Theor Appl Genet (1988) 76:93–96

The cytogenetics of a triploid *Hordeum bulbosum* and of some of its hybrid and trisomic derivatives

H. M. Thomas and R. A. Pickering *
Welsh Plant Breeding Station, Plas Gogerddan, Aberystwyth, SY23 3EB, UK

Received December 7, 1987; Accepted February 6, 1988
Communicated by G. Wenzel

Summary. The progeny from a cross between diploid *H. vulgare* and triploid *H. bulbosum* were mostly triploid (VBB) hybrids, the other progeny were haploid (V) barley (*H. vulgare*). From a cross between diploid and triploid *H. bulbosum,* four of the seven possible trisomic lines were isolated. The Giemsa banded karyotype of *H. bulbosum* was produced, and two of the lines were identified as trisomic for chromosomes 6 and 7. The cytology and transmission rates of the trisomics were examined.

Key words: *Hordeum bulbosum* triploid, trisomic, haploid barley

Introduction

H. bulbosum is a weed species widely distributed around the Mediterranean and into the Middle East, and exists in both diploid, $2n=2x=14$, and tetraploid forms, $2n=4x=28$ (Jørgensen 1982). Populations collected eastwards from Greece were all tetraploid while those from western Greece to Morocco were all diploid with the exception that one plant collected in Spain was triploid, $2n=3x=21$, and Jørgensen (loco citato) considered this plant to be an auto-triploid *H. bulbosum.*

In the present study, the triploid *H. bulbosum* described by Jørgensen (1982) was crossed with both diploid *H. bulbosum* and diploid *H. vulgare* and the different progeny types were recorded. The trisomic plants of *H. bulbosum* were studied further.

Materials and methods

The plant materials used were: Triploid *H. bulbosum* (Cb 3685) (kindly supplied by: N. Jacobsen, Botanical Institute, Royal Veterinary and Agricultural University, Copenhagen, Denmark), diploid *H. bulbosum* (Cb 2929/1), and *H. vulgare* cultivars Tyra, Vada, Emir.

Cb 3685 and Cb 2929/1 were allowed to cross naturally in isolation and seed harvested from both plants. The crosses between the *H. vulgare* cultivars and Cb 3685 were made by the method described by Thomas and Pickering (1983) and involved embryo culture. Cb 3685 was used as the male parent only. Trisomics of *H. bulbosum* recovered from the $3x-2x$ cross were used as female parents in crosses with Cb 2929/1 and the immature embryos were rescued and cultured (Thomas and Pickering, loco citato).

For mitotic chromosome counts root-tips were taken from seedlings germinated on moist filter paper or from vegetative tillers grown on a culture tank (Morgan 1976). After pretreatment in distilled water at 1 °C for 24 h or in an aqueous saturated solution of α-bromonaphthalene for 1½ h the root-tips were fixed in ethanol-acetic acid (3:1) and stained by the Feulgen method. Squashed preparations were made in 1% aceto-carmine.

For Giemsa banding, root-tips were pre-treated and fixed as before, then hydrolysed in 1N HCl for 1 min and squashed in 45% acetic acid. Preparations were scanned under phase contrast and well spread cells photographed. Each good preparation was then frozen in liquid nitrogen and the cover slip removed. The preparation almost invariably adhered to the slide and this was stored in absolute alcohol for 1 wk.

The slides were air-dried and placed in a saturated solution of Barium hydroxide for 30 min at room temperature, rinsed in several changes of tap water and placed in 2 × SSC at room temperature for 5 min, and then transferred in 2 × SSC at 60 °C for 45 min. After a brief rinse, the slides were stained in 5% Giemsa solution (Gurr's R66 improved) in Sorensen's buffer at pH 6.9 for between 10 min and 2 h, and monitored frequently under the microscope until the right intensity of staining was obtained.

For meiotic analyses, inflorescences were taken before emergence, fixed in Carnoy's solution (6:3:1) and anthers squashed in 1% aceto-carmine.

---

* Present address: Crop Research Division, Lincoln, DSIR, Private Bag, Christchurch, New Zealand

Results

The meiosis of the triploid *H. bulbosum* (Cb 3685) was analysed in 50 PMCs at metaphase I and the mean chromosome pairing per cell was: 4.78 trivalents, 1.84 ring bivalents, 0.32 rod bivalents and 2.28 univalents. These figures tested according to the method described by Alonso and Kimber (1981) confirmed that the three genomes were nearly identical, and that the plant therefore was an auto-triploid.

The progeny of crosses between *H. bulbosum* (3x) as the pollen parent and three varieties of *H. vulgare* (2x) were either chromosomally unstable triploid hybrids (VBB) or haploid barley (V) (Table 1); no diploid hybrids were recovered. The progenies from the reciprocal crosses between triploid *H. bulbosum* and the diploid *H. bulbosum,* Cb 2929/1, are presented in Table 2. Six trisomic plants (2n + 1) were found plus one plant with 16 chromosomes.

The six trisomic plants were designated Tris A to Tris F (Table 3). Morphologically, three resembled diploid *H. bulbosum,* while a fourth was equally vigorous but the leaves were darker in colour with a blue-green hue. The other two trisomics, Tris D and Tris E were weak, dwarf, and low tillering, Tris E more so than Tris D. The 16 chromosome plant was also weak and dwarf, but high tillering.

The Giemsa banded karyotype of diploid *H. bulbosum* is presented in Fig. 1; it is based on a composite of photographs of cells of the diploid *H. bulbosum* Cb 2929/1. Although an attempt has been made to use the same order of numbering of chromosomes as used by Vosa (1976), as the two karyotypes differ somewhat in arm ratios and banding patterns, one cannot with certainty relate one to the other. Intercalary bands were stained in the long arm of at least one of the larger chromosomes but these were not found with any degree of consistency and have not been included in the karyotype.

By comparison with this karyotype the extra chromosomes of Tris A and Tris B have been positively identified as chromosome 6 (Fig. 2a) while Tris C, because few cells have been successfully stained, has been only tentatively identified as trisomic for chromosome 6. Tris D is trisomic for the satellite chromosome, i.e. chromosome 7 (Fig. 2b). The trisome of the remaining two plants has not been identified, though Tris F is trisomic for either chromosome 1 or 2 but it has not been possible to determine which. Where the trisome has been identified, the chromosome number has been appended to the designating letter (Table 3).

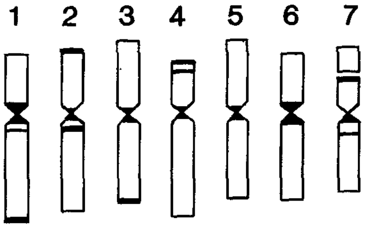

Fig. 1. The Giemsa banded karyotype of *H. bulbosum* Cb 2929/1

Table 1. Progeny of the triploid *H. bulbosum* (Cb 3685) when crossed with three diploid varieties of *H. vulgare*

H. vulgare variety (♀)	No. of plants	
	3× (hybrid)	1× (haploid)
Tyra	8	1
Vada	5	0
Emir	5	4
Totals	18	5

Table 2. Progeny from reciprocal crosses between *H. bulbosum* (3x) and *H. bulbosum* (2x)

No. of chromosomes	3x (♀) × 2x (♂)		2x (♀) × 3x (♂)	
	No. of plants	(%)	No. of plants	(%)
2n	3	33.3	26	96.3
2n+1	5	55.6	1	3.7
2n+2	1	11.1	0	–
Totals	9	100.0	27	100.0

Table 3. Chromosome pairing in 6 primary trisomic plants of *H. bulbosum*

Trisomic	Type	No. of cells			
		1III+6II	7II+1I	6II+3I	xta/cell
A (6)	Vigorous	28	22	0	13.80
B (6)	Vigorous	17	32	1	13.54
C (6?)	Vigorous	22	26	2	13.54
D (7)	Weak	33	17	0	13.82
E	Weak	18	32	0	13.28
F	Blue-green	23[a]	21	6	13.50

[a] Includes 1 cell with 1III + 5II + 2I

Chromosome pairing at metaphase I was scored in 50 PMCs from each of the six trisomic plants (Table 3). The mean chiasma frequency per cell of 13.28 recorded for Tris E was significantly lower ($P < 0.01$) than the chiasma frequency of the other trisomic plants; they did not differ significantly from each other.

In the 300 PMCs analysed the trisomic chromosomes paired as a trivalent in 47% of cells, as a bivalent and univalent in 50% of cells and remained unpaired as three

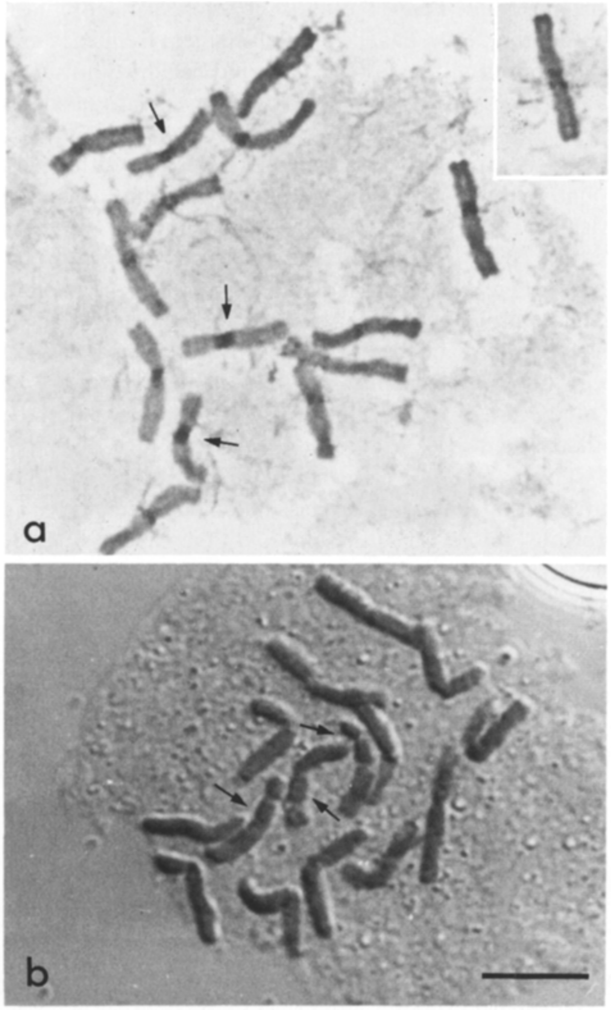

Fig. 2. a Tris A (arrows indicate chromosomes 6) — Giemsa; b Tris D (arrows indicate chromosomes 7) — Feulgen; bar represents 10 μm

univalents in 3% of cells. The last type were found mostly in Tris F (Table 3). A χ^2 test showed a significant difference ($P < 0.02$) between the trivalent frequencies of the six plants.

The six trisomic plants were crossed with diploid *H. bulbosum*, and the number of euploid and trisomic types recovered are shown in Table 4; no seed set was obtained in the cross with Tris E. The transmission rates through the female side of the extra chromosome did not differ significantly among the five plants tested.

Table 4. Contingency table of transmission rate of the trisome through the female side in five trisomic plants pollinated by diploid *H. bulbosum*, Cb 2929/1; $\chi^2 = 0.517$; df = 4; N.S.

Trisomic ♀	Frequency of progeny types		
	2n	2n+1	Total
A (6)	27	7	34
B (6)	23	7	30
C (6?)	38	8	46
D (7)	13	4	17
F	27	7	34
Totals	128	33	161

Discussion

Although triploids are theoretically capable of producing 2x gametes the rare occurrence of triploid progeny shows that 2x gametes have a very low functioning ability (Khush 1973). In the relatively small number of progeny produced by reciprocally crossing triploid *H. bulbosum* with diploid *H. bulbosum*, 2n + 2 was the highest chromosome number found. According to Khush (1973) higher chromosome numbers are not recovered because gametophytes and zygotes of most species with higher chromosome numbers cannot survive because of imbalance caused by the extra chromosomes.

The progeny of the crosses between diploid *H. vulgare* and triploid *H. bulbosum* are therefore especially interesting. Here 80% of the progeny arose from fertilization by 2x *bulbosum* pollen, though the 2x pollen must have represented only a very small proportion of the pollen produced. To have found as many VBB hybrids in *H. vulgare* (2x) × *H. bulbosum* (3x) suggests that the few 2x pollen produced had some selective advantage over haploid and aneuploid pollen. Differences between rates of growth of x and 2x pollen tubes have been found in other plants, e.g. in *Solanum tuberosum* 2x pollen grew more quickly than x pollen (Breukelen 1982). If 2x *bulbosum* pollen grows faster than x *bulbosum* pollen on *vulgare* styles then this could explain why so many fertilizations were by 2x pollen in this experiment when it represented a small proportion of the pollen available.

The high proportion of triploid hybrid progeny may also be a reflection of the relative viability of these two types of embryo. The superior viability of VBB embryos over V embryos is amply illustrated by the data of Lange (1971): from the cross VV × BB 450 embryos were cultured from 487 spikes pollinated, and only 3 of these embryos grew into plants. From the cross VV × BBBB 801 embryos were cultured from just 391 spikes pollinated and 146 of these embryos developed into plants. It is likely, therefore, that the large number of 3x (VBB) progeny reflects the relative viability of VBB and V embryos.

The two types of progeny in this interspecific cross demonstrates the effect of genome balance in *H. vulgare* × *H. bulbosum* crosses. Lange (1971), and Kasha and Sadasiviah (1971) found that *bulbosum* chromosomes were not eliminated from the combination of two genomes of *bulbosum* and one of *vulgare*. In the present interspecific cross when 2x *bulbosum* pollen was involved in fertilization, triploid hybrids were produced with the genomic constitution VBB. When x *bulbosum* pollen was involved the *bulbosum* chromosomes were eliminated during subsequent embryogenesis thereby producing *H. vulgare* haploids.

No interspecific hybrid progeny was recorded containing an aneuploid set of *bulbosum* chromosomes. If a n + 1 gamete was involved in any of the fertilizations of *H. vulgare* then either the zygote was inviable or all 8 *H. bulbosum* chromosomes were eliminated to produce a *vulgare* haploid. If the gene(s) responsible for the genome balance effect seen in VBB hybrids are on one chromosome then the n + 1 *bulbosum* gamete carrying the extra dose of that chromosome should produce a viable, chromosomally stable zygote with the constitution VB + 1B. A larger experiment may have recovered such a combination.

The number of trisomic plants recovered from the cross between the triploid *H. bulbosum* and diploid *H. bulbosum* corresponds to that found by Tsuchiya (1963) from the cross between 3x and 2x *H. spontaneum* v. *transcaspicum*. It appears that three of the six trisomic plants are trisomic for the same chromosome, namely chromosome 6. The transmission of this trisome through the female side is obviously disproportionately high: it featured in 2 out of 5 trisomic plants recovered, and it so happens that it is the one trisomic found when the triploid was used as the male parent. It is not unusual for one trisomic to be more prevalent than others. On selfing a triploid *H. spontaneum* the trisomic 'Pale' represented 28% of the trisomics recovered while only 8.69% were of the 'Pseudonormal' type (Tsuchiya, loco citato).

In the trisomics of tomato, Khush (1973) found that the female transmission rate of long extra chromosomes was lower than that for short extra chromosomes. His interpretation of this was that long chromosomes caused greater imbalance in the gametophyte and this was as a result less likely to function. The extra chromosome in Tris A, Tris B, and probably Tris C is one of the smallest, based on measurements at mitosis. This may have been responsible for the disproportionately high recovery of this chromosome from the triploid, that is, if chromosome size affects the fitness of either the aneuploid gametophyte or zygote in *H. bulbosum*.

Tsuchiya (1963) identified the 'Pale' and 'Pseudonormal' trisomics of *H. spontaneum* as trisomic for chromosomes 1 and 5 respectively, and these, according to Vosa (1976) are the longest and shortest chromosomes in the complement. In this case the longest chromosomes had the highest transmission rate. The longer chromosomes had the highest transmission rates in maize also (Einset 1943), this was because the longer chromosomes paired more frequently as trivalents than the shorter chromosomes. From the data of Tsuchiya (loco citato) we find that 'Pale' has the highest trivalent frequency and 'Pseudonormal' the lowest. It should be added however, that the pattern does not hold for all seven chromosomes or indeed for the 3x × 2x in the *H. spontaneum*.

In the present experiment there were no differences found in the transmission rates of the trisomes when the trisomic plants were crossed with a diploid *H. bulbosum* but the embryos from these crosses were artificially cultured whereas in the original cross between the triploid and the diploid, the progeny was recovered by seed. It may, therefore, be that if the progeny of the trisomics had been obtained conventionally the shorter chromosome would again have had a higher transmission rate than the other trisomes.

Tsuchiya (1967) describes a trisomic series in cultivated barley that could be classified by the same types as previously used for *H. spontaneum* (Tsuchiya 1963). All the trisomics from both series were viable; all had high female fertility and a 'fairly high' transmission rate. In the case of the four trisomic types studied here two types, namely 'Vigorous' and 'Blue-green' are very strong growing, the other two types are weak and in the case of Tris E no seed set has been obtained by pollination with a diploid and it has been difficult to maintain it by vegetative propagation.

References

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

Breukelen EWM von (1982) Competition between 2x and x pollen in styles of *Solanum tuberosum* determined by a quick in vivo method. Euphytica 31:585–590

Einset J (1943) Chromosome length in relation to transmission frequency of maize trisomes. Genetics 28:349–364

Jørgensen RB (1982) Biosystematics of *Hordeum bulbosum* L. Nord J Bot 2:421–434

Kasha KJ, Sadasiviah RS (1971) Genome relationships between *H. vulgare* L. and *H. bulbosum* L. Chromosoma 35:264–287

Khush GS (1973) Cytogenetics and aneuploids. Academic Press, New York London

Lange W (1971) Crosses between *Hordeum vulgare* L. and *H. bulbosum* L. I. Production, morphology and meiosis of hybrids, haploids and dihaploids. Euphytica 20:14–29

Morgan WG (1976) A technique for the production of polyploids in grasses. Euphytica 25:443–446

Thomas HM, Pickering RA (1983) Chromosome elimination in *Hordeum vulgare* × *H. bulbosum* hybrids. 1. Comparisons of stable and unstable amphidiploids. Theor Appl Genet 66:135–140

Tsuchiya T (1963) Chromosome trisomics and aneuploids. Barley Genetics I. Pudot, Wageningen, pp 116–150

Tsuchiya T (1967) The establishment of a trisomic series in a two-rowed cultivated variety of barley. Can J Genet Cytol 9:667–682

Vosa CG (1976) Chromosome banding patterns in cultivated and wild barleys (*Hordeum* spp.). Heredity 37:395–403