase I/anaphase I cells were scored to determine their chromosome pairing and assortment behaviour.

Four of the synthetic amphiploids (*Durococcum* I–IV) synthesized in 1983–1984, along with their four durum wheat parents, *T. monococcum* and a relatively high-yielding, well adapted bread wheat (cv WL 711) as the check were grown at Gurdaspur and Ludhiana in a randomized block design with three replications at each location in 1984–1985. A border row of a universal rust susceptible variety Agra Local was planted on either side of each replication and was also used as an infector row. Due to limited seed of the newly synthesized amphiploids, each plot consisted of one 1.5 m row with 10 plants spaced 15 cm apart. The observations were recorded from five competitive plants per plot on plant height, number of tillers per plant, days to 50% flowering, 100-grain weight, grain yield per plant, threshability and percent of seed protein content. The incidence of Karnal bunt was recorded in a separate replicated trial at Ludhiana after artificial inoculation at the boot stage following the method of Aujla et al. (1982). Karnal bunt infected and healthy seeds were counted from the inoculated spikes and the incidence of Karnal bunt was expressed as the percentage of diseased seed. The percentages of protein and Karnal bunt infection were converted by angular transformation before analysis of variance. The data on yellow rust incidence was recorded on line basis as per modified Cobb's scale after creating artificial epiphytotic conditions by spraying on the infector rows inoculum containing predominant races of yellow rust viz; 13, 14, 14A, 20A, 38, 38A, A, G and K.

Results and discussion

No cross incompatability was observed in any of the cross combinations involving tetraploid and diploid wheats. The F_1 hybrid seeds were viable in both directions whether the female parent was tetraploid wheat or diploid wheat. All the F_1 hybrids, however, were sterile. The parents, *T. monococcum* and tetraploid wheats showed regular meiosis with 7 and 14 bivalents, respectively.

The data of meiotic metaphase chromosome pairing of six triploid hybrids originating from *T. durum* × *T. monococcum* is given in Table 1. The F_1 hybrids ($2n=3x=21$) showed univalents and bivalents without multivalent formation. The number of univalents ranged from 7–13 and bivalents from 4–7 per PMC in different hybrids. In the present study, the mean number of univalents ranged from 7.40–8.68 while the mean bivalent frequency varied from 6.16–6.80 per cell. There was, however, a higher frequency of rod bivalents than ring bivalents in the triploid hybrids, indicating that the A genome of the tetraploid wheat may not be completely homologous with the A^m genome of *T. monococcum*. Driscoll et al. (1979) observed 9.90+5.55 II with no multivalents in F_1 hybrid of *T. turgidum* × *T. monococcum*. Johnson and Dhaliwal (1978) and Vardi and Zohary (1967) also reported an average of 9.0 I+5.7 II+0.19 III in *T. dicoccum* × *T. boeoticum* and 9.88 I+5.22 II+0.16 III in *T. durum* × *T. boeoticum* triploid hybrids, respectively.

Only 15 out of 20 triploid hybrids, including reciprocals, were treated with colchicine and amphiploidized heads or sectors were obtained in only 9 hybrids involving durum cultivars as the female parents with *T. monococcum* as the male. No amphiploid head or sector was obtained in the triploid hybrids where *T. monococcum* was the female parent, which might be due to the difference in the cytoplasm of *T. monococcum* and *T. durum*. The cytoplasm of the tetraploid wheat is contributed by the B genome donor and not by the A genome donor (Maan and Lucken 1970; Suemoto 1973). It is also known that *T. monococcum*, like *T. timopheevi* and *T. zhukovskyi*, possesses cytoplasm that induces male sterility when *T. aestivum* nucleus is substituted into its cytoplasm. It is possible that the doubled sectors in the triploid hybrids with *T. monococcum* as female parents remained sterile like the triploid hybrids due to cytoplasmic male sterility induced by monococcum cytoplasm, and could not be recovered.

Table 1. Mean and range (in *parenthesis*) of various meiotic metaphase configurations in *T. durum* × *T. monococcum* hybrids ($2n=3x=21$)

Triploid bybrid	No. of cells	Configuration per cell			
		I	II		
			Ring	Rod	Total
Produra × *T. monococcum*	50	8.56 (7–13)	2.78	3.44	6.22 (4–7)
Mexi. C75 × *T. monococcum*	50	8.68 (7–13)	2.80	3.36	6.16 (4–7)
Yavaros 79 × *T. monococcum*	50	7.52 (7–9)	3.40	3.55	6.75 (6–7)
Boy 's' × *T. monococcum*	50	7.40 (7–11)	2.70	4.10	6.80 (5–7)
Cocorit × *T. monococcum*	49	8.47 (7–13)	2.78	3.49	6.27 (4–7)
DWL 5031 × *T. monococcum*	50	8.24 (7–11)	3.12	3.26	6.38 (6–7)

Table 2. Diakinesis/metaphase I chromosome association in *T. durum* × *T. monococcum* amphiploids

Amphiploid ($2n=6x=42$)	No. of cells		Frequency of I	II Ring	II Rod	II Total	III	IV	1IV+19IIs Ring	1IV+19IIs Chain	2IV+17IIs 2 rings	2IV+17IIs 1 ring +1 chain	2IV+17IIs 2 chains	3IV+15IIs 3 rings	3IV+15IIs 2 rings +1 chain	Total no. of cells
x *Durococcum* I (PCD 57 × *T. mono.*)	100	Mean	0.48	11.28	8.01	19.29	0.30	0.51	53.66	13.01	19.51	8.94	0.81	4.07	0.0	123
		Range	0–6	–	–	15–21	0–2	0–3								
x *Durococcum* II (DWL 5031 × *T. mono.*)	100	Mean	0.92	10.47	8.53	19.00	0.06	0.63	62.01	9.10	16.00	9.00	0.60	2.20	1.10	179
		Range	0–5	–	–	15–21	0–1	0–3								
x *Durococcum* III (WC 6001 × *T. mono.*)	100	Mean	0.47	10.67	8.85	19.52	0.07	0.57	36.36	9.09	24.24	14.14	6.56	5.05	4.04	198
		Range	0–6	–	–	15–21	0–1	0–3								
x *Durococcum* IV (Boy's × *T. mono.*)	99	Mean	0.33	6.36	13.20	19.56	0.13	0.54	53.78	21.97	11.36	8.33	3.03	1.52	0.00	132
		Range	0–5	–	–	15–21	0–1	0–3								

Nine amphiploids were synthesized and named as ×*Durococcum* I – ×*Durococcum* IX. These amphiploids involved nine different *T. durum* cultivars viz. PCD 57, DWL 5031, WC 6001, Boy'S', Mexi. C75, Produra, PBW 34, PBW 117 and Yavaros 79, respectively, and one spring growth habit type *T. monococcum.* Five amphiploids, *Durococcum* V–IX, were synthesized in 1984–1985. No diploidized sector could be obtained from the triploid hybrid of Cocorit × *T. monococcum.* The results of chromosome association of four *T. durum* × *T. monococcum* amphiploids (×*Durococcum* I–IV) are given in Table 2. The amphiploids had a high frequency of bivalents and a lower frequency of univalents and multivalents (including trivalents and quadrivalents). On an average, 15–21 bivalents per cell were recorded in these amphiploids with mean ranging from 19.00 in ×*Durococcum* II to 19.56 in ×*Durococcum* IV. A maximum of three quadrivalents per cell was observed. In all the amphiploids the PMC's with 19 II + 1 IV were most frequent followed by PMC's with 17 II + 2 IV. The PMC's with 15 II + 3 IV had a very low frequency.

On the basis of chromosome pairing in triploid hybrids, a high frequency of PMC's with up to six quadrivalents would be expected in the amphiploids. High preferential diploid pairing in the synthetic amphiploids ($AABBA^mA^m$) with two A genomes strongly suggests that the A genome of *T. durum* had significantly diverged from the A^m genome of *T. monococcum.* A low frequency of quadrivalents in the synthetic amphiploids *T. dicoccoides* – *T. boeoticum* (Bell 1950; Bell and Sachs 1953), *T. araraticum* – *T. boeoticum* (Dhaliwal and Johnson 1976; Driscoll et al. 1979) and *T. zhukovskyi* (Upadhya and Swaminathan 1963) with two A genomes strengthens the view that the A genome of tetraploids probably diverged following its incorporation in the tetraploids due to the buffering effect of the other genomes in the polyploids while the A genome of the diploids stayed relatively unmodified.

The data on chromosome distribution in these amphiploids (Table 3) showed that in spite of some quadrivalents a majority of the cells had 21–21 chromosome assortment at anaphase I and as a result the amphiploids had balanced gametes and a high seed set. However, in some of the PMC's there were 1–2 laggard univalents. On an average, the amphiploids had 95.2% fertility as compared to 90.8% and 95.6% seed set in *T. monococcum* and *T. durum,* respectively the triploid being completely sterile.

The mean observations on four amphiploids for various morphological traits, yield components, grain protein content and incidence of yellow rust and Karnal bunt along with their parents and a high-yielding, well adapted *T. aestivum* cv WL 711 at Gurdaspur and Ludhiana are given in Table 4. The amphi-

Table 3. Chromosome distribution at anaphase I in amphiploids. Laggard chromosomes are in *parenthesis*

Amphiploid	Frequency (%) of PMC's with different chromosome distribution							
	21–21 (0)	20–22 (0)	20–1–21 (1)	20–2–20 (2)	19–2–21 (2)	19–3–20/ more (3)	19–1–22 (1)	Total no. of PMC's –analysed
x *Durococcum* I (PCD 57 × *T. mono.*)	60.86	14.13	17.39	2.17	5.43	0.0	0.0	92
x *Durococcum* II (DWL 5031 × *T. mono.*)	71.82	6.36	10.00	9.09	2.73	0.0	0.0	110
x *Durococcum* III (WC 6001 × *T. mono.*)	63.27	10.88	14.97	8.16	1.36	1.36	0.0	147
x *Durococcum* IV (Boys's' × *T. mono.*)	47.12	5.24	20.94	17.80	3.14	5.24	0.52	191

Table 4. Mean values of different characters over two environments of diploid, tetraploids parents and four synthetic amphiploids. F = free, H = hard

Variety/ amphiploid	Plant ht. (cm)	Tiller no.	Days to 50% flowering	No. of spikelets	100-grain wt. (g)	Grain yield/ plant (g)	Grain protein (%)	Incidence		Threshability
								Yellow rust	Karnal bunt	
T. monococcum	102.6	29.3	103	23.4	2.42	17.9	12.2	0	0.0	H
T. durum										
cv PCD 57	91.9	15.7	102	21.2	4.36	33.9	14.6	5 S	32.7	F
cv DWL 5031	84.9	12.1	99	20.7	4.60	22.0	12.0	5 S	10.6	F
cv WC 6001	74.2	13.0	98	21.1	3.70	23.1	12.1	0	22.6	F
cv Boy's'	86.4	15.9	98	22.4	4.59	40.8	11.8	5 S	11.5	F
x *Durocccum* I (PCD 57 × *T. mono.*)	110.4	17.3	117	25.0	5.25	21.0	13.2	0	0.0	H
x *Durococcum* II (DWL 503 × *T. mono.*)	112.0	14.0	119	26.6	4.40	21.9	14.2	5 MR	0.0	H
x *Durococcum* III (WC 6001 × *T. mono.*)	97.3	12.7	125	25.4	4.59	12.8	16.4	5 MR	0.0	H
x *Durococcum* IV (Boy's' × *T. mono.*)	120.1	17.0	109	27.3	5.25	31.8	15.1	10 MR	0.0	H
T. aestivum										
cv WL 711	102.8	15.9	94	20.5	3.86	26.3	12.2	100 S	45.5	F
C. D. at 0.05	3.68	3.67	–	1.03	0.13	8.99	2.30	–	4.23	–
0.01	4.93	4.92	–	1.39	0.18	12.06	3.08	–	7.74	–

ploids ×*Durococcum* I, II and IV were significantly taller than *T. monococcum* and their respective *T. durum* parents. The amphiploid had a numerically higher tiller number than the respective durum parents but a significantly lower tiller number than *T. monococcum*. The *T. monococcum* parent contributed this character to the amphiploids. All the amphiploids flowered later than both parents. All the amphiploids had a significantly higher number of spikelets per spike than the parents and *T. aestivum* check. *T. durum* is known to have high 100-grain weight whereas *T. monococcum* has very low 100-grain weight because of small and flattened grains. The amphiploids, however, had very bold and long grains as compared with their respective durum parents. Three amphiploids (×*Durococcum* I, III and IV) had significantly higher 100-grain weight than their respective parents and cv WL 711. For grain yield, some of the amphiploids were comparable to certain *T. durum* lines and cv WL 711. ×*Durococcum* IV gave the highest mean yield and also had the highest mean 100-grain weight among all the other amphiploids, *monococcum* and cv WL 711 studied in two environments. Two amphiploids (×*Durococcum* III and IV) had a significantly higher grain protein content than all the lines evaluated at 5% level of significance, although their durum parents had a lower protein content than the other durum parents.

The amphiploids had a very low incidence of yellow rust and the reaction was mostly of the resistant type. Their resistance to rust is attributed to the higher level of resistance of *T. monococcum* (Dhaliwal and Gill 1982). All the amphiploids and *T. monococcum* were completely free from Karnal bunt incidence, whereas it ranged from 10.6%–32.7% in tetraploid wheats and 45.7% in bread wheat cv WL 711. *T. monococcum,* therefore, possesses dominant genetic resistance to Karnal bunt.

The comparison of amphiploids and their parents indicates that the amphiploids showed hybrid vigour for plant height, number of tillers per plant, 100-grain weight and protein content, which can be permanently fixed in amphiploids and exploited in the form of a new manmade cereal. The basis of hybrid vigour of the amphiploids for most of the character could be attributed to the additive and over-dominance effects of different genes of the A^m genome of *T. monococcum* from those of the A genome of durum wheat.

The amphiploids have certain defects such as excessive height, late flowering and maturity and hard threshing attributed here to *T. monococcum.* These defects should be overcome before they can be exploited commercially. The chromosome 5A of *T. monococcum* carrying genes for hard threshing (Mac Key 1954) and late flowering can be substituted by corresponding homoeologous chromosomes of D or R genomes by crossing amphiploids with *T. aestivum* or triticale, respectively. A limited substitution of the D and R genome chromosomes for the corresponding A^m chromosomes might also help in eliminating the limited multivalent formation in the amphiploids for better stability and diploid meiotic pairing. However, raising a large amphiploid population may also be helpful to select the free threshing form of an amphiploid which might occur due to multivalent pairing among the four AAA^mA^m genome chromosomes carrying the gene for threshability.

Triticum monococcum var. *sinskajae* possesses free threshing (Waines 1983) and newly induced free threshing mutants of *T. monococcum* (H. S. Dhaliwal, personal communication) are also available. Free threshing lines of *T. monococcum* should be utilized for the synthesis of amphiploids. Several accessions of *T. boeoticum* that are known to have high protein content and two functional florets per spike instead of one in *T. monococcum* are available in the germplasm collections. The new amphiploids involving such accessions might be more vigorous for yield components and possess still higher protein content than the ones synthesized and evaluated here.

References

Aujla SS, Grewal AS, Gill KS, Sharma I (1982) Artificial creation of Karnal bunt disease of wheat. Cereal Res Commun 10:171–176

Bell GDH (1950) Investigations in the *Triticinae.* I. Colchicine techniques for chromosome doubling in interspecific and intergeneric hybridization. J Agric Sci 40:9–19

Bell GDH, Sachs L (1953) Investigations in *Triticinae.* II. The cytology and fertility of intergeneric and interspecific hybrids and their derived amphiploids. J Agric Sci 43: 105–115

Dhaliwal HS, Johnson BL (1976) Origin of *Triticum zhukovskyi.* Wheat Inf Serv 41–42:33–35

Dhaliwal HS, Gill KS (1982) Screening and utilization of wild-wheat germplasm for rust resistance. Wheat Inf Serv 54:39–42

Driscoll CJ, Bielig LM, Darvey NL (1979) Analysis of frequencies of chromosome configurations in wheat and wheat hybrids. Genetics 91:755–767

Gill BS, Broder LE, Hatchett JH, Harvey TL, Martin TJ, Raupp WJ, Sharma HC, Waines JG (1983) Disease and insect resistance in wild wheats. Proc 6th Int Wheat Genet Symp. Kyoto, Japan pp 785–792

Johnson BL, Dhaliwal HS (1978) *Triticum urartu* and genome evolution in the tetraploid wheats. Am J Bot 65:907–918

Mac Key J (1954) Neutron and X-ray experiments in wheat and a revision of the speltoid problem. Hereditas 40:65

Maan SS, Lucken KA (1970) Inheritance of *T. boeoticum* cytoplasm and genomes of *T. aestivum* and *T. durum.* Restoration of male fertility and plant vigour. Euphytica 19:498–508

Sharma HC, Waines JG, Forster KW (1981) Variability in primitive and wild wheats for useful genetic characters. Crop Sci 21:555–559

Suemoto H (1973) The origin of cytoplasm of tetraploid wheats. In: Proc 4th Int Wheat Genet Symp. Columbia, Mo (USA), pp 109–113

Upadhya MD, Swaminathan MS (1963) Genome analysis of *T. zhukovskyi,* a new hexaploid wheat. Chromosoma 14: 589–600

Upadhya MD, Swaminathan MS (1965) Studies on the origin of *Triticum zhukovskyi* and the mechanism regulating chromosome pairing. Indian J Genet 25:1–13

Vallega V (1978) Useful genetic characters in diploid *Triticum* species. Proc 5th Int Wheat Genet Symp. New Delhi, India, pp 156–162

Vardi A, Zohari D (1967) Introgression in wheat via triploid hybrids. Heredity 22:541–560

Waines JG (1983) Genetic resources in diploid wheats: The case for diploid commerical wheats. Proc 6th Int Wheat Genet Symp. Kyoto, Japan, pp 115–122

Watanabe YK, Mukade K, Kokobun K (1956) Studies on the production of amphiploids as the source of resistance to leaf rust in wheats. II. Cytological studies on the F_1 hybrids and the amphiploids *T. timopheevi* × *T. monococcum* L. Jpn J Breed 6:23–31