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A genetic linkage map of hexaploid naked oat constructed with SSR markers

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ABSTRACT

Naked oat is a unique health food crop in China. Using 202 F_2 individuals derived from a hybrid between the variety 578 and the landrace Sanfensan, we constructed a genetic linkage map consisting of 22 linkage groups covering 2070.50 cM and including 208 simple sequence repeat (SSR) markers. The minimum distance between adjacent markers was 0.01 cM and the average was 9.95 cM. Each linkage group contained 2–22 markers. The largest linkage group covered 174.40 cM and the shortest one covered 36.80 cM, with an average of 94.11 cM. Thirty-six markers (17.3%) showing distorted segregation were distributed across linkage groups LG5 to LG22. This map complements published oat genetic maps and is applicable for quantitative trait locus analysis, gene cloning and molecular marker-assisted selection.

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1. Introduction

Naked oat (*Avena nuda* L., AACCDD, $2n = 21$, $x = 7$) is an important health food crop characterized by high protein and β-glucan contents [1,2]. Oats are the basis of various food products including oatmeal, oat milk, and oat chocolate. Oats also serve as feed in some regions. In recent years, molecular marker-based genetic maps have been developed in diploid [3,4], tetraploid [5], and hexaploid [6–14] oats, based primarily on RFLP, AFLP, and DArT markers.

Following the first genetic linkage map of Kanota × Ogle (KO) published in 1995 [6], there have been continued efforts to increase the density of the map with various kinds and numbers of markers. The most comprehensive map of hexaploid hulled oat with 1166 markers covers 1890 cM in the latest reports [7,8,12]. Several other mapping populations were developed from either Kanota or Ogle to enrich the linkage maps, such as the KM (Kanona × Marine), OT (Olge × TAM) and OM (Olge × MAM) maps which cover 736–2049 cM [8–10]. Other mapping populations were developed from Terra × Marion, MN841801-1 × Noble-2 and UFRGS7 × UFRGS910906 [13,15,16]. The first doubled haploid (DH) population used to construct a genetic linkage map in oat was produced in 2008 [14]. The map was improved by increasing the number of markers from 625 to 1058, and eventually covered 1688 cM [17]. More and new kinds of markers, such as SNPs, were used in constructing more recent genetic maps [18,19]. However, the map is still lacking in SSR markers. In this study, our aim was to use a new mapping population of 202 F_2 individuals from a cross of the naked variety 578 and the landrace Sanfensan to

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construct a genetic linkage map based on SSR markers. Our objective was to produce a genetic reference map in a Chinese hexaploid naked oat population.

2. Materials and methods

2.1. Establishing a mapping population

Naked oat variety 578 (large grain) from Bashang, Hebei Province, as male parent was crossed with the landrace Sanfensan (small grain) from Datong, Shanxi, China in 2009. The F_1 was grown in 2010 and an F_2 mapping population containing 202 individuals was generated in 2011. The population showed clear segregation for agronomic traits, particularly grain size.

2.2. Sources of simple sequence repeat (SSR) primers

A total of 4024 pairs of simple sequence repeat (SSR) primers were selected, of which 3600 pairs were developed in the Minor Crop Laboratory, Institute of Crop Science, Chinese Academy of Agricultural Sciences [18], 200 pairs were based on the hexaploid oat transcriptome [19], 124 pairs were from the literature, and 100 primer pairs were from wheat.

2.3. Screening SSR primers and detecting genotypes by PCR

Genomic DNA was extracted from young leaves of field-grown plants by the CTAB method [20]. The PCR equipment was manufactured by Bio-Rad. In a 20 μL system for PCR amplification, the reaction solution contained 50 ng of DNA,

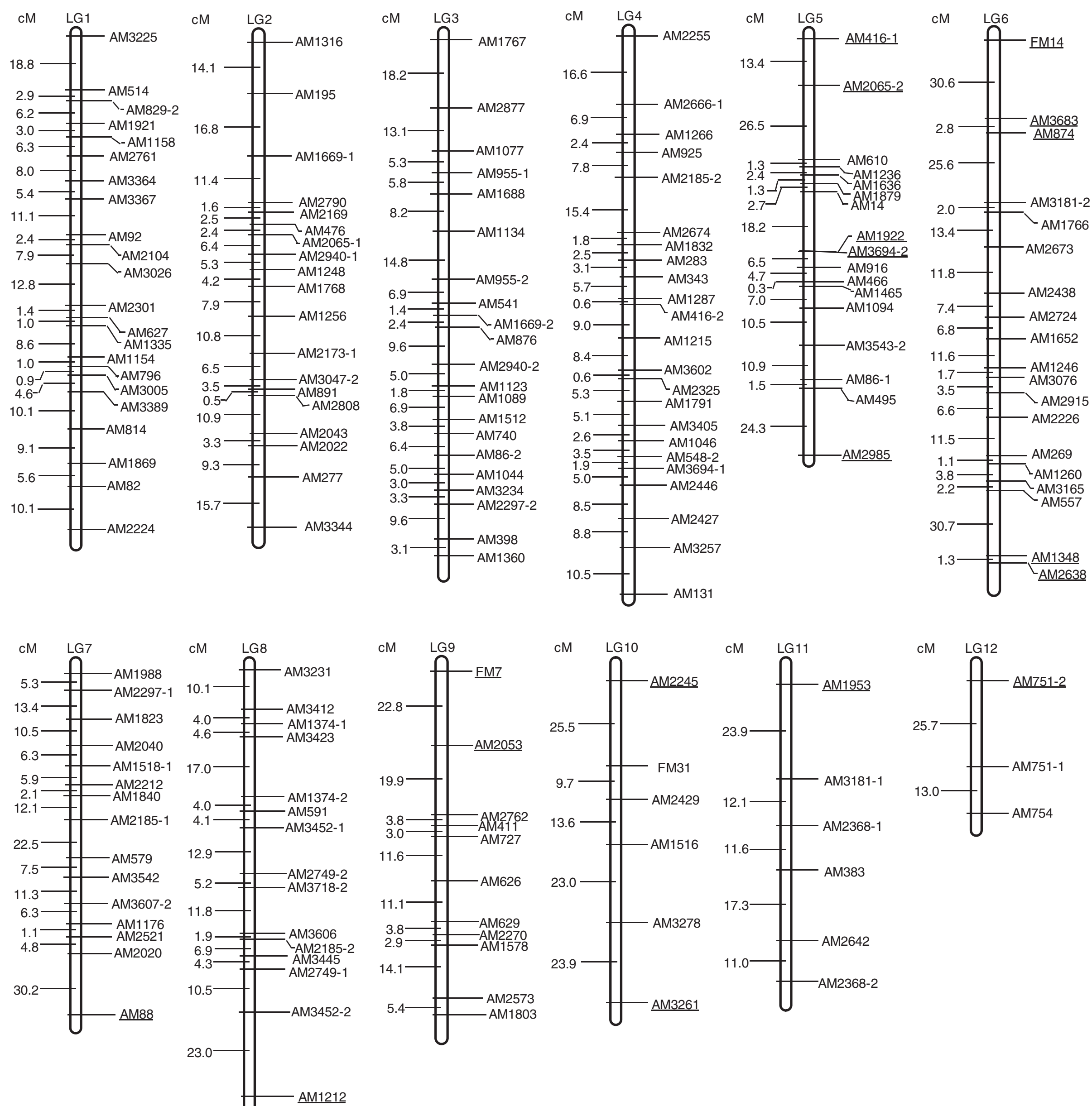

Fig. 1 – Genetic linkage map of naked oat constructed with SSR markers. Markers with distorted segregation are underlined.

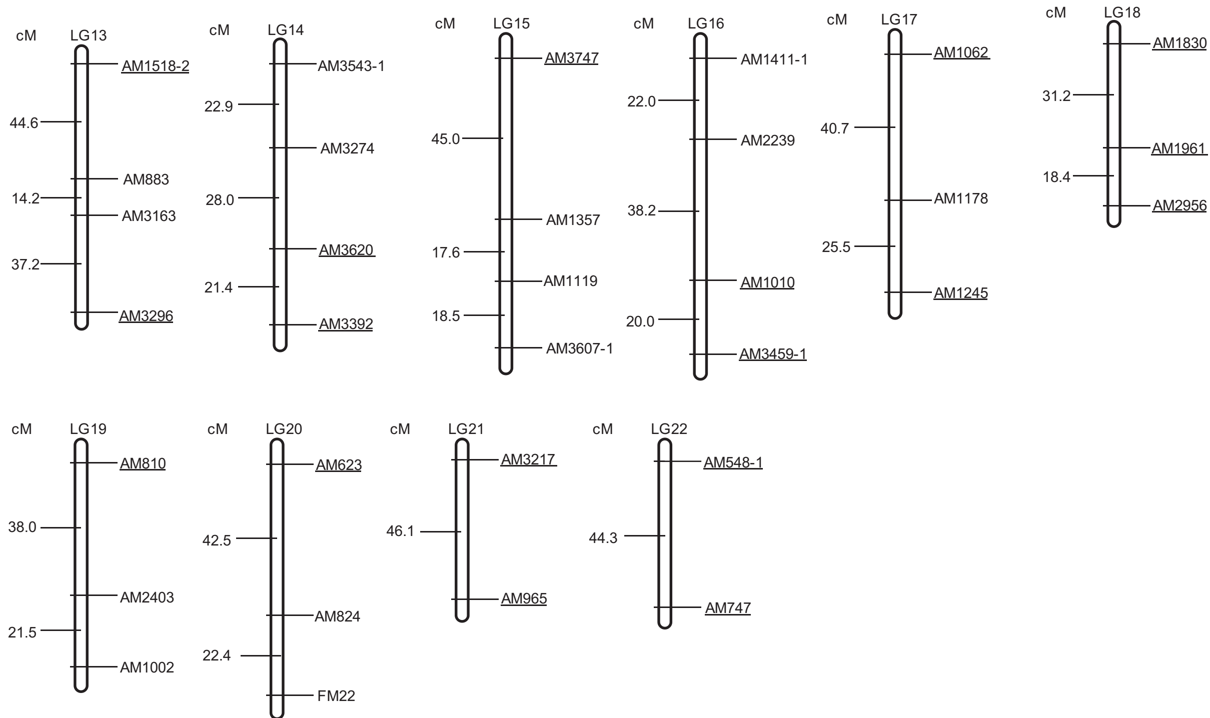

Fig. 1 (*continued*).

1.5 mmol L^{-1} $MgCl_2$, 0.2 mmol L^{-1} dNTP, 1.0 U *Taq* DNA polymerase, and 0.1 μmol L^{-1} primer. The program for PCR was denaturation at 94 °C for 5 min, followed by 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 60 s. The cycle was repeated 30 times followed by extension at 72 °C for 10 min, and then cooling at 4 °C. Amplified products were detected on 8% non-degenerating polyacrylamide gels (PAGE). Electrophoresis was run for 2.5 h at 200 V with an electric current of 500 mA, and staining with silver nitrate. Polymorphic primers were chosen to genotype the F_2 individuals. For analysis the male parent allele was encoded as “A” and the contrasting allele from the female parent as “B”. The heterozygote was recorded as “H”. Missing or null alleles were recorded as “–”.

2.4. Data analysis and map construction

Chi-squared tests for goodness-of-fit to expected codominant Mendelian segregation ratios (1:2:1) were applied to segregation data for each SSR marker. JoinMap 4.0 software was used to construct the genetic linkage map by estimating the recombination values and map distances [21]. Linkage groups were established using a minimum LOD score of 3.0. Loci with skewed genetic ratios were not included in map construction. MapDraw was used to produce the genetic map [22].

3. Results

Of 4024 pairs of SSR primers screened, 277 were polymorphic between the two parental genotypes. Among them, 229 were polymorphic between individuals of the F_2 population. A total of 256 polymorphic loci were detected, as 27 primers each detected two loci. A genetic linkage map was constructed using data for these polymorphic loci. The map, containing 208 loci and comprising 22 linkage groups (LGs), was 2070.5 cM in length. The largest linkage group covered 174.40 cM and the shortest 36.80 cM, and the mean length was 94.11 cM. Among 208 loci mapped, 4 were detected by SSR markers from a previous study, 10 were from the oat seed transcriptome, and 194 were from markers developed in our laboratory. None of the wheat SSR markers was polymorphic.

The two largest linkage groups (LG1 and LG4) contained 22 loci and the two smallest groups (LG21 and LG22) contained only two. The average number of loci in each linkage group was 9.45. The minimum distance between two loci was 0.01 cM. The average map distance between two loci was 9.95 cM (Fig. 1). Thirty six loci segregated with distorted genetic ratios. They were distributed across LG5 to LG22 (Table 1). The large LG5 had loci with distorted ratios not only in terminal but in central regions. Fifteen of the 27 two-locus SSRs were mapped in the linkage groups, 4 pairs of loci in the same linkage group and 11 in different linkage groups (Table 2).

4. Discussion

4.1. Distorted segregation

Distorted segregation has been commonly found in previous studies in oats and other crops and could have been caused by chromosomal rearrangements associated with introgression

Table 1 – Distribution of markers showing segregation distortion across linkage groups.

Linkage group	Number of markers	Number of markers showing distorted segregation	Percentage (%)	Marker name
LG5	17	5	29	AM416-1, AM2065-2, AM1922, AM3694-2, AM2985
LG6	19	5	26	FM14, AM3683, AM1348, AM2638, AM874
LG7	15	1	7	AM88
LG8	15	1	7	AM1212
LG9	11	2	18	FM7, AM2053
LG10	6	2	33	AM2245, AM3261
LG11	6	1	17	AM1953
LG12	3	1	33	AM751-2
LG13	4	2	50	AM1518-2, AM3296
LG14	4	2	50	AM3620, AM3392
LG15	4	1	25	AM3747
LG16	4	2	50	AM1010, AM3459-1
LG17	3	2	67	AM1062, AM1245
LG18	3	3	100	AM830, AM1961, AM2956
LG19	3	1	33	AM810
LG20	3	1	33	AM623
LG21	2	2	100	AM3217, AM966
LG22	2	2	100	AM548-1, AM747

of alien segments or with gametophytic genes preference [13,23–25]. In the genetic linkage map of hexaploid oat developed by Zhu and Kaeppler, 9.0% of AFLP and RAPD markers showed distorted segregation [10]. Among 208 loci mapped on the present linkage groups, 36 (17.3%) showed significantly distorted segregation ratios. The distribution of loci with distorted segregation on LG5 to LG22 of the present map (Table 1), was somewhat different from the more clustered arrangement reported by de Koeyer et al. [13]. This difference may be associated with the SSR markers used in the current study not being targeted to particular chromosomes or regions of the oat genome.

4.2. The size of the current linkage map

Following the development of molecular technologies in major crops such as corn, wheat and soybean, several genetic linkage maps have been constructed for diploid and hexaploid oats. In most previous oat linkage maps, the size ranged from 1396.7 to 1688.0 cM, and the maps comprised 19–34 linkage groups with an average length of 49.65–88.31 cM [9,10,17,26,27]. Our map containing 22 linkage groups and covering 2070.5 cM is larger than most previous maps, except that produced by Barbosa et al. [16], which covers 3000 cM on 37 linkage groups with an average length of 81.08 cM. A larger linkage map may cover more of the genome.

4.3. SSR markers and linkage groups

SSR markers proved effective in constructing a genetic linkage map for naked oat in an F_2 population. Of the 4024 SSRs, 5.1% (208) were mapped on linkage groups, including 15 two-locus SSRs (Table 2). Marker duplication on different chromosomes may be indicative of homoeologous genes. However, four such duplications in the same linkage group in the present study were not suggestive of duplicated loci in different linkage groups. Markers were not evenly distributed across linkage groups, a feature also noted in other studies. The largest linkage groups, LG1 and LG4, each had 22 markers, and the smallest, LG21 and LG22, each had only two. This disparity may reflect the size of the oat genome and the limited number of markers on the map. Some parts of the genome may also be more gene-rich than others. The interval between markers on the map developed by Wight et al. [12] was 0.76 cM, based on 1176 markers. The large gaps in the present map will need to be reduced with additional markers. The present study was the first attempt to construct a linkage map for naked oat using SSR markers. Eight pairs of SSR markers based on the

Table 2 – Primers with two polymorphic loci and their distribution in linkage groups.

Marker name	Linkage group	Marker name	Linkage group	Marker name	Linkage group
AM416-1	LG5	AM1669-1	LG2	AM3181-2	LG6
AM416-2	LG4	AM1669-2	LG3	AM3452-1 & -2	LG8
AM548-1	LG22	AM2065-1	LG2	AM3543-1	LG14
AM548-2	LG4	AM2065-2	LG5	AM3543-2	LG5
AM751-1 & -2	LG12	AM2185-1	LG7	AM3607-1	LG15
AM955-1 & -2	LG3	AM2185-2	LG8	AM3607-2	LG7
AM1374-1 & -2	LG8	AM2297-1	LG7	AM3694-1	LG4
AM1518-1	LG7	AM2297-2	LG3	AM3694-2	LG5
AM1518-2	LG13	AM3181-1	LG11		

oat seed transcriptome and four markers from a previous study were added to the present map. These markers may be useful in studies of grain traits and for integrating different genetic linkage maps. No marker developed in wheat gave useful polymorphisms when used in oats, owing presumably to the lack of a sufficiently close genetic relationship.

5. Conclusion

The first genetic linkage map involving 208 SSR markers was developed in hexaploid naked oat using the Sanfensan × 578 F_2 population. The map contains 22 linkage groups and covers 2070.5 cM and can be used in QTL mapping, gene cloning, and marker-assisted breeding in naked oat.

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