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Mutants and duplication in chromosome 7 (syn. 5H) in the barley line HA21: duplications may enhance QTLs and serve to make constant linear *cis*-heterozygosity

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Cytological and linkage data indicate a duplication in the short arm of chromosome 7 (syn. 5H) in the mutant line HA21 (barley, *Hordeum vulgare*, cv. 'Pirkka'). The associated mutant (*ha21*) shows a weighted average linkage of 22.1 cM with *pld*, hitherto an ignored anthocyaninless gene, of cv. Pirkka. Some crosses produce F_2 segregants with an exaggerated *ha21* phenotype which may represent position effect or increased dosage of the mutant gene through recombination. Compared with cv. Pirkka, HA21 has changes in grain chemistry (α- and β-amylase, β-glucanase), which may be caused by changed QTL dosage or QTL position effect due to duplication. The use of duplication in creating constant $+m/+m$ or $m+/m+$ linear *cis*-heterozygotes is suggested. Linear *cis*-heterozygotes may produce stable heterosis or attenuate the undesired effects of drastic mutants.

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A morphological variant was found in a field of cv. 'Pirkka', a six-rowed barley (*Hordeum vulgare* L.), by the first author when he was a schoolboy in 1965. This truebreeding variant, HA21 (as a mutant *ha21*), has small, sessile to subsessile lateral florets that may remain sterile under some conditions. It sometimes has an abrupt tapering lemma, fine awns, a tendency to pistillody, and semidwarfism that does not respond to gibberellic acid (GA) and GA_3 (AHOKAS 1973). HA21 has an increased protein content (AHOKAS 1977). HA21 has also been classfied as a *fus* (fusiform or pyramid-shaped spike) mutant (FRANCKOWIAK and PECIO 1992). Two different F_2s, with HA21 as one parent, showed rare segregants of dwarfed, sterile plants with exaggerated *ha21* characteristics. These segregants were hypothesized to have been due to an increase in gene dosage through recombination, HA21 itself possibly carrying a duplication.

Meiosis in the HA21 × Pirkka F_1 hybrid showed a heteromorphic bivalent of a satellite pair, which supports the duplication explanation (H.A., unpublished). When HA21 was studied as part of a genetics laboratory excercise, it was found that HA21 has, of the two possible satellite chromosomes, somewhat longer short arm of chromosome 7 [syn. 5H according to the newest revision (LINDE-LAURSEN et al. 1997)] than cv. Pirkka at mitotic metaphase (STENIUS et al. 1976). Linkage data of translocation testers with the duplication of chromosome 7 (5H) are presented below.

The use of duplication in making fixed linear *cis*-heterozygotes through recombination is indicated. Linear *cis*- heterozygosity, arranged by a duplication, may result in a fixed heterosis, or it may attenuate the adverse effects of drastic mutant genes represented by both the mutant and wild allele. Dosage of quantitatative trait loci (QTL) may also be increased by duplication. Constant heterosis arranged with duplications of other type has recently been suggested by GRAMATIKOVA (1995).

MATERIAL AND METHODS

Plants. — Translocation lines were obtained from Dr. P. Hagberg (The Swedish University of Agricultural Sciences, Svalöv, Sweden). The non-mutant genotype of cv. 'Ko A' was a segregant from the genetic stock Kmut 184a obtained from Dr. T. Tsuchiya (Colorado State University, USA). The line carrying the *msg44cx* gene (male sterile) was a hybrid derivative from the cross (Pirkka × AHOR 2680) × (AHOR 2680 × 'Paavo'). Paavo SCS1 is a segregating cytoplasmic streak mutant (AHOKAS 1976). Cv 'Otra' and Paavo were from a commercial seed lot. A single plant of each was used for crosses, except from Pirkka, where several plants have been used without variable results. Segregations were recorded under field conditions.

Grain enzyme activities. — HA21 (also accessioned as PI 349682) and its original cv. Pirkka (Tammisto line a4459) were planted in single rows, side by side, boarded by rows of other barleys in the field on silty clay fertilized with 400 kg ha^{-1} of 20-10-10 (NPK) in

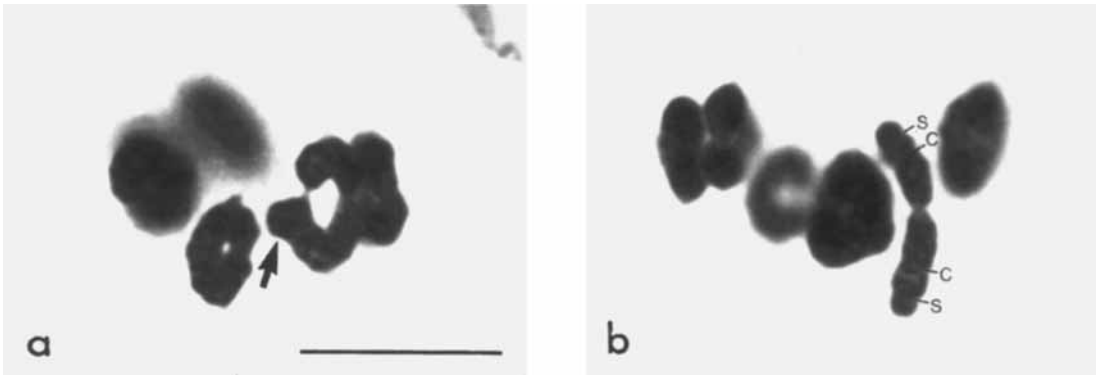

Fig. 1. a and **b**. Metaphase I in the F_1 hybrid of HA21 × Pirkka. **a** A heteromorphic loose ring bivalent (arrow). **b** A heteromorphic rod bivalent. The short arm of the longer chromosome has two major coils between the centromere (c) and the secondary constriction (s), while the shorter has one. Scale = 10 μm.

Elimäki (in 1990 and 1993) and on sandy clay with 550 kg ha^{-1} of 20-4-8 in Jokioinen (in 1996) in southern Finland.

Extracts for enzyme activities were made of husked, surface sterilized grain aseptically germinated for 5 days at 15°C, as described previously (AHOKAS and NASKALI 1990). The protein in extracts was measured with BSA as a standard by the UV method of AHOKAS (1978), α-amylase by Ceralpha, β-amylase by Betamyl, and endo-β-glucanase by azo-barley glucan (Biocon, Megazyme) as described previously (AHOKAS and NASKALI 1990; AHOKAS and ERKKILÄ 1992).

Linkage and cytology. — Linkage was analyzed with MAPMAKER and is reported in Haldane cM (LANDER et al. 1987). Cytological meiotic material was fixed in aceto ethanol (1:3) and stained using the Feulgen procedure.

RESULTS

Cytology. — The meiotic division of HA21 × Pirkka showed a heteromorphic bivalent, supporting the hypothesis that HA21 is the result of a duplication. Special cases showed a double-coil dimension on the short arm of a satellite-carrying chromosome, i.e., no. 6 (6H) or 7 (5H) (Fig. 1a and b). The heteromorphic bivalent appeared as a rod with a higher proportion (8.1 %) than all the other bivalents (2.4 %) in 246 metaphase I PMCs studied.

Segregation and linkage. — The cross of HA21 × Pirkka, segregated at F_2 *ha21* and wild type fitting a 1:3 ratio (Table 1). Crosses of HA21 with cv. Pirkka, Paavo and with Ko A did not produce exaggerated segregants at F_2. These exaggerated segregants were only observed in the cross with cv. Otra and the line HA72-62. The pale auricle colour (*pla*) of HA21 and cv. Pirkka was found to be linked with the *ha21* phenotype. Linkage estimates ranged from 10.8 to 38.2 cM (Table 1), with a weighted average of 22.1 cM. No linkage was evident between *ha21* and *v* (six-rowed, syn. *vrs1*), *s* (short rachilla hair, syn. *srh*, *msrh*), or *msg44cx* (male sterile) nor between *pla* and *s*.

The association of *ha21* with the translocation break points was tested in the F_2 of each cross. The data support linkage to the short arm of chromosome 7 (5H) (Table 2). The *ha21* locus shows linkages with the break-points of T3-7d, T6-7ae, and T6-7k on the short arm, and that of T1-7f apparently close to the centromere of the chromosome 7 (5H).

Grain enzyme activities. — HA21 had a higher seed mass. The activity of β-amylase, a stored seed protein, was significantly higher in HA21 than in cv. Pirkka (Table 3). In contrast, the activities of α-amylase and β-glucanase, which appear during germination, were lower in HA21 than in cv. Pirkka after five days of germination. The enzyme activity differences are consistent on both soluble protein and grain mass basis.

DISCUSSION

Linkage. — The locus *ha21* is loosely linked to the pale auricle (*pla*) gene of cv. Pirkka (Table 1). The *pla* mutation makes the epigeal organs free from anthocyanins, or highly reduces the contents. Absence of anthocyanins, caused by various loci, is a feature in many cultivars, though a locus assigned to chromosome 7 (5H) is unknown (JENDE-STRID 1993, 1995). The exaggerated segregants of two of the HA21 crosses could be ascribed to dosage or positional effects. The mutant *ha21* is probably associated with the duplication.

Table 1. *F_2 segregation of ha21 and other genes*

Pedigree	Other genes (m_2)	Number of plants	Phenotype numbers of *ha21* and other mutant				Fit to 3:1 for *ha21*	Number of exaggerated *ha21* segregants	*ha21-pla* linkages (cM)
			ha21,m_2	*ha21*,+	+,m_2	+,+			
HA21/Pirkka	–	852	–	204	–	648	$P > 0.30$	0	–
Paavo/HA21	*Pla*	353	72	17	16	248	$P > 0.90$	0	10.8
Paavo SCS1/HA21	*Pla*	39	6	5	4	24	$P > 0.50$	0	34.9
HA21/Ko A	*Pla*	95	13	7	13	62	$P > 0.50$	0	32.9
Ditto	*S*		9	11	22	53			
Ditto	*V*		8	12	22	53			
HA21/Otra	*Pla*	154	26	14	25	89	$P = 0.02$[a]	16	38.2
HA72-62/HA21	*msg44cx*	104	2	23	17	62	$P > 0.90$	2	–
Ditto	*V*		5	20	15	64			

[a] With the exaggerated 16 excluded, $P > 0.90$

Three of the four translocation break-points showing linkage with *ha21*, viz. T3-7d (KASHA and BURNHAM 1965; PERSON 1969; LINDE-LAURSEN 1988), T6-7ae (HAGBERG et al. 1978), and T6-7k (HAGBERG et al. 1978; LINDE-LAURSEN 1988) have been indicated to be on the short arm of chromosome 7 (5H), while that of T1-7f has been indicated to be on the long arm between *S* and the centromere (PERSSON 1969).

The locus *S* was mapped with intervening markers on the long arm of chromosome 7 (5H) to be 49.1 Kosambi cM from the centromere (KLEINHOFS et al. 1993: Fig. 7) and apparently somewhat less in the integrated map of QI et al. (1996). The observed free recombination of *S* with *ha21*, and with *pla*, is in accordance with the maps.

Grain enzyme activities. — Though cv. Pirkka has a high β-amylase activity (SIMBERG 1950; ALLISON and SWANSTON 1974), the β-amylase level is significantly higher in HA21 (Table 3) in samples germinated for five days. Beta-amylase reaches its maximum activity level after about five days of germination (GRIME and BRIGGS 1995; EVANS et al. 1997). The seed-expressed β-amylase locus is on the chromosome 4 (4H) (KREIS et al. 1988).

The 2/3 or 3/4 level of α-amylase activity in HA21, compared with Pirkka, after five days of germination, may be attributed to several causes. HA21 may have an elevated level of α-amylase inhibitor or an altered time-scale for the induction of α-amylase during germination. The coding loci of α-amylase appear on chromosomes 1 (7H) and 6 (6H) (BROWN and JACOBSEN 1982).

The short arm of chromosome 7 (5H) has QTLs for α-amylase, soluble protein, grain protein (TINKER and MATHER 1994; OZIEL et al. 1996), grain mass/volume, and plant height (TINKER and MATHER 1994). QTLs for diastatic power, which closely correlates with β-amylase activity (ALLISON and SWANSTON 1974; SANTOS and RIIS 1996), appear in the short arm of chromosome 7 (5H) (OZIEL et al. 1996; THOMAS et al. 1996). Other reports also show that chromosome 7 (5H) has QTLs i.a. for α-amylase and grain protein (HAYES and IYAMABO 1994; HAYES et al. 1993; HAN and ULLRICH 1994; OZIEL et al. 1996; MATHER et al. 1997) or grain nitrogen content (BEZANT et al. 1997; MATHER et al. 1997), grain mass, and soluble protein (MATHER et al. 1997). Grain size and protein, plant height, α- and β-amylase are also affected in HA21 as compared with cv. Pirkka (Table 3 and references in the introduction). Duplication or position effects of QTLs in HA21 may be a reason for the changes. There appears to be quantitative differences in grain protein bands as revealed by SDS-PAGE of fractionated proteins in HA21 and cv. Pirkka (unpublished).

Table 2. *F_2 segregation of ha21 and partial fertility of translocation tester crosses*

Translocation	Fertile		Partially fertile		Breakpoint linkage to *ha21* (cM)
	(+)	*ha21/ha21*	(+)	*ha21/ha21*	
T1-3b	77	48	78	34	
T1-7f	44	31	69	15	30.0
T2-3g	93	40	97	42	
T2-5a	98	34	84	42	
T2-7b	95	36	89	40	
T3-7c	68	44	82	27	
T3-7d	89	37	87	20	33.1
T4-5e	63	35	62	48	
T4-7b	78	12	37	11	
T6-7ae	84	29	82	20	36.5
T6-7i	84	35	76	25	
T6-7k	36	7	63	15	34.9

Table 3. *Activities of enzymes after five days of germination in the extracts of HA21 and the original cultivar Pirkka. Means of harvests of three seasons, 1990 and 1993 in Elimäki, and 1996 in Jokioinen*

Barley	Mean grain mass (mg)	Extracted protein (%)	α-Amylase (U)		β-Amylase (U)		β-Glucanase (U)	
			(g grains)$^{-1}$	(g extracted protein)$^{-1}$	(g grains)$^{-1}$	(mg extracted protein)$^{-1}$	(kg grains)$^{-1}$	(g extracted protein)$^{-1}$
HA21	49.3 ± 1.7	2.9 ± 0.7	96.6 ± 2.3	2166 ± 13	1005 ± 9	22.1 ± 1.2	1221 ± 4.3	28.8 ± 2.0
Pirkka	41.8 ± 1.0	2.3 ± 0.2	127.7 ± 1.8	3443 ± 11	698 ± 9	18.6 ± 1.4	1446 ± 6.2	39.1 ± 1.3
Test	F = 8.362	U = 3	Sign test		Sign test		Sign test	
P	P = 0.01	P = 0.35	P < 0.01		P < 0.02		P < 0.01	

Duplication induction. — Duplications have been produced by crossing partially overlapping translocations (HAGBERG and HAGBERG 1992). A special characteristic, level of root-associated bacteria, of a duplication carrier has been found in barley (HAGBERG and HAGBERG 1987; LILJEROTH and BÅÅTH 1988; LILJEROTH et al. 1994). While duplications may increase the dosage of desired loci, control of the transcriptional level by DNA methylation may occur in association with duplications (SUBRAHMANYAM et al. 1994; PRADHAN and SUBRAHMANYAM 1995). A triplication with fixed heterozygosity for the esterase 4 locus may have occurred in an Israeli *H. vulgare* ssp. *spontaneum* (C. Koch) A. & Gr. (KAHLER et al. 1981; SOLIMAN and ALLARD 1989).

Radiation can induce duplications in cereals (MACKEY 1954). Cv. Pirkka was released in 1952, and HA21 was found in 1965, during the busy nuclear testing period. Of the formed and monitored radionuclides, ^{90}Sr and ^{137}Cs depositions in the 1961–1965 pentad were, respectively, 81 and 67 times that detected in 1981–1985; the maximum occurred in 1962, when the average rate was 850 Bq m^{-2} of $^{90}Sr + ^{137}Cs$ in Finland (PAAKKOLA 1988). Significant differences exist between barley cultivars at accumulating of ^{137}Cs (ØHLENSCHLÆGER et al. 1993). Moreover, the original Pirkka field was in an area (Elimäki) where the external, mostly rock-emitted radiation shows a relatively high mean dose rate, 17.5 μR h^{-1} at 1 m height (LEMMELÄ 1984).

Breeding with duplications. — Linear *cis*-heterozygotes may be produced by arranging different allele composition in the duplicated segments. This might result in favourable cases of fixed heterosis or possibly attennuate the effect of drastic mutants. Stable arrangements of the constitutions $+m/+m$ or $m+/m+$ could be useful for breeding. The relationship of HA21 duplication with *lys3* (high lysine) locus is being studied. The *lys3* locus shows a tight linkage with the T3-7d breakpoint (JENSEN 1979). It may also be possible to increase the QTL number affecting other loci with duplication. In HA21, changed levels in α- and β-amylase, β-glucanase, grain mass and plant height may have occurred by QTLs.

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