**Genomic relationships among sixteen Avena species based on (ACT)\textsubscript{6} trinucleotide repeat FISH**

<table>
<thead>
<tr>
<th>Journal</th>
<th>Genome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manuscript ID</td>
<td>gen-2017-0132.R2</td>
</tr>
<tr>
<td>Manuscript Type</td>
<td>Article</td>
</tr>
<tr>
<td>Date Submitted by the Author</td>
<td>06-Nov-2017</td>
</tr>
</tbody>
</table>
| Complete List of Authors | Luo, Xiaomei; Sichuan Agricultural University  
Tinker, Nicholas; Ottawa Research and Development Centre  
Zhou, Yong-Hong; Sichuan Agricultural University  
Wight, Charlene; Ottawa Research and Development Centre  
Liu, Juncheng; Sichuan Agricultural University  
Wan, Wenlin; Sichuan Agricultural University  
Chen, Liang; Sichuan Agricultural University  
Peng, Yuanying; Triticeae Research Institute of Sichuan Agricultural University, |
| Is the invited manuscript for consideration in a Special Issue? | N/A |
| Keyword | Genetic diversity, Oat, Oligonucleotide, Chromosome markers |
Genomic relationships among sixteen Avena species based on (ACT)$_6$ trinucleotide repeat FISH

Xiaomei Luo¹*, Nick A. Tinker², Yonghong Zhou³, Charlene P. Wight², Juncheng Liu¹, Wenlin Wan¹, Liang Chen¹, Yuanying Peng³

¹ College of Forestry, Sichuan Agricultural University, Huimin Road 211, Wenjiang District 611130, Chengdu City, Sichuan Province, China;
² Ottawa Research and Development Centre, Agriculture and Agri-Food Canada, KW Neatby Bldg., Central Experimental Farm, 960 Carling Avenue, Ottawa, ON K1A 0C6, Canada;
³ Triticeae Research Institute, Sichuan Agricultural University, Huimin Road 211, Wenjiang District 611130, Chengdu City, Sichuan Province, China

* Corresponding author: Phone: 86-028-86291456 E-mail: xiaomei_luo@sicau.edu.cn
Abstract Knowledge of the locations of repeat elements could be very important in the assembly of genome sequences and assignment to physical chromosomes. Genomic and species relationships among sixteen species were investigated using fluorescence in situ hybridization (FISH) with the Am1 and (ACT)_6 probes. The Am1 oligonucleotide probe was particularly enriched in the C genomes, whereas the (ACT)_6 trinucleotide repeat probe showed a diverse distribution of hybridization patterns in the A, AB, C, AC, and ACD genomes but might not be present in the B and D genomes. The hybridization pattern of *A. sativa* was very similar to that of *A. insularis*, indicating that this species most likely originated from *A. insularis* as a tetraploid ancestor. Although the two FISH probes failed to identify relationships of more species, this proof-of-concept approach opens the way to the use of FISH probes in assigning other signature elements from genomic sequence to physical chromosomes.

Keywords: Genetic diversity; Oat; Oligonucleotide; Chromosome markers
Introduction

The genus *Avena* comprises 29 species with three ploidy levels (Baum 1977; Baum and Fedak 1985a, b). This genus includes two divergent diploid genomic groups (the A and C genomes). Based on genomic research, \( A_c, A_l, A_d, A_s, A_p, C_p, \) and \( C_v \) are used to provide detailed descriptions of subtle chromosomal alterations (Rajhathy and Thomas 1974). The A, B and D genomes are closely related to one another (Jellen et al. 1994; Katsiotis et al. 1997; Linares et al. 1998). Additionally, the B and D genomes seem to be derivatives of the A genome (Rajharthy and Thomas 1974). Hybridization and chromosome doubling has produced polyploid species with AB, AC and ACD genomes (Rajhathy 1991). Different hypotheses have been advanced regarding the potential diploid and tetraploid progenitors of these polyploid species.

The AB genome species have two possible origins. The first possibility is \( A_x \) genome duplication of an *A. hirtula*, *A. wiestii*, or *A. lusitanica* diploid progenitor (Ladizinsky and Zohary 1968; Sadasivaiah and Rajharthy 1968; Katsiotis and Forsberg 1995; Nikoloudakis et al. 2008). AB genomes with this origin include *A. barbata*, *A. vaviloviana*, and *A. abyssinica* because these three species hybridize with both one another and other *Avena* species (Leggett and Markland 1995). The second possibility is the hybridization of an allopolyploid of \( A_x A_y \) genome species, in which case the A and B genomes remained distinct (Rajharthy and Morrison 1959; Fominaya et al. 1988). This origin of the AB genome includes only *A. agadiriana*. This species shares several morphological similarities with *A. canariensis*, *A. magna* and *A. murphyi* as well as with some hexaploids; furthermore, the species crosses easily with these hexaploids, which might indicate that *A. agadiriana* participated in hexaploid evolution (Thomas 1989; Badaeva et al. 2010a).

The ACD genome species also have two possible origins. The first possibility is the fusion of
ancestral C genome diploids with an ancestral A genome diploid to create ancestral AxC tetraploids.


The Avena species relationships and origins have been widely studied by the above molecular and cytological approaches. Fluorescence in situ hybridization (FISH) has also been successfully used to identify the C genome of Avena species (Fominaya et al. 1995; Nikoloudakis and Katsiotis 2008; Chew et al. 2016; Liu et al. 2017). Probable diverged A (D) genome ancestors include A. longiglumis, A. canariensis, A. wiestii, and A. atlantica (Luo et al. 2014; Chew et al. 2016; Liu et al. 2017).

The Avena species relationships and origins have been widely studied by the above molecular and cytological approaches. Fluorescence in situ hybridization (FISH) has also been successfully used to identify the C genome of Avena species (Fominaya et al. 1995; Nikoloudakis and Katsiotis 2008), to detect A genomes (Linares et al. 1998), to discriminate AB genomes (Katsiotis et al. 1997; Irigoyen et al. 2001; Badaeva et al. 2010a), to analyse AC genomes (Shelukhina et al. 2007), to compare species with A genomes (Shelukhina et al. 2008), to explore D genome origins (Luo et al. 2014, 2015), to distinguish ACD genomes (Irigoyen et al. 2002; Sanz et al. 2010), to characterize A. macrostachya (Badaeva et al. 2010b), and to examine genomic and species relationships among Avena taxa (Katsiotis, 2000; Tomas et al. 2016). These FISH probes are usually several hundred base pairs in length, such as

Oligonucleotides, except for (AC)$_{10}$, have rarely been used as FISH probes in Avena species (Fominaya et al. 2017), but they have been widely used for species of other genera, including Triticum aestivum (Cuadrado et al. 2008; Tang et al. 2014), Secale cereale (Fu et al. 2015), Leymus mollis (Yang et al. 2015), Aegilops triuncialis (Mirzaghaderi et al. 2014), and Thinopyrum intermedium (Li et al. 2016). Since none of the genomes in Avena have been published, knowledge of the locations of repeat elements could be very important in the assembly of genome sequences and assignment to physical chromosomes. To develop more chromosome markers to describe the relationships among Avena species within the diploid, tetraploid and hexaploid groups, we have undertaken a FISH analysis testing the oligonucleotide Am1 and trinucleotide repeat (ACT)$_6$ probes.

Materials and methods

Plant materials and chromosome preparation

Details of the species, geographic distributions, and numbers of accessions and genomic constitutions of the sixteen Avena species are given in Table 1. Seeds were germinated on sterile wet filter paper under controlled temperature and light conditions (25 °C, 14 h, light and 20 °C, 10 h, dark). When the seedling were 1.5-2.0 cm in length, the root tips were excised, fully immersed in nitrous oxide for 4 h and then stored in 70% ethanol (Kato et al. 2004). Approximately 1 mm of the root-tip meristem was cut and treated with cellulose and pectinase; then, the suspension was dropped onto slides (Komuro et al. 2013). After air drying, the slides were examined using an Olympus CX21 (Olympus, Japan) and then stored at -20 °C prior to use.
DNA probe preparation

Two probes were used for the FISH analysis. The first probe was oligonucleotide Am1, which contained a 51-bp fragment with the sequence 5’
GATCCATGTGTTTGTGGAAGACACACATGCAATGACTCTAGTGGTT 3’ and was described by Fominaya et al. (1995). The second probe was the trinucleotide repeat (ACT)$_6$, which contained an 18-bp fragment with the sequence 5’ACTACTACTACTACTACT 3’ and was described by Cuadrado et al. (2008). The oligonucleotide probes were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). The synthetic oligonucleotide Am1 was 5’-end-labelled with 6-carboxytetramethylrhodamine (TAMRA), whereas the other synthetic trinucleotide repeat (ACT)$_6$ was 5’-end-labelled with 6-carboxyfluorescein (FAM). These synthesized probes were diluted using 1× TE solution; the concentration was maintained at 10 µM, and the solution was stored at -20 °C.

FISH analysis

FISH with two probes was perfected as described by Hao et al. (2013). The chromosome preparations were fixed with 4% (w/v) paraformaldehyde, washed with 2× saline sodium citrate (SSC), and dehydrated using an ethanol series before air drying. Deionized formamide (FA; 60 µL) was added to the chromosome preparations, which were then denatured for 2 min at 80 °C and placed in an ethanol series at -20 °C before air drying. A hybridization mixture (10 µL) containing 0.35 µL trinucleotide repeat (GAA)$_6$, 4.825 µL of 2× SSC and 4.825 µL of 1× TE was applied to each chromosome preparation. The preparations were covered with glass coverslips, and the chromosomes and probes were hybridized at 37 °C in a humidity chamber for 1-2 h. The preparations were then rinsed twice for 5 min with 2× SSC at room temperature and finally with ddH$_2$O. The preparations were counterstained with 4, 6-diamidino-2-phenylindole (DAPI, Vector Laboratories, Inc., Burlingame, VT, USA).
slides were examined using an Olympus BX-51 microscope coupled to a Photometric SenSys Olympus DP70 CCD camera (Olympus, Japan). The raw images were processed using Photoshop ver. 7.1 (Adobe Systems Incorporated, San Jose, CA, USA) using only functions that affected the entire image equally. Three to five spreads were observed from each species with corresponding signal results.

Results

FISH analysis of mitotic metaphase plates from sixteen *Avena* species

FISH analysis of the mitotic metaphase plates of six (AA) *Avena* diploids illustrated in Fig. 1. The trinucleotide repeat probe (ACT)$_6$ is visible in Figs. 1a-1f (green). The (ACT)$_6$ probe produced single intercalary bands (arrows). These bands were relatively weak in intensity but were reproducible near, in the middle or at the termini of the chromosomes. Two signals were observed near the termini of the chromosomes, with one each in the A$_s$ genome (Fig. 1a, *A. wiestii*) and A$_l$ genome (Fig. 1b, *A. longiglumis*). Four signals were observed near or in the middle of the A$_s$ genome chromosomes (Fig. 1c, *A. nuda*), whereas two signals were observed near or in the middle of the A$_c$ genome chromosomes (Fig. 1d, *A. nuda*; Fig. 1e, *A. strigosa*) and in the A$_c$ genome chromosomes (Fig. 2f, *A. canariensis*). A summary of these results is as follows: (i) two signals were detected near the chromosome termini in the A$_l$ genome; (ii) two signals were detected near or in the middle of the A$_c$ genome chromosomes; and (iii) two and four signals were detected near or in the middle of the chromosomes and two signals were detected near the chromosome termini in the A$_s$ genome. These results suggest that the distribution of (ACT)$_6$ differs among the A$_l$, A$_c$ and A$_s$ genomes. Additionally, the results suggest that the A$_s$ genome is diverse.

FISH analysis of the mitotic metaphase plates of three (AABB) *Avena* tetraploids is illustrated in Fig. 2. The probe (ACT)$_6$ is visible in Figs. 2a-2c (green). The (ACT)$_6$ probe produced single intercalary
bands (arrows). These bands were relatively weak in intensity but were reproducible near the middle or at the termini of the chromosomes. Two signals were observed at the chromosome termini, and two signals were observed in the middle of the chromosomes in the AB genome (Fig. 2a, A. barbata). Four signals were observed near the middle of chromosomes in the AB genome (Fig. 2b, A. abyssinica; Fig. 2c, A. vaviloviana). These results suggest that the distribution of (ACT)$_6$ differs between the A. barbata genome and the A. abyssinica and A. vaviloviana genomes. Furthermore, the results suggest that the AB genome is variable.

FISH analysis of the mitotic metaphase plates of two (CC) Avena diploids is illustrated in Fig. 3. The probe (ACT)$_6$ is visible in Figs. 3a-3c (green). The (ACT)$_6$ probe produced single intercalary bands (white arrows). These bands were particularly intense but were reproducible near the middle or at the termini of the chromosomes. Two signals were observed near the chromosome termini, and four signals were observed near the middle of the chromosomes in the C genome (Figs. 3a, A. ventricosa; Figs. 3b-3c, A. eriantha). These results suggest that the distribution of (ACT)$_6$ is similar between A. ventricosa and A. eriantha. Interestingly, two abnormal chromosomes were observed in Fig. 3c (A. eriantha, red arrows). The long chromosome was approximately five times longer than the short chromosome. One obvious constriction was observed near the terminus of the long chromosome arm, and the other obvious constriction was observed near the terminus of the short chromosome arm. The abnormal chromosomes were observed in only one metaphase cell, but this observation still suggests that the A. eriantha chromosome structure is unstable.

FISH analysis of the mitotic metaphase plates of three (AACC) Avena tetraploids is illustrated in Fig. 4. The probe (ACT)$_6$ is visible in Figs. 4a-4f (green), whereas the oligonucleotide probe Am1 is visible in Figs. 4b, 4d, and 4f (red). The (ACT)$_6$ probe produced single intercalary bands (arrows), whereas the
Am1 probe produced signals that were particularly enriched in the C genome chromosomes. Fourteen signals were observed near or in the middle of the AC genome chromosomes (Figs. 4a, *A. insularis*). Eight signals were observed near or in the middle of the A genome chromosomes, whereas six strong signals were observed near or in the middle of the C genome chromosomes (Fig. 4b). Two signals were observed at the chromosome termini, and ten signals were detected near or in the middle of the AC genome chromosomes (Fig. 4c, *A. magna*). Four signals were observed near or in the middle of the A genome chromosomes, two signals were observed at the A chromosome termini, and six signals were observed near or in the middle of the C genome chromosomes (Fig. 4d, *A. magna*). Two signals were observed at the chromosome termini, and ten signals were detected near or in the middle of the AC genome chromosomes (Fig. 4e, *A. murphyi*). Four signals were detected near or in the middle of the A genome chromosomes, whereas six signals were observed near or in the middle of the C genome chromosomes, and two weak signals were observed at the C genome chromosome termini (Fig. 4f, *A. murphyi*). Interestingly, two signal loci were observed close to the middle or at the terminus of one chromosome (Figs. 4c-4d, *A. magna*; 4e-4f, *A. murphyi*). These results suggest that the distribution of (ACT)$_6$ differs among the *A. insularis*, *A. magna* and *A. murphyi* genomes. Furthermore, the results suggest that the AC genome is variable.

FISH analysis of the mitotic metaphase plates of two (AACCDD) *Avena* hexaploids is illustrated in Fig. 5. The probe (ACT)$_6$ is visible in Figs. 5a-5b (green), whereas the probe Am1 is visible in Figs. 5b and 5d (red). The (ACT)$_6$ probe produced single intercalary bands (arrows). These bands were particularly intense but were reproducible in the middle of the chromosomes. Fourteen signals were observed in the middle of the ACD genome chromosomes (Figs. 5a, *A. sativa*). Eight signals were observed in the middle of the AD genome chromosomes, while six were observed in the middle of the
C genome chromosomes (Figs. 5b, *A. sativa*). Twelve signals were observed in the middle of the ACD

| genome chromosomes (Figs. 5c, *A. fatua*). Six signals were observed in the middle of the AD genome chromosomes, whereas six were observed in the middle of the C genome chromosomes (5d, *A. fatua*).

| These results suggest that the distribution of (ACT)$_{10}$ is similar between *A. sativa* and *A. fatua*.

Furthermore, the results suggest that the ACD genome is conserved.

**Summary of the FISH signal patterns of sixteen *Avena* species**

A summary of the FISH signal patterns of sixteen *Avena* species based on the results shown in Fig. 1 to Fig. 5 is illustrated in Fig. 6. The sixteen species were sorted into five groups (A, AB, C, AC, and ACD) based on the genome constitution of the species in the group. To visually display the relationships among the species, the probed chromosomes are presented with the species. The signal loci were
distributed near or in the middle of the chromosomes (M) or at the chromosome termini (T). Group A includes three subgroups: (i) 2AT, including *A. wiestii* (A$_s$) and *A. longiglumis* (A$_l$); (ii) 2AM, including *A. nuda* (A$_s$), *A. strigosa* (A$_s$), and *A. canariensis* (A$_c$); and (iii) 4AM, including *A. brevis* (A$_s$). Group AB includes two subgroups: (i) 2M2T, including *A. barbata*, and (ii) 4M, including *A. abyssinica* and *A. vaviloviana*. Group C includes *A. ventricosa* and *A. eriantha* (4CM2CT). Group AC includes three subgroups: (i) 8AM6CM, including *A. insularis*; (ii) 4AM2AT6CM, including *A. magna*; and (iii) 4AM6CM2CT, including *A. murphyi*. Group ACD includes *A. sativa* and *A. fatua* (8ADM6CM and 6ADM6CM). Interestingly, two signal loci were observed close to the middle or at the terminus of one chromosome each in *A. magna* and *A. murphyi* (2 signals on 1 chromosome). These results suggest that the distribution of the (ACT)$_{10}$ signal loci among the sixteen *Avena* species is diverse.

The results from groups A and AB suggest that (i) 2M2T in *A. barbata* may have originated from 2AT in *A. wiestii* (A$_s$) and *A. longiglumis* (A$_l$) as well as 2AM in *A. nuda* (A$_s$), *A. strigosa* (A$_s$), and *A.
canariensis (A<sub)c</sub>), (ii) 4M in *A. abyssinica* and *A. vaviloviana* may have originated from 4AM in *A.

brevis* (A<sub>s</sub>), and (iii) probe (ACT)<sub>6</sub> might not be present in the B genomes. The results from groups C

and AC suggest that (i) 4CM in *A. insularis*, *A. magna* and *A. murphyi* may have originated from 4CM

in *A. ventricosa* and *A. eriantha* and (ii) 2CT in *A. murphyi* may have originated from 2CT in *A.

ventricosa* and *A. eriantha*. The results from groups A and AC suggest that (i) 2AM in *A. insularis*, *A.

magna* and *A. murphyi* may have originated from 2AM in *A. nuda* (A<sub>s</sub>), *A. strigosa* (A<sub>s</sub>), and *A.

canariensis* (A<sub>c</sub>) and (ii) 4AM in *A. insularis*, *A. magna* and *A. murphyi* may have originated from

4AM in *A. brevis* (A<sub>s</sub>). The AC and ACD results suggest that 8ADM6CM in *A. sativa* may have

originated from 8AM6CM in *A. insularis*, and (iii) probe (ACT)<sub>6</sub> might not be present in the D

genomes.

In total, the hybridization patterns for the Am1 and (ACT)<sub>6</sub> probes showed that (i) probe Am1 was

particularly enriched in the C genome, and (ii) probe (ACT)<sub>6</sub> showed large loci in the C genome and

was distributed in the A, AB, C, AC, and ACD genomes but might not be present in the B and D

genomes.

**Discussion**

Oligonucleotide probes Am1 and (ACT)<sub>6</sub> revealed diverse patterns among the A, AB, C, AC, and ACD

genomes. This result provides key information that can be used in the physical assignment of genome

sequences to chromosomes. Moreover, this proof-of-concept approach opens the way to the use of

FISH probes in assigning other signature elements from genomic sequence to physical chromosomes.

Further discussion will focus on the following points: (i) the diverse patterns of the A, AB, C, AC, and

ACD genomes and (ii) the genetic relationships between *Avena* species and the hexaploid oat origins.

**Diverse patterns of the A, AB, C, AC, and ACD genomes**
Recently, FISH has been widely shown to be an important and effective tool for the characterization
and assessment of intraspecific and interspecific genetic relationships in *Avena* (Irigoyen et al. 2001;
Badaeva 2010a, b; Luo et al. 2014, 2015). The oligonucleotide probe Am1 was particularly enriched in
the C genome. This probe contained only 51 base pairs but could still identify the C genome in the
present study, thus functioning nearly the same as the 464 bp pAm1 (Solano et al. 1992; Sanz et al.
2010). The trinucleotide repeat probe (ACT)$_6$ also showed large loci in C genome. Fominaya et al.
(2017) reported a preferential distribution of (AC)$_{10}$ in the pericentromeric and interstitial regions of A
and D genome chromosomes and in the pericentromeric heterochromatic regions of C genome
chromosome. The location of the trinucleotide repeat probe (ACT)$_6$ loci at either the middle or termini
(or even at both the middle and terminus of an individual chromosome) show the diverse distribution of
hybridization patterns in *Avena* species.

The (ACT)$_6$ signal pattern was relatively constant in the C and ACD genomes. The C and ACD
genomes contained only two species each in this study, which may make the (ACT)$_6$ signal pattern an
easy trend to recognize. However, the observation of abnormal chromosomes in *A. eriantha* indicates
that structurally unstable chromosomes still retain the 6CM signal loci. Chew et al. (2016) also found
that all hexaploids and C genome diploids might remain unchanged and form two major clusters, which
was consistent with our results. The (ACT)$_6$ signal pattern was more diverse in the A, AB and AC
genomes. Three (ACT)$_6$ signal patterns were observed in the A genomes and even in the A$_s$ genome.
Differences among the A genomes of diploid species have also been characterized using cytological
studies (Rajhathy and Thomas 1974; de la Hoz and Fominaya 1989; Thomas 1992) and molecular
studies (Fu and Williams 2008; Chew et al. 2016; Liu et al. 2017). Two (ACT)$_6$ signal patterns were
observed in the AB genomes. Irigoyen et al. (2001) found that *A. vaviloviana* gave a strong smear,
whereas *A. barbata* gave a weak smear in which pronounced bands were found. Badaeva et al. (2010a) found that *A. abyssinica* and *A. vaviloviana* had a clear C band in the middle of the short arm, which was either weaker or absent in *A. barbata*. The difference between *A. abyssinica*, *A. vaviloviana* and *A. barbata* is consistent with our results. Three (ACT)$_6$ signal patterns were observed in the AC genomes. Differences were found among *A. insularis*, *A. magna* and *A. murphyi* in the present study. Previously, *A. insularis* was distinguished from *A. magna* and *A. murphyi* (Yan et al. 2016a), and *A. magna* was shown to be distinct from *A. murphyi* (Drossou et al. 2004), which is mostly consistent with our results.

The (ACT)$_6$ probe loci were distributed in the A, AB, C, AC, and ACD genomes but might not be present in B and D genomes based on the intensity and number of (ACT)$_6$ signals. The (ACT)$_6$ signals were weak and few in the A and AB genomes but strong and many in C, AC, ACD genomes. Comparing A and AB, the few differences in the signals indicate that (ACT)$_6$ loci might not be present in the B genome. Comparing AC and ACD, the few differences in the signal indicate (ACT)$_6$ loci might not be present in the D genome. Even if the (ACT)$_6$ signal present in the B and D genomes, it should be very weak and few in number. (ACT)$_6$ probe loci are found in the A genome but not the B or D genome, indicating that the A genome is different from the B and D genomes. Minor genetic differences have been discerned between the A, B and D genomes (Oinuma, 1953; Katsiotis et al. 1997). The A$_s$ 120a satellite DNA sequence may discriminate the A genome from the B and D genomes in AB genomes (Irigoyen et al. 2001) and ACD genomes (Linares et al. 1998; Sanz et al. 2010), which is consistent with our results. Based on the distribution of (ACT)$_6$ loci on the C genome chromosomes of diploid and polyploid *Avena* species, we propose a model for the chromosomal alterations that have occurred during the evolution of *Avena* species. This perspective is also supported by Badaeva (2010b) based on rDNA locus distributions.
Genetic relationships of *Avena* species and the hexaploid oat origin

Based on the (ACT)$_6$ signal patterns of the A and AB genomes, *A. barbata* may have originated from *A. nuda*, *A. strigosa*, and *A. canariensis* as well as *A. wiestii* and *A. longiglumis*, while *A. abyssinica*, *A. vaviloviana* may have originated from *A. brevis*. Genetic homogeneity existed between *A. barbata*, *A. wiestii* and *A. longiglumis* (Liu et al. 2017, Alicchio et al. 1995). Liu et al. (2017) inferred that *A. brevis* was related to *A. abyssinica* and *A. vaviloviana*. This result mostly agrees with our results. Nevertheless, Li et al. (2000) indicated that *A. strigosa* and *A. longiglumis* participated in the formation of *A. abyssinica*. This result contradicts our result. Based on the (ACT)$_6$ signal patterns of the A, C, and AC genomes, *A. insularis*, *A. magna* and *A. murphyi* may have originated from *A. brevis*, *A. wiestii*, *A. longiglumis*, *A. ventricosa* and *A. eriantha*. Liu et al. (2017) inferred that *A. murphyi* was related to *A. ventricosa* and *A. eriantha*. Nikoloudakis and Katsiotis (2008) revealed the close proximity of *A. ventricosa* to an active role in the evolution of tetraploid and hexaploid oats. This result supports our result. However, Leggett (1998) indicated that *A. eriantha* was unlikely to have participated in the formation of *A. magna*. Liu et al. (2017) inferred that *A. nuda* was related to *A. murphyi*. This result is different from our result. Based on the (ACT)$_6$ signal patterns of the AC and ACD genomes, *A. sativa* may have originated from *A. insularis*. This result is confirmed by cytological and fertility data (Ladizinsky 1998), cytogenetic analysis (Badaeva et al. 2010b), and phylogenetic analysis (Liu et al. 2017). However, doubts were cast on these findings by Shelukhina et al. (2007), Nikoloudakis et al. (2008), Peng et al. (2010), Yan et al. (2014).

The (ACT)$_6$ signal loci are observed at the chromosome termini in the A genome (*A. wiestii* and *A. longiglumis*), C genome (*A. ventricosa* and *A. eriantha*), AC genome (*A. magna* and *A. murphyi*), and AB genome (*A. barbata*) but not in the ACD genome (*A. sativa* and *A. fatua*), which indicates that
chromosome construction has exhibited gradual variation during evolution. Kellogg et al. (2004) put forward that this variation was the outcome of a set of highly active processes, including gene duplication and deletion, chromosomal duplication followed by gene loss, amplification of retrotransposons separating genes, and genome rearrangement; the latter process often follows hybridization or polyploidy. Rodrigues et al. (2017) suggested that the loss of the C genome sequence might be correlated with differences in the parental genome size in *Avena* polyploid species. Indeed, the C genome is larger than the A genome (Bennett 2012). Yan et al. (2016b) demonstrated that most of the polyploid species in *Avena* had experienced genome downsizing in relation to their diploid progenitors. Therefore, the (ACT)₆ signal loci at the chromosome termini are possibly lost during the evolution of *Arena* species hybridization or polyploidy.

**Conclusion**

The hybridization patterns of *A. sativa* are very similar to the pattern of *A. insularis*. Hence, the present results suggest that *A. insularis* might be the AC genome progenitor of *A. sativa*. Our study using only two FISH probes failed to indicate the relationships of more species. However, other oligonucleotide and trinucleotide repeat probes have been used to label *Avena* species with the aim of revealing the species and genomes of the *Avena* taxa. Our results provide key information that can be used in the physical assignment of genome sequences to chromosomes. Moreover, this proof-of-concept approach opens the way to the use of FISH probes in assigning other signature elements from genomic sequences to physical chromosomes.

**Acknowledgements**

This study was supported by the Natural Science Foundation of China (003Z1001). The authors greatly appreciate the American National Plant Germplasm System (Pullman, WA, USA) and Plant Gene
Resources of Canada (Saskatoon, SK, Canada) for providing the material for the investigation.

Compliance with Ethical Standards

Conflict of Interest: The authors declare that they have no conflict of interest.

References:


Legends

Fig. 1. FISH analysis of the mitotic metaphase plates from six AA *Avena* diploids: (a) *Avena wiestii*, (b) *Avena longiglumis*, (c) *Avena brevis*, (d) *Avena nuda*, (e) *Avena strigosa*, and (f) *Avena canariensis*. The chromosomes were probed with a 5’-FAM-labelled (ACT)$_6$ trinucleotide repeat (green) in all images. The (ACT)$_6$ signals are indicated with white arrows. All chromosomes were counterstained with DAPI. Scale bar = 5 µm.

Fig. 2. FISH analysis of the mitotic metaphase plates from three AABB *Avena* tetraploids: (a) *Avena barbata*, (b) *Avena abyssinica*, and (c) *Avena vaviloviana*. The chromosomes were probed with a 5’-FAM-labelled (ACT)$_6$ trinucleotide repeat (green) in all images. The (ACT)$_6$ signals are indicated with white arrows. All chromosomes were counterstained with DAPI. Scale bar = 5 µm.

Fig. 3. FISH analysis of the mitotic metaphase plates from two CC *Avena* diploids: (a) *Avena ventricosa* and (b-c) *Avena eriantha*. The chromosomes were probed with a 5’-FAM-labelled (ACT)$_6$ trinucleotide repeat (green) in all images. The (ACT)$_6$ signals are indicated with white arrows, whereas abnormal chromosomes are indicated with red arrows. All chromosomes were counterstained with DAPI. Scale bar = 5 µm.

Fig. 4. FISH analysis of the mitotic metaphase plates from three AACC *Avena* tetraploids: (a-b) *Avena insularis*, (c-d) *Avena magna*, and (e-f) *Avena murphyi*. The chromosomes were probed with a 5’-FAM-labelled (ACT)$_6$ trinucleotide repeat (green) in all images and with a 5’-TAMRA-labelled *Am1* oligonucleotide (red) in b, d and f. The trinucleotide repeat (ACT)$_6$ signals are indicated with white arrows. All chromosomes were counterstained with DAPI. Scale bar = 5 µm.

Fig. 5. FISH analysis of the mitotic metaphase plates from two AACCDD *Avena* hexaploids: (a-b) *Avena sativa* and (c-d) *Avena fatua*. The chromosomes were probed with a 5’-FAM-labelled (ACT)$_6$
trinucleotide repeat (green) in all images and with a 5’-TAMRA-labelled Am1 oligonucleotide (red) in
b and d. The (ACT)$_n$ signals are indicated with white arrows. All chromosomes were counterstained
with DAPI. Scale bar = 5 µm.

Fig. 6. Genetic relationships between the *Avena* species based on FISH signal patterns. Sixteen species
were sorted into five groups (A, AB, C, AC, and ACD) based on the genome constitution of the species
in the group. To visually display the relationships among the species, the probed chromosomes are
presented with the species. The signal loci are summarized into two types. One type has a signal
location near or in the middle of a chromosome (M), whereas the other type has a signal location near
or at the terminus of a chromosome (T). Additionally, the capital letters A and C indicate the genomes
of *Avena* (e.g., 2AM indicates signal loci near the middle of 2 chromosomes in the A genome).

Captions

Table 1 Summary of the sixteen *Avena* species included in the current study.
Table 1 Summary of sixteen *Avena* species included in current study.

<table>
<thead>
<tr>
<th>Species</th>
<th>Geographic distribution</th>
<th>No. of accessions</th>
<th>Genome</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Avena brevis</em></td>
<td>Canada, Ontario</td>
<td>Clav 9013</td>
<td>A_0A_0</td>
</tr>
<tr>
<td><em>Avena nuda</em></td>
<td>Netherlands</td>
<td>PI 401795</td>
<td>A_0A_0</td>
</tr>
<tr>
<td><em>Avena strigosa</em></td>
<td>Spain</td>
<td>PI 73584</td>
<td>A_0A_0</td>
</tr>
<tr>
<td><em>Avena wiestii</em></td>
<td>Egypt, Giza</td>
<td>PI 53626</td>
<td>A_0A_0</td>
</tr>
<tr>
<td><em>Avena canariensis</em></td>
<td>Spain</td>
<td>CN 6195</td>
<td>A_0A_0</td>
</tr>
<tr>
<td><em>Avena longiglumis</em></td>
<td>Canada, Ontario</td>
<td>Clav 9071</td>
<td>A_0A_1</td>
</tr>
<tr>
<td><em>Avena eriantha</em></td>
<td>Spain, Madrid</td>
<td>PI 367381</td>
<td>C_0C_0</td>
</tr>
<tr>
<td><em>Avena ventricosa</em></td>
<td>Morocco</td>
<td>PI 657337</td>
<td>C_0C_1</td>
</tr>
<tr>
<td><em>Avena abyssinica</em></td>
<td>Ethiopia</td>
<td>Clav 2519</td>
<td>AABB</td>
</tr>
<tr>
<td><em>Avena barbata</em></td>
<td>Israel</td>
<td>PI 287199</td>
<td>AABB</td>
</tr>
<tr>
<td><em>Avena vaviloviana</em></td>
<td>Ethiopia, Tigre</td>
<td>PI 412729</td>
<td>AABB</td>
</tr>
<tr>
<td><em>Avena insularis</em></td>
<td>Tunisia</td>
<td>CN 108634</td>
<td>AACC</td>
</tr>
<tr>
<td><em>Avena magna</em></td>
<td>Morocco</td>
<td>PI 657552</td>
<td>AACC</td>
</tr>
<tr>
<td><em>Avena murphy</em></td>
<td>Morocco</td>
<td>PI 657364</td>
<td>AACC</td>
</tr>
<tr>
<td><em>Avena sativa</em></td>
<td>Australia</td>
<td>PI 584783</td>
<td>AACCDD</td>
</tr>
<tr>
<td><em>Avena fatua</em></td>
<td>Russian Federation, Leningrad</td>
<td>Clav 1779</td>
<td>AACCDD</td>
</tr>
</tbody>
</table>

*Avena magna* is synonym of *Avena maroccana.*