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The Karyotype of Tuleen 346 Barley

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Summary. Tuleen 346 barley is a triple homozygote for 3 unequal interchanges, T1-5v, T2-6y and T3-7d, which were induced independently in the variety, 'Bonus'. It has great potential value in studying differences in behaviour and position among chromosomes within the same cell in barley and its hybrids since at least 5 and usually all 7 chromosome types can be identified in Feulgen preparations, compared with only 3 types in normal barley. Measurement of chromosome arm and satellite lengths in 10 haploid root-tip metaphases showed that in all comparisons except of the longest with the next longest, the 7 chromosome types were distinct in total length ($P < 0.001$–0.05) and in 8 of the 10 cells, the longest chromosome had a smaller arm ratio than the next longest. In these preparations, each of the 5 shortest chromosomes was easily identifiable by size and morphology. The longest chromosome was about 2.2 times as long as the shortest. C-banding showed that each of the 7 chromosome types had a clearly unique band pattern. The C-band patterns of interchange chromosomes confirmed and increased the precision of previously published breakpoint locations, viz. the short arms of chromosomes 1, 2, 3 and 7 and the long arms of chromosomes 5 and 6.

Key words: Barley – Interchange – C-banding

Introduction

In much cytogenetical work, it is essential to identify individual chromosomes within a cell. This is particularly important in certain barley hybrids where chromosomes from one species may be gradually eliminated at mitosis (Subrahmanyam and Kasha 1973; Bennett et al. 1976) or take up different positions in the cell from those of the other species (Finch et al. 1981). Even in non-hybrid barley cells, identification of individual chromosomes has proved essential for such purposes as the production of trisomic sets (Tsuchiya 1967) and it has been of great assistance in gene mapping (Tuleen and Gardenhire 1974). However, identification of individual chromosomes in normal barley is often difficult since chromosomes 1 to 4 are similar in size and shape, and older studies have needed significant revision (Tuleen 1973; Künzel 1976; Noda and Kasha 1978). Future work will require more precise identification of chromosomes by cytological techniques and one simple way to achieve this is to use abnormal karyotypes with bigger differences among chromosome types (Nicoloff et al. 1979). An outstanding example of such a karyotype is a stock produced by Professor N. A. Tuleen, Texas A&M University Department of Soil and Crop Sciences, in which all seven chromosome types are individually identifiable owing to unequal interchanges involving six of them. This stock was briefly mentioned by Ramage (1975) but no name or detailed description is available. It was synthesized from three different interchange lines within the variety, 'Bonus', but we propose to call the triple homozygote Tuleen 346. The present paper provides details of its karyotype and seeks to establish that each chromosome can be identified individually with reasonable certainty in light microscope studies.

Materials and Methods

Genotypes

Tuleen 346 is a homozygote for 3 interchanges induced in the variety 'Bonus', namely T1-5v and T2-6y (Ramage 1975) and T3-7d (Ramage et al. 1961). According to A. Hagberg (pers. comm.), induction was by 9,300 dis neutron iradiation (see Mac Key 1951) in 1949 (T3-7d) and "acute" γ ray treatment (800 r) of plants at meiosis for 1 hour on 27 June 1961 (T2-6y) and 24 hours on 21 June 1962 (T1-5v). During 1970–1972, N. A. Tuleen extracted a double interchange homozygote from

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the F_2 of each of the crosses, T1-5v×T3-7d and T2-6y×T3-7d, crossed them together (using T1-5v+T3-7d as female) and found in their F_2 a plant homozygous for all three interchanges together (N. A. Tuleen pers. comm.). Prof. Tuleen kindly sent us seed from his F_3 plant number 346 from this F_2 triple homozygote. – Haploid Tuleen 346 was produced by pollinating the diploid with *Hordeum bulbosum* clone J1 (Simpson et al. 1980) and raising plants after embryo culture using methods similar to those described by Jensen (1975). *H. bulbosum* chromosomes are preferentially eliminated from hybrid cells early in seed development (Subrahmanyam and Kasha 1973).

Feulgen Microdensitometry

Tuleen 346 and control Sultan barley were germinated in the dark at 20 °C on moist filter paper in petri dishes. The tips of roots 1–2 cm long were excised, fixed for 2 h in Carnoy's fluid rinsed briefly in distilled water, hydrolysed in 1 mol/l HCl for 12 min at 60 °C, washed in distilled water for 1 min, stained in leuco-basic Fuchsin for 2 h at 20 °C, washed in SO_2 water for 10 min thrice and washed in distilled water for at least 5 min. Root-tips were then gently tapped flat in 45% v/v aqueous acetic acid under coverslips on slides and squashed, one tip per slide. Between excision and squashing, all roots of both varieties were treated together in the same vial. The DNA amounts of 10 mid-prophase nuclei from each of 5 roots from each variety were measured in arbitrary units using a Vickers M86 integrating microdensitometer. Three readings were taken from each nucleus and all 10 slides were prepared and scored in rapid sequence to maximise comparability.

Feulgen Karyotype Preparations

Haploid plantlets were taken from embryo culture into hydroponics. The solution, vessels and aeration routine were similar to those described by Finch et al. (1981) but cultures were kept for up to three weeks in the growth room as described (Finch et al. 1981) and in similar but less controlled conditions in the laboratory. Actively growing root-tips were excised, pretreated for 4 h in saturated, aqueous 1-bromo-naphthalene at 20 °C to accumulate metaphases, fixed for 1–12 h in Carnoy's fluid or Farmer's fluid, hydrolysed for 12 min in 1 mol/l HCl at 60 °C and stained for 2 h in leuco-basic Fuchsin. The meristem of each of 4 stained root-tips was gently teased out with a needle, mainly as single cells or few-celled clumps into a small drop of 45% acetic acid on a slide, one meristem per slide, and gently squashed under a coverslip. The meristem of a fifth stained root-tip was cut off and gently tapped flat and squashed in 45% acetic acid under a coverslip. Metaphase cells with a complete, undamaged chromosome set lying flat in focus were photographed and prints made at a final magnification of about ×2,000. Prints were a viewed at a magnification of ×10 and the lengths of chromosome arms (excluding primary and secondary constrictions) were measured to the nearest 0.4 mm with a ruler. The data in Figure 1 are means of measurements made on one cell in the fifth root and two or three cells in the other roots. The tips of diploid roots 1–2 cm long of seeds germinated as described for microdensitometry were pretreated, fixed and stained similarly to haploid roots and gently tapped flat under coverslips in a 1 : 1 mixture of 45% acetic acid and saturated propionic orcein. Metaphase chromosomes were then measured as described for haploid roots. – Each interchange chromosome was named after both chromosomes involved, with the chromosome contributing the centromere written first. Thus chromosome T1-5v and T5-1v from interchange T1-5v have the centromeres from chromosomes 1 and 5, respectively.

C-banding

Diploid roots were taken from germinating seeds pretreated as described earlier, fixed in Farmer's fluid for 24 h, hydrolysed for 1 h in 0.2 mol/l HCl at 20 °C and washed in distilled water. Meristem cells from each root-tip were gently teased out into a drop of 45% acetic acid on a slide, one meristem per slide, and squashed under a coverslip. The coverslip was removed after freezing in liquid N_2 and the slides were air dried for 1 h, put into ethanol for 24 h, air dried for 1 h, put into saturated aqueous $Ba(OH)_2$ in distilled water at 60 °C for 5.5 min, washed in water at 45 °C for 1 min twice, put into 2×SSC at 20 °C for 15 min and into fresh 2×SSC at 52 °C for 1.5 h, rinsed in distilled water at 20 °C, stained for about 1 h in 20% v/v Leishman stain in aqueous 0.067 mol/l $Na_2H\ PO_4$, rinsed in distilled water, air dried for 24 h, rinsed in xylene and mounted in Gurr's DPX. All solutions were made up immediately before use. – Eleven metaphases from 3 diploid roots were studied and C-band positions and maximum sizes in 3–6 chromosomes of each type were measured from photographs and mean positions inserted on the ideogram derived from Feulgen preparations of 10 haploid roots (Fig. 1). Measurements of C-band sizes were adjusted to bring the total length of non-telomeric C-bands to 6.88% of total genome length as in Bonus (Linde-Laursen 1978a, Fig. 2) while keeping relative C-band lengths in proportion to those measured in Tuleen 346.

Results and Discussion

DNA Amount

The means and standard deviations of total DNA measurements of 50 diploid mid-prophase nuclei each were 5.3 ± 0.3 arbitrary units in both Tuleen 346 and Sultan ($P > 0.99$). Therefore the $4C$ DNA amount of Tuleen 346 is the same as in Sultan, i.e. 22.2 pg (Bennett and Smith 1976), despite the changed karyotype.

Karyotype in Feulgen Preparations

Figure 1 gives mean chromosome lengths and arm ratios of Tuleen 346 measured in 10 haploid root-tip metaphases and Figs. 2 and 3 show haploid and diploid metaphases, respectively. In Tuleen 346, the longest chromosome is about 2.2 times as long as the shortest. In 'Bonus', the longest chromosome is about 1.3 times as long as the shortest (Linde-Laursen 1978a). Chi-squared tests showed the 10 haploid cells of Tuleen 346 to be homogeneous for the relative and absolute lengths and arm ratios of each chromosome type. Variance analysis of the lengths of the 7 chromosome types showed significant differences ($P < 0.05$) between

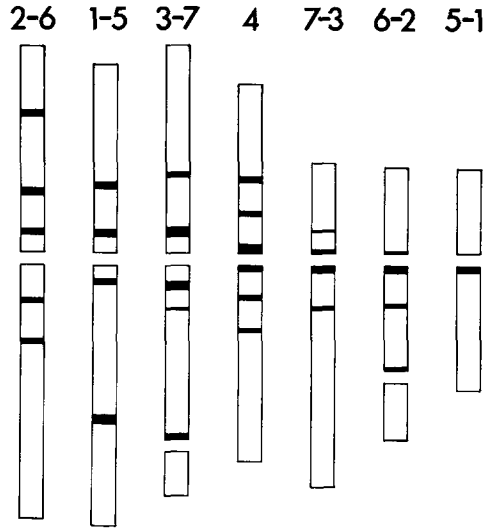
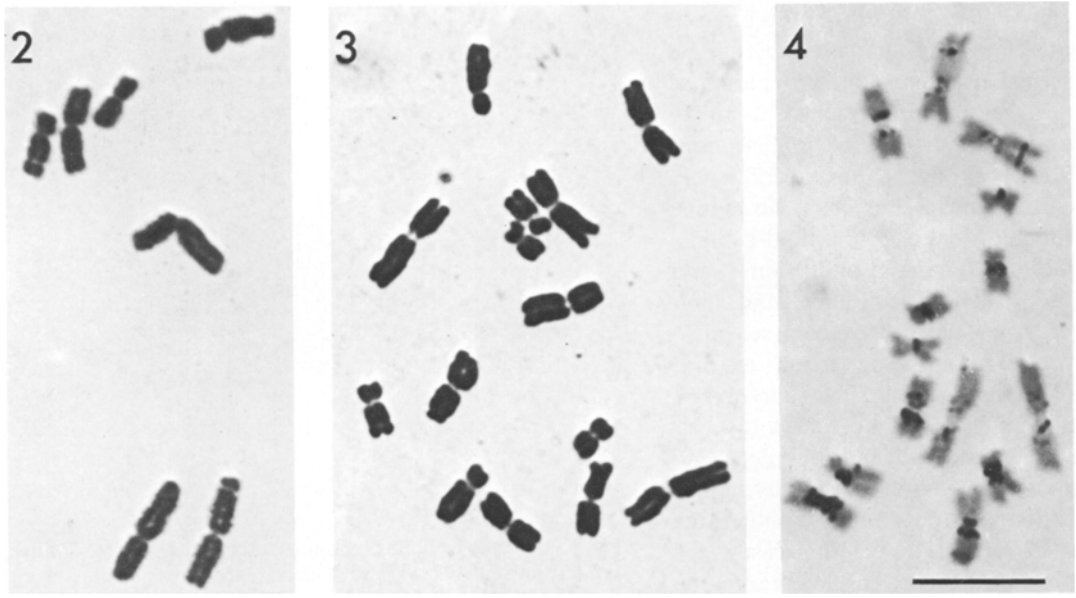


Fig. 1. Idiogram of Tuleen 346 based on 10 haploid metaphases, with C-bands found in 11 diploid metaphases. Mean length as percentage of total genome length (L%) and in μm (L μm) and arm ratio (AR) are given for each chromosome. Standard deviations are in brackets

means in all comparisons except chromosome T2-6y with chromosome T1-5v. Measurements of all chromosome arms in 5 metaphases from diploid roots gave very similar results to those in Fig. 1 ($P > 0.99$). The only difficulty in identifying chromosome types in both haploid and diploid metaphases is usually that chromosomes T2-6 and T1-5v are similar to each other. Rarely, as in one cell used for Fig. 1, chromosome T3-7d is longer than these two, but the satellite and low arm ratio usually identify chromosome T3-7d at once. As in normal barley, the satellite from chromosome 6 on chromosome T6-2y is usually more obvious than that from chromosome 7 on chromosome T3-7d. In haploid cells, confusion between chromosomes T2-6y and T1-5v is less than in diploid cells, since each chromosome is represented only once. Thus in 8 of 10 cells used for Fig. 1, the arm ratio of the longest chromosome was less than that of the next longest chromosome, but in 1 cell the reverse was true and in another cell, the longest two chromosomes were the same length but differed in arm ratio. In these 2 cells, chromosome T2-6y was identified by its lower arm ratio. In 2 cells used for Fig. 1 the satellite arm of chromosome T3-7d was the short arm. Had the satellite not been visible, the chromosome T3-7d arm ratio would have been estimated as 1.10 ± 0.08 instead of 1.05 ± 0.11. Probably, therefore, the arm ratio of chromosome 4, which is also nearly metacentric, is slightly overestimated and its standard diviation slightly underestimated, owing to similar but undetected reversal of relative arm lengths.



Figs. 2–4. Metaphase cells in root tips of Tuleen 346 stained in Feulgen (**2, 3**) or Leishman (**4**). **2** shows a haploid cell. **3** and **4** show diploid cells. Bar, 10 μm

Table 1. Relative lengths (% of whole genome) of corresponding chromosome segments in 'Bonus' and Tuleen 346

'Bonus' Chromosome segment	Tuleen 346 Chromosome segment	Length	
		'Bonus'	Tuleen 346
1 long arm	T1–5v short arm	7.50	7.69
2 long arm	T2–6y short arm	8.67	8.39
3 long arm	T3–7d short arm	8.67	8.41
4 short arm	4 short arm	5.95	6.86
4 long arm	4 long arm	7.89	7.92
5 short arm	T5–1v long arm	5.15	5.01
6 short arm (excl. sat.)	T6–2y long arm (excl. sat.)	4.40	4.22
6 satellite	T6–2y satellite	2.33	2.37
7 long arm	T7–3d long arm	9.18	8.93
7 satellite	T3–7d satellite	1.68	1.76
1+5 all arms	T1–5v+T5–1v	26.78	26.67
2+6 all arms	T2–6y+T6–2y	29.62	28.76
3+7 all arms	T3–7d+T7–3d	29.75	29.80

Table 1 shows that estimates of the relative lengths (% of whole genome) of Tuleen 346 chromosome segments not affected by interchange and of whole chromosomes summed in pairs from the same interchange agree very well ($P > 0.99$ in χ^2 test) with the corresponding relative lengths in 'Bonus' measured in Fig. 2 of Linde-Laursen (1978 a).

C-bands and Interchange Breakpoints

Figure 4 shows a C-banded metaphase and Fig. 5 shows the mean positions and relative sizes of the C-bands found at metaphase in 'Bonus' (Linde-Laursen 1978 a) and Tuleen 346. The complete absence of telomeric C-bands from Leishman-stained preparations of Tuleen 346 (Fig. 4) and their presence at all 14 telomeres in Giemsa-stained 'Bonus' preparations (Linde-Laursen 1978 a) is probably due to differences between the stains in their interactions with barley telomeric heterochromatin. In Fig. 5, the total genome length is drawn equal to 100 units in both 'Bonus' and Tuleen 346, and the C-bands of 'Bonus' and their presumed counterparts in Tuleen 346 are given arbitrary designations to facilitate comparison of arm lengths and C-band patterns in the two karyotypes. There is good agreement between the sizes and positions of the 29 non-telomeric C-bands of Tuleen 346 and the 30 non-telomeric C-bands of 'Bonus', given that Tuleen 346 was derived from 'Bonus' by the three interchanges, T1-5v, T2-6y and T3-7d. The reasons for the minor deviations of the Tuleen 346 karyotype found from that expected on this basis, such as the apparent loss of band 6e, are unknown, but may be due

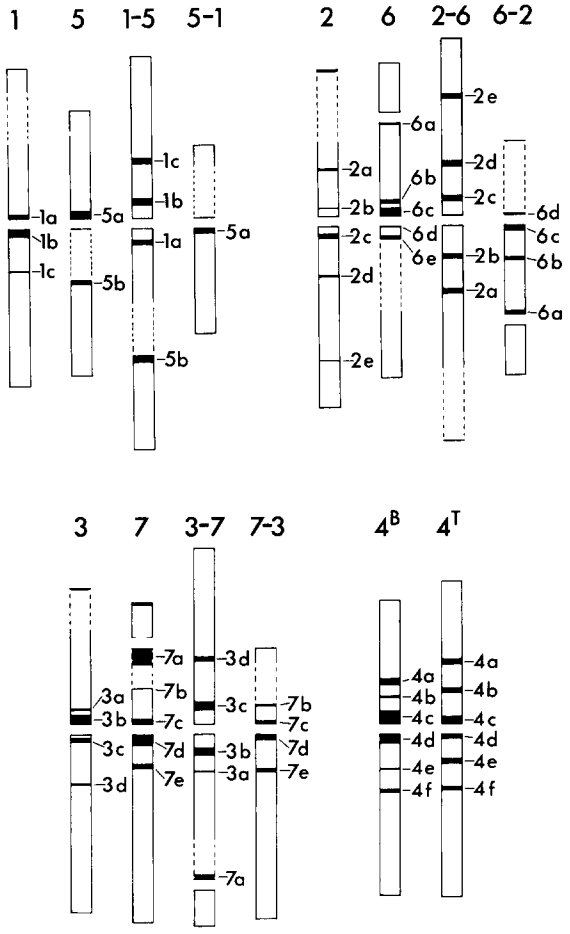


Fig. 5. C-bands in Bonus (chromosomes 1 to 3, 4^B, 5 to 7, after Linde-Laursen 1978a) and Tuleen 346 (chromosomes 1–5, 2–6, 3–7, 4^T, 5–1, 6–2, 7–3). Corresponding C-bands are given the same arbitrary number and letter. Broken lines show possibles sites of interchange breakpoints

either to the more contracted state of the Tuleen 346 chromosomes (Fig. 4) compared with those of 'Bonus' (Linde-Laursen 1978a, Fig. 1), or to differences in developing and measuring C-bands. Alternatively, band 6e may have been deleted by the irradiation treatment used to produce T2-6y.

Comparison of the C-band patterns of 'Bonus' and Tuleen 346 can be used to aid location of the breakpoints of T1-5v, T2-6y and T3-7d as follows.

T1-5v

Tuleen and Gardenhire (1974) deduced from karyotype studies in the cross of T1-5v with T1-7f that the T1-5v breakpoint in chromosome 1 is the arm which does not carry the br locus. The br locus maps on the short arm of chromosome 1 (Tsuchiya 1980) and is not carried by the telotrisomic designated 1L by Tsuchiya (1972) in which the only C-band is band 1a in Fig. 4 (Linde-Laursen 1978b). The data of Tuleen and Gardenhire (1974) therefore place the T1-5v breakpoint on chromosome 1 in the arm drawn above the centromere by Linde-Laursen (1978a, b) and in Fig. 4. This arm is referred to as the short arm of chromosome 1 in the present paper. Tuleen and Gardenhire (1974) located the T1-5v breakpoint in chromosome 5 in the long arm, the translocated segment of 5 being longer than that from chromosome 1. The arm lengths of chromosomes T1-5v and T5-1v, together with the unchanged position of band 1c relative to bands 1a and 1b and the new position of band 5b (Fig. 5), confirm that the breakpoints are in the short arm of chromosome 1 and the long arm of chromosome 5. Furthermore, as interchange separated band 5b from the chromosome 5 centromere 2.4 units away, the breakpoints are in the segments 2.4 units long shown on each chromosome in Fig. 5.

T2-6y

Tuleen (pers. comm. 1977) located the T2-6y breakpoint on the short arm of chromosome 2 and the long arm of chromosome 6, with a long piece of 6 exchanged for a short piece of 2. The arms lengths and unchanged band patterns (apart from the seeming loss of band 6e) of chromosomes T2-6y and T6-2y confirm this. Band 6e was unclear in Tuleen 346, but the band on the short arm of chromosome T6-2y may be bands 6d and 6e fused owing to the contracted state of the chromosomes studied. As the short arm of chromosome T6-2y is about 3.4 units long and the breakpoint may be anywhere on it, the breakpoints are in the segments 3.4 units long shown on each chromosome in Fig. 5.

T3-7d

Tuleen (1973) located the breakpoints of T3-7d on the short arm of chromosome 7 between the centromere and the satellite and probably on the short arm of chromosome 3. The arm lengths of chromosomes T3-7d and T7-3d, together with the unchanged relative positions of bands 3a-d and 7b-e and the new position of the satellite and band 7a on chromosome T3-7d confirm this (Fig. 5). Furthermore, as interchange separated the satellite from band 7b 1.9 units away, the breakpoints are in segments 1.9 units long shown on each chromosome in Fig. 5.

Interestingly, band 7a, which is easily identifiable by its position beside the smaller satellite, is much smaller in chromosome T3-7d than in 'Bonus' chromosome 7 (Figs. 4, 5). This band is also reduced in size in 'Pallas', an X-ray mutant of 'Bonus' (Linde-Laursen 1978a). If 'Bonus' had two karyotypes at the time 'Pallas' was induced, as suggested by Linde-Laursen (1978a), T3-7d may have been induced from the same variant line of 'Bonus' as 'Pallas' was. However, band 7a is in a relatively unstable segment (Nicoloff et al. 1979) and the variants may therefore have independent origins.

The Value of Tuleen 346

Tuleen 346 is a vigorous and stable line which is as fertile as normal barley. The identity of each chromosome is readily apparent in Feulgen stained preparations except for occasional confusion between chromosomes T2-6y and T1-5v which are easily distinguishable by C-banding. It has great potential value in studies of chromosome behaviour in both barley and its hybrids. Nicoloff et al. (1981) studied differences in response to mutagens among cytologically marked segments of barley chromosomes using 2 multiple interchange stocks similar to Tuleen 346. Also, in individual Feulgen stained metaphases and anaphases of Tuleen 346 × *H. bulbosum* hybrid embryos, where 5 of the 7 Tuleen 346 chromosomes are easily identifiable, it is often possible to observe that only *H. bulbosum* chromosomes are being eliminated (Finch unpublished). With normal barley × *H. bulbosum* hybrids, this is impossible to observe directly because only barley chromosomes 5 and 6 and chromosome 7 of both species can be identified (Kasha and Sadasivaiah 1971). Tuleen 346 thus increases precision in elimination studies in such hybrids.

Tuleen 346 is also very suitable for studies of chromosome position. Detailed relative chromosome positions have been elucidated under the light microscope in gently squashed preparations of *Crepis*

(Wagenaar 1969) and *Ornithogalum* (Ashley 1979) where there were only 3 chromosome types, each clearly identifiable by shape or C-band pattern, respectively. However, in many genotypes, chromosome types are not distinguishable in the light microscope without squashing enough to risk disturbing their relative in vivo positions. The study of serial sections in the electron microscope reveals relative in vivo chromosome positions but does not allow easy identification of chromosomes that are similar in size and shape such as chromosomes 1–4 of normal barley. Detailed relative in vivo positions of barley chromosomes have been observed by electron microscope studies of Tuleen 346, however (Bennett unpublished).

Thus in two important areas of current cytological enquiry, viz. differential behaviour and differential positioning among chromosomes within the same cell, Tuleen 346 offers an important gain in precision over normal stocks in a species already well favoured for cytogenetical research. Its use in these areas can therefore be expected to yield a greatly increased understanding of chromosome behaviour.

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