Appendix 1. The *Hordeum spontaneum* map

**AFLP and SSR markers**

Seventeen primer combinations were selected: E33M54, E33M61, E35M48, E35M54, E35M61, E38M55, E38M58, E42M51 and E45M55 (Qi and Lindhout, 1997), E37M32, E37M33, E40M38, E41M32, E42M40, E42M32, E42M40 (Becker et al. 1995) and E31M55. DNA was isolated from two-week-old leaves of the 233 F2 plants, using the CTAB method (Ausubel et al. 1999). The AFLP protocol was essentially as described in Vos et al. (1995). The DNA was double digested with the restriction enzymes EcoRI and MseI. The EcoRI specific primers were labeled with either 700 or 800 nm infrared dye (IRD700, IRD800) for detection with an automated laser sequencer (Li-Cor Inc., Lincoln, NE, U.S.A.). In addition, markers generated by the primer combinations E32M61, E33M55, E39M61, E42M48 and E38M54 (Qi and Lindhout 1997) were genotyped co-dominantly by Keygene B.V., Wageningen, The Netherlands.

Separately, a set of 13 SSR markers was used (Ramsay et al. 2000). The primers were labeled with either IRD700 or IRD800 for the Li-Cor sequencer. Approximately 20 ng of template DNA was used in the PCR reaction mixture, which consisted further of 1 x PCR buffer, 0.5 U of AmpliTaq polymerase (Perkin Elmer Inc., Wellesley, MA, U.S.A.), 200 µM dNTPs and 1 pmol of forward and reverse primer. The reaction volume was 10 µL. Four different PCR programs were used for amplification as described by Ramsay et al. (2000).

**Map construction**

The AFLP markers from 17 primer combinations were scored dominantly as the absence or presence of an amplification product. The scoring was done by eye with the help of the program Cross Checker (Buntjer 1999). The markers from five primer combinations generated and scored by Keygene B.V. were scored co-dominantly using the Quantar™Pro software (http://www.keygene-products.com/html/index_products.htm), which enables a distinction between the homozygous and heterozygous state based on band intensity. The AFLP marker names were designated from the primer combination and size of the amplification product. SSRs were scored co-dominantly.

During the crossing process we kept track of the structure of the mapping population. Inspection of the data revealed that some of the F2 sub-families did not segregate for a number of markers, due to partial homozygosity of the parental F1-plant. This resulted from heterozygosity at such a marker for one of the parents of our cross. Neglecting this would affect the mapping results, i.e. by segregation distortion of these markers and an overestimation of recombination frequency...
between ‘affected’ and ‘non-affected’ markers (P. Stam, unpublished results). Therefore, for each marker we checked its segregation in each F₂ sub-family and removed data from non-segregating sub-families.

With the corrected data set a linkage map was constructed using the JoinMap 3.0 software package (Van Ooijen and Voorrips 2000). Linkage groups were assigned using a LOD threshold of 5.0. Kosambi’s mapping function was used to calculate map distances. To assign the linkage groups to known barley chromosomes, SSR loci (Ramsay et al. 2000) as well as AFLP markers in common with earlier maps from several cultivated barley populations L94 x Vada’ (Qi et al. 1998), ‘L94 x 115-6’ (P. Lindhout, personal communication), ‘Apex x Prisma’ (Yin et al. 1999) and ‘Proctor x Nudinka’ (Becker et al. 1995) were used.

**Linkage map**

The marker data revealed that the ‘Ashqelon’ parent must have been heterozygous: 59% of the ‘Ashqelon-specific’ markers did not segregate in at least one F₂ sub-family. Heterozygosity was less prominent in the ‘Mehola’ parent; with 7% of the ‘Mehola’-specific markers not segregating in at least one F₂ sub-family. As a consequence, 45% of the markers contained no useful linkage information. After adjustment 202 markers (196 AFLP and 6 SSR) could be mapped without problems.

The resulting linkage map is shown in Fig. A1. The markers are distributed over 11 linkage groups. Except for group U1, each linkage group contains both dominant and co-dominant markers. These co-dominant markers provided sufficient anchors to enable integration of the parental maps. The total map length equals 445 cM. This makes an average of 18 markers per linkage group, the range being from two to 36. The average distance between two markers was 2.2 cM. No gaps between two adjacent markers were larger than 20 cM.

The assignment of linkage groups to barley chromosomes is based on AFLPs and SSRs that are in common with other linkage maps of cultivated barley (Table A1). Seven of the eleven linkage groups could unambiguously be assigned to known barley chromosomes. Three groups (2A, U1 and U2) did not contain any common markers, and one group (6) contained markers that mapped to different chromosomes in other mapping populations. Groups 2A and 6 were tentatively assigned to chromosomes based on weak linkage of some markers that were not mapped in our population, but have been mapped in at least one of the other populations. Linkage groups U1 and U2 remained ‘unassigned’, due to lack of markers shared with other maps.

The chi-square values for goodness-of-fit ranged from 0.83 to 1.60 for the 11 linkage groups, indicating a good overall fit. Therefore, even though the current map had to be assembled
by removing a substantial part of the markers, the remaining data still resulted in a reliable map
that can serve as a basis for further linkage and QTL mapping.

References

2.11-2.12 in Short protocols in molecular biology: a compendium of methods from current

markers in barley. Mol Gen Gen 249: 65-73

Buntjer JB (1999) Cross Checker Fingerprint analysis software v2.9, Wageningen University and
Research Centre, The Netherlands


molecular map in barley. Theor Appl Gen 96: 376-384

Ramsay L, Macaulay M, degli Ivanissevich S, MacLean K, Cardle L, Fuller J, Edwards KJ,
1997-2005

linkage maps. Plant Research International, Wageningen, The Netherlands

Research 23: 4407-4414

Fig. A1. The linkage map of wild barley, *Hordeum spontaneum*. Assignment of linkage groups to
barley chromosomes 1H to 7H as described in the text. Linkage groups U1 and U2 are unassigned.
AFLP marker identifiers are composed of primer combinations and estimated length of the
amplification product. Co-dominant markers are indicated in boldface, and markers used in
identification of chromosomes are indicated in italics. Clusters of markers mapping to the same
position (within 1 cM) are indicated by vertical bars to the left of clusters.
Table A1. The linkage groups, the chromosomes to which they are assigned, number of markers in each linkage group, length of linkage group, the relevant maps the chromosomes were based on and common markers linking the map of *H. spontaneum* ‘Ashqelon’ x ‘Mehola’ to other *Hordeum* maps.

<table>
<thead>
<tr>
<th>Linkage group</th>
<th>Hordeum chromosome</th>
<th>Number of markers</th>
<th>Length (cM)</th>
<th>Assignment based on</th>
<th>No. of common markers</th>
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<tr>
<td>1</td>
<td>1 (7H)</td>
<td>28</td>
<td>45</td>
<td>L94 x Vada&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1</td>
</tr>
<tr>
<td>2A</td>
<td>2 (2H&lt;sup&gt;6&lt;/sup&gt;)</td>
<td>25</td>
<td>66</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>2B</td>
<td>2 (2H)</td>
<td>11</td>
<td>51</td>
<td>L94 x Vada&lt;sup&gt;1&lt;/sup&gt;</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L94 x 116-5&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>3A</td>
<td>3 (3H)</td>
<td>36</td>
<td>72</td>
<td>Lina x HS&lt;sup&gt;3&lt;/sup&gt;</td>
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<td></td>
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<td></td>
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<td>L94 x Vada&lt;sup&gt;1&lt;/sup&gt;</td>
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</tr>
<tr>
<td>3B</td>
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<td>6</td>
<td>24</td>
<td>Lina x HS&lt;sup&gt;3&lt;/sup&gt;</td>
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<tr>
<td>4</td>
<td>4 (4H)</td>
<td>24</td>
<td>43</td>
<td>L94 x Vada&lt;sup&gt;1&lt;/sup&gt;</td>
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<td></td>
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<td>Proctor x Nudinka&lt;sup&gt;4&lt;/sup&gt;</td>
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<td>67</td>
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<tr>
<td>6</td>
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<td>13</td>
<td>20</td>
<td>Lina x HS&lt;sup&gt;3&lt;/sup&gt;</td>
<td>-</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td>L94 x Vada&lt;sup&gt;1&lt;/sup&gt;</td>
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<td></td>
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<td></td>
<td></td>
<td>Prisma x Apex&lt;sup&gt;5&lt;/sup&gt;</td>
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<td>7</td>
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<td>19</td>
<td>36</td>
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<td>Total</td>
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<sup>1</sup> Qi *et al.* 1998; <sup>2</sup> P. Lindhout, pers. comm.; <sup>3</sup> Ramsay *et al.* 2000; <sup>4</sup> Becker *et al.* 1995; <sup>5</sup> Yin *et al.* 1999; <sup>6</sup>Tentative assignment (see text).