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RFLP mapping of the barley homeotic mutant *lax-a*

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Abstract The *lax-a* homeotic mutant of barley has flowers in which lodicules are replaced by stamens (giving five stamens per flower). RFLP mapping of an F_2 population from a Bonus *lax-a*^{1} × *H. spontaneum* cross showed that the mutation was on the short arm of chromosome 7(5H), closely linked to the centromere. An additional F_2 population was used to show that the *lax-a* mutation gave the five-stamen phenotype in all flowers of 6-rowed spikes and that hoods were elevated and reduced in size in *lax-a*/*Hooded* double-mutant plants.

Key words Barley · RFLP · Linkage map · homeotic mutant · *lax-a* mutant

Introduction

Mutations causing changes in the fate of organ primordia are central to studies of flower development. They are best characterized in dicotyledons, especially *Antirrhinum majus* (snapdragon) and *Arabidopsis thaliana* (see recent reviews by Coen 1991; Drews et al. 1991; Okamura et al. 1993). Mutations affecting plant development in monocotyledons are also attracting increased attention, particularly in crop species such as barley (*Hordeum vulgare*) (Bossinger et al. 1992, 1993) and maize (*Zea mays*) (Veit et al. 1993). Analyzing the developmental biology of monocot crops will give new insights into crop evolution and offers novel opportunities for the directed manipulation of plant form in breeding programmes.

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In order to identify cloned genes corresponding to specific mutants it is valuable to have map locations of the latter. The *lax-a* mutant of barley has several distinct effects on plant phenotype including a homeotic alteration of flower structure, with modified stamens appearing in place of lodicules. Its effects on all aerial parts of the plant (roots have not been studied) show that the *LAX-A* gene product must be necessary for normal development of vegetative as well as floral structures.

Fifteen alleles of the *lax-a* mutant, produced by a variety of radiation or chemical treatments, are known in barley (Larsson 1985a). Seven are in the variety Bonus and eight in the variety Foma. The *lax-a* mutation has previously been mapped in relation to morphological markers and translocation break-points and is known to be located on barley chromosome 7(5H) (Persson 1969a,b; Larsson 1985b). The present paper describes the genetic map location of the *lax-a* mutation in relation to RFLP markers and provides additional information on its pleiotropic effects.

Materials and methods

Seeds of 15 lines homozygous for *lax-a* mutations (a^1, a^4, a^8, a^{20}, a^{37}, a^{39}, a^{54} and a^{92} in the variety Bonus, and a^{208}, a^{218}, a^{222}, a^{229}, a^{256}, a^{278} and a^{286} in the variety Foma) were kindly provided by Dr. Udda Lundqvist, Svalöf AB, Svalöv, Sweden.

The genetic map location and pleiotropic effects of *lax-a* were determined using three F_2 populations derived from crosses in which Bonus, homozygous for the *lax-a*^{1} allele, was used as the female parent. The first cross was with the variety Betzes and the second with an accession of wild barley (*Hordeum spontaneum*). The F_2 populations contained 216 and 169 plants respectively. The effect of the *lax-a*^{1} allele on the development of 6-rowed spikes and its interaction with the *Hooded* (*K*) mutation were analyzed in a third F_2 population from a Bonus *lax-a*^{1} × Colsess (BGS152) cross. All populations were grown in a glasshouse with 18 h light. DNA for RFLP analysis was extracted from seedlings using a CTAB method (Murray and Thompson 1980). Southern blotting, probe labelling and hybridization was as described in Devos et al. (1992) except that probe denaturation was by the addition of a 1/10 vol of 3 M NaOH to the labelling reaction. Recombination frequencies were calculated using MAPMAKER (Lander et al. 1987) and converted to cM using the Kosambi mapping function.

Results and discussion

Morphological characteristics of *lax-a*

Glasshouse-grown plants homozygous for any one of the 15 *lax-a* alleles tested had distinctive phenotypic difference from Bonus or Foma. The *lax-a* mutant plants were shorter, due to a reduction in the length of the upper vegetative internodes. They also showed a strong tendency to produced curled leaves, particularly in the flag leaf. The tillers tended to be curved, and more tillers were produced in the *lax-a* plants than in their respective wild-types. Ear length was increased due to greater internode length in the rachis, but the length of the lemma (including the awn) was reduced (Fig. 1, Table 1). The transition from the blade of the lemma to the awn was broadened in *lax-a* mutants and the length of the palea was increased (Fig. 2). Fertility was reduced, and the seeds were longer and thinner (with lower 100-grain weights) than those of wild-type plants. These observations were in agreement with descriptions of field-grown plants given by Larsson (1985a). In the glasshouse, the *lax-a* mutants flowered 1–2 weeks later than their respective parents.

All *lax-a* lines consistently showed replacement of lodicules with stamens (Fig. 2c and 3), or occasionally carpelloid stamens. The two additional stamens were smaller than normal and typically had two loculi instead of the usual four (Bossinger et al. 1992, 1993) (Fig. 3). Pollen grains in the extra anthers appeared normal, but the anthers failed to dechisce under our glasshouse conditions and no seed was set in spikes from which the three normal anthers were removed. Crosses were attempted by crushing the additional anthers to release pollen, but no seeds were obtained.

Morphological characteristics of the Bonus *lax-a*^{1} × Betzes F_2 population

Six F_1 plants from a Bonus *lax-a*^{1} × Betzes cross were grown, all of which appeared normal. The normal appearance of seeds from F_1 plants, and from F_2 plants subsequently found to be heterozygous for the *lax-a*^{1} mutation, showed that seed shape and seed size were controlled by the maternal genotype of the plant. An F_2 population from a single F_1 plant was grown which segregated 159 wild-type to 57 mutant plants. This was not significantly different from the expected 3:1 ratio (χ^2 $1df = 0.22$ ns). F_2 plants showed segregation of all *lax-a* characteristics except for delay in flowering time (Table 1) and curling of the flag leaf.

In order to determine if any effects of the *lax-a* mutation were detectable in heterozygotes, F_3 families were grown from the F_2 plants with normal phenotypes. Comparisons of data from putative wild-type

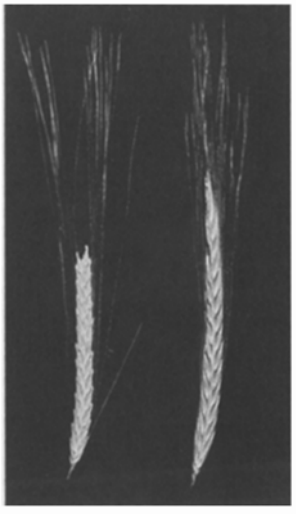


Fig. 1 Spikes of wild-type (left) and *lax-a*^{1} mutant (right) barley cv Bonus. Spikes with equal numbers of florets are shown

Table 1a Mean values of plants in the Bonus *lax-a*^{1} × Betzes F_2 population. The significance (*P*) of differences between wild-type and mutant plants was determined by analyses of variance

Character	Wild-type	*lax-a*	%Change	*P*
Spike characters				
Spike length (mm)	88.5	105.4	19.1	**
Number of spikelets[a]	25.1	26.4	5.2	**
Mean spike internode length[b] (mm)	3.5	4.0	14.3	***
Mean spike internode length[c] (mm)	3.3	3.6	18.2	***
Lemma length[d] (mm)	141.7	125.8	−11.2	***
Palea length (mm)	10.3	13.1	27.2	***
Mean grain weight (mg)	30.8	25.0	−18.8	***
Vegetative characters				
Plant height[e] (mm)	768.1	614.4	−20.0	***
Topmost internode (mm)	225.7	170.8	−24.3	***
Internode-1 (mm)	172.7	145.5	−15.8	***
Internode-2 (mm)	131.5	107.2	−18.5	***
Internode-3 (mm)	114.7	90.2	−21.4	***
Internode-4 (mm)	86.6	61.0	−29.6	***
Internode-5 (mm)	33.4	36.0		ns
Internode-6 (mm)	3.0	3.8		ns
Number of internodes	5.7	5.9		ns
Flowering time				
Ear emergence time[f] (days)	6.4	7.2	2.9	ns

Table 1b Mean values for characters showing significant differences between putative homozygous wild-type F_2 plants and wild-type/*lax-a* F_2 plants. Wild-type plants were those with flowers having three stamens and normal lodicules. *lax-a* plants had five anthers and no lodicules

Character	wt/wt	wt/*lax-a*	% Change	*P*
Spike length (mm)	86.5	90.6	4.7	<0.10
Mean spike internode length[b] (mm)	3.5	3.6	2.9	*
Mean spike internode length[c] (mm)	3.2	3.4	6.3	*
Palea length (mm)	10.1	10.4	3.0	***

[a] *lax-a* plants tend to have more sterile spikelets at the top of the spike, and hence have fewer grains than wild-type plants
[b] Total spike length/number of florets
[c] Ten internodes measured from the centre of the spike
[d] Base of the lemma to the tip of the awn
[e] Soil surface to the base of the spike (collar). Internodes are numbered from the top of the plant
[f] Days from ear emergence of the earliest flowering F_2 plant

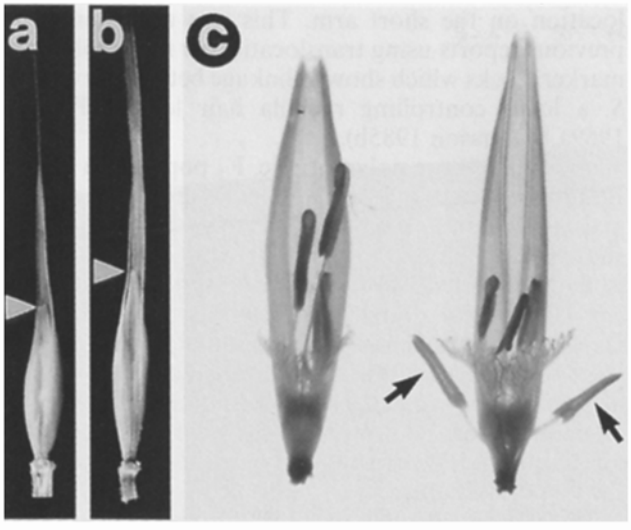


Fig. 2 Florets of wild-type (**a**) and *lax-a*^{1} mutant (**b**) cv Bonus. The *arrowheads* illustrate the difference in the length of the palea. This is also seen in (**c**) which shows flowers of wild-type (left) and *lax-a*^{1} mutant (right) cv Bonus from which the lemmas have been removed. The two additional stamens in the *lax-a*^{1} mutant are *arrowed*

homozygous F_2 plants (no *lax-a* mutant plants in F_3 families of 4–12 plants) with data from confirmed heterozygous F_2 plants showed three significant differences. These were the two estimates of spike internode length and palea length (Table 1). The increase in total spike length in the heterozygotes was of borderline significance. The significant characters did not have intermediate values but were close to the values of wild-type plants. Thus, while the homeotic transformation of the lodicules behaved as a truly recessive character, heterozygosity for the *lax-a*^{1} mutation had small quantitative effects on some other spike characters.

Morphological characteristics of the Bonus *lax-a*^{1} × Colsess (*K*) cross

The effect of homozygosity of the *lax-a*^{1} allele was also examined in a cross with a 6-rowed barley carrying the *Hooded* (*K*) mutation. Awned 6-rowed F_2 plants either had florets with a normal phenotype or showed the typical *lax-a* phenotype in all three florets at each node. In *lax-a*^{1}/*K* double-mutant plants the broadening of the lemma to awn transition, which tended to elevate the hood and reduce its size (Fig. 4), was still seen. The effect on the hood was variable within individual spikes.

Fig. 3 Scanning electron micrographs of wild-type (**a**) and *lax-a*^{1} mutant (**b**) flowers of cv Bonus from which the lemmas have been removed. *G* glume, *S* normal stamen, *L* lodicule, * additional stamens which have two loculi instead of the normal four. *Bars* represent 100 μm

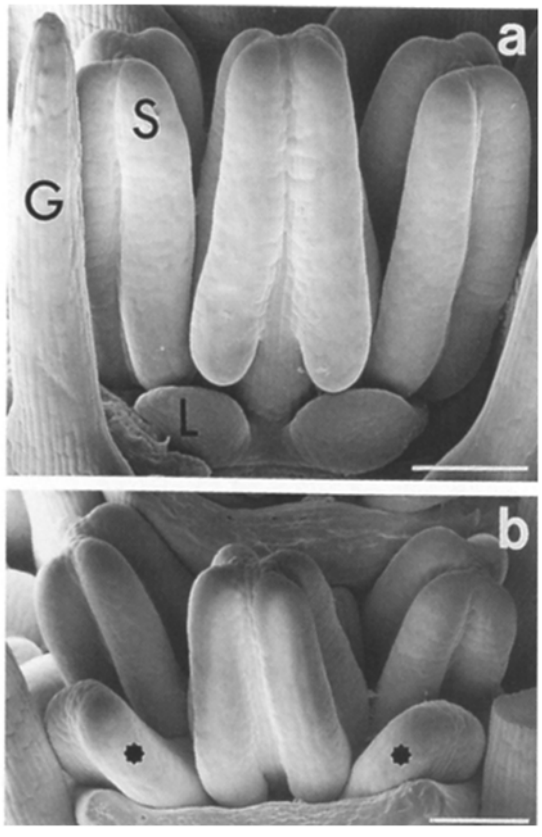
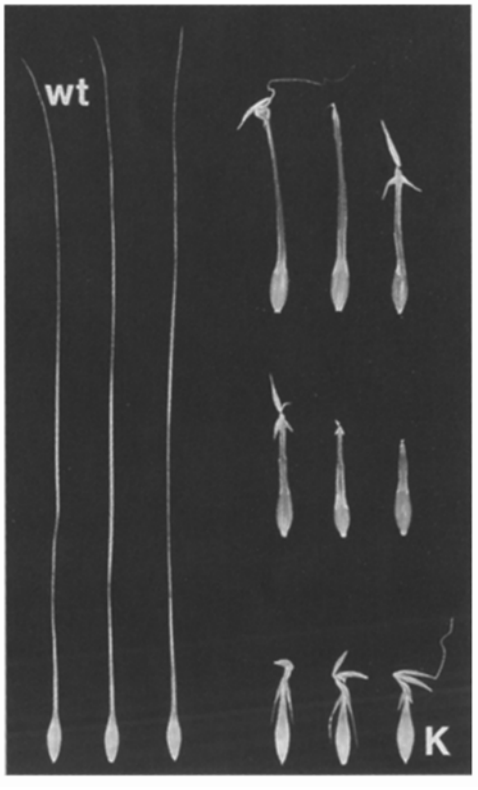


Fig. 4 Left – three florets of wild-type (wt) cv Bonus (normal awns). Bottom right – three florets showing the *Hooded* (*K*) mutant phenotype in cv Colsess. Top right – six florets from a *lax-a*^{1}/*Hooded* spike illustrating the range of lemma form in double-mutant plants



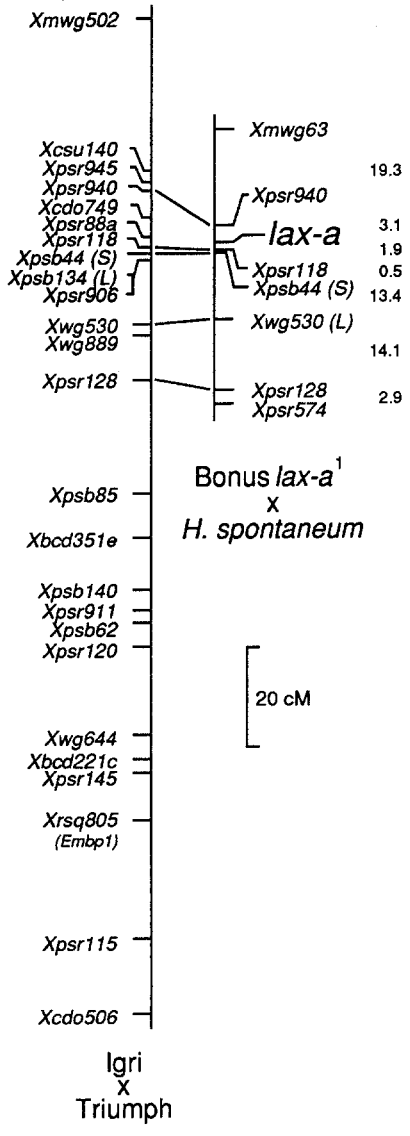
Genetic map location of the *lax-a* mutation in relation to RFLP markers

The Bonus *lax-a*^{1} × Betzes population used for phenotypic characterization proved to have a low level of polymorphism for markers on chromosome 7(5H), the known location of the *lax-a* mutation. However, *lax-a* was weakly linked to *Xmwg63* (23 cM) and *Xwg530* (33 cM), suggesting that it had a proximal location on the short arm. This was consistent with previous reports using translocation or morphological-marker stocks which showed linkage between *lax-a* and *S*, a locus controlling rachilla hair length (Persson 1969a, b; Larsson 1985b).

A second, more polymorphic, F_2 population from a Bonus *lax-a*^{1} × *Hordeum spontaneum* cross was used to map the mutation more accurately. This cross also showed segregation that was not significantly different from the expected 3:1 ratio (138 wild-type : 31 mutant, χ^2 $1df = 0.50$ ns) for all spike characters associated with the *lax-a* mutation, including the development of stamens in place of lodicules, allowing unambiguous identification of *lax-a*^{1} homozygotes. The pleiotropic effects of *lax-a*^{1} on plant height and flowering time were much less clear due to a high level of variability for these characters in the F_2 population.

MAPMAKER analysis of RFLP scores for probes previously mapped in the *Xmwg63-Xwg530* interval showed that *lax-a* was on the short arm of chromosome 7(5H) in the *Xpsr940-Xpsb44* interval (Fig. 5). There was one recombinant between *Xpsb44* and *Xpsr118*, and the two possible placements for *Xpsr118* relative to *Xpsb44* were of similar likelihood. The order shown is *Xpsr940-lax-a-Xpsr118-Xpsb44* as this was consistent with the most likely order of the RFLP markers in a separate barley cross (Laurie et al. 1995). The genetic map location was close to the centromere but chromosome 7(5H) shows compression of the genetic map in proximal regions of the arms (Kleinhofs et al. 1993), as do other barley chromosomes. Thus, the gene may not be physically close to the centromere.

Fig. 5 Map location of the *lax-a*^{1} mutant. Positioning of the Bonus × *H. spontaneum* map in relation to the rest of chromosome 7 (5H) is by comparison to a map from an Igri × Triumph barley cross (Laurie et al. 1995). Probes marked (*S*) or (*L*) were the most proximal markers on the short or long arms, respectively. The arm locations of probes were determined using wheat/barley telosomic addition lines (Islam 1983). The *Xbcd*, *Xcdo*, *Xwg* and *XcsuBG* markers were previously mapped in barley by Heun et al. (1991). The *Xmwg* markers were previously mapped by Graner et al. (1991). The *Xpsr* and *Xpsb* markers were from the John Innes Centre wheat or barley libraries respectively



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References

Bossinger G, Lundqvist U, Rohde W, Salamini F (1992) Genetics of plant development in barley. In: Munck L (ed) Barley genetics VI. Proc 6th Int Barley Genet Symp. Munksgaard International Publishers Ltd., Copenhagen, pp 989–1022

Bossinger G, Rohde W, Lindqvist U, Salamini F (1993) Genetics of barley development: mutant phenotypes and molecular aspects. In: Shewry PR (ed) Barley: genetics, biochemistry, molecular biology and biotechnology. CAB International, Oxford, pp 231–263

Coen ES (1991) The role of homeotic genes in flower development and evolution. Annu Rev Plant Physiol Plant Mol Biol 42:241–279

Devos KM, Atkinson MD, Chinoy CN, Liu CJ, Gale MD (1992) RFLP-based genetic map of the homoeologous group-3 chromosomes of wheat and rye. Theor Appl Genet 83:931–939

Drews GN, Weigel D, Meyerowitz EM (1991) Floral patterning. Curr Opin Genet Dev 1:174–178

Graner A, Jahoor A, Schondelmaier J, Siedler H, Pillen K, Fischbeck G, Wenzel G, Herrmann RG (1991) Construction of an RFLP map of barley. Theor Appl Genet 83:250–256

Heun M, Kennedy AE, Anderson JA, Lapitan NLV, Sorrells ME, Tanksley SD (1991) Construction of a restriction fragment length polymorphism map for barley (*Hordeum vulgare*). Genome 34:437–447

Islam AKMR (1983) Ditelosomic additions of barley chromosomes to wheat. In: Sakamoto S (ed) Proc 6th Int Wheat Genet Symp. Mazuren Co. Ltd., Kyoto, pp 233–238

Kleinhofs A, Kilian A, Saghai Maroof MA, Biyashev RM, Hayes PM, Chen FQ, Lapitan N, Fenwick A, Blake TK, Kanazin V, Ananiev E, Dahleen L, Kudrna D, Bollinger J, Knapp SJ, Liu B, Sorrells M, Heun M, Franckowiak JD, Hoffman D, Skadsen R, Steffenson BJ (1993) A molecular, isozyme and morphological map of the barley (*Hordeum vulgare*) genome. Theor Appl Genet 86:705–712

Lander ES, Green P, Abrahamson JA, Barlow A, Daly MJ, Lincoln S, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Larsson HEB (1985a) Morphological analysis of *laxatum* barley mutants. Hereditas 103:230–253

Larsson HEB (1985b) Linkage studies with genetic markers and some *laxatum* barley mutants. Hereditas 103:269–279

Laurie DA, Pratchett N, Bezant JH, Snape JW (1995) RFLP mapping of five major genes and eight quantitative trait loci controlling flowering time in a winter × spring barley (*Hordeum vulgare* L.) cross. Genome 38:575–585

Murray MG, Thompson WF (1980) The isolation of high-molecular-weight plant DNA. Nucleic Acids Res 8:4321–4325

Okamura JK, den Boer BDW, Jofuku KD (1993) Regulation of *Arabidopsis* flower development. Plant Cell 5:1183–1193

Persson G (1969a) An attempt to find suitable genetic markers for dense ear loci in barley. I. Hereditas 62:25–96

Persson G (1969b) An attempt to find suitable genetic markers for dense ear loci in barley. II. Hereditas 63:1–28

Veit B, Schmidt RJ, Hake S, Yanofsky MF (1993) Maize floral development: new genes and old mutants. Plant Cell 5:1205–1215