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JENS SANDFAER: High frequency of spontaneous triploids in barley

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Mostly from crosses between autotetraploids and diploids, various authors (KERBER 1954; TSUCHIYA 1958; DERENNE 1967; surveys by SMITH 1951 and NILAN 1964) have obtained triploid plants ($2n = 3x = 21$) of cultivated barley (*Hordeum vulgare* L.). The number of triploids derived from such crosses has been low.

DERENNE (1967) and TSUCHIYA (1960) have described the development and meiotic cytology of barley triploids which produce many aneuploids, the most frequent of which were primary trisomics ($2n = 2x + 1 = 15$). Triploids are thus valuable sources of trisomics, which are becoming increasingly useful in gene localization in barley. This paper describes a selection procedure that has led to a high yield of triploids in two barley varieties.

Materials and methods

In Svalöf Freja and Tystofte Prentice, both two-rowed spring varieties, seed samples of four subjectively defined size classes were selected by hand picking (Table 1) from unthreshed heads of material originating from one of our competition studies (SANDFAER 1968). The aim was a sample consisting of 200 seeds, but class 2 was so rare in Freja that only 37 seeds were obtained.

Root tips for chromosome counting were pretreated for 4 hours at room temperature in a saturated α-monobromonaphthalene solution, fixed in 99 parts of 30 per cent ethanol to 1 part of lactic acid at 0° C (JACOBSEN 1954), hydrolyzed for 8 min in 1 N HCl at 60° C, cooled, and washed in water. Softening was done by cellulase (30 mg/ml, Aspergillus niger, Light) for 30 min at room temperature, followed by rinsing in distilled water. The root tips were stained in basic fuchsin for 15 min and squashed in aceto-orcein. Permanent mounts were made by quick-freezing of the squash preparation by means of a CO_2 squash-slide freezer (JACOBSEN 1965) followed by mounting in Canada balsam.

Results and conclusions

The germination in size class 1, Table 1, was very low for both varieties, but in class 2 92.4 per cent of the seeds of T. Prentice and 48.6 per cent of those of Freja germinated. Barley seeds with a 1000-kernel weight of 10—12 g are very thin and shrivelled, and they can easily be classified as unviable. In a normal seed-cleaning procedure they will be discarded.

While the plants in classes 3 and 4 were vigorous and uniform, those in classes 1 and 2 varied in size and morphology. Some of the plants were of a small, slender type (Fig. 1). These plants were usually a few days later in germination and were in the initial growth stages characterized by narrow leaves, which gave them a slender appearance. Since they did not represent a clear-cut

Table 1. The germination percentage in different seed-size classes and the distribution of the plants in morphological sub-classes

Size class no.	1000-kernel weight, g	Number of seeds	Germination %	Number of plants	Sub-class[1]	Number of plants
T. Prentice						
					a	0
1	3.2	200	1.5	3	b	0
					c	3
					a	46
2	12.3	198	92.4	183	b	100
					c	37
					a	194
3	27.4	200	97.5	195	b	1
					c	0
					a	200
4	52.1	200	100.0	200	b	0
					c	0
Freja						
					a	0
1	3.5	173	0.6	1	b	1
					c	0
					a	6
2	10.5	37	48.6	18	b	4
					c	8
					a	198
3	28.2	200	100.0	200	b	2
					c	0
					a	199
4	54.5	200	100.0	200	b	1
					c	0

[1] See text

type, the plants were grouped into three sub-classes: *a* normal, *b* intermediate, and *c* small, slender plants.

The chromosome numbers were determined in a small number of the plants in classes 3 and 4; all plants in these classes were found to be diploid. All plants in classes 1 and 2 were analyzed cytologically; the results appear in Table 2. A very high frequency of triploids, namely 89 per cent in T. Prentice and 75 per cent in Freja, was found in the 2-c class of small, slender plants. In the intermediary class, 2-b, a lower, but still considerable percentage of triploids was found, whereas no triploids were found in class 2-a, consisting of plants classified as morphologically normal. The results clearly demonstrate that a great number of plants raised from seeds with a 1000-kernel weight of about 10 g are triploids in both varieties. In T. Prentice 51 per cent of all plants grown from seeds of size class 2 were triploids, and in Freja the percentage was 39. The results also show that within that class it is possible, by selection, according to plant height and morphology to separate a sub-class with an even higher frequency of triploids. The plants were grown to maturity, and all triploid and aneuploid plants were found to have a very high percentage of sterile flowers.

The frequency of seeds of the four size classes was determined for both varieties in material from the same plots as used in the experiment described. Size class 2, in which most of the triploids were found, constituted about 5 per cent of the T. Prentice seeds, whereas this size class was much rarer in Freja, viz. about 0.2 per cent. The frequency of triploids in the T. Prentice seed lot is estimated to be 0.05×0.92 (germination in class 2) $\times 0.51$ (triploids among germinated seeds) which equals 2.3 per cent, an the corresponding frequency was 0.04 per cent in Freja. It is obvious

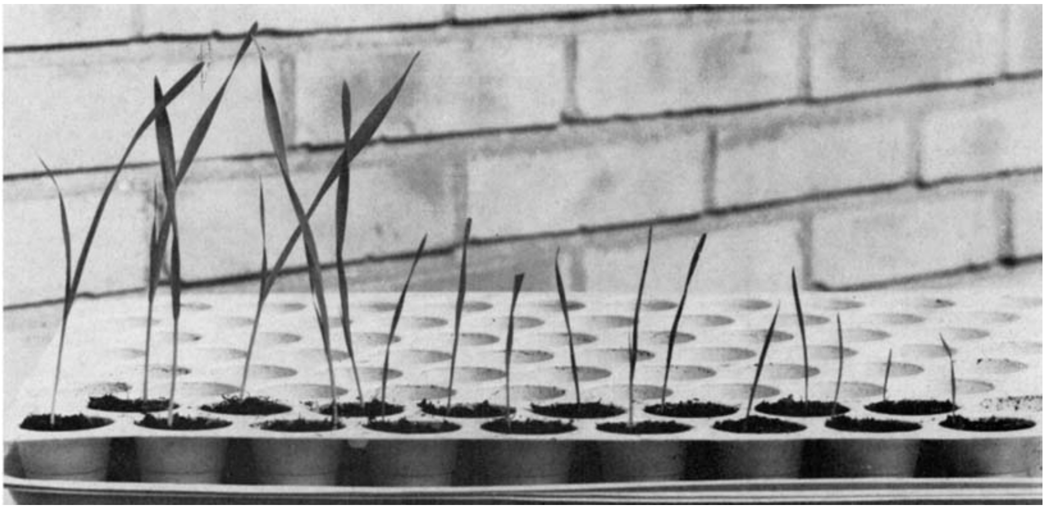

Fig. 1. From left to right: Normal, intermediate, and small slender plants.

that the frequency of triploids differed in the two seed lots.

To test the selection procedure suggested by the observations described, material of T. Prentice originating from the same plot as the material used in the experiment reported was threshed, as far as possible without discarding any of the light seeds by blowing. The seeds were then grouped in different seed-size classes on a seed cleaner in which an air stream fractionated the seeds according to specific gravity. Only two fractions were sown: Fraction I, containing 192 seeds with a 1000-kernel weight of 9.8 g, gave 156 plants; fraction II, containing 900 seeds with a 1000-kernel weight of 14.5 g, gave 840 plants. A strict morphological selection was carried out, yielding 40 and 70 plants of the small, slender type in fractions I and II, respectively. This sub-class constituted 26 per cent of the plants in fraction I and only 8 per cent in fraction II, which shows a clear decrease in the frequency of small, slender plants as a consequence of even a rather moderate increase in the 1000-kernel weight from 9.8 to 14.5 g. Out of the 110 small plants 105 were grown to maturity. Only two plants, presumably diploids, had normal fertility. The remaining 103 plants showed extensive flower sterility, and according to the cytological results already reported the majority of these plants were presumably triploids. Hence, in the material tested the method described permits the separation of a large number of triploids without any great effort.

The T. Prentice line used carries the barley stripe mosaic virus, BSMV (SANDFAER 1970). Freja is normally free from BSMV, but the material used in this experiment had for some years been grown on plots next to T. Prentice, and a low infection rate was found in Freja. Since BSMV increases the frequency of sterile flowers (INOUYE 1962; MCKINNEY and GREELEY 1965), it is possible that the triploids originated solely or primarily from virus-induced, so-called sterile flowers. This question cannot yet be finally settled. However, six triploids have been found in another sample of Freja and a virus-free line of T. Prentice has yielded four triploids. In these cases it was possible to trace the triploids back to their mother-plants. The triploids as well as about 50 other seeds from each mother plant were virus tested, and no virus infection was revealed. Four triploids were isolated from another spring barley variety, Abed Bomi, and no virus infection was found in triploids or in other plants from the same seed lot. These results indicate that triploid plants occur in virus-free as well in virus-infected material of some barley varieties. Other data indicate that the frequency of triploids is much higher in virusinfected than in virus-free material of T. Prentice.

Table 2. The frequency of diploids, triploids and aneuploids in different classes according to seed size and plant morphology

Class no.[1]	Total number of plants	Number of plants: Diploids $2n = 2x = 14$	Triploids $3n = 3x = 21$	Aneuploids[2]	Triploids in %
T. Prentice					
1—c	2	0	2	0	100.0
2—a	46	45	0	1 ($2n = 2x + 1 = 15$)	0.0
2—b	100	37	61	2 ($2n = 2x + 1 = 15$; $2n = 3x + 2 = 23$)	63.0
2—c	37	2	33	2 ($2n = 2x + 1 = 15$; $2n = 3x + 1 = 22$)	89.2
Freja					
1—b	1	1	0	0	0.0
2—a	6	6	0	0	0.0
2—b	4	3	1	0	25.0
2—c	8	1	6	1 ($2n = 2x + 1 = 15$)	75.0

[1] See Table 1
[2] Observed chromosome number; most likely interpretation given in brackets

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Summary

In the two barley varieties Tystofte Prentice and Svalöf Freja, the frequency of spontaneous triploids was found to be 2.6 and 0.04 per cent respectively. The triploids originated from light, shrivelled seeds. By selection, according to seed size, a group of plants was obtained in which the frequency of triploids was 51 per cent in T. Prentice and 39 per cent in Freja. Within this group the frequency of triploids could be further increased by selection according to plant morphology. In a sub-group of small, slender plants the frequency of triploids was 89 per cent in T. Prentice and 75 per cent in Freja. The material used of the two varieties was found to be infected with the barley stripe mosaic virus, but available data indicate that triploids also occur in the progeny of virus-free plants of these varieties, though the frequency of triploids was much higher in the virus-infected than in the virus-free material.

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